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Variations in the median sacral crest and angulation of the first sacral spinous process associated with sacrocaudal fusion in greyhounds

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Abstract: The current study aimed to investigate the association between the morphology of the median sacral crest, variation in the angle of the spinous process of the first sacral vertebra, and the occurrence of sacrocaudal fusion in greyhounds. In this study, 167 sacra from cadavers of greyhounds (previously euthanized for reasons unrelated to this study) were collected, classified into standard and fuses sacra (based on the number of fused vertebrae and type of fusion), and then classified based on the morphology of the median sacral crest into three different types: type F (full crest), type N (when the median sacral crest is incomplete, and type R (when the median sacral crest is present but it was short or reduced). Among the 167 sacra, 91 sacra were used to measure the angle of the spinous process of the first sacral vertebrae (1st SPA). Each of the sacra was digitally photographed using a Nikon D3100 digital camera (Tokyo, Japan) in which the camera was positioned laterally with the lens parallel to the central part of the lateral aspect of the sacrum and on a flat surface of the laboratory bench. Image-Pro Express Version 5.0 imaging software (Media Cybernetics, Silver Spring, Maryland) was used to draw and measure the angles. The angle of the 1st SPA was defined as the angle formed dorsally at the intersection of two lines. The first line was drawn to represent the cranial ridge of the spinous process of the 1st SPA, and the second line was drawn across the most dorsal surface points of the spinous processes of the 1st (S1) and 3rd (S3) sacral vertebrae. Significant ($p < 0.001$) morphological differences (F, R, N) were found in the median sacral crest, and the prevalence of median sacral crest type R in standard sacra was 35.1% compared to 10% in fused sacra, type F was 41.2% in standard sacra and 10% in fused sacra, and type N was 23.7% in standard sacra and 80% in fused sacra. The angle 1st SPA with median sacral type N was statistically significantly less (more upright) than those in sacra with median sacral type F ($P < 0.042$). Differences have been found in the median sacral crest and angulation of the spinous process of S1 vertebra in sacra with different types of median sacral crest.

Key words: Greyhounds, median sacral crest, sacral spinous process, sacrocaudal fusion

1. Introduction

Sacra are morphologically diverse among animal species [1]. Back in the 1940s, Slijper (1946) developed many body-axis models and indicated that there was a relation between the length of the spinous process of vertebrae and body size in many animal species [2]. Moreover, variations in the morphology of different parts of the vertebrae have been described and studied in different species [3–5]. Santinelli et al. (2016) found an association between the shape of the spinous process of the C7 vertebra and the breeds of horses, also an association between the shape of the spinous process of the C7 vertebra and the shape of T1 vertebra.

In the canine sacrum, which is usually formed from the fusion of three sacral vertebrae, a median sacral crest is present between the spinous processes of the sacral vertebrae, and those spinous processes stand in an upright position, almost perpendicular to the sacrum [6].

Sacral spinous processes are fused to form the median sacral crest [7]. In sheep, the sacrum consists of four sacral vertebrae of which the last was found to be either incompletely or partially fused [8]. The median sacral crest in the sacrum in dog exists between the three spinous processes of all sacral vertebrae. The objective of this study was to investigate the factors, which might affect the morphology of the median sacral crest in greyhounds, such as body weight, sex, sacrum weight, length and width, and the occurrence of sacrocaudal fusion.

2. Materials and methods

2.1. Subjects

In this study, 171 greyhound cadavers were used from Melbourne, Australia, 94 of which were males and 77 were females. The ages of greyhounds were more than two years old. The age was estimated by examining the teeth of each cadaver. For studying the median sacral crest, 167 sacra (97 standard and 70 fused) were used. Among the same

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167 sacra collected, 91 sacra were used to measure the angle of the spinous process of the first sacral vertebrae. The samples used in this experiment were collected from cadavers donated to the university, the animals having been previously euthanized for reasons not associated with this study.

2.2. Classification of sacra

Based on the number of fused vertebrae and type of fusion, the sacra were first classified into standard and fused sacra based on the occurrence and types of sacrocaudal fusion [9]. However, in the current study, the following letter classifications were used: (A) for the standard sacrum (three fused vertebrae), (B) fused sacrum (either the complete fusion occurred between the 3rd sacral (S3) the and 1st caudal (Cd1) vertebrae, only fusion between the transverse processes of the S3 and Ca1 vertebrae, or only a body fusion between the bodies of the S3 and Cd1 vertebrae). Then, they were classified based on the morphology of the median sacral crest into three different types: type F (standard type) when a complete fusion (full crest) was present between the spinous processes of the 1st and 2nd sacral vertebrae, type N when fusion was incomplete between the spinous processes of the 1st and 2nd sacral vertebra making the median sacral crest incomplete, and type R when the median sacral crest was present but it is short or reduced between the spinous processes of the 1st and 2nd sacral vertebrae. Sacra were independently classified twice by the same investigator (with the investigator blinded as to the previous classification) and one more time by another investigator.

2.3. Measuring the angle of the spinous process of the first sacral vertebra (1st SPA)

The angle of the cranial edge of the spinous process of the 1st (S1) sacral vertebra (1st SPA) was defined as the angle formed dorsally at the intersection of two lines. The first line (line ac) was drawn to represent the cranial ridge of the spinous process of the 1st sacral vertebra, and the second line (line ab) was drawn across the most dorsal surface points of the spinous processes of the 1st (SPS1) and 3rd (SPS3) sacral vertebrae (Figure 1). Image-Pro Express Version 5.0 imaging software (Media Cybernetics, Silver Spring, Maryland) was used to draw the lines.

2.4. Imaging

Each of the sacra was digitally photographed using a Nikon D3100 digital camera (Nikon, NIKON Corp., Japan) with the camera positioned laterally and the len parallels to the central part of the lateral aspect of the sacrum and on a flat surface of the laboratory bench. The distance between the camera and the sacra was kept constant for all photographs (Figure 2).

Image-Pro Express Version 5.0 imaging software (Media Cybernetics, Silver Spring, Maryland) was used to draw and measure the angles. The angle formed between the two drawn lines from the spinous process of the 1st sacral vertebra was measured and then subtracted from 180° to get the angle (denoted *) of the cranial edge of the spinous process of the 1st sacral vertebra. Two left lateral photographs were taken for each sacrum, and two repeated measurements were taken for the angle.

2.5. Statistical analyses

Data were analyzed using the SPSS statistical tool version 23 (IBM Corp., Armonk, NY, USA). For continuous measurements, intra-rater and inter-rater reliability (test-retest reliability) were assessed using Lin's concordance correlation coefficient [10,11]. Concordance correlation coefficient values range from –1 to +1, with +1 indicative of perfect agreement. While, for continuous nominal (categorical) variables, the Kappa statistics test was performed, and the strength of agreement was evaluated in

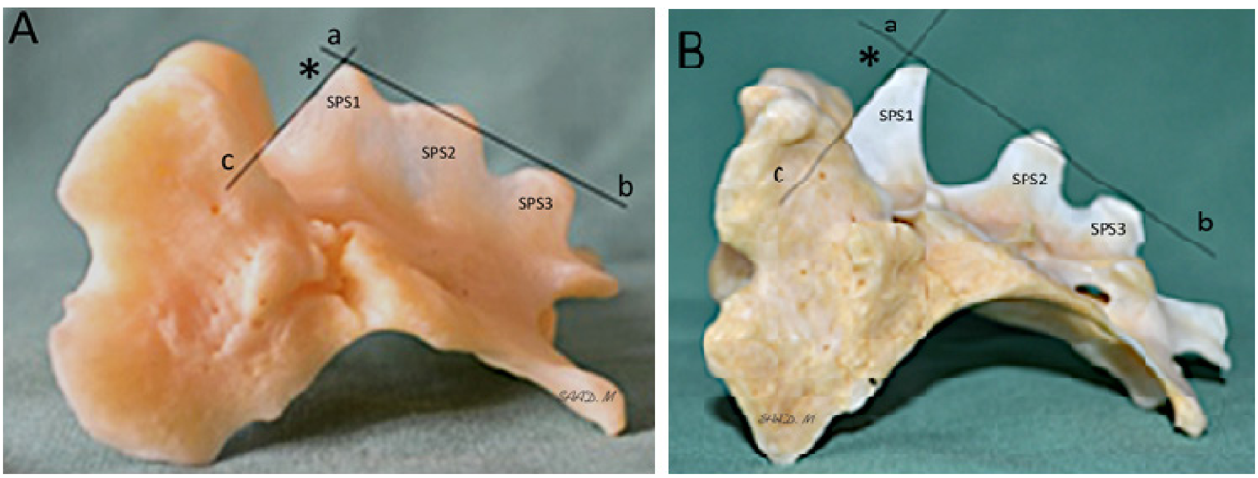

Figure 1. Measurement of the angle of the cranial edge of the spinous process of the first sacral vertebra in lateral aspects of standard (A) and fused (B) sacra in greyhounds.

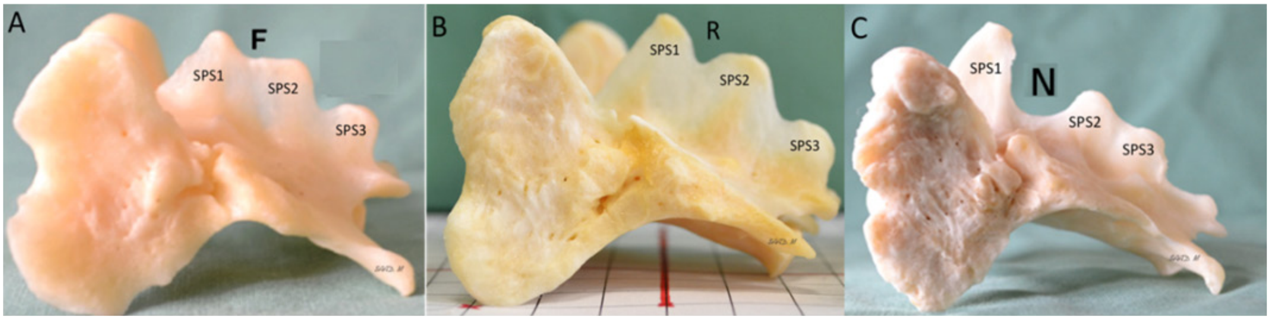

Figure 2. Classification of the median sacral crest in greyhounds. A: Standard type; Type F: when a complete fusion (full crest) is present between the spinous processes of the 1st and 2nd sacral vertebrae. B: Type R: When the median sacral crest is present, but it is short or reduced between the spinous processes of the 1st and 2nd sacral vertebrae. C: Type N: when fusion is incomplete (incomplete median sacral crest) between the spinous processes of the 1st and 2nd sacral vertebrae, making the median sacral crest incomplete. SPS1, SPS2, and SPS3 are spinous processes of the 1st, 2nd, and 3rd sacral vertebrae, respectively.

accordance with Landis and Koch [12]. The strength of the agreement was scaled as follows: poor agreement (when the Kappa value is less than zero), a slight agreement (when the Kappa range is between 0.00–0.20), fair agreement (when Kappa is between 0.21–0.40), moderate (when Kappa is between 0.41–0.60), substantial (when Kappa is between 0.61–0.80), and almost perfect agreement when Kappa is 0.81–1.00. Descriptive statistics of sacrum measurements for all greyhounds and for greyhounds stratified by median sacral classification (R, F, and D) are provided. Histograms of sacrum measurements were plotted to confirm that the data follows a normal distribution.

The normality of each plotted distribution was assessed using the Shapiro–Wilk test. The equality of the variances for each of the measurements for each of the sacrum types (R, F, and N) was assessed using Levene's test.

Multiple linear regression analysis was used to quantify the association between sacral weight (as the outcome variable) and sacral type (standard or fused), median sacral crest classification (R, F, and N), body mass, and sex (as explanatory variables). Median sacral crest type F was considered as the standard type. Multiple linear regressions provided estimates of sacral measurements for the three types of the median sacral crest (R, F, and N), and adjusted for the confounding effects of sacrum's type (standard or fused), body mass, and sex. Median sacral crest type F was set as a reference when implementing the regression tests because it was frequently found in standard sacra. Our linear regression model took the form:

$$S.Weight_i = \beta_0 + \beta_1\ type\ of\ sacrum_i + \beta_2\ type\ of\ median\ sacral_i + \beta_3\ sex_i + \beta_4\ body\ mass_i$$

In Equation 1, S. Weight, represents the sacral weight for i^{th} greyhound, β_0 is the intercept term, β_1 is the regression coefficient for sacrum type (a categorical variable comprised of two levels: fused and standard), β_2 is the regression coefficient for the median sacral crest type (a categorical variable comprised of three levels; presence (F), reduction (R), and absence (N) of the median sacral crest), β_3 is the regression coefficient for sex (a categorical variable comprised of two levels; male and female) and β_4 is the regression coefficient for body mass.

Similar linear regression models (Equation 2) were developed for sacral length and sacral width. Logistic regression analysis was used to quantify the association between the morphology of the median sacral crest (as an outcome variable) and body mass and sex (as explanatory variables). This allowed the estimation of the association between sex and the presence of sacral fusion, to adjust for the confounding effect of body mass. Our logistic regression model took the form:

$$logit(p_i) = \beta_0 + \beta_1\ sex_i + \beta_2\ body\ mass_i$$

Where $logit(p_i)$ represents the logit of the probability of i^{th} greyhound having a fused sacrum, β_0 is the intercept term, β_1 is the regression coefficient for sex (a categorical variable comprised of two levels; male and female) and β_2 is the regression coefficient for body mass. In all analyses, a P value of < 0.05 was declared statistically significant.

3. Results

3.1. Reproducibility/ repeatability of data

This novel classification of the median sacral crest was evaluated by taking the records twice by the same investigator and once more by another investigator. Intra-rater and inter-rater reliability (test-retest reliability) were assessed and showed almost perfect agreement between the 1st and 2nd classifications of the same investigator as the values of Kappa was equal to 0.90 (p < 0.001) as shown in Table (1) and also almost perfect agreement between the classifications of the first investigator and the second investigator (Kappa = 0.91 (p < 0.001)) as show in Table (1).

Regarding the angle of the spinous process of the 1st (S1) sacral vertebra, intra-rater and inter-rater reliability (test-retest reliability) was assessed and showed a high level of concordance between the 1st and 2nd readings of the same investigator and between the readings of the first and the second investigator (Table 2). The descriptive statistics of sacral measurements stratified by classification of the median sacral crest for all greyhounds were combined and presented in Table (3). The highest values for the weight, length, and width of the sacrum were for those sacra with median sacral crest type N, while the lowest values were for those with median sacral type F (the standard type).

3.2. Median sacral crest in greyhounds with standard and fused sacra

Among the greyhounds' sacra used for the median sacral crest study, the prevalence of median sacral crest type R in standard sacra was 35.1% compared to 10% in fused sacra, type F was 41.2% in standard sacra and 10% in fused sacra, and type N was 23.7% in standard sacra and 80% in fused sacra (Table 4). Chi-square test showed that there was a statistically significant difference in the prevalence of different types of median sacral crest by type of sacrum ($\chi 2$ text statistic 51.72; df (degrees of freedom) 2; $p = 0.001$). The results showed that greyhounds with standard sacra were more likely to have a higher prevalence of median sacral crest types F and R, compared to those with fused sacra. However, greyhounds with fused sacra were more likely to have a higher prevalence of median sacral crest type N. Among the 165 greyhound cadavers, 89 cadavers were those of males (53.9%) and 76 were those of females (46.1%). The prevalence of each type of median sacral crest across males and females is presented in Table 4. Chi-square test showed that there was no statistically significant difference in the prevalence of fused sacra by sex ($\chi 2$ text statistic 0.36; df 2; $p = 0.84$).

Table 1. Intra-rater and Inter-rater reliability tests of the different types of the median sacral crest (F, R, and N) in greyhounds.

Classification		2nd Classification of the same investigator/ Intra-rater reliability test			Kappa value	p value
		R	F	N		
		N (%)	N (%)	N (%)		
1st Classification	R	38 (86.4)	3 (6.8)	1 (1.3)	0.90	< 0.001 *
	F	6 (13.6)	41 (93.2)	0 (0.0)		
	N	0 (0.0)	0 (0.0)	78 (98.7)		
	Total	44 (100)	44 (100)	79 (100)		
		Classification of the second investigator/ Inter-rater reliability test				
		R	F	N		
		N (%)	N (%)	N (%)		
1st Classification	R	37 (88.1)	5 (9.8)	0 (0.0)	0.91	< 0.001 *
	F	1 (2.4)	46 (90.2)	0 (0.0)		
	N	4 (9.5)	0 (0.0)	74 (100)		
	Total	42 (100)	51 (100)	74 (100)		

F: Presence, R: reduction, and N: the absence of median sacral crest between the spinous process of S1 and S2 vertebrae.
*: statistically significant as $p < 0.05$

Table 2. Intra-rater and inter-rater correlation of measurements of 1st SPA in greyhounds.

	Intra-rater correlation			Inter-rater correlations		
Measurement	CCC	p value	95% C.I	CCC	p value	95% C.I
1st SPA	0.963	< 0.001 *	.94 to .98	0.98	< 0.001 *	0.97 to 0.98

C.I: Confidence interval, CCC: Concordance correlation coefficient.
*: statistically significant as $p < 0.05$.
1st SPA: angle of the spinous process of S1 vertebra.

Table 3. Descriptive statistics of each of the sacral measurements described for the median sacral crest study, stratified by median sacral crest classification (R, F, and N).

Classification	Measurement	n	Mean ± SD	Median (Q1, Q3)	Min - Max
R	S. Weight (g)	39	27.79 ± 5.39	27.41 (23.30, 30.75)	(20.11–43)
	S. Length (mm)	41	48.12 ± 4.90	47 (45, 49.50)	(38–64)
	S. Width (mm)	41	58.20 ± 3.42	59 (56.50, 60)	(50–65)
	Body mass (kg)	36	29.80 ± 3.54	29.61 (27.04, 32.72)	(23.57–36.76)
F	S. Weight (g)	45	26.30 ± 5.12	26.07 (22.16, 29.58)	(17.82–40.6)
	S. Length (mm)	47	47.40 ± 4.87	46 (44, 49)	(41–62)
	S. Width (mm)	44	57.84 ± 3.37	58 (55, 60)	(51–65)
	Body mass (kg)	42	29.05 ± 4.42	29.33 (26.51, 31.66)	(12.48–36.5)
N	S. Weight (g)	75	29.09 ± 5.84	28.95 (25.19, 32.28)	(15.58–42.23)
	S. Length (mm)	78	56.40 ± 6.75	59 (48.75, 61)	(41–66)
	S. Width (mm)	76	59.54 ± 3.63	60 (58, 61)	(51–70)
	Body mass (kg)	73	29.09 ± 3.44	29.25 (26.37, 31.6)	(21.43–38.23)
Total	S. Weight (g)	159	27.98 ± 5.63	27.66 (23.93, 30.84)	(15.58–43)
	S. Length (mm)	166	51.81 ±7.25	48.50 (46, 59)	(38–66)
	S. Width (mm)	161	58.73 ± 3.57	59 (56.50, 61)	(50–70)
	Body mass (kg)	151	29.25 ± 3.74	29.38 (26.61, 31.73)	(12.48–38.23)

F: Presence, R: Reduction, and N: Absences of median sacral crest between the spinous process of S1 and S2 vertebrae.

Table 4. Comparison of the prevalence of different types of the median sacral crest (F, R, and N) between the spinous processes of S1 and S2 vertebrae in male and female greyhounds with standard and fused sacra.

Type of median sacral crest	Type of sacrum		Chi-square	p value
	Standard	Fused		
	N (%)	N (%)		
R	34 (35.1)	7 (10)	51.72	< 0.001 *
F	40 (41.2)	7 (10)		
N	23 (23.7)	56 (80)		
Total	97 (100)	70 (100)		
Type of median sacral crest	Sex		Chi-square	p value
	Male	Female		
	N (%)	N (%)		
R	23 (25.8)	17 (22.4)	0.36	0.84
F	24 (27)	23 (30.3)		
N	42 (47.2)	36 (47.4)		
Total	89 (100)	76 (100)		

F: Presence, R: Reduction and N: Absences of median sacral crest fusion between the spinous process of S1 and S2 vertebrae.
*: statistically significant as $p < 0.05$.

3.3. Association between the morphology of sacrum and type of sacrum, type of median sacral crest, sex, and body mass

Estimated regression coefficients and their standard errors for the linear regression model of the association between type of sacrum, type of median sacral crest, sex, and body mass and sacral length, sacral weight, and sacral width are shown in Table 5. Histograms of the residuals from the sacral length, sacral weight, and sacral width showed normally distributed behavior. After adjusting for the effect of type of median sacral crest, sex, and body mass, sacra with median sacral crest type N were 2.18 gm (95% CI (confident interval) 0.213 to 4.15) heavier than the standard median sacral crest type F (t-test statistic 2.19; $p < 0.03$). After adjusting for the effect of type of median sacral crest, sex, and body mass, sacra with median sacral

Table 5. Regression coefficients and their standard errors from a linear regression model of factors influencing sacral weight, length, and width in greyhounds.

Explanatory Variable	Coefficient (SE)	t	p value	95% CI
S. Weight				
Intercept	8.01 (4.1)	1.95	< 0.053	
Median Sacral Crest:				
R	0.94 (1.18)	0.801	0.425	−1.38 to 3.26
N	2.18 (0.99)	2.19	< 0.030 *	0.213 to 4.15
F	Reference			
Sex:				
Female	Reference			
Male	1.33 (1.11)	1.20	0.232	−0.858 to 3.52
Body mass (kg):	0.62 (0.151)	4.09	< 0.001 *	0.319 to 0.914
S. Length				
Intercept	36.95 (4.67)	7.91	< 0.001	
Median Sacral Crest:				
R	0.153 (1.33)	0.115	0.909	−2.5 to 2.8
N	8.43 (1.14(	7.42	< 0.001 *	6.18 to 10.7
F	Reference			
Sex:				
Female	Reference			
Male	0.94 (1.3)	0.731	0.466	−1.6 to 3.5
Body mass (kg):	0.35 (0.172)	2.04	< 0.043 *	0.012 to 0.69
S. Width				
Intercept	47.9 (2.73)	17.57	< 0.001 *	
Median Sacral Crest:				
R	−0.03 (0.78)	−0.033	0.974	−1.58 to 1.52
N	1.56 (0.67(	2.31	< 0.022 *	0.23 to 2.9
F	Reference			
Sex:				
Female	Reference			
Male	−0.05 (0.75(	−0.067	0.946	−1.53 to 1.43
Body mass (kg):	0.35 (0.10)	3.48	< 0.001 *	0.151 to 0.55

SE: Standard error; CI: Confidence Interval. R2 = 0.24.
*: statistically significant as $p < 0.05$. F: Presence, R: reduction, and N: absence of median sacral crest between the spinous process of S1 and S2 vertebrae.

crest type N were 8.43 mm (95% CI 6.18 to 10.7) longer than the standard median sacral crest type F (t-test statistic 7.42; p < 0.001). The median sacral crest type R was similar to the standard median sacral crest type F (t-test statistic 0.115; p = 0.909). After adjusting for the effect of type of median sacral crest, sex, and body mass, sacra with median sacral crest type N were 1.56 mm (95% CI 0.23 to 2.9) wider than the standard median sacral crest type F (t-test statistic 2.31; p < 0.022). The median sacral crest type R was similar to the standard median sacral crest type F (t-test statistic −0.033; p = 0.974).

3.4. Association between type of median sacral crest, sex, and body mass

The estimated regression coefficients and their standard errors for the logistic regression model of the association between type of sacrum, sex, and body mass on the presence of each type of median sacral crest (R and N) are shown in Table 6. Median sacral crest type F was considered as a standard type because it is associated with the standard type of sacrum (three fused vertebrae).

After adjusting for the effect of type of sacrum and body mass, no statistically significant association was identified between sex and median sacral type R (z statistic 0.055; p = 0.814). After adjusting for the effect of sacrum's type and sex, no statistically significant association was identified between body mass and the sacral type R (z statistic 0.178; p = 0.673). After adjusting for the effect of sacrum's type and body mass, no statistically significant association was identified between sex and the median sacral crest type N (z statistic 0.190; p = 0.663). Similarly, no statistically significant association was identified between body mass and the median sacral crest type N (z statistic 0.238; p = 0.595).

3.5. Angle measurement of the spinous process of the first sacral vertebra (1st SPA)

The descriptive statistics for the measurements of the sacrum and angle of the spinous process of the 1st (S1) sacral vertebra in greyhounds were combined and presented in Table 7.

3.6. Association between type of sacrum, type of median sacral crest, sex and body mass

The estimated regression coefficients and their standard errors for the linear regression model of the association between sacrum's type, type of median sacral crest, sex, and body mass, and the angle of the spinous process of the 1st (S1) sacral vertebra are shown in Table 8. Histograms of the residuals from the angle of the spinous process of the 1st sacral vertebra were normally distributed.

After adjusting for the effect of sacrum's type, sex, and body mass, the angle of the spinous process of the 1st sacral vertebrae of sacra with median sacral type N was 6.3 degree (95% CI −12.4 to −0.24) and statistically significantly less

Table 6. Regression coefficients and their standard errors from a logistic regression model of factors influencing the presence of fused sacra in greyhounds.

Explanatory Variable	Median sacral crest type		Total	Coefficient (SE)	z	p value	95% CI
Intercept	R	41	167	1.2 (2.3)	0.284	0.59	
	N	79	167	−3.17 (2.4)	1.81	0.179	
Type of Sacrum							
Standard	R	34	97	0.05 (0.62)	0.006	0.940	1.05 (0.31 to 3.54)
	N	23	97	2.41 (0.49)	24.24	< 0.001 *	11.13 (4.3 to 29)
Fused	R	7	70	Reference			
	N	56	70	Reference			
Sex:							
Female	R	17	76	Reference			
	N	36	76	Reference			
Male	R	23	89	−0.15 (0.65)	< 0.055	0.814	0.86 (0.242 to 3.05)
	N	42	89	−0.27 (0.63)	0.190	0.663	0.76 (0.22 to 2.6)
Body Mass (kg):	R	41	167	−0.03 (0.08)	0.178	0.673	0.97 (0.83 to 1.13)
	N	79	167	0.05 (0.09)	0.283	0.595	1.05 (0.89 to 1.24)

SE: Standard error; OR: odds ratio; CI: confidence interval.

*: statistically significant as p < 0.05. F: Presence, R: Reduction and N: Absences of median sacral crest between the spinous process of S1 and S2 vertebrae.

Table 7. Descriptive statistics of each of the sacral measurements described in the study of the angle of the spinous process of the 1st sacral vertebra in greyhounds.

Measurement	n	Mean ± SD	Median (Q1, Q3)	Min - Max
S. Weight (g)	88	27.62 ± 4.82	27.11 (24.4, 30.3)	19.02–42.23
S. Length (mm)	91	51.95 ± 6.68	49 (46, 59)	43–64
S. Width (mm)	90	58.81 ± 3.13	59 (57, 61)	51–68
Body Mass (kg)	77	29.4 ± 3.33	29.46 (27.08, 31.6)	22.4–38.23
1st SPA (°)	91	80.43 ± 10.2	81.30 (72.85, 88.24)	47.2–98.4

S. Weight: weight of sacrum, S. Length: length of sacrum, S. Width: width of sacrum, and 1st SPA: angle of the spinous process of the 1st sacral vertebra.

Table 8. Regression coefficients and their standard errors from a linear regression model of factors influencing sacral weight (1st SPA) in greyhounds.

Explanatory Variable	Coefficient (SE)	t	p value	95% CI
Intercept	73.2 (12.5)	5.86	< 0.001 *	
Median Sacral Crest				
R	–0.49 (3.35)	–0.15	0.884	–7.2 to 6.2
N	–6.3 (3.05)	–2.1	< 0.042 *	–12.4 to –0.24
F	Reference			
Type of Sacrum:				
Standard	–2.5 (2.9)	–0.87	0.387	–8.3 to 3.26
Fused	Reference			
Sex:				
Female	Reference			
Male	–1.1 (2.8)	–0.37	0.711	–6.7 to 4.6
Body mass (kg):	0.46 (.42)	1.09	0.278	–0.38 to 1.3

SE: Standard error; CI: Confidence Interval. R2 = 0.033.
*: statistically significant as p < 0.05. F: Presence, R: Reduction and N: Absence of median sacral crest between the spinous process of S1 and S2 vertebrae.

(more upright) than those in sacra with the median sacral type F (t-test statistic 2.1; p < 0.042). Also, the angle of the spinous process of the 1st sacral vertebrae of sacra with the median sacral type R was similar to those in sacra with the median sacral type F (t-test statistic 0.15; p = 0.884). After adjusting for the effect of sacrum's type, type of median sacral crest, and body mass, the angle of the spinous process of the 1st sacral vertebra in male greyhounds was similar to those of females (t-test statistic 0.37; p = 0.711). One kilogram increase in body mass was associated with a 0.46 degree (95% CI –0.38 to 1.3) increase in the angle.

4. Discussion

This paper studied the morphological differences in the median sacral crest and its association with the angle of the spinous process of the 1st (S1) sacral vertebra in greyhounds with no apparent pathology and damage-free sacra (standard and fused). The lack of literature about the morphology of the sacrum, median sacral crest, and sacral spinous processes in greyhounds and dogs in general made the interpretations of the findings of this study difficult. However, in this study, a novel anatomic classification system for sacra based on the morphology of the median sacral crest between the spinous process of the S1 and S2 vertebrae in greyhounds is proposed.

The results of this study proved that variations in the morphology of the median sacral crest influenced sacral measurements such as weight, length, and width. These variations in the measurements of the sacrum (weight, length, and width) might affect its function and are recommended to be taken into consideration in clinics; these differences are particularly associated with the occurrence of sacrocaudal fusion. For example, when palpating the region prior to accessing the lumbosacral space for epidural injection, the space between the spinous process of the first and second sacral vertebrae in type N sacra might easily be mistaken for the lumbosacral space between the 7th (L.7) lumbar and the cranial edge of the sacrum greyhound.

The two processes of bone resorption and formation are balanced during remodelling in the skeleton of mature animals [13]. Any change and increased loading on bone results in an increase in body mass and any decrease in loading causes a reduction in the mass of bone [14,15]. There is a linear relationship between the amount of loading and bone formation; if the loading exceeds the yield point, then a permanent deformation or damage might occur [16]. These increases in the prevalence of reduction (R) or absence (N) of the median sacral crest, in association with the increase in weight, length, and width of the sacrum, might reflect the adaptation of the sacrum to certain biomechanical influences. It suggests that the distribution of load through the sacrum is different in sacrocaudally fused sacra.

There was almost no relevant literature or anatomic studies that have addressed the morphological variation in the median sacral crest or the spinous processes of the sacrum in greyhounds or dogs in general. Sex was reported to influence the morphology of vertebrae. For example, it has been reported that the high frequency of variation in the morphology of some vertebrae such as cervical or thoracic vertebrae in the horse is associated with sex [17]. In another study, variations in the morphology in the spinous processes of human lumbar vertebrae between males and females have been found. However, in this study, it has been found that the variation in the sacral morphology and prevalence in variations in the median sacral crest of greyhounds was not influenced by sex as shown in Table (6). These variations in the morphology of the median sacral crest might relate to this breed of dog, but this needs an investigation for the morphology of median sacral crests in other breeds of dogs comparing them with those in greyhounds.

In this study, a new classification of sacra based on the morphology of median sacral crest has been suggested and validated. One of the morphological variations that was noticed in association with the occurrence of sacrocaudal fusion, was the reduction (R) or absence (N) of the median sacral crest between the spinous processes of the S1 and S2 vertebrae. This novel classification of sacra, using the morphology of the median sacral crest, has been validated by repeating the classification by the first investigator and then by the second investigator, and showed almost perfect agreement between both classifications based on statistical tests [12].

Establishing a new valid method to classify sacra by median sacral crest, clearly shows that the morphology of sacra in the greyhound could differ (usually in literature reporting that sacrum consists of only three fused vertebrae) from what has been described in the literature [6], and this ensured that, besides the number of fused vertebrae forming the sacrum in dogs or any other animal species, other morphological characteristics can be used to distinguish the sacra. In fact, the occurrence of sacrocaudal fusion in greyhounds has created a clear variation and influenced the morphology of the sacrum.

Another important finding of this study was that the angle of the spinous process of the 1st sacral vertebra was more perpendicular, in association with a reduction (R) or absence (N) of the median sacral crest and tended to be more upright in fused sacra compared with standard ones.

Although, there was no association between the angle of the 1st (S1) sacral vertebra and sex, body mass, or type of sacrum (standard and fused sacra), significant differences in the angle were found between those sacra with median sacral crest type N and those with median sacral crest type F. In those with type N, the angle tended to be more upright and closer to perpendicular.

The reason for this variation in angle is not clear yet and has not been addressed in any literature. However, in humans, it has been reported that some spinous processes become relatively perpendicular in those patients suffering from scoliosis, which is a lateral curvature of the spine [18]. This difference in the angle is proposed to be related to the position and action of the supraspinous and interspinous ligaments. Furthermore, it was proposed that the flexion and extension of vertebral column parts would influence the ligaments stretching proportionally to their distances from the center of rotation of that specific part of the vertebral column [19]. Regarding the current study and as the supraspinous and interspinous ligaments are considered to be a part of the passive system which assist in spine stability [20], the spinous process of the S1 vertebra and the variation in the median sacral crest between the S1 and S2 vertebrae might be affected by the function of related ligaments.

The variation in the angle of the spinous process of the 1st sacral vertebra might have clinical applications. For example, it may be useful for potential instrumentation within the sacrum region, because the sacrocaudal junction is susceptible to clinical complications such as fracture and luxation [21]. Also, a study done by Feeney and Oliver (1980) showed that sacral fractures were the most common type of pelvic fractures in dogs and cats [22]. The spinous processes of lumbar or sacral vertebra have been used as a site for fixation for tension band tools in many surgical approaches [23]. In addition, in dogs and cats, the spinous process of the S1 has been used for sacral fracture and luxation fixation [21].

The variation in the angle between standard sacra, which was associated with complete fusion (F) of the median sacral crest, and fused sacra, which is characterized by a reduction (R) or absence (N) of the median sacral crest, would help to suggest how this kind of variation in the median sacral crest might have developed. As the presence of fusion (F) was so prevalent in standard sacra, it is proposed that the process of reduction in the sacral crest starts was in this type of sacrum (standard with a full fusion of the median sacral crest), then was followed by the beginning of a reduction of the median sacral crest in association with sacrocaudal fusion, and this process ends by having a completely fused sacrum with an absence of the median sacral crest (N) between the S1 and S2. The type R in the standard sacra was considered as an intermediate stage between the other two types (F and N) of the median sacral crest.

The reasons for these variations in the median sacral crest, in association with sacrocaudal fusion, are not clear. It has been reported that if the loading exceeds the yield point, then permanent deformation or damage might occur [16], and this might be the reason for what is happening at different rates during the biological processes of sacrocaudal fusion. Also, according to Wolf`s Law, the absence of loading might cause the absence of the crest. This interpreted mechanism is based on the fact that the fusion between the Ca1 and S3 vertebrae might push the sacrum cranially, and this might subsequently alter the sacroiliac alignment and then the biomechanics of the sacrum. This interpretation is supported by a recent study by Lazennec et al., (2017) which showed that spinal fusion in humans enhanced the hip-spine biomechanics [24]. An increase in the roughness of the auricular surface of the sacrum's wing that was observed in association with the sacrocaudal fusion in these dogs, suggests an increase in the strength of the connection between the sacrum and ilium, possibly due to the requirement to withstand greater forces across that joint. Another observation was that the fused sacra had more concave surface, which suggests that the pelvic surface would suffer higher compression forces. This leads to an increase in the tensile force on the median sacral crest between the spinous processes of the S1 and S2 vertebrae, and subsequently influencing the reduction or absence of the median sacral crest there. However, this suggested mechanism needs more investigations and still lacks proof.

According to the aforementioned suggested mechanism, the absence or reduction in the median sacral crest, which is mainly based on the increase in the length and width of the sacrum in association with the increase in the roughness of the articular surface of the sacrum's wing, caused the sacrum to be positioned more cranially and ventrally in association with an increase in the curvature of the pelvic surface. All these observed events emphasized the need to study the angulation of the spinous process of S1 vertebra.

The reasons for the variation in the morphology of the spinous processes of different parts of the vertebral column are unclear. It has been reported that the spinous process of the vertebrae might act as levers for force transmission, and that their direction is determined by the muscles and forces of ligaments acting on them [2]. Slijper (1946) suggested that the upright position of the spinous processes of the sacral vertebrae is due to the force of the muscles of the hind limbs, which originates from the sacral region. The reason why the reduction and absence of the median sacral crest would be associated with sacrocaudal fusion is not clear.

This study has some limitations as the greyhounds under this study were adults and more than two years old, so these variations in the median sacral crest need to be investigated in younger greyhounds and in other breeds of dog, too, with different ages to help discovering whether the morphological variations are associated with changes in the loading environment and developed over time, or they are a direct reason of causing the increased occurrence of sacrocaudal fusion. The biomechanical properties of the spinous process of S1 vertebra were not investigated in this study either, such an investigation would help to explain the possible causes of the observed variations.

5. Conclusion

In this study, a novel method was used and proposed to classify the sacra in greyhounds based on the morphology of the median sacral crest. This study showed that there are differences in the angulation of the spinous process of S1 vertebra with different types of median sacral crest.

Though it is understood that this kind of differences cannot determine causation or provide a connection between the morphology of the median sacral crest and the occurrence of sacrocaudal fusion, it would help to identify biological or mechanical connections that should be investigated in future researches. The reason for the reduction or absence of the median sacral crest between the spinous processes of S1 and S2 vertebrae, in association with the occurrence of sacrocaudal fusion in greyhounds, is not clear. It is not clear if this change in morphology of the sacrum has been a result of genetics through evolution, or a kind of adaption for certain behaviors that affected the development. In clinics, it would be interesting to separate greyhounds into groups by type of sacra and investigate if greyhounds with sacrocaudal fusion are prone to develop particular spine, hind limbs muscle injuries or locomotor related diseases.

Furthermore, the findings of this study reflect the need for further clinical and biomechanical investigations for the morphology of the spinous processes of sacra in greyhounds with different types and ages and its potential role in locomotion in greyhounds and other athletic dog breeds.

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Conflict of Interest

No conflict of interest is declared for this work.

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