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Structural characteristics and predicted functional capacities of epaxial muscles in chondrodystrophic and non-chondrodystrophic dogs with and without suspected intervertebral disc herniation- a preliminary study

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ABSTRACT

Epaxial muscle atrophy is related to spinal diseases in dogs. However, the influence of intervertebral disc herniation (IVDH) on the functional capacity of epaxial muscles has not been investigated. We aimed to estimate force and power-generating capacity of epaxial muscles in chondrodystrophic Dachshunds and non-chondrodystrophic Border terriers bred for similar purposes. Further we aimed to compare these features in Dachshunds with and without IVDH. Cadavers of Dachshunds (n = 16) and Border terriers (n = 7) were investigated with MRI. In the absence of clinical information, MRI findings were used to categorize the Dachshunds into affected (n = 8) and non-affected (n = 8). Epaxial muscle mass, muscle belly length, fascicle length, architectural index and physiological cross-sectional area (PCSA) were obtained through dissections, pain and exercise history through questionnaires. Difference between groups and effect of covariates were assessed with ANCOVA models. Dachshunds had greater muscle mass in M. splenius, *M. longissimus* capitis and M. iliocostalis thoracis (all $P < .05$). Dachshunds had higher PCSA in M. semispinalis complexus ($P = .004$) and M. iliocostalis lumborum ($P = .016$) than Border terriers, which had longer muscle fascicles in these muscles ($P = .004$ and $P = .002$, respectively). Affected Dachshunds had longer muscle fascicles than non-affected Dachshunds in *M. longissimus* thoracis et lumborum ($P = .004$) and *M. longissimus* cervicis ($P = .011$). Body weight had a significant impact on all muscle variables, but pain and exercise had none. Dachshund epaxial muscles have greater potential for force production than those of the Border terrier. This may imply that Dachshunds, due to predisposition to IVDH, require more spinal stability provided by the epaxial muscles.

1. Background

Signs of atrophy, decreased size and increased fat infiltration, in the epaxial muscles have been reported in dogs with intervertebral disc herniation (IVDH) and with lumbosacral stenosis (Boström et al., 2014; Cain et al., 2016; Henderson et al., 2015; Lerer et al., 2015). Intervertebral disc herniation is the most frequently treated spinal disease in dogs, with the Dachshund being the most commonly affected breed (Brisson, 2010). Currently, physiotherapy is mainly used as a supportive treatment in dogs after IVDH surgery, with the aim of restoring hind limb function after paralysis (Hodgson et al., 2017; Olby et al., 2005; Sims et al., 2015). In human medicine, patients with different causes and severity of back pain are managed with physiotherapy with an emphasis on targeted training of paraspinal muscles to enhance the recovery of functional ability (Danneels et al., 2001; Falla and Hodges, 2017; Hides et al., 2001) and to prevent recurrence of injury (Goubert et al., 2016). This potential has not been investigated in small animal physiotherapy.

The canine epaxial muscles consist of three longitudinal muscular systems, each with multiple fibers overlapping several segments (Evans, 1993). These are, from medial to lateral: the transversospinalis, the longissimus and the iliocostalis systems (Evans, 1993; Fig. 1). The known functions of epaxial muscles depend on spinal segment and may vary at different gaits (Sharir et al., 2006; Schilling and Carrier, 2009, 2010). In addition to producing movements of the spine, the epaxial muscles are also important for maintaining the position and integrity of

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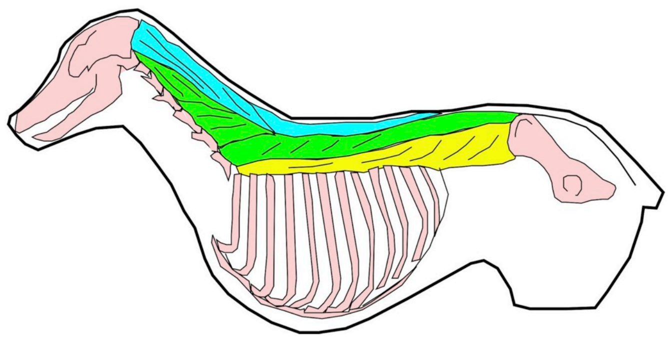

Fig. 1. Schematic drawing of epaxial muscles in the dog, adapted from Webster et al. (2014). Spinalis et semispinalis muscle group in light blue, longissimus muscle group in green and iliocostalis muscle group in yellow.

the vertebral column (postural/static stability) and for controlling and resisting movements of the spine (dynamic stability) (Ritter et al., 2001; Webster et al., 2014). Muscle architecture is defined as the arrangement of muscle fibers within the muscle, relative to the axis of force generation. It is described using the following five parameters: muscle belly and tendon length, muscle fibre length, muscle physiological cross-sectional area (PCSA) and pennation angle (the angle between the internal tendon and muscle fibers; Lieber and Blevins, 1989). Muscle architecture can be used to predict functional capacity in terms of force production and power generation and is essential for rough estimations of specific individual muscle function (Hudson et al., 2011; Ward et al., 2009; Williams et al., 2008a, 2008b). In principle, a muscle with short pennate fibers and large PCSA has high force production capacity, hence potential for providing postural and dynamic stability (Webster et al., 2014). Muscles with long parallel fibers are often involved in production of large movements and muscles with long muscle fibers and large PCSA have capacity for power generation (large amount of force with high shortening velocity over a wide range) (Webster et al., 2014). Information on muscle architecture allows the comparison of muscle functional capacity and specialized adaptations between different species and breeds (Webster et al., 2014) and is utilized in human medicine to plan surgical procedures and physical training regimes (Ward et al., 2009).

Studies on muscles in canine and equine limbs (Pasi and Carrier, 2003; Williams et al., 2008a, 2008b; Crook et al., 2008) and in the backs of dogs (Webster et al., 2014) suggest breed-specific differences in muscle architecture due to selective breeding of animals for a particular purpose. Knowledge about muscle architecture in breeds prone to back problems is critical in small animal veterinary medicine in order to target exercise regimes to spinal musculature in dogs recovering from back pain and spinal surgery. The authors know of only one previous report on epaxial muscle architecture in dogs (Webster et al., 2014), and the possible implications of disease on the muscular architectural design have not yet been investigated.

The aim of this study was to evaluate the influence of breed on the estimated functional roles of epaxial muscles in chondrodystrophic Dachshunds and non-chondrodystrophic Border terriers and the influence of IVDH on the functional capacity of the epaxial muscles in chondrodystrophic Dachshunds. Specifically, the objectives were to estimate the force and power-generating capacity of epaxial muscles in Dachshunds and Border terriers and to clarify the relationship between these characteristics and IVDH in Dachshunds.

1.1. Hypotheses

We hypothesized that the epaxial muscle architecture in Dachshunds would possess higher potential for spinal muscle force production than the Border terrier.

We also hypothesized that the muscle architecture of the epaxial muscles would differ between Dachshunds and Border terriers because of their differing geometry, i.e. the long back and short legs of Dachshunds compared with the short back and long limbs of Border terriers (Fig. 2).

Finally, we hypothesized that the epaxial muscle mass PCSA and fascicle length would be decreased in Dachshunds with IVDH.

2. Materials and methods

2.1. The animals

This study was an anatomical study comparing two breeds (Dachshunds and Border terriers) and comparing affected dogs to non-affected within one breed (Dachshunds). The study protocol was approved by the Viikki Campus Research Ethics Committee, University of Helsinki (7/2013). Client-owned Dachshunds and Border terriers euthanized for any reason between fall 2013 and spring 2016 at the Veterinary Teaching Hospital of the University of Helsinki were included. Seventeen Dachshunds and 7 Border terriers were donated to the hospital for research and teaching purposes. The dog owners made all donations voluntarily and written consent was provided. Exclusion criteria were postmortem deterioration of muscle tissue limiting reliable data collection. The dogs' age, gender and breed and the reason for euthanasia were obtained from patient records and the pain and exercise histories were derived via owner questionnaires after euthanasia.

2.2. MRI evaluation

After euthanasia, the cadavers were immediately frozen at −20 °C with the spine in a straight position until further procedures. Prior to dissection, the cadavers were defrosted at 4 °C for a maximum of 48 h and the spine of each cadaver was MRI scanned (3.0 Tesla, Tesla Siemens, Siemens (first 14 dogs), 3.0 Tesla, Magnetom Skyra, Siemens (final 10 dogs)). The cadavers were placed supine in a foam cradle to ensure straightness of the spine. T1 sagittal (TE 9.4, TR 701) and transverse (TE 2.46, TR 7) and T2 sagittal (TE 106, TR 3000) and transverse (TE 80–82, TR 4020–4300) sequences were obtained from spinal levels Th1 – S1 using 3 mm slice thickness and 10% gap between slices.

An ECVN diplomate (TSJ) evaluated the images in random order blinded to all background data of the dogs. A Pfirrman grade (1–5) was given for each intervertebral disc (T1-S1) based on evaluation of sagittal T2-weighted images (Bergknut et al., 2011) and averages of the Pfirrman grades were calculated for the thoracic spine and lumbar spine. Additionally, the type of intervertebral disc disease was determined as 1) normal or disc degeneration only, 2) bulging of the intervertebral disc (symmetric uniform extension of the outer margin of the disc circumferentially), 3) disc protrusion (focal disruption of the annulus) and 4) disc extrusion based on evaluation of T1 and T2-weighted transverse images (Besalti et al., 2006). The dog was categorized as affected if the intervertebral disc disease grade was 3 or 4 in at least one disc space. The lesion site, lesion side and number of affected intervertebral discs were recorded (Table 1).

2.3. Pain history

To account for possible pain history affecting physical activity (and therefore, muscle parameters), the owners answered a questionnaire regarding pain-related characteristics (Lappalainen et al., 2014). Owners were asked to report episodes such as reluctance to jump, neck or back pain, unexplained pain episodes and paralysis, as well as whether veterinary advice was sought for the reported problems and whether the reported problems affected the animal's daily life. Based on these answers, a sum variable (pain score) from 0 to 6 was generated, with higher scores indicating greater owner-reported pain. The questions related to ‘reluctance to jump’, ‘pain in neck or back’ and

Table 1
Descriptive statistics for the dogs.

Dog	Gender	Age	Body weight	BMI	Back length	Pain score	Duration of walks (min)	Pfirrman grade	IVDD type	Lesion site	Reasons for euthanasia
Dsh 1*	F	13.7	7.4	13.8	40.5	5	36.4	3.1	4	T9–10	Old age
Dsh 2*	F	13.7	7.8	15.8	40.0			3.0	3	L5–6	Old age
Dsh 3*	F	4.2	7.9	16.2	40.3			2.3	3	L7-S1	Owner's request
Dsh 5*	F	15.7	11.3	21.8	42.5	0	45.0	3.0	4	**T13-L1**, L1–2, L4–5	Old age
Dsh 6*	F	13.9	9.7	20.5	41.8	0	45.0	3.7	4	T11–13, **T13-L1**, L6–7	Old age
Dsh 9*	F	16.0	8.9			5	15.0	3.5	4	**T12–13, T13-L1**	Old age, hind limb paralysis
Dsh 10*	M	15.0	17.7	31.4	45.5	3	45.0	3.3	4	T9–10, **T13-L1**	Old age, hind limb paralysis
Dsh 15*	F	9.2	5.6	11.2	36.6	3	15.0	3.0	3	L2–3	Chronic back pain
		13.9 (3.4)	**9.06 (3.2)**	**16.18 (6.5)[a]**	**38.49 (4.4)[a]**	**2.78 (1.8)**	**31.66 (13.5)**	**3.06 (0.4)**	**3.5 (0.6)[ab]**		
Dsh 4	M	7.2	9.1	18.5	40.8			2.6	1		Owner's request
Dsh 7	F	16.0	8.3	15.5	35.6	3	15.0	3.2	1		Old age
Dsh 8	M	1.0	6.6	12.9	40.5	0	45.0	2.2	1		Surgery complication
Dsh 11	M	19.0	4.6	9.6	31.2	5	15.0	2.8	2		Old age
Dsh 12	F	3.0	11.5	21.8	40.7	2	57.9	2.0	1		Suspected intoxication
Dsh 13	F	16.0	9.7	17.9	39.0	4	15.0	3.7	1		Old age
Dsh 14	F	2.0	9.5	19.3	43.2	0	23.6	2.1	1	L7-S1	HBC, L7 fracture and luxation
Dsh 16	F	9.1	12.1	19.7	40.8	6	45.0	3.1	1		Mammary gland tumour
		8.9 (4.9)	**8.47 (1.9)**	**14.56 (4.5)[b]**	**37.69 (33.68)[b]**	**2.36 (1.5)**	**32.51 (15.3)**	**2.6 (0.5)**	**1.17 (0.5)[b]**		
BT 3*	F	14.0	6.8	9.0	36.2	2	23.6	3.1	4	L2–3	Old age, hind limb paralysis
BT 5*	M	14.4	10.5	13.8	35.0	2	15.0	3.8	3	T10–11, T11–12, L3–4	Old age
BT 7*	F	13.6	7.2	9.9	32.0	4	45.0	3.6	4	T13-L1	Balance deficits
BT 1	M	8.1	7.2	9.3	34.8	2	45.0	1.8	1		Intracranial tumour
BT 2	F	11.6	6.8	8.5	33.3	2	45.0	2.8	1		Intracranial tumour
BT 4	F	9.0	8.5	11.3	34.0	2	36.4	2.7	1		Intracranial tumour
BT 6	F	15.0	6.8	8.8	33.2	4	15.0	3.1	1		Balance deficits, hind
		12.24 (2.5)	**7.67 (1.2)**	**10.07 (1.7)[ab]**	**34.06 (1.3)[ab]**	**3.0 (1.2)**	**32.14 (14.1)**	**2.96 (0.6)**	**2.14 (1.3)[a]**		Limb paralysis

Gender, age, body weight, body mass index (BMI), back length, Pfirrman grade, type of IVDD, pain score, duration of walks for each dog as well as mean and standard deviation (bolded) and the reason for euthanasia are presented. For dogs with multiple lesion sites, the type of IVDD is given according to the most severe lesion. The most severe lesion site is bolded. Dachshund (Dsh), Border terrier (BT), hit by car (HBC). The dogs indicated with an asterisk were classified as affected. Mean values with superscript corresponding letters indicate significant differences between the groups ([a]significant difference between affected Dachshunds and Border terriers, [b]significant difference between non-affected Dachshunds and Border terriers).

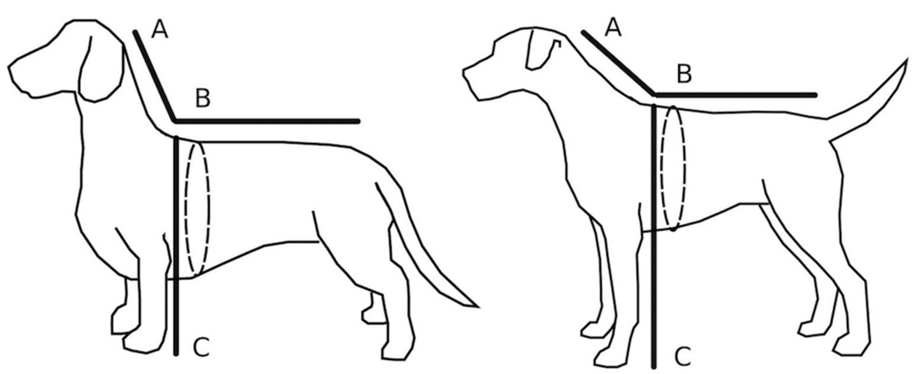

Fig. 2. Different geometry of the Dachshund (left) and the Border terrier (right). A: measurement from the occipital protuberance to the base of tail (cm), B: back length (cm), C: height at withers. The dotted oval indicates the girth circumference measurement (cm).

'unexplained pain episodes' were kept as separate variables because of their associations with intervertebral disc disease in a previous publication (Lappalainen et al., 2014).

2.4. Exercise level

To account for the potential influence of physical exercise history on the muscle parameters, the amount of exercise for each dog during its last two years of life was categorized using an exercise questionnaire (Boström et al., 2018). Owners were asked about amount, duration, intensity and frequency of physical exercise. The responses were averaged to account for possible variations in exercise on workdays and weekends, and the following variables were generated: number of walks/day, distance of walks/day (km), duration of walks/day (min) and from the last two, we calculated mean walk velocity/day (m/s).

2.5. Subject morphology

Gross anatomy measurements (cm) all taken by the same researcher (AB) with the intact cadaver in right lateral recumbency using a flexible tape measure included girth circumference, distance from occipital protuberance to base of tail, distance from midpoint between dorsal border of scapula to base of tail (back length) and height at the withers (height). Body weight was obtained using a commercial scale with an accuracy to 20 g (Soehnle S20 2763, Soehnle Professionals, Germany). The body mass index (BMI) was calculated for each dog as follows: Body weight (BW) $_{kg}$/(height at the withers $_{cm}$ × length from occipital protuberance to base of tail $_{cm}$) (Mawby et al., 2004; Fig. 2).

2.6. Muscle morphology

One researcher (AB) collected the muscle architectural data according to a previous study (Webster et al., 2014). The same routine procedure was performed on all cadavers. The cadavers were skinned and the front limb removed. The dissection started on the right-hand side. The epaxial muscles in the cervical, thoracic and lumbar spine were isolated systematically, carefully removing external tendons. The following muscles were investigated: M. multifidus cervicis, thoracis and lumborum, M. semispinalis complexus and biventer, M. spinalis et semispinalis cervicis and thoracis, *M. longissimus* capitis, cervicis, thoracis and lumborum, and the M. iliocostalis thoracis and lumborum. *M. longissimus* and M. iliocostalis lumborum were removed as one muscle as they were difficult to dissect separately (Webster et al., 2014).

Muscle mass was determined using an electronic balance accurate to 0.01 g (KERN EMS 3000-2, Kern, Germany). For the smallest muscle, M. multifidus thoracis, the mass was additionally confirmed with an electronic analysis balance (Mettler AE 240, Mettler Toledo AF, Switzerland) with an accuracy of 0.001 g. The repeatability of the balances was tested before each data collection session.

The muscle belly length was measured from origin to insertion with a flexible plastic tape measure, accurate to 1 mm. If a muscle had multiple insertions, the length was measured to the insertion point furthest from the origin. This allowed the muscle belly length to represent the whole line of action for that particular muscle. Muscle fascicle length determines the range of lengths over which a muscle can generate an active force (Zajac, 1992). An incision was made through the muscle belly, longitudinally to the muscle fascicles (bundles of individual muscle fibers that are visible to the naked eye). A minimum of 5 and a maximum of 10 fascicles were selected randomly and the length was measured using a digital caliper (Alpha Tools, Germany). Where the muscle overlapped several spinal segments, fascicle lengths were sampled throughout the entire length of the muscle belly. The architectural index (AI) was calculated by dividing mean fascicle length by muscle belly length (Webster et al., 2014). The AI normalizes fascicle length for muscle belly length and reflects the number of sarcomeres in series in a muscle, and thus, the potential velocity of a muscle contraction (Sharir et al., 2006).

The resting pennation angle was defined as the angle between the internal tendon and the muscle fascicle or where no internal tendon was present, the angle of fascicles from the external aponeurosis of the muscle. The pennation angle was recorded using a clear plastic protractor to an accuracy of 1°.

Muscle volume was estimated by dividing muscle mass by muscle density of 1.06 g/cm^3 (Mendez and Keys, 1960). Muscle volume and muscle fascicle length data together can provide an estimate of the physiological cross-sectional area (PCSA). The PCSA in this study was calculated for each muscle as muscle volume divided by mean fascicle length (Payne et al., 2004, 2005; Webster et al., 2014). The PCSA reflects the number of sarcomeres in parallel within a muscle, hence also capacity for maximum isometric force (F_{max}) generation (Payne et al., 2005; Sharir et al., 2006). The maximum isometric force generation capacity was estimated based on the PCSA: an established method used in previous studies (Webster et al., 2014). The F_{max} is directly proportional to PCSA so that a muscle with larger PCSA has greater F_{max}. The maximum isometric stress is similar for all vertebrate skeletal muscle, 0.2–0.3 MPa (Wells, 1965), while the maximum contraction velocity, required producing exact F_{max} values can vary between species and in different fibre types (Payne et al., 2004).

The maximum power that a muscle can generate is a direct function of the number of active cross bridges within the muscle and is directly proportional to muscle volume or mass, hence larger muscles will have an increased capacity for powerful contraction (Payne et al., 2004; Zajac, 1992). Information of fascicle length combined with muscle volume and mass, makes it possible to speculate about the velocity of contraction and range of motion over which the muscle can develop force (Payne et al., 2005).

2.7. Scaling

Allometry is the study of the relationship between body size and shape and describes the regular manner in which certain morphological or physiological variables change in relation to body mass (Myatt et al., 2011; Schmidt-Nielsen, 1984). Allometric scaling is common in biology when comparing two animals of different sizes or the same animal at different sizes during growth. As allometric scaling of muscle architectural data permits comparison between individuals of different size (Webster et al., 2014; Schmidt-Nielsen, 1984), it was considered necessary in our comparison of Dachshunds and Border terriers to scale the data accordingly. Parameters were scaled to dog mass: muscle mass scaled as (muscle mass/dog mass in grams); muscle belly length as (muscle length/dog mass $^{0.33}$); muscle fascicle length as (fascicle length/dog mass $^{0.33}$) and PCSA as (PCSA/dog mass$^{0.66}$) (Webster et al., 2014).

In the case of dogs with such different height/length proportions as the Dachshund and Border terrier, allometric scaling of length measures based on mass may not be the most appropriate method of scaling. Therefore, both muscle belly and fascicle lengths were also scaled to back length as an alternative method of normalizing these muscle characteristics across dogs with differing body form.

2.8. Statistical analysis

The sample size was estimated based on power analysis using the main outcome variables (muscle mass, muscle belly length, mean fibre length and PCSA) determined in a similar study on dogs (Webster et al., 2014). The power analysis indicated that a sample of 6–8 dogs in each group was required. Data were tested for normality using the Shapiro-Wilk test and normal QQ-plots. No concerns of violations of the normality assumptions were raised. Mean and standard deviation (SD) were used to describe body mass, BMI, back length, height and girth circumference, gender, age, Pfirrman grade, type of intervertebral disc disease, pain score, exercise and the muscle variables in the dogs. Independent samples and Student's *t*-tests (continuous) and the non-parametric Mann-Whitney test (non-continuous) were used to compare the means of the descriptive variables between affected and non-affected Dachshunds and between Dachshunds and Border terriers, respectively. The statistical analysis was done using SAS® System for Windows, version 9.3 (SAS Institute Inc., Cary, NC, USA) and SPSS IBM statistics, version 24. The level of significance was set at < 0.05 in all analyses. Due to the exploratory nature of the analyses, all presented p-values are raw p-values, not adjusted for multiple testing.

2.8.1. Dachshunds and Border terriers

All Dachshunds were compared to all Border terriers. All investigated muscles were used in the analysis between Dachshunds and Border terriers and a within-dog average was calculated for each architectural variable, muscle mass, muscle belly length, muscle fascicle

Table 2
Muscle data for Dachshunds and Border terriers.

Muscle	Mass		Muscle belly length (mm)		Fascicle length (mm)		PCSA (mm^2)	
	DSH	BT	DSH	BT	DSH	BT	DSH	BT
Splenius	26.9 (8.5) ^{ma}P = .043*	19.2 (3.4)	218.2 (25.9) ^{ma}P = .195 ^{ble}NS	197.1 (5.6)	136.6 (26.1) ^{ma}NS ^{ble}NS	128.8 (15.3)	1.91(0.62) ^{ma}NS	1.41 (0.20)
Semispinalis complexus	14.6 (5.4) ^{ma}P = .218	10.4 (2.1)	172.4 (22.7) ^{ma}P = .954 ^{ble}P = .025*	162.0 (9.65)	82.8 (17.0) ^{ma}P = .004* ^{ble}P = .0003*	99.8 (17.4)	1.68 (0.52) ^{ma}P = .004*	1.00 (0.21)
Semisipnalis Biventer	21.6 (6.59) ^{ma}P = .486	17.1 (2.1)	207.2 (24.8) ^{ma}P = .252 ^{ble}NS	186.8 (4.7)	112.2 (35.4) ^{ma}NS ^{ble}NS	122.4 (25.9)	2.12 (0.97) ^{ma}NS	1.38 (0.36)
Longissimus capitis	6.6 (2.3) ^{ma}P = .048*	6.6 (1.4)	167.2 (25.9) ^{ma}NS ^{ble}P = .048*	151.4 (10.1)	112.1 (26.2) ^{ma}NS ^{ble}NS	98.3 (20.8)	0.62 (0.23) ^{ma}NS	0.71 (0.29)
Longissimus cervicis	14.4 (4.8) ^{ma}P = .260	10.6 (1.4)	159.9 (19.1) ^{ma}NS ^{ble}P = .473	136.1 (2.33)	52.1 (10.6) ^{ma}NS ^{ble}P = .940	45.9 (7.7)	2.68 (0.82) ^{ma}P = .688	2.25 (0.42)
Multifidus cervicis	9.0 (2.9) ^{ma}P = .175	6.3 (0.98)	122.8 (15.8) ^{ma}P = .033 ^{ble}NS	105.5 (6.2)	35.1 (4.8) ^{ma}P = .154 ^{ble}P = .800	30.9 (4.2)	2.44 (0.76) ^{ma}P = .175	1.95 (0.95)
Spinalis et semispinalis cervicis	5.6 (1.5) ^{ma}P = .461	4.2 (1.0)	121.6 (11.2) ^{ma}NS ^{ble}P = .009*	114 (8.8)	83.8 (19.0) ^{ma}NS ^{ble}P = .085	87.0 (7.4)	0.66(0.24) ^{ma}NS	0.50(0.09)
Multifidus thoracis	0.7 (0.23) ^{ma}P = .220	0.5 (0.05)						
Spinalis et semispinalis thoracis	18.5 (6.3) ^{ma}P = .457	15.5 (1.6)	182.9 (27.1) ^{ma}NS ^{ble}P = .049*	173.6 (7.6)	58.1 (12.0) ^{ma}P = .052 ^{ble}NS	47.9 (6.3)	3.04 (0.69) ^{ma}P = .032*	3.1 (0.56)
Longissimus thoracis et lumborum	64.9 (24.2) ^{ma}P = .653	52.4 (10.7)	342.2 (37.5) ^{ma}NS ^{ble}P = .134	315.6 (31.4)	47.6 (8.9) ^{ma}NS ^{ble}P = .473	43.2 (4.4)	12.9 (3.84) ^{ma}P = .551	11.5 (2.36)
Iliocostalis thoracis	8.0 (3.0) ^{ma}P = .001*	4.9 (0.7)	198.1 (26.0) ^{ma}P = .041* ^{ble}NS	169.3 (8.5)	25.9 (7.4) ^{ma}NS ^{ble}NS	21.5 (2.9)	3.02 (0.78)) ^{ma}NS	2.22 (0.36)
Multifidus lumborum	15.1 (6.7) ^{ma}P = .941	11.6 (2.2)	217.3 (33.0) ^{ma}NS ^{ble}NS	201.6 (19.9)	20.9 (4.2) ^{ma}NS ^{ble}NS	20.4 (2.3)	7.01 (2.94) ^{ma}NS	5.45 (1.25)
Iliocostalis lumborum	53.9 (21.1) ^{ma}P = .284	37.4 (11.4)	195.9 (31.0) ^{ma}P = .963 ^{ble}P = .090	183.2 (18.2)	33.2 (8.7) ^{ma}P = .002* ^{ble}P = .004*	40.5 (5.3)	15.53 (5.67 ^{ma}P = .016*	9.41 (3.22)

The mean and (SD) for unscaled data for all investigated muscles are compared between the two breeds. P-values are based on ANCOVA analysis of scaled data. An asterisk indicates a significant difference after scaling for body mass/back length. Small letters indicate scaled to mass^{ma} and scaled to back length^{ble}. Dachshund (DSH) (n = 16), Border terrier (BT) (n = 7)
NS (not shown) indicates a poor fit of the statistical model, thus, results are considered unreliable and are not presented.

length, architectural index and PCSA, using the data from both the left and right sides. These average variables were used in all analyses. The differences in the muscle variables between the two breeds were investigated in the data scaled to body mass and scaled to back length in all muscles and analysed using an Analysis of Covariance (ANCOVA) model, with breed as the only fixed factor and dog age as a covariate. To maintain sample size, all included Dachshunds were compared with all included Border terriers.

2.8.2. Affected and non-affected Dachshunds

As MRI imaging was available from the first thoracic vertebrae to the sacrum, only the 7 muscles in the thoracic and lumbar spine (i.e. *M. longissimus* cervicis, M. multifidus thoracis, *M. longissimus* thoracis et lumborum, M. spinalis et semispinalis thoracis, M. iliocostalis thoracis, M. multifidus lumborum, M. iliocostalis lumborum) were included in the analysis between affected and non-affected Dachshunds. Differences in the muscle variables between groups were investigated using ANCOVA models on unscaled data. Body weight, back length, Pfirrman grade, age, pain score and duration of walks were used as possible covariates. The number of walks/day, distance of walks/day (km) and duration of walks/day (min) correlated strongly with each other, and therefore, only the duration of walks was included in the list of possible covariates.

2.8.3. Statistical modelling

To be able to utilize the complete set of dogs, two different statistical modelling strategies were used. The first strategy on the full set of dogs excluded pain score and duration of walks from the covariate list, whereas the second strategy (for dogs with no missing covariate information) used all possible covariates: body weight, Pfirrman grade, age, back length, pain score and duration of walks. The statistical modelling was started by inclusion of all possible covariates in the ANCOVA model with both analysis strategies. However, all of the models had significant multicollinearity (significant correlation between explanatory variables), present and thus, all of the models have been simplified from the full model. The multicollinearity was caused mainly by three issues: 1) strong correlation between body weight and back length variables, 2) strong correlation between age and Pfirrman grade variables and 3) inclusion of duration of walks as a covariate (with the second analysis strategy). To reduce multicollinearity of the models, one of the strongly correlated variable-pairs (back length and age) was excluded from the analysis. With the second analysis strategy, in case multicollinearity remained after these exclusions, the duration of walks variable was also excluded from the model.

With the first analysis strategy, a sensitivity analysis was also conducted, where the two variables previously excluded from the model (back length and age) were kept in the model and the other two variables (body weight and Pfirrman grade) were excluded. If a proper model fit was still not achieved after the described exclusions, the modelling was terminated and the results are not shown due to poor model fit to the data. In all of the fitted models, multicollinearity of the model was assessed based on the tolerance values of the model. Heteroscedasticity of the models were investigated using White's test.

3. Results

Twenty-four dogs were donated to the study. One Dachshund was excluded from the analysis on postmortem evaluation due to poor muscle condition caused by severe diabetes. Three owners did not return the pain and exercise questionnaires. The dogs were divided into two groups for each analysis: the breed analysis 1) Dachshunds (n = 16) and Border terriers (n = 7) and 2) and based on the MRI findings, Dachshunds affected with IVDH and (n = 8) Dachshunds not affected with IVDH (n = 8). Of the Border terriers, also three were affected with IVDH (Table 1). Descriptive data used in the analysis are presented in Table 1 and additional details are provided in Table 4, Supplementary data.

3.1. Differences between Dachshunds and Border terriers

Relative to Border terriers, Dachshunds had significantly longer back lengths (34.0 ± 1.3 cm vs. 39.9 ± 3.4 cm, P < .0001), lower height (37.2 ± 2.1 cm vs. 28.4 ± 3.6 cm, P < .0001) and higher BMI (10.0 ± 1.8 vs. 17.7 ± 5.3, P = .002). There were no significant differences in the other descriptive variables between the two breeds (Table 1, Table 4 Supplementary data). All of the thirteen muscles were investigated. The means and standard deviations are presented in Table 2, with an asterisk indicating a significant difference after scaling to body mass/back length. The *M. longissimus* dorsi was caudally fused to M. iliocostalis lumborum and dissecting them apart was difficult. The data of these two muscles are therefore presented as one functional unit in Figs. 3–6.

The analysis of data scaled to body mass showed that, relative to Border terriers, Dachshunds have significantly greater muscle mass in M. splenius (P = .044), *M. longissimus* capitis (P = .049) and M. iliocostalis thoracis (P = .001), longer muscle belly in M. ilocostalis thoracis (P = .0412) and higher PCSA in M. semispinalis complexus (P = .005) and M. iliocostalis lumborum (P = .017) (Table 2, Fig. 2). Border terriers, in turn, showed significantly longer muscle fascicles in M. semispinalis complexus (P = .004) and M. iliocostalis lumborum (P = .003) and higher PCSA in M. spinalis et semispinalis thoracis (P = .032) than Dachshunds (Table 2, Fig. 3).

The analysis of data scaled to back length showed that Border terriers have longer muscle bellies in the M. semispinalis complexus (P = .025), *M. longissimus* capitis (P = .048) and M. spinalis et semispinalis thoracis (P = .049) than Dachshunds (Table 2, Fig. 3). Border terriers also had significantly longer fascicle lengths in M. semispinalis complexus (P = .0003) and M. ilicostalis lumborum (P = .0004) than Dachshunds (Table 2, Fig. 4). Border terriers had higher AI for M. semispinalis complexus and biventer and M. iliocostalis lumborum, although these differences did not reach statistical significance (Fig. 5). Fig. 6 illustrates the functional roles of muscles by plotting average fascicle length against PCSA for each muscle in both breeds. The functional roles for M. splenius, M. semispinalis complexus and biventer, M. semispinalis et spinalis cervicis and *M. longissimus* capitis were mainly production of large movements. The *M. longissimus* thoracis et lumborum and M. iliocostalis lumborum were force producers in both breeds, with Dachshunds' M. iliocostalis lumborum being superior to that of Border terriers. When the *M. longissimus* thoracis et lumborum and M. iliocostalis lumborum were considered together as a functional unit, this unit had the greatest force production capacity in the Dachshund. M. multifidus lumborum in both Dachshunds and Border terriers had clearly less capacity to generate force than *M. longissimus* thoracis et lumborum or M. iliocostalis lumborum (Fig. 6).

3.2. Effect of covariates on the difference between Dachshunds and Border terriers

When scaled to body mass, there was a negative effect of age on muscle mass for M. iliocostalis lumborum (P = .004), *M. longissimus* cervicis (P = .001), *M. longissimus* thoracis et lumborum (P < .0001), M. multifidus lumborum (P < .0001), M. spinalis et semispinalis thoracis (P = .024) and M. multifidus thoracis (P = .047). No effect of age was present on muscle belly length or fascicle length for any of the investigated muscles, but there was a significant effect of age on the PCSA for M. multifidus cervicis (P = .031) and *M. longissimus* thoracis et lumborum (P = .007). After scaling to back length, age had a significant effect on muscle belly length for *M. longissimus* cervicis (P = .017) and M. spinalis et semispinalis thoracis (P = .010).

3.3. Difference between affected and non-affected Dachshunds

Affected Dachshunds were significantly older (13.4 ± 3.4 years) than non-affected Dachshunds (8.9 ± 4.9 years, P = .014). Although affected Dachshunds had higher Pfirrman grade, body weight and BMI as well as longer back length than non-affected dogs, these differences were not significant (Table 1). Pain score and duration of walks were similar for both affected and non-affected dogs (Table 1).

No significant differences emerged for affected vs. non-affected Dachshunds in muscle mass, belly length or PCSA (Table 3). The analysis encompassing the pain score and exercise variables showed affected dogs (n = 6) to have longer fascicle lengths than non-affected dogs (n = 7) in *M. longissimus* cervicis (P = .012). In the analysis on the full set of data (covariates Pfirrman grade/body weight or back length/age), the affected dogs had longer fascicle lengths than non-affected dogs in *M. longissimus* thoracic et lumborum (P = .005, Table 3).

3.4. Effect of covariates on the difference between affected and non-affected Dachshunds

The analysis of the full set of data with only body weight and Pfirrman grade as covariates revealed a significant effect of body weight on muscle mass, belly length, fascicle length and PCSA for the following muscles: *M. longissimus* cervicis, M. spinalis et semispinalis thoracis, *M. longissimus* thoracis et lumborum, M. multifidus lumborum, M. iliocostalis thoracis and M. iliocostalis lumborum (all P < .05), and on muscle mass for M. multifidus thoracis (P = .026).

In the analysis with all covariates included, the effect of body weight was still significant on muscle mass for M. iliocostalis lumborum (P = .058), M. iliocostalis thoracis (P = .005), *M. longissimus* cervicis (P = .010), *M. longissimus* thoracis et lumborum (P = .042), M. multifidus lumborum (P = .010), M. spinalis et semispinalis (P = .006) and M. multifidus thoracis (P = .032). The effect on fascicle length was significant for M. iliocostalis lumborum (P = .021), *M. longissimus* thoracis et lumborum (P = .047) and M. spinalis et semispinalis (P = .030) and on PCSA for *M. longissimus* cervicis (P = .021) and *M. longissimus* thoracis et lumborum (P = .046).

In the sensitivity analysis, with back length and age as covariates, back length had a significantly increasing effect on all response variables in the following muscles: *M. longissimus* cervicis, M. spinalis et semispinalis thoracis, M. multifidus thoracis, *M. longissimus* thoracis et lumborum, M. multifidus lumborum, M. iliocostalis thoracis and M. iliocostalis lumborum (all P < .05). Back length had no significant effect on *M. longissimus* cervicis fascicle length. None of the investigated covariates had any effect on differences detected between affected and non-affected Dachshunds.

4. Discussion

This study has inferred functional roles for epaxial muscles in the Dachshund and Border terrier breeds based on quantitative anatomical

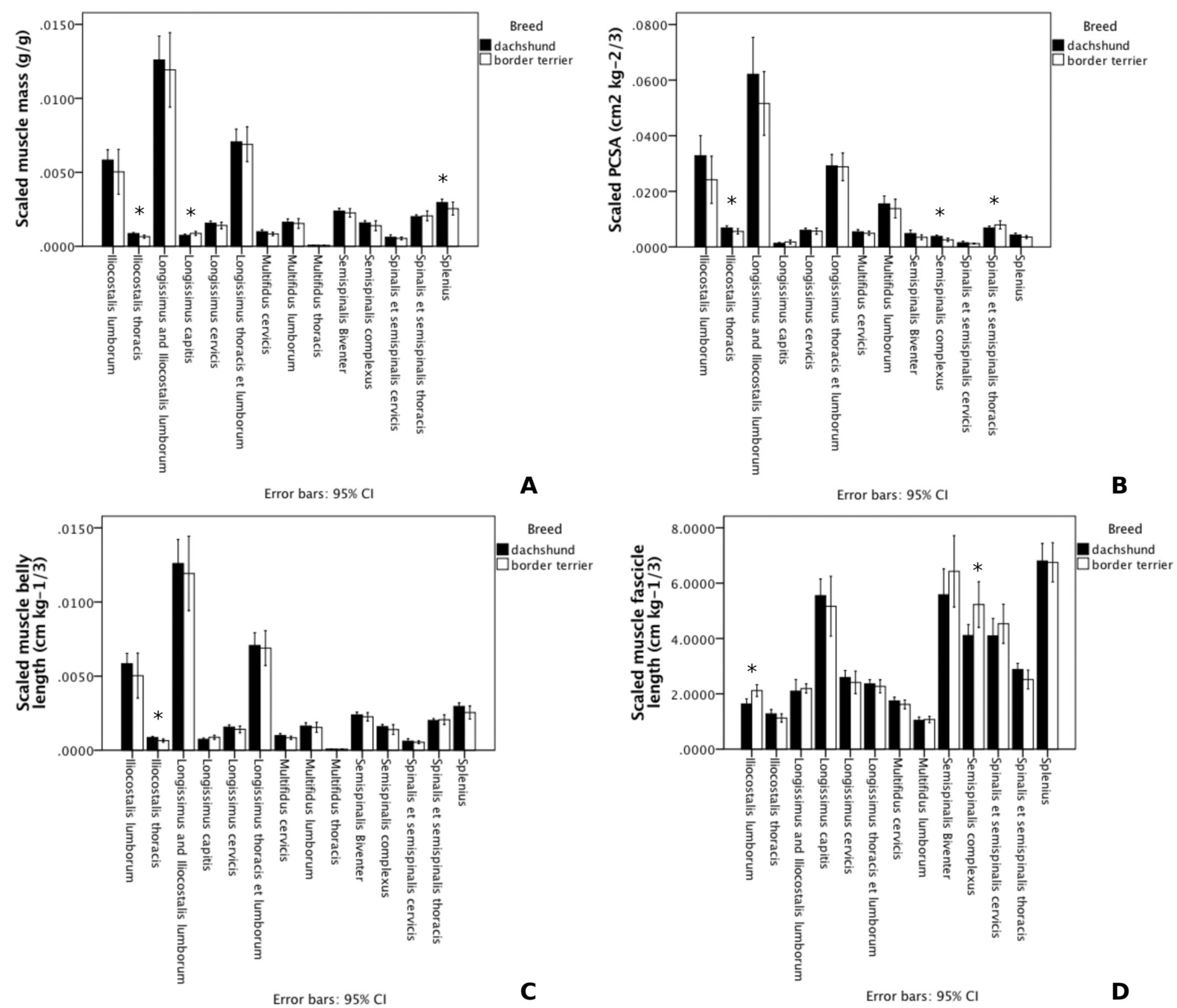

Fig. 3. Differences in muscle parameters of A) muscle mass, B) physiological cross-sectional area, C) muscle belly length, D) muscle fascicle length, scaled to body mass, between the two breeds. The bars represent the mean with 95% confidence interval for Dachshunds (black) and Border terriers (white). Significant differences ($P < .05$) are indicated with an asterisk.

variables. The results suggest that lumbar epaxial muscles in particular may play an important role as force producers in the stabilization of the spine. From an anatomical point of view, the stabilizing function of a muscle can be estimated based on its force production capacity using PCSA (Webster et al., 2014; Zwambag et al., 2014). As explained in the methods, the maximum isometric force (F_{max}) is directly related to PCSA, i.e. a muscle with large PCSA will also have higher F_{max} (Myatt et al., 2011; Williams et al., 2008a; Webster et al., 2014). Extrapolation from PCSA to precise values of F_{max} requires knowledge of muscle specific tension values (Payne et al., 2004, 2005) and such values do not exist for the muscles in these studied breeds. Also, to get accurate values for power generating capacity, the contraction velocity need to be known (Payne et al., 2004, 2005). Therefore the presented results should be taken as estimates, not exact quantitative F_{max} values. The M. iliocostalis lumborum has high potential for force production in both breeds, but significantly more in the Dachshund (Fig. 6). As a broad principle, this may suggest a higher requirement for both postural and dynamic stability provided by the epaxial muscles to compensate for the long spine. The muscle fascicles were longer in the longissimus muscle in non-affected versus affected Dachshunds, but the absence of any other differences between the two groups suggest that muscle morphology may not be altered significantly by IVDH.

4.1. Differences between Dachshunds and Border terriers

We hypothesized that epaxial muscles in Dachshunds would possess high potential for spinal muscle force production. In particular the large M. iliocostalis lumborum, which is known to be important in generating high force also in other breeds (Webster et al., 2014), is similarly adapted for force production in Dachshunds (Fig. 6). One line of thought is that Dachshunds require more muscle force to compensate for the long vertebral column due to chondrodystrophic conformation (Verheijen and Bouw, 1982). Dachshunds' M. iliocostalis lumborum seem better suited for force production (providing stability of the spine) than that of Border terriers. In the latter breed, where it has longer fascicles, it is likely to contribute more to allowing large movements in addition to providing stability. This suggests that we can accept our first hypothesis. The long Dachshund spine, susceptible to disc degeneration

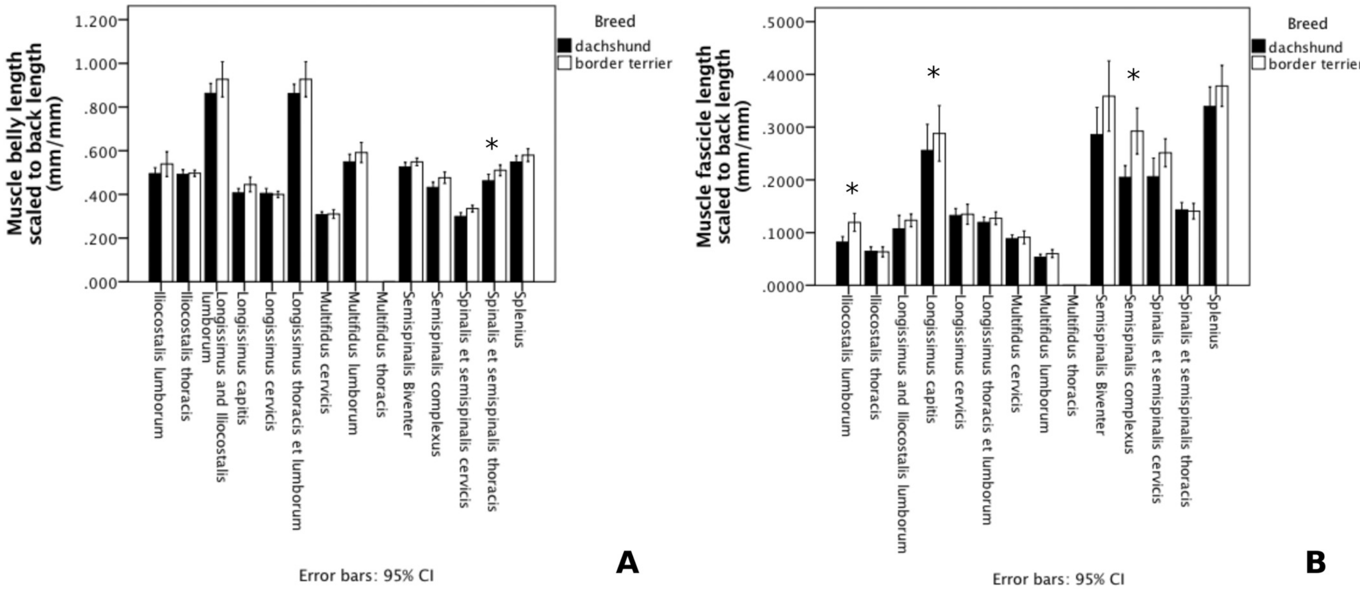

Fig. 4. Differences in muscle parameters between the two breeds, scaled to back length. Parameters comprise A) muscle belly length and B) muscle fascicle length. The bars represent the mean with 95% confidence interval for Dachshunds (black) and Border terriers (white). Significant differences ($P < .05$) are indicated with an asterisk.

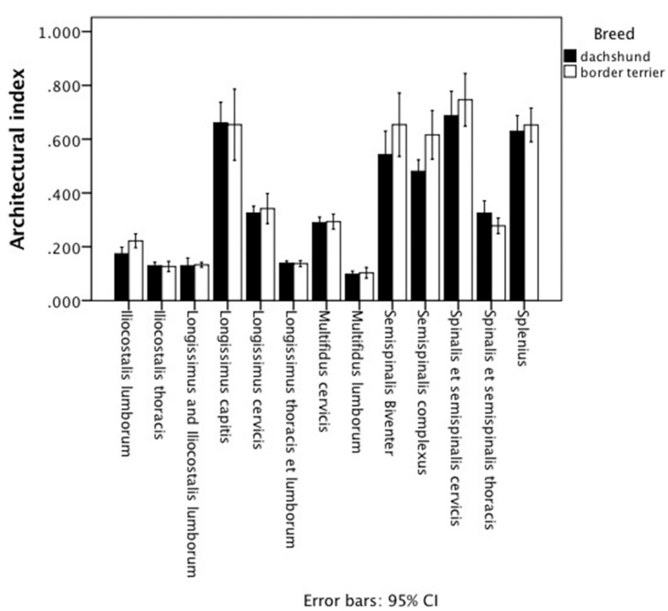

Fig. 5. Architectural index (AI; fascicle length/muscle length) for Dachshunds (black) and Border terriers (white). Bars represent the mean with 95% confidence interval.

and IVDH, may require more dynamic stabilization provided by muscles.

Previously, estimated functional roles of epaxial muscles have been considered in the Greyhound, selectively bred for sprinting, and the Staffordshire bull terrier, bred for fighting. The M. iliocostalis lumborum in our studied breeds (Fig. 6) appear to have similar stabilizing functions to the Staffordshire bull terrier (Webster et al., 2014), with the Dachshund and the Staffordshire bull terrier being more alike than the Border terrier. When M. iliocostalis lumborum is considered together with *M. longissimus* thoracis et lumborum as a combined functional unit, the estimated function changes more towards power production in all breeds.

Border terriers showed longer muscle fibers in M. semispinalis complexus and M. iliocostalis lumborum, regardless of the scaling method (Figs. 3d and 4b). The longer muscle fascicles in these muscles would suggest that they likely function over a wider range than the same muscles in the Dachshund, and may be involved in production and controlling large movements of the spine. However, three of the Border terriers also had IVDH, and it may be that the fascicle length was influenced by the disease (discussed later in the text for the longissimus muscle in affected Dachshunds). The motion of the Dachshund spine may be altered due to the predisposition of disc degeneration or due to a long and mobile spine being more vulnerable than a shorter spine. The biomechanical function of the healthy intervertebral disc is to transfer compressive forces between vertebrae and to provide both movement and stability to the spinal segments (White and Panjabi, 1978). In the degenerated disc, the annulus fibrosus becomes stiffer and weaker, preventing it from resisting tensile forces sufficiently (Bergknut et al., 2013; Johnson et al., 2010). This suggests that the stabilizing function of the intervertebral disc decreases with increased degeneration (Bergknut et al., 2013), which would require increased work from surrounding muscles to compensate for the lost stability. This is further supported by research showing that the more degenerative and fibrocartilaginous chondrodystrophic disc is less stiff and incurs greater displacement during spinal motion than the non-chondrodystrophic, non-degenerated disc (Erwin et al., 2015). It could therefore be an important adaptation for a chondrodystrophic breed to have higher spinal stiffness provided by force-producing muscles.

Our second hypothesis was that the muscle architecture of epaxial muscles would differ between Dachshunds and Border terriers because of their differing geometry. Based on our results, we can accept this hypothesis for M. semispinalis complexus, M. spinalis et semispinalis thoracis and M. iliocostalis lumborum. The longer fascicles in M. semispinalis complexus and M. ilicostalis lumborum as well as the higher AI in M. semispinalis complexus and biventer and M. iliocostalis lumborum in the Border terrier support the suggestion that these muscles contribute to large movements of this breed's spine. This may be related to Border terriers having longer legs, hence greater leverage, causing more movement through the spine from the hind limbs during locomotion (Hudson et al., 2011; Williams et al., 2008a). Unfortunately, the authors are not aware of any kinematic studies on the spinal motion in these two breeds, and such data are required to fully develop our argument.

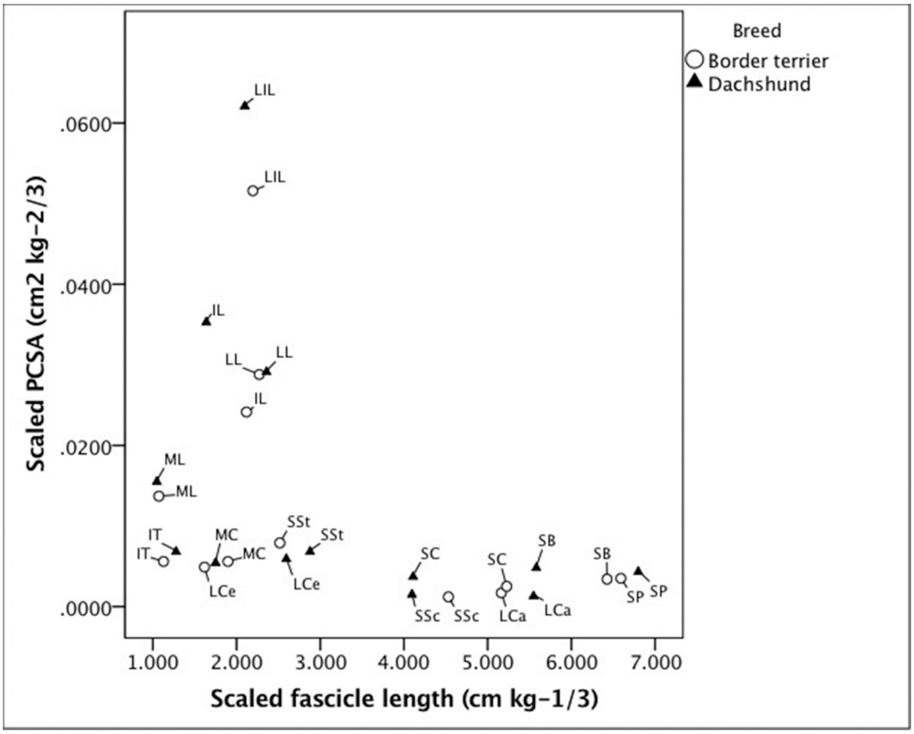

Fig. 6. Mean scaled PCSA plotted against mean scaled fascicle length. Muscles in the bottom right of the plot are muscles working over a wide range of motion. Muscles towards the top right of the plot would be suited for power production, and muscles to the top left of the plot have high capacity for generating force. For both Dachshunds and Border terriers, *M. longissimus* thoracis et lumborum, M. iliocostalis lumborum and M. multifidus lumborum have high capacity for force generation. SP = splenius, SB = semispinalis biventer, SC = semispinalis complexus, LCa = longissimus capitis, SSc = spinalis et semispinalis cervicis, SSt = spinalis et semipsinalis thoracis, LCe = longissimus cervicis, MC = multifidus cervicis, IT = iliocostalis thoracis, IL = iliocostalis lumborum, LL = longissimus thoracis et lumborum, ML = multifidus lumborum, LIL = longissimus thoracis et lumborum and iliocostalis lumborum combined.

Table 3
Muscle data for affected versus non-affected Dachshunds.

Muscle	Mass (g)		Muscle belly length (mm)		Fascicle length (mm)		PCSA (mm^2)	
	Affected	Non-affected	Affected	Non-affected	Affected	Non-affected	Affected	Non-affected
Longissimus cervicis	14.3 (5.94) P = .855[a] P = .369[b] P = .368[c]	14.4 (3.94)	160.9 (22.91) P = .975[a] P = .063[b] NS[c]	159.0 (16.06)	56.4 (12.71) P = .160[a] P = .011[b]* P = .265[c]	47.8 (6.10)	2.5 (0.84) P = .273[a] P = .256[b] P = .169[c]	2.9 (0.79)
Multifidus thoracis	0.7 (0.27) P = .343[a] P = .641[b] P = .944[c]	0.7 (0.21)						
Spinalis et semispinalis thoracis	18.8 (7.52) P = .414[a] P = .832[b] P = .611[c]	18.1 (5.23)	190.6 (28.05) P = .406[a] P = .585[b] P = .753[c]	175.2 (25.52)	62.0 (10.50) P = .085[a] P = .619[b] NS[c]	54.3 (12.86)	2.9 (0.78) P = .195[a] P = .458[b] P = .112[c]	3.2 (0.55)
Longissimus thoracis et lumborum	61.0 (25.52) P = .303[a] P = .278[b] P = .443[c]	68.8 (23.90)	353.1 (39.60) P = .217[a] P = .737[b] P = .560[c]	331.3 (34.36)	51.1 (10.84) P = .004[a]* P = .179[b] NS[c]	44.1 (5.04)	11.5 (3.28) P = .155[a] P = .071[b] P = .210[c]	14.5 (3.96)
Iliocostalis thoracis	8.7 (3.84) P = .083[a] P = .158[b] P = .848[c]	7.3 (1.87)	208.5 (28.61) P = .074[a] P = .667[b] P = .207[c]	187.7 (19.63)	28.2 (8.85) P = .158[a] P = .512[b] P = .554[c]	23.7 (5.28)	3.0 (0.73) P = .554[a] P = .513[b] NS[c]	3.1 (0.87)
Multifidus lumborum	14.9 (8.17) P = .672[a] P = .411[b] P = .838[c]	15.4 (5.55)	213.3 (39.71) P = .820[a] P = .843[b] NS[c]	221.3 (27.02)	21.2 (5.82) NS[a,b,c]	20.8 (1.82)	6.8 (3.19) P = .876[a] P = .781[b] P = .896[c]	7.2 (2.87)
Iliocostalis lumborum	53.4 (23.45) P = .931[a] P = .473[b] P = .606[c]	54.6 (20.05)	196.9 (39.06) P = .729[a] P = .318[b] P = .795[c]	194.9 (23.08)	34.7 (7.44) P = .468[a] P = .532[b] NS[c]	31.7 (10.16)	13.8 (4.95) P = .499[a] P = .676[b] NS[c]	16.1 (5.95)

Unscaled mean and (SD) for muscle mass, muscle belly length, muscle fascicle length and PCSA averaged from the left and right side in affected and non-affected Dachshunds.

NS (not shown) indicates a poor fit of the statistical model, thus, results are considered unreliable and are not presented.

[a] P-values are based on ANCOVA analysis of the full set of data with body weight and level of degeneration as covariates (affected n = 8, non-affected n = 8). An asterisk indicates significant difference between the two groups.

[b] P-values are based on ANCOVA analysis with body weight, level of degeneration, pain sum and duration of walks as possible covariates (affected =6, non-affected n = 7). An asterisk indicates significant difference between the two groups.

[c] P-values are based on ANCOVA analysis with back length and age as covariates (affected n = 7, non-affected n = 8).

In Border terriers, the M. spinalis et semispinalis had higher PCSA and longer belly length than in Dachshunds. In particular the higher PCSA would suggest higher force production in this muscle, hence greater capacity for stabilization, in the Border terrier, despite the shorter back. The reasons for these differences are not known, but it is clinically noteworthy that this muscle is large in relation to other epaxial muscles and spans two major motion segments in the spine, the cervicothoracic and thoracolumbar junctions (Evans, 1993). Nevertheless, the M. spinalis et semispinalis rarely receives attention in clinical rehabilitation practice.

In the neck, the M. splenius and M. semispinalis biventer appear to have the potential to produce or control and allow large movements in both breeds, slightly more so in the Border terrier. This is in agreement with previous research stating that the function of cervical epaxial muscle is to maintain posture against gravity, stabilize the cervical spine and thoracic segments and produce a wide range of movements (Sharir et al., 2006). It must be noted that scaling by back length may not be suitable for the neck muscles, specifically as this region is not included in the “back length” measurement, thus neck length may be independent of total back length. However, our results are consistent regardless of the scaling method used, giving a good degree of certainty that clear patterns of muscle morphology are present.

We could not accept our second hypothesis for two of the more caudal spinal muscles, M. multifidus lumborum and *M. longissimus* thoracis et lumborum, as muscle parameters were similar for these two muscles in each breed. Perhaps selective breeding in Dachshunds and Border terriers has not been as extreme in terms of muscle functional anatomy as it has been in the sprinting Greyhound and the fighting Staffordshire bull terrier. The *M. longissimus* had higher mass and PCSA in the Greyhound than in the Staffordshire bull terrier (Webster et al., 2014) and when *M. longissimus* was combined with M. iliocostalis lumborum the propensity for power production was clearly higher in the Greyhound. This indicates suitability for rapid and powerful extension of the spine during sprinting tasks (Webster et al., 2014). Both the Dachshund and the Border terrier were originally bred and used for the same purpose: hunting under the ground in burrows (digging and crawling) and on the ground (running). The muscle architecture may be influenced more by artificial selection for particular locomotor purpose and workload, rather than by simply the geometry of the animal.

