EVALUATION OF EPAXIAL MUSCLE STRUCTURE IN DOGS WITH SPINAL DISEASE

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Cover picture, the Dachshund Harmi Armi by Vesa-Ville Välimäki

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To my boys
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Abstract

Epaxial muscle atrophy, decreased cross-sectional area (CSA) and increased fat infiltration has been found in dogs with spinal pathology. However, methods to quantify muscle structure have not been validated, and breed differences in the epaxial muscle architecture of dogs have not been considered in relation to spinal pathology.

We aimed to validate a method to quantify epaxial muscle size and composition in dogs using magnetic resonance imaging (MRI) and computed tomography (CT) and to estimate functional roles of the epaxial muscles in Dachshunds and Border terriers. Further aims were to investigate the influence of intervertebral disc disease (IVDD) on epaxial muscle size, composition and internal architecture in dogs.

Two blinded observers evaluated the CSA, fat content and muscle attenuation of the Mm. multifidi, M. spinalis et semispinalis and M. longissimus from low field MR and CT images of 10 healthy Dachshunds. The measurement method was reliable, showing substantial to almost perfect intra- and inter-rater reliability for all variables.

We used the muscular architecture parameters muscle mass, muscle belly length, fascicle length and physiological cross-sectional area (PCSA) to estimate the force and power production capacity of the epaxial muscles in Dachshunds and Border terriers and in Dachshunds with IVDD. We found significantly higher PCSA and shorter fascicles in M. semispinalis complexus and M. iliocostalis lumborum in Dachshunds compared to Border terriers. The IVDD affected Dachshunds had significantly longer muscle fascicles than non-affected Dachshunds in M. longissimus cervicis and thoracis et lumborum.

We also investigated the CSA and fat infiltration of the Mm. multifidi and M. longissimus from high-field MR images in Dachshunds with IVDD and in dogs with fibrocartilaginous embolism (FCE). The FCE dogs had significantly greater CSA and less fat infiltration in the Mm. multifidi and M. longissimus than Dachshunds.

Our findings showed that the epaxial muscle CSA and fat content can be reliably measured both on MRI and CT. The Dachshund epaxial muscles have greater potential for force production than Border terriers, suggesting a greater requirement for spinal stability in the Dachshund. The longer fascicles in the M. longissimus of affected Dachshunds may suggest compensation due altered position of the spine. The decreased CSA and increased fat infiltration may be related to compressive IVDD in Dachshunds.

We conclude that breed differences must be considered when evaluating muscle structure. Further, our results supports previous evidence that the epaxial muscle structure may be compromised in Dachshunds with IVDD.
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List of original publications

This thesis is based on the following publications, which are referred to in the text by their Roman numerals:


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Abbreviations

AI  Architectural Index
ANCOVA  Analysis of Covariance
ANOVA  Analysis of Variance
CI  Confidence Interval
CSA  Cross-sectional area
CT  Computed tomography
DICOM  Digital Imaging and Communication in Medicine
EMG  Electromyography
FCE  Fibrocartilaginous embolism
Fmax  Maximal isometric force
HU  Hounsfield Units
ICC  Intra-class correlation coefficient
IVDD  Intervertebral Disc Disease
IVDH  Intervertebral Disc Herniation
LBP  Low Back Pain
MPa  Megapascal
MRI  Magnetic Resonance Imaging
PACS  Picture Archive and Communication System
PCSA  Physiological cross-sectional area
ROI  Region of Interest
SD  Standard Deviation
TE  Time to Echo
TR  Time to Repeat
WL  Window Level
WW  Window Width
1 Introduction

In dogs, the function of epaxial muscles is to produce extension and lateral bending of the spine, to maintain posture of the spine, to counteract gravity and forces exerted on the spine by the hind limbs providing local and dynamic stabilisation to the spine (Ritter et al., 2001; Schilling and Carrier, 2009; Webster et al., 2014). Skeletal muscle function depends on anatomical location, architecture, electrical activity, metabolic profile and possible degenerative changes in the muscles related to disease or inactivity.

Muscle architecture, the arrangement of muscle fibres within the muscle relative to the axis of muscle force generation, can be used to estimate the force and power production capacity of a muscle. Studies on muscles in the limbs (Pasi and Carrier, 2003; Williams et al., 2008a; 2008b) and the back of dogs (Webster et al., 2014) suggest breed-specific differences in muscle architecture due to selective breeding of the animal for a certain purpose.

In humans, low back pain (LBP) is one of the most frequent causes of extended sick leave and early retirement (Hoy et al., 2014). The human paraspinal muscles, equivalent to the canine epaxial muscles and the Mm. multifidi in particular, are considered important muscles for stabilization and sensorimotor control of the human spine (Moseley et al., 2002; 2003; MacDonald et al., 2006). Low back pain results in muscle dysfunction of particularly the Mm. multifidi and the M. erector spinae (Hodges and Tucker 2011) as well as altered muscle structure, such as decreased cross-sectional area (CSA) or volume, and increased fat infiltration. Such muscular degeneration may compromise a muscle’s contractile ability and force production (D’Hooge et al., 2012). The CSA may be decreased and fat infiltration increased locally near the painful segment, on the side of the symptoms and generally in back pain patients compared with controls (Hides et al., 1994; Batté et al., 2012; D’ Hooge et al., 2012). Dysfunction and altered muscle structure may persist, although pain has resolved or the injury healed (Hides et al., 1996; Hodges and Tucker 2011). Therefore human back pain management emphasizes active therapeutic exercise based on thorough clinical assessment and clinical reasoning with the aim of restoring optimal function of the paraspinal muscles (Falla and Hodges 2017). Knowledge about how muscle structure is altered due to pain or spinal pathology is important in the development of treatment and prevention of back pain (Goubert et al., 2016).

In humans, paraspinal muscle degeneration has been investigated for years using MRI and CT (Danneels et al., 2000; Barker et al., 2004; Hides et al., 2007; Batté et al., 2012; Crawford et al., 2017). The CSA of a muscle can reliably be measured from MRI or CT transverse images, and the fat infiltration can be estimated using the signal intensity on MRI and the muscle attenuation on CT (Danneels et al., 2000; Battie’ et al. 2012; D’Hooge et al., 2012; Valentin et al., 2015a). Unfortunately, large variety in quantification methods used causes inconsistency in the results and makes it difficult to draw conclusions across studies (Goubert et al., 2016). Scanning parameters and image view settings are often insufficiently reported along with poorly described measurement protocols and inadequate observer training and experience (Valentin et al., 2015a; Crawford et al., 2017).

Intervertebral disc degeneration is very common in Dachshunds due to genetic factors related to its chondrodystrophic conformation (Hansen 1951; Bergknut et al., 2013). The disc degeneration frequently leads to thoracolumbar intervertebral disc disease (IVDD), with extrusion of the nucleus pulposus into the spinal canal, causing acute spinal cord
compression, which results in pain and different grades of paraparesis (Langerhuus and Mills 2017). The recommended treatment is surgical decompression for non-ambulatory patients, and mild cases are treated conservatively with strict cage rest (Moore et al., 2016; Langerhus and Mills 2017). Although physiotherapy is often used postoperatively with the focus on restoring the motor function in the hind limbs (Hodgson et al., 2017; Zidan et al., 2018), new research did not confirm the benefits of physiotherapy in dogs with acute thoracolumbar IVDH (Zidan et al. 2018).

Very little is known about epaxial muscles in dogs in relation to spinal disease, chondrodystrophic conformation, ageing, pain or exercise. Also, validated methods to quantify muscle structure in dogs are lacking. Information about epaxial muscles in chondodystrophic dogs is required for a more accurate clinical reasoning process in physiotherapy and to design exercises targeting the epaxial muscles specifically. When this thesis was initiated, no information existed on epaxial muscle structure in connection with spinal disease in dogs. This work focuses on epaxial muscle architecture and degenerative changes concerning IVDD in dogs and reflects upