Turkish Journal of Veterinary & Animal Sciences

Volume 48 | Number 2 Article 4

2024

The stereological and morphometrical investigation of pars lumbalis of the spinal cord inNew Zealand rabbit

BERNA DUMAN
Burdur Family Health Center, bernaduman@ogr.mehmetakif.edu.tr

İFTAR GÜRBÜZ
Burdur Mehmet Akif Ersoy Universitesi, iftar@msn.com

TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2024) 48: 107-116

doi:10.55730/1300-0128.4343

The stereological and morphometrical investigation of pars lumbalis of the spinal cord in New Zealand rabbit

Berna DUMAN[1], **İftar GÜRBÜZ**[2,*]

[1]Burdur Family Health Center, Burdur, Turkiye

[2]Department of Anatomy, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur, Turkiye

Received: 27.09.2023 • **Accepted/Published Online:** 05.03.2024 • **Final Version:** 02.04.2024

Abstract: In the study, 12 (6 females, 6 males) healthy adult New Zealand rabbits were used to examine the morphometrical and stereological values. White matter, gray matter and total segment volume of the segments in the pars lumbalis were calculated by Cavalieri's principle. The findings were grouped according to sexand statistically evaluated. As a result, 38% of the length of the spinal cord in rabbits belonged to the pars lumbalis. The morphometric parameters were not statistically different between male and female rabbits ($p > 0.05$). The segment with the biggest volume was L5. White matter's greatest volume was the L4 segment. The volume of gray matter was enormous in the L6 segment in both genders. When the average volume of lumbal segments was compared according tosex, there was no statistically significant difference ($p > 0.05$). However, the volume of each segment was greater in male rabbits than in females. In bothsexes, when the correlation values of the volume were compared with the weight and head-tail length of the rabbit, it was observed that this value was not statistically significant ($p > 0.05$). As a result, this study is thought to be a reference to the researchers to review the neuroanatomy of the lumbal part of the spinal cord in New Zealand rabbits and to localize the neurological studies to be conducted in this type.

Key words: Lumbal segment, morphometry, spinal cord, stereology

1. Introduction

The spinal cord *(Medulla spinalis)* has particular importance for the central nervous system because of its diseases and poor prognosis. The volumetric changes in the spinal cord are physiopathological changes occurring as a result of injury. Paralysis may emerge due to loss of coordination and sensation depending on the rate of lesion resulting from these physiopathological changes [1]. Particularly lumbal segment of the spinal cord is more prone to the formation of physiopathological changes because of its mobility. There are studies that have been conducted to assess and characterize such microstructural damage in the spinal cord using morphometric, histopathological, or medical diagnostic imaging methods [2, 3]. The rabbits are one of the commonly used laboratory animals in these studies [4–6]. The spinal cord is also an organ that is macroscopically or microscopically affected by factors such as age and disease just like other organs. It has been reported that quantitative data are more effective than qualitative data in the evaluation of the morphological changes in this organ [7].

The stereological and morphometrical methods based on effective sampling of biological tissues have been frequently used since 1980 for efficient calculation of volume, surface area, length, and count parameters of the tissue without prejudice [8–10]. These techniques are applied for calculation of volume, surface area and cell count [11] particularly in the brain [12,13] and spinal cord [14]. However, more detailed data have been obtained using stereological research methods in poultries [15–19], horses [7] and rats [20]. On the other side, morphometric studies [21,22] and microscopic developmental studies [11] have been conducted on the spinal cord in rabbits. However, no study that has determined the volume rates of gray matter and white matter in the lumbal segments of the spinal cord based on sex in the New Zealand rabbits has been encountered in a detailed literature review. Thus, the aim of this study is to identify the volume values of pars lumbalis of the spinal cord in terms of sexin New Zealand rabbits.

2. Materials and methods

2.1. Materials

Totally 12 New Zealand rabbits (Oryctolagus cuniculus) (6 females and 6 males) were used as experimental materials in the study to determine the morphometric and

* Correspondence: iftargurbuz@mehmetakif.edu.tr

stereological values of the lumbal segments of the spinal cord. The age range of the rabbits was 9–12 months. The reliability of the data was assured by preferring the rabbits that were raised under the same circumstances and were healthy. The animals were euthanized in accordance with ethical rules. The biometric data (body weight, head-tail length,sex) of all the animals were taken before starting the dissection procedure. In the dissection procedure, primarily soft tissues that stretch from the atlas to the first caudal vertebra and were located on the dorsal of the vertebral column (*columna vertebralis*) were removed. Then, the rabbits were kept in the fixation solution (10% formaldehyde solution) for 6 days. Following this procedure, the vertebral column was carefully broken from the region arcus vertebra and the spinal cord was exposed completely, and removed from the trunk.

2.2. Segmentation and tissue monitoring

Before starting the segmentation procedure, the overall length of the spinal cord removed from the vertebral channel or canal (*canalis vertebralis*) was measured by digital caliper and the volume of pars lumbalis was calculated by Archimedes' principle. Then, segmentation of the lumbal region of the spinal cord was performed. In the segmentation process, the vertebral canal in which each segment was located was determined as the boundary. Following the segmentation procedure, each segment was placed into a cassette and recorded. Next, primarily macroscopic measurements (weight, length, and diameter) were conducted. The length of the segment was measured from the median line, and its width was measured from the median line in a laterolateral direction with a digital caliper. The segments were fixed within 10% formol-alcohol solution, and dehydrated in a graded series of ethanol (70%, 80%, 96%, 100%: 1 h each). Then, the segments were passed through a series of methyl benzoate and benzene, and then completely blocked by paraffin [23]. The segments were subjected to a routine histological process and then completely blocked by paraffin. Following the blockade process, the method that will be applied in stereological volume calculations was determined by conducting a pilot study.

2.3. Pilot study

The coefficient of error is 0.05 and below this value is an important parameter for the reliability of the research [24]. The pilot study determined the number of the required sections and sampling technique to obtain an approximately 0.05 or a lower coefficient of error (CE) in the stereological studies. Then, the microtome sectioning procedure of segments was continued based on the sampling technique. In the preliminary study, 5µm-thick tissue sections were taken from each lumbal segment. The sampling procedure was randomly and systematically performed at a rate of 1/200 by beginning from a randomly selected section among the first 30 sections and taking the following 200th section. The method determined in the pilot study was applied in all the lumbal segments. Accordingly, 10–16 sections were obtained by sampling at the rate of 1/200 and the numbers of the sections taken from each segment were also recorded (average 2550.65 ± 485 sections for each segment).

2.4. Staining of the sections

The sections were stained with Luxol Fast Blue (Figure 1) [25] and Hematoxylin Eosin (Figure 2) [17] and stains.

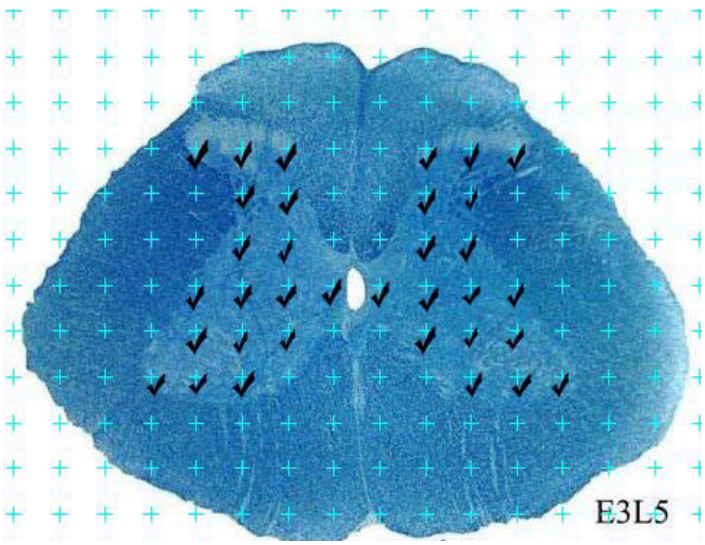

Figure 1. The male rabbit (E3) in the L5 segment, the point count process in the Gray matter (painting with Luxol Fast Blue).

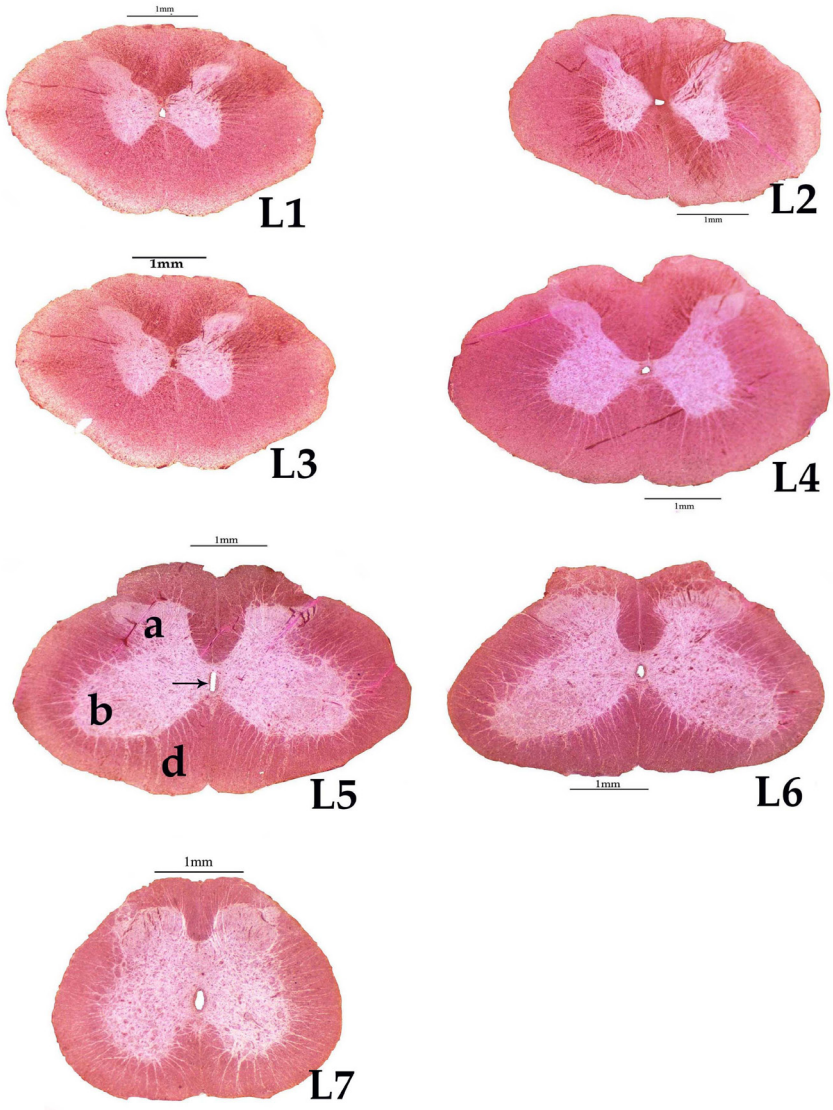

Figure 2. The images of the sections that paint with Hematoxylin Eosin taken under 2 magnification in the dissection microscope in male rabbit, **a.** Dorsal horn (Cornu dorsale, gray matter), **b.** Ventral horn (Cornu ventrale, gray matter), **Arrow.** Central channel (Canalis centralis), **d.** White matter

2.5. Microscopic measurements and volume calculation

Each segment was photographed under 2X magnification of the light microscope (Leica RM, 2135, Nussloch, Germany). The photographs were transferred to the Image J software and primarily morphometric data (laterolateral diameter and dorsoventral diameter) of the central channel of the segments were measured. The values were recorded in the Microsoft Office Excel program. Following, volumetric measurements were performed by Cavalieri's Principle (Figure 1) [24].

2.6. Statistical analysis

The descriptive statistical values of the data and comparison between sexand segment values were calculated by using the SPSS (Version 20.0) software package. The comparison between the sexwas carried out by applying the "Independent Samples T" test. The significance level of the difference between the segments was determined using "The Kruskal Wallis Test" while the difference between the mean values was determined by applying the "Tukey" test as the "Post Hoc" test. "Pearson's correlation test" was

performed between biometric values of the rabbits and white matter, gray matter and volume values of the lumbal segments in terms ofsex.

3. Results

Table 1 shows the descriptive parameters of the rabbits. As shown in the table, mean values of the descriptive parameters belonging to the male and female rabbits were similar and no statistically significant difference was present between these values ($p > 0.05$). In the rabbits, 38% of the spinal cord length belonged to pars lumbalis. The segments L1, L2, L3, L4, L5 and L6 of pars lumbalis were located towards to cranial of the same lumbal vertebra channel, however, these segments slightly shifted towards the cranial. On the other side, approximately 1/3 of the segment L7 shifted towards the cranial of the same vertebra channel. Conus medullaris terminated at the level of the second sacral vertebra.

Table 2 shows mean of the length, diameter and weight of the segments. Accordingly, the segments L1, L2, L3, L4 and L5 were long whereas the segments L6 and L7 were remarkably short. The diameters of the segments increased from L1 to L6 whereas the diameter of L7 relatively decreased. Intumescentia lumbalis was located at the segments L5, L6 and L7. However, the diameter of segment L7 got increasingly narrowed toward the caudal. It was observed that the weights of the segments increased from L1 to L5 while the weights of segments L6–L7 relatively decreased. The difference between morphometric parameters of the male and female rabbits was not statistically significant ($p > 0.05$).

Table 3 shows the volume of the lumbal segments. The highest and the lowest volume belonged to the segments L5 and L7, respectively. It was observed that the volume of the lumbal segments increased from L1 to L5 in both sexeswhereas these values declined from L5 to L7. The

Table 1. The average and standard deviation values of the descriptive parameters of the female and male New Zealand rabbits.

Descriptive parameters	Female rabbit	Male rabbit	p values
Body weight (gr)	1816.66 ± 416.7	1900 ± 497.9	0.760
Head-tail length (mm)	365.83 ± 21.39	368.00 ± 32.07	0.893
Total length of the spinal cord (mm)	275.33 ± 27.36	275.83 ± 38.44	0.980
The length of pars lumbalis of the spinal cord (mm)	106.61 ± 5.47	105.51 ± 4.01	0.700
The weight of pars lumbalis of the spinal cord (gr)	1.65 ± 0.32	1.78 ± 0.21	0.412

Comparision between gender: $p > 0.05$

Table 2. Mean and standard deviation of morphometric values of lumbal segments.

Segment	Morfometric parameters	Female rabbit Ort ± Sd	Male rabbit Ort ± Sd	p values
	Length (mm)	16.43 ± 1.10	16.57 ± 1.84	0.875
L1	Diameter (mm)	4.24 ± 0.59	4.63 ± 0.43	0.209
	Weight (g)	0.20 ± 0.04	0.21 ± 0.03	0.528
	Length (mm)	16.33 ± 0.83	16.98 ± 1.50	0.380
L2	Diameter (mm)	4.48 ± 0.43	4.77 ± 0.26	0.191
	Weight (g)	0.21 ± 0.04	0.24 ± 0.04	0.294
	Length (mm)	16.61 ± 0.94	16.60 ± 1.02	0.721
L3	Diameter (mm)	4.71 ± 0.40	4.76 ± 0.37	0.835
	Weight (gr)	0.23 ± 0.06	0.25 ± 0.04	0.481
	Length (mm)	16.48 ± 0.87	16.57 ± 1.36	0.901
L4	Diameter (mm)	5.05 ± 0.35	5.10 ± 0.38	0.826
	Weight (gr)	0.26 ± 0.06	0.27 ± 0.05	0.776
	Length (mm)	16.08 ± 1.42	15.32 ± 1.09	0.324
L5	Diameter (mm)	5.53 ± 0.49	5.67 ± 0.64	0.681
	Weight (gr)	0.30 ± 0.06	0.30 ± 0.06	0.864
	Length (mm)	13.16 ± 2.11	12.53 ± 2.65	0.657
L6	Diameter (mm)	5.93 ± 0.46	6.14 ± 0.14	0.323
	Weight (gr)	0.26 ± 0.05	0.27 ± 0.04	0.915
	Length (mm)	10.98 ± 1.65	11.20 ± 2.46	0.863
L7	Diameter (mm)	5.48 ± 0.59	5.83 ± 0.47	0.294
	Weight (gr)	0.17 ± 0.04	0.22 ± 0.09	0.280

Comparision between genders: $p > 0.05$

Table 3. The average volume and standard deviation of pars lumbalis of the spinal cord in female and male rabbits. (p values for male and female comparison).

Segment	Female rabbits (mm^3)	Male rabbits (mm^3)	p values (between genders)
L1	112.20 ± 25.47	126.16 ± 27.71	0.376
L2	120.94 ± 31.25	139.24 ± 33.34	0.359
L3	127.94 ± 35.84	145.55 ± 28.30	0.342
L4	134.23 ± 34.97	154.29 ± 34.53	0.526
L5	150.30 ± 43.16[b]	161.24 ± 40.17[a]	0.626
L6	140.32 ± 35.14[b]	134.31 ± 20.19	0.768
L7	73.73 ± 25.25[a]	96.06 ± 54.38[b]	0.374
Total	867.18 ± 188.34	956.89 ± 135.93	

Letters indicate statistical significance resulting from within-group segment comparison ($p < 0.05$). No statistical differences between sex ($p > 0.05$).

difference between the volume of the lumbal segments in terms of sexwas not statistically significant ($p > 0.05$).

Table 4 shows the mean volume and standard deviation values of the white matter. As given, the highest and the lowest volume of the white matter belonged to the segments L4 and L7, respectively. It was observed that the mean volume of the white matter increased from the segment L1 to L4 in both sexes whereas these values decreased from the segment L4 to L7. The volume of white matter was higher in the male rabbits, however, this difference was not statistically significant ($p > 0.05$).

The evaluation of the data given in Table 4 revealed that the highest and the lowest volume of the gray matter belonged to the segments L6 and L1 in both sexes, respectively. The volume of gray matter increased from segment L1 to L6 while its volume relatively declined in segment L7. Accordingly, there was no statistical difference between males and females.

The comparison between the volumes of gray matter and white matter of the same sexaccording to their segments revealed the presence of statistically significant differences ($p < 0.05$) (Table 4). However, there was no difference in the volume of the same segment according to sex($p > 0.05$).

The highest and lowest volume rate of gray matter/segment shown in Table 4 belonged to the segments L7 and L1, respectively. It was also detected that the volume rate of gray matter/segment increased significantly towards the caudal ($p < 0.05$).

According to Table 5, the largest laterolateral diameters of the central channel were measured in the segments L5 and L6 in the female rabbits while the largest laterolateral diameters were determined in the segments L4 and L5 in the male rabbits. The largest dorsoventral diameter of this canal was assessed in the segments L6 and L7. When male and female rabbits were compared in terms of dorsoventral diameter of the central channel, it was observed that the value of the segment L4 was statistically significant ($p < 0.05$).

Table 6 compares the mean volume of pars lumbalis of the spinal cord, which was removed from canalis vertebralis after fixation process using formaldehyde, calculated with Archimedes' principle and the mean volume calculated according to Cavalieri's principle after histological procedures. The shrinkage ratios were 17.93% and 18.56% in female and male rabbits, respectively.

Table 7 shows the correlation values of some parameters taken from male and female rabbits. Accordingly, the ratio of gray matter to pars lumbalis generally showed a negative correlation with the given values in the lumbal segments of the spinal cord in both male and female rabbits. In bothsex, the correlation between volume (volume of pars lumbalis, volume of gray matter and white matter) and weight was not statistically significant ($p > 0.05$). Likewise, the correlation between the values of head-tail length and volume of pars lumbalis was not statistically significant ($p > 0.05$). There was a positive correlation between the overall weight and volume of the lumbal segments ($p < 0.05$). However, no significant correlation was detected between the volume of gray matter and segment weight or rabbit weight ($p > 0.05$).

4. Discussion

The gray and white matter of the central nervous system are characteristics measured and compared between animal species. Gray matter is the region of input-output processing units (neuronal somato). White matter consists of myelin tracts that connect neurons. Decreased gray and white matter volumes serve as a diagnostic measurement in neurological disorders [26, 27]. For example, in neurodegenerative diseases of the spinal cord, death of motor neurons may cause a decrease in gray matter volume

Table 4. The average volume and standard deviation of the white matter, gray matter of the lumbal segments and gray matter's ratio of total segment in female and male rabbits. (p values for male and female comparison) (S: segment, T: total).

S	Female rabbit	Male rabbit	p	Female rabbit	Male rabbit	p	Female rabbit	Male rabbit	p
	White matter (mm^3)			Gray matter (mm^3)			Gray matter's ratio (%)		
L1	91.65 ± 23.6[a]	105.57 ±24.83[a]	0.330	20.19 ± 4.38[a]	20.58 ± 3.10[a]	0.982	18.37 ± 3.49[ab]	16.54 ± 1.61[ab]	0.164
L2	98.48 ± 26.98[a]	115.31 ± 30.07[a]	0.349	22.05 ± 5.06[a]	23.92 ± 4.16[a]	0.485	18.45 ± 2.20[ab]	17.50 ± 2.67[abc]	0.593
L3	102.73 ± 33.19[a]	116.52 ± 25.21[a]	0.417	24.82 ± 5.05[ac]	28.57 ± 3.95[ac]	0.145	19.95 ± 3.64[ab]	19.86 ± 2.30[abc]	0.922
L4	109.34 ± 30.1[a]	117.90 ± 27.70[a]	0.559	32.04 ± 8.64[ad]	35.69 ± 8.33[a]	0.450	21.71 ± 3.40[ca]	23.22 ± 2.68[abc]	0.784
L5	106.38 ± 32.03[a]	113.27 ± 26.44[a]	0.622	43.07 ± 13.38[bd]	47.44 ± 17.74[bc]	0.658	28.90 ± 3.82[c]	29.03 ± 6.47[dc]	0.934
L6	85.28 ± 27.83[a]	79.44 ± 19.98[ab]	0.716	54.46 ± 8.14[b]	54.25 ± 13.42[b]	0.898	39.69 ± 4.29[d]	40.81 ± 10.77[d]	0.763
L7	34.36 ± 14.04[b]	49.89 ± 39.79[b]	0.417	38.95 ± 12.54[bcd]	45.68 ± 16.04[bc]	0.364	52.88 ± 6.09[e]	54.47 ± 11.78[e]	0.938
T	628.11 ± 161.47	697.93 ± 111.31		235.60 ± 30.36	256.17 ± 35.02		27.98 ± 3.04	26.8 ± 2.84	0.164

Letters indicate statistical significance resulting from within-group segment comparison ($p < 0.05$). No statistical differences between sex ($p > 0.05$).

Table 5. The average and standard deviation of the diameter of central channel of pars lumbalis in female and male rabbits.

Segment	Diameter (mm)	Female rabbit	Male rabbit	p values
L1	Laterolateral	0.08 ± 0.04	0.09 ± 0.04	0.617
	Dorsoventral	0.15 ± 0.02	0.17 ± 0.05	0.450
L2	Laterolateral	0.08 ± 0.04	0.08 ± 0.02	0.352
	Dorsoventral	0.13 ± 0.03	0.13 ± 0.01	0.841
L3	Laterolateral	0.08 ± 0.02	0.10 ± 0.05	0.415
	Dorsoventral	0.12 ± 0.03	0.12 ± 0.02	0.614
L4	Laterolateral	0.09 ± 0.02	0.11 ± 0.02	0.319
	Dorsoventral	0.10 ± 0.01*	0.13 ± 0.02*	0.022
L5	Laterolateral	0.10 ± 0.04	0.11 ± 0.01	0.585
	Dorsoventral	0.12 ± 0.04	0.15 ± 0.03	0.347
L6	Laterolateral	0.10 ± 0.04	0.09 ± 0.01	0.497
	Dorsoventral	0.22 ± 0.04	0.21 ± 0.04	0.665
L7	Laterolateral	0.08 ± 0.02	0.09 ± 0.008	0.282
	Dorsoventral	0.21 ± 0.03	0.22 ± 0.03	0.594

*: $p < 0.05$

Table 6. Average volume values and shrinkage rates determined by Archimet and Cavalieri principle in pars lumbalis of the spinal cord.

New Zealand rabbit	Archimet principle (mm^3)	Cavalieri principle (mm^3)	Shrinkage rates
Female	1056.66	867.18	17.93%
Male	1175	956.89	18.56%

Table 7. Correlation values between some parameters of the spinal cord in female and male rabbits.

Female→ Male↓	W	HTL	TLM	TLL	VL	TVSA	TVSG	WL	VRSG
W		.992**	.903*	.882*	.684	.721	.347	.868*	-.808
HTL	.897*		.901*	.860*	.755	.791	.415	.918**	-.871*
TLM	.880*	.931**		.617	.599	.625	.329	.821*	-.763
TLL	.668	.756	.810		.646	.680	.345	.725	-.679
VL	.734	.793	.673	.565		.996**	.864*	.939**	-.895*
TVSA	.775	.827*	.742	.718	.978**		.815*	.952**	-.927**
TVSG	.370	.444	.253	-.065	.771	.621		.705	-.568
WL	.966**	.941**	.865*	.661	.873*	.887*	.557		-.945**
VRSG	-.573	-.535	-.658	-.934**	-.363	-.548	.311	-.498	

*p < 0.05, **p < 0.01. W. weight, HTL. head-tail length, TLM. total length of spinal cord, TLL. Total length of pars lumbalis, VL. volume of pars lumbalis, TVSA. total volume of white matter of pars lumbalis, TVSG. total volume of gray matter of pars lumbalis, WL. Weight of pars lumbalis, VRSG. volume ratio of gray matter to pars lumbalis.

[28]. The lumbal segments of the spinal cord are important in that the spinal nerves branching from here form the plexus lumbosacralis, which provides innervation to the hind legs and pelvic regions [29].

In this study, it was aimed to determine the morphometric values of the lumbal segments of the spinal cord. The morphometric values such as the diameter or length of the segments in the spinal cord are the parameters used as a diagnostic tool in the studies conducted using postmortem [30, 31] or medical imaging methods [32, 33]. The overall length of the spinal cord in rabbits has been reported to be 27.4 cm [21]. Also in the present study, the length of the spinal cord in the New Zealand rabbit was determined to be 27.5 cm, which is similar to the value reported by Kahvecioglu et al. [21]. The ratio of the length of pars lumbalis of the spinal cord to the overall length of the spinal cord in the rabbit was reported to be 34.36% [11] and 34% [22]. In the present study, this rate was found to be 38% in female and male rabbits.

The longest lumbal segment of the spinal cord was L2 (20 mm) in rabbit [22], while it was L1 in horse [14], donkey [34], human [31] and monkey [35]. In the study, the longest segment was L3 (16.61 ± 0.94) in female rabbits and L2 (16.98 ± 1.50) in male rabbits. It has been reported that L3 is the longest segment in the Angora goat [21] consistent with the female New Zealand rabbit. The findings of this study are inconsistent with Farag et al. [22]'s report. This difference is thought to be due to the different breeds of rabbits (Chincillas and Angora rabbits). The shortest lumbal segment was L6 (11.5 mm) in rabbits [22] and (the last lumbal segment) in horses [14]. In the New Zealand rabbit, the shortest segment was L7 (10.98 mm and 11.20 mm in females and males). When the data were compared with the literature, it was seen that the shortest segment was the last lumbal segment and it was similar to the horse.

The vertical diameter of the central channel is significantly larger than the transversal diameter in pars lumbosacralis of the spinal cord in Leghorn chickens [16]. In addition, when the volume ratios of the central channel were compared according to sex, it was statistically significantly larger ($p < 0.05$) in male chickens [16]. Similar to the literature [16] in New Zealand rabbits, the vertical diameter of the central channel was significantly larger than the transversal diameter, and the diameter of the central channel was generally larger in male rabbits than in female rabbits. In biometric studies, differences in morphological structure are detected according to sex. It is thought that the morphometric change in central canal diameter is caused by hormonal differences during growth.

The length and diameter of the lumbal segments in the rabbits are compared in Table 8. According to the data in Table 8, the length of L1, L2, L3, L4, L5 and L7 in female and male New Zealand rabbits was smaller and the the length of L6 was relatively larger than the rabbits reported by Farag et al. [22]. The lateral diameter of the L1, L2, and L3 (4 mm) was the same in rabbits. Kahvecioglu et al. [21] reported that the lateral diameter in rabbits was the same in the L1 and L2 segments (4.1 mm), and it expanded from L2 to L7. Unlike studies [21,22], it was found that the lateral diameter tended to widen from L1 to L6 in New Zealand rabbits, while it was relatively narrow in the L7 segment. However, the lateral diameter of the L1, L2, L3, L4, L5, and L6 in female and male New Zealand rabbits was relatively close to the references [21, 22]. The lateral diameter of the L7 was smaller, unlike the literature [21,

Table 8. Comparison of the data of lumbal segments of spinal cord in some rabbit breeds with the findings data in the male and female New Zealand rabbits used in the study.

Segment	Morphometrical parameters	Female (New Zealand Rabbit)	Male (New Zealand Rabbit)	Chincillas and Angora rabbits [22]	Rabbit [21]
L1	Length (mm)	16.43	16.57	18.9	-
	Lateral diameter (mm)	4.24	4.63	4.0	4.1
L2	Length (mm)	16.33	16.98	20.0	-
	Lateral diamater (mm)	4.48	4.77	4.0	4.1
L3	Length (mm)	16.61	16.60	19.2	-
	Lateral diameter (mm)	4.71	4.76	4.0	4.3
L4	Length (mm)	16.48	16.57	16.9	-
	Lateral diameter (mm)	5.05	5.10	5.0	5.0
L5	Length (mm)	16.08	15.32	18.9	-
	Lateral diameter (mm)	5.53	5.67	6.0	5.4
L6	Length (mm)	13.16	12.53	11.5	-
	Lateral diameter (mm)	5.93	6.14	6.0	6.2
L7	Length (mm)	10.98	11.20	12.8	-
	Lateral diameter (mm)	5.48	5.83	6.6	6.5

22]. In this study, it was determined that the diameter of the L7 segment gradually narrowed caudally. It is thought that the reason for the difference from the findings of the researchers [21,22] is that the measurement location of the L7 segment is different. Because the width of the L7 segment in the cranial was larger than L6, then narrowed to the caudal.

Similarly with literature data [16], volumes of white matter and gray matter were remarkably higher in the males than females in the New Zealand rabbits and Leghorn breed chickens [16] while the ratio of gray matter to overall volume was relatively higher in females than males.

The gray matter of the spinal cord is part of the autonomic nervous system. In addition, it forms the somatomotor center of neurons that provide sensory and motor activities of the legs and trunk. Therefore, the amount of gray matter volume is associated with the function of the region [36]. To interpret the calculated gray matter volume capacity, the function of the region in which it is located should be taken into account [36]. Accordingly, the volume of gray matter varies in line with the sensorimotor needs of the spinal nerves leaving the lumbal segment. In light of literature findings [7, 14, 37, 38], the volume rate of gray matter to white matter relatively increased from cranial to caudal in each segment of the spinal cord. Also in the present study, the volume of gray matter increased from segment L1 to segment L7 whereas the volume of white matter decreased, which is compatible with the literature data [7, 14, 38]. It has been reported that in the New Zealand rabbit, the plexus lumbosacralis is formed by the last four lumbal spinal nerves and the radix ventralis of the first caudal spinal nerve [39]. Nerves leaving the plexus lumbosacralis provide the motor activity of the hind leg. Therefore, the backward increase in gray matter volume may be a functional requirement to ensure motor activity of the hind leg.

In the study conducted by Bakıcı et al. [40] on the spinal cord of chicken and quails it was stated that Cavalieri's Principle was an objective, accurate and effective method for estimation of the volume. In that study [40], it was reported that the volumetric shrinkage rates were 39.16% and 41.32% before and after the fixation procedure, respectively, 14.97% and 12.41% after the fixation procedure and application of Cavalieri's Principle, respectively, and 48.29% and 48.5% before the fixation procedure and after application of Cavalieri's Principle, respectively. Selcuk and Bahar [14] reported that the shrinkage rate was 26.2% in the lumbal segments of the spinal cord after the histological procedures in horses. On the other hand, in the present study, shrinkage rates in the lumbal segments after the histological procedures were determined to be 17.93% and 18.56% in the female and male New Zealand rabbits, respectively. Shrinkage rates (17%–18%), which may be a practical problem for those working on live animals, should be taken into consideration. However, this study

has some limitations concerning the shrinkage rate of the spinal cord. Because of the fragility of the study material, lumbal spinal segments could not be removed as a single part through canalis vertebralis from the fresh cadaver without impairing tissue integrity. The segmentation procedure of the cadavers could be completed properly after the formaldehyde fixation procedure. Thus, volume could not be measured before fixation (in fresh cadaver) and hence shrinkage ratio data could not be compared with the fresh cadaver.

The results obtained with the Cavalieri principle were compared with the archimental principle in some studies, and realistic data were obtained [20, 40, 41]. Therefore, the interpretations made based on the data obtained as a result of stereological studies are correct, reliable and acceptable [42].

As a consequence, it is thought that this study would allow reviewing the clinical neuroanatomy of pars lumbalis of the spinal cord in New Zealand rabbits (Oryctolagus cuniculus) and lumbal spinal cord volume ratio enables evaluation of spinal cord atrophy with lumbal spinal lesions without being affected by the interindividual variations. This study could be a reference for the researchers in the localization of the neurological investigations in this species.

Acknowledgment
We sincerely thank the Burdur Mehmet Akif Ersoy University Scientific Research Projects Coordinator (project number: 0585–YL–19) for supporting this research work. This study is summarized from the Master Thesis titled "The Stereological and Morfometrical Investigation of Pars Lumbalis of Spinal Cord in New Zealand Rabbit" written by Berna Duman.

Conflict of interest
We declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Ethical approval
The approvals were obtained from the Animal Experiments Local Ethics Committee of Mehmet Akif Ersoy University (Date: 13.02.2019, Number: 490) before the study.

References

1. Leener BD, Taso M, Cohen-Adad J, Callot V. Segmentation of the human spinal cord. Magnetic Resonance Material in Physics 2016; 29: 125-153. https://doi.org/10.1007/s10334-015-0507-2
2. Ozyurt B, Kesici H, Alıcı SK, Yılmaz S, Odacı E et al. Prenatal exposure to diclofenac sodium changes the morphology of the male rat cervical spinal cord: A stereological and histopathological study. Neurotoxicology Teratology 2011; 33: 282-287. https://doi.org/10.1016/j.ntt.2011.01.002.
3. Arıkanoglu A. Tavşanlarda epidural aralığa verilen magnezyum sülfat medulla spinalis'te hasar oluşturur mu? Phd Thesis, Dokuz Eylül University, İzmir, Turkey, 2015 (in Turkish).
4. Lang-Lazdunski L, Matsushita K, Hirt L, Waeber CH, Vonsattel, JPG, et al. Spinal cord ischemia. Development of a model in the mouse. Stroke 2000; 31: 208-213. https://doi.org/10.1161/01.str.31.1.208
5. Demircioglu İ, Demiraslan Y, Gurbuz I, Dayan MO. Examination of the topography and morphometry of hypophysis (Glandula pituitaria) by computed tomography in new zealand rabbits. Atatürk Universitesi Veteriner Bilimleri Dergisi 2021; 16 (2): 170-175. https://doi.org/10.17094/ataunivbd.870886
6. Oktay T, Demiraslan Y. Intracranial arteries of New Zealand rabbits; identification, three-dimensional modelling, and morphometry by computed tomography angiography. Anatomia Histologia Embryologia 2021; 50: 707-715. https://doi.org/10.1111/ahe.12679
7. Bahar S, Bolat D, Selcuk ML. The segmental morphometric properties of the horse cervical spinal cord: A study of cadaver. Scientific World Journal 2013; 734923: 9. https://doi.org/10.1155/2013/734923
8. Howard CV, Reed MG. Unbiased Stereology: Three dimensional measurement in microscopy. BIOS Scientific Publishers. Oxford, 1998.
9. Mouton, PR. Principles and Practices of Unbiased Stereology, John Hopkins University Press, Baltimore, Md, USA, 2002.
10. Jashari T, Duro S, Gundemir O, Szara T, Ilieski V et al. Morphology, morphometry and some aspects of clinical anatomy in the skull and mandible of Sharri sheep. Biologia, 2021; 77: 423-433.
11. Al- Saffar FJ, Al-Haaik AG. Gross and microscopic developmental study of the local rabbits spinal cord. Journal of Entomology and Zoology Studies 2017; 5 (6): 2555-2562.
12. Bahar S, Dayan MO. Volumetric estimations of the gray matter, white matter and lateral ventricles on the brain hemispheres in horses using cavalieri principle. Eurasian Journal of Veterinary Sciences 2014; 30 (2): 102-107. https://doi.org/10.15312/EurasianJVetSci.201425927
13. Yucel F, Unal N, Ercakır M, Guven G. Determination of age-related volume changes in rat brain by Cavalieri's Method. Erciyes Medical Journal 2003; 25 (4): 179-185.
14. Selcuk ML, Bahar, S. The morphometric properties of the lumbar spinal cord segments in horses. Journal of Animal and Veterinary Advances, 2014; 13 (11): 653-659.

15. Turgut M, Tunc AT, Aslan H, Yazici AC, Kaplan S. Effect of pinealectomy on the morphology of the chick cervical spinal cord: A stereological and histopathological study. Brain Research, 2007; 1129 (1): 166-173.

16. Bolat D. Leghorn ırkı kanatlılarda medulla spinalis'in stereolojik metotlar ile incelenmesi. Selçuk Üniversitesi Sağlık Bilimleri Enstitüsü, Konya, Turkey, 2011 (in Turkish).

17. Cakmak G, Soyguder Z, Ragbetli MC. A morphological and stereological study on cervical segment of spinal cord of quails. Anatomia Histologia Embryologia 2017; 46: 258-266. https://doi.org/10.1111/ahe.12265

18. Cakmak G, Karadag H. A morphological and stereological study on calculating volume values of thoracic segments of geese. Folia Morphologica 2019; 78 (1): 145-152. https://doi.org/10.5603/FM.a2018.0115

19. Cakmak G, Soyguder Z. A stereological study on lumbosacral segments of spinal cord in the geese. Turkish Journal of Veterinary and Animal Science 2022; 46 (4): 647-658. https://doi.org/10.55730/1300-0128.4236

20. Candan M, Cakmak G. A morphological and stereological study on cervical spinal cord of one and five months age male rat. Bitlis Eren University Journal of Science 2020; 1: 143-156. https://doi.org/10.17798/bitlisfen.657678

21. Kahvecioglu KO, Ozcan S, Cakır M. Tiftik keçisinde medulla spinalis üzerinde anatomik çalışmalar. Yüzüncü Yıl Universitesi Veteriner Fakultesi Dergisi, 1995; 6 (1-2): 76-78.

22. Farag FM, Elayat MA, Wally YR, Elkarmoty AF. Morphometric studies on the spinal cord segments of the domestic rabbit. Journal of Veterinary Anatomy 2012; 5 (2): 33-47.

23. Öztop M, Özbek M, Beyaz F, Köknur S, Ergün E et al. Expression patterns of natriuretic peptides in pre-hibernating and hibernating Anatolian ground squirrel (Spermophilus xanthoprymnus) kidney. Veterinary Research Communications 2019; 43: 249-259. https://dx.doi.org/10.1007/s11259-019-09767-7

24. Gundersen HJ, Jensen EB. The efficiency of systematic sampling in stereology and its prediction. Journal of Microscopy 1987; 147: 229-263. https://doi.org/10.1111/j.1365-2818.1987.tb02837.x

25. Charles J, Churukian BA. 2009. Method of the histochemical stains and diagnostic application. University Of Rochester Medical Center Rochester, New York Second Web Edition; 2009.

26. Gilmore CP, DeLuca GC, B€o L, Owens T, Lowe J et al. Spinal cord atrophy in multiple sclerosis caused by white matter volume loss. Archives of Neurology 2005; 62: 1859–1862. https://doi:10.1001/ archneur.62.12.1859

27. Kearney H, Miller DH, Ciccarelli O. Spinal cord MRI in multiple sclerosis– diagnostic, prognostic and clinical value. Nature Reviews Neurology 2015; 11: 327-338. https://doi:10.1038/nrneurol.2015.80

28. Paquin ME, El Mendili MM, Gros C, Dupont SM, Cohen-Adad J et al. Spinal cord gray matter atrophy in amyotrophic lateral sclerosis. American Journal of Neuroradiology 2018; 39: 184–192. https://doi:10.3174/ajnr.A5427

29. Demiraslan Y, Tıpırdamaz S. Systema Nervosum. In: Veteriner Sistematik Anatomi. Edt: Demiraslan Y, Dayan MO, Nobel Tıp Kitabevleri, İstanbul; 2021.

30. Kameyama T, Hashizume Y, Sobue G. Morphologic features of the normal human cadaveric spinal cord. Spine 1996; 21 (11): 1285-1290.

31. Ko HY, Park JH, Shin YB, Baek SY. Gross quantitative measurements of spinal cord segments in human, Spinal Cord 2004; 42 (1): 35-40.

32. Choi D, Carroll N, Abrahams P. Spinal cord diameters in cadaveric specimens and magnetic resonance scans, to assess embalming artefacts. Surgical and Radiologic Anatomy 1996; 18 (2): 133-135.

33. Costa RC, Parent JM, Partlow G, Dobson H, Holmberg DL et al. Morphologic and morphometric magnetic resonance imaging features of Doberman Pinschers with and without clinical signs of cervical spondylomyelopathy. American Journal of Veterinary Research 2006; 67 (9): 1601-1612.

34. Hazıroglu M, Ocal MK. Merkebin (Equus Asinus L.) medulla spinalis'i üzerinde komparatif morfolojik araştırmalar II. Segmentlerin Topografik incelenmesi. Ankara Universitesi Veteriner Fakültesi Dergisi 1988; 35 (2-3): 476-487.

35. Thomas CE, Combs CM. Spinal cord segments. B. Gross structure in adult monkey. The American Journal of Anatomy 1965; 116: 205-216.

36. Henmar S, Simonsen EB, Berg RW. What are the gray and white matter volumes of the human spinal cord? Journal of Neurophysiolgy 2020; 124: 1792-1797. https://doi:10.1152/jn.00413.2020.

37. Rahmanifar F, Mansouri S, Ghazi S. Histomorphometric study of the spinal cord segments in the chick and adult male ostrich (Struthio camelus). Iranian Journal of Veterinary Research 2008; 9 (4): 336-340. https://doi.org/10.22099/IJVR.2008.2615

38. Arkac Toyran A, Cakmak G. A morphological and stereological study on thoracal spinal cord of one and five months age male rat. Bitlis Eren Universitesty Journal of Science 2021; 10 (1): 67-81.

39. Çiftçi N. Yerli kedi (Felis domestica L.) ve Yeni Zelanda tavşanının (Oryctologus cuniculus L.) Plexus lumbosacralis'li üzerinde komparatif mikroanatomik ve subgros araştırmalar. Ankara Universitesi Sağlık Bilimleri Enstitüsü, Ankara, Turkey, 1993 (in Turkish).

40. Bakıcı C, Oto Ç, Ekim O, Ahlat O, Ozen D et al. Comparison of the volume fraction values of gray matter on the cervical enlargement of the spinal cord in chicken and quail. Ankara Universitesi Veteriner Fakultesi Dergisi 2019; 66: 1-6.

41. Turkmenoglu I, Kocak GK, Akosman MS. Volume estimation of the kidneys by stereological methods. Kocatepe Veterinary Journal 2016; 9 (4): 304-307. https://doi.org/021110800027930

42. İkinci Keleş A. Sağlık alanında kullanılan kantitatif yöntem, Stereoloji. Dicle Universitesi Tıp Fakültesi Dergisi 2019; 46 (3): 615-621 (in Turkish).