The estimated force production capacity of M. multifidus lumborum was small relative to the capacity of *M. longissimus* thoracis et lumborum and M. ilocostalis lumborum. Muscles with high force production capacity, short muscle fascicles and high PCSA are adapted towards stabilizing the spine (Webster et al., 2014). Electromyography studies show that *M. longissimus* lumborum and M. multifidus lumborum perform stabilizing work against large movements and forces at different gaits (Schilling and Carrier, 2009, 2010; Ritter et al., 2001). M. multifidus lumborum has been previously presented as an important muscle for stabilization and motor control of the spine in humans (Macdonald et al., 2006; Ward et al., 2009). Also in pigs, horses and dogs, M. multifidus lumborum is considered a key muscle for dynamic stabilization of the spine (Hodges et al., 2006; Stubbs et al., 2010; Schilling and Carrier, 2010). These studies investigated M. multifidus using CSA measurements on MRI, histology and EMG, but they did not consider force production capacity in terms of muscular PCSA. In our Dachshunds and Border terriers, however, the relatively small predicted force production capacity of M. multifidus lumborum compared with other lumbar epaxial muscles illustrated in Fig. 6 gives rise to the question: why would the force production capacity of M. multifidus lumborum not differ between groups in two breeds, given their potentially different physical demands for postural and dynamic stabilization of the spine? The M. multifidus lumborum is known to restrict and control motion between individual spinal segments (Evans, 1993; Ritter et al., 2001; Ward et al., 2009), hence is considered a postural stabilizer of the vertebral column. When looking at force production capacity, it seems that both *M. longissimus* thoracis et lumborum and M. iliocostalis lumborum in our dogs could provide both postural and dynamic stability to the spine. Further information on muscle fibre type would be useful in order to confirm these suggestions, but unfortunately this was outside of the scope of this study.

In rehabilitation of dogs with spinal disease, we tend to consider that findings in the human spine are directly transferable to the quadruped spine (Boström et al., 2014; Cain et al., 2016; Henderson et al., 2015). This may be inappropriate considering the different gaits in dogs (Schilling and Carrier, 2010) and the possible effects of gravity on the horizontal spine in quadrupeds compared with the vertical spine in bipeds. There may be a need to review current rehabilitation routines and consider more targeted species- or breed-specific approaches, including stimulation of force production of the epaxial muscles.

4.2. Difference between affected and non-affected Dachshunds

Our last hypothesis was that the mass, PCSA and fascicle length of epaxial muscles would be decreased in Dachshunds affected by IVDH. Our results indicated that the Pfirrman grade or IVDH did not affect the muscle architecture in the thoracolumbar epaxial muscles, and therefore, this hypothesis was rejected for these muscles. The affected Dachshunds were older and had higher Pfirrman grades. These findings are in line with previous research stating that disc degeneration increases with age (Bergknut et al., 2011; Hansen, 1951). Although the Pfirrman grading system itself is considered reliable (Bergknut et al., 2011), the MRI evaluation shows only the current situation and it remains unknown how long the disc degeneration or the IVDH has been present. Additionally, the severity of spinal cord compression does not always correspond to clinical signs. (Besalti et al., 2006). Several of the affected Dachshunds had only mild spinal cord compression and four had multiple affected intervertebral discs. Whether the muscles in these dogs were affected by disuse due to prolonged pain or discomfort remains unknown, and future studies should aim to confirm our findings in dogs with established clinical signs of IVDH.

The fascicle lengths were significantly shorter in non-affected than affected Dachshunds in *M. longissimus* cervicis and *M. longissimus* thoracis et lumborum. Decreased fascicle length in addition to decreased PCSA and volume would be expected in sarcopenia, i.e. the loss of muscle mass associated with ageing (Narici and Maffulli, 2003), and in disuse atrophy (De Boer et al., 2007; Narici and Maffulli, 2003). If the affected Dachshunds in this study were presenting with disuse atrophy, one would expect these individuals to have decreased fascicle length as well as reduced muscle PCSA and volume. However, the fascicles were longer in the affected Dachshunds and there was no difference in PCSA between the groups, suggests the change in fascicle length in the longissimus muscle occurred independently of muscle size. It is therefore unlikely that disuse atrophy would be the major cause of these differences. One theory is that the longissimus muscle in the non-affected Dachshunds has reduced working range, but the reasons remain unknown. Another explanation may be that the muscles in the affected Dachshunds are compensating to maintain a certain posture of the spine that would allow for pain-free and economical locomotion despite the disease. Dachshunds with IVDH frequently show kyphosis of the thoracolumbar spine (Coates, 2014). It is known that muscles adapt to load and eccentric work increases fascicle length (Franchi et al., 2014; Narici and Maffulli, 2003; Narici et al., 2016). That said, the epaxial muscles in a kyphotic spine would have to work eccentrically and adapt to working in an elongated position by increasing their fascicle length. Information from a clinical examination prior to euthanasia would be needed to confirm this theory. Another line of thought suggests that the affected, older dogs with more disc degeneration have a less stable spine, causing the longissimus muscle to work over a greater range of motion, with longer muscle fascicles as a result of adaptation to the abnormal load.

Age as a covariate had no effect on any response variables in this population. Interestingly, however, during the dissections, macroscopic fat infiltration was observed in the lumbar iliocostalis and longissimus muscles in the oldest dogs. Unfortunately these observations were too few and too heterogeneous in nature to include in the analysis. However, considering that fat infiltration increases in both disuse and neurogenic muscle atrophy (Kamath et al., 2008; Narici and Maffulli, 2003), this may have influenced the results and muscular changes related to ageing could be an interesting topic for future research in dogs. Still, it must be kept in mind that body weight had an effect on most of the response variables. The use of ANCOVA models with body weight as a covariate is considered a robust alternative to scaling (Myatt et al., 2011), and we cannot exclude that body weight in combination with the small sample may have influenced the results. Interestingly, both the affected and non-affected Dachshunds had very similar pain scores and exercise levels. This provides another potential explanation for why there was no decreased mass or PCSA detected in the affected Dachshunds.

4.3. Limitations

The data collection relied on dog owners donating their pets to research after euthanasia. This resulted in very old dogs and dogs with possible underlying diseases as well as a small sample size. Twenty-one of the 24 owners completed the questionnaires despite the emotional circumstances related to the donations. The questionnaires were not validated, but they provided valuable information about pain history and exercise regimes that have not been accounted for in previous studies on canine muscle architecture (Webster et al., 2014; Williams et al., 2008a, 2008b). The questionnaires were answered retrospectively, sometimes several months after euthanasia. This may have decreased the reliability of the replies, and previous research highlights the subjectivity in pain evaluations reported by owners (Brown et al., 2007).

We were able to relate the clinical signs to the MRI findings in only three dogs, as they were euthanized because of hind limb paralysis, and postmortem MRI confirmed the IVDH. This is a flaw in the study design, but data collection from busy clinical practice and emergency duty made reliable clinical evaluation pre euthanasia impossible and thus the determination of affected versus non-affected status was based on MRI evaluation only. In terms of the breed analysis, it must be noted that three Border terriers were also classified as affected. This presence of disc degeneration or IVDH may have influenced the results of the breed comparison and interpretation of the results should be done with caution. Considering the high age and IVDH in the studied dogs as well as the observed macroscopic fat infiltration, this has certainly influenced our results and may partly explain why no other differences were detected in the response variables. Although the power analysis suggested our sample size to be sufficient, the large variation in descriptive statistics may have required a larger sample of affected and non-affected Dachshunds to detect changes in muscular architecture.

5. Conclusions

We have estimated functional roles of the epaxial muscles of Dachshunds and Border terriers. The M. iliocostalis lumborum in the Dachshund seems more suitable for generating force and stabilization, while in the Border terrier it appears better adapted for contributing large movements. The Dachshund, susceptible to disc degeneration and IVDH, may require more stabilization for its ertebral column, but whether the M. iliocostalis meets the demand for stabilization sufficiently remains unknown. The longer fascicle lengths in the longissimus muscle of affected Dachshunds may suggest a consequence of compensation due to lost stability or altered position of the spine. Based on this research, we suggest considering targeted, controlled, breed-specific retraining exercises that stimulate force production in the epaxial musculature of dogs recovering from spinal disease.

Declarations of interest

None

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Appendix A. Supplementary data

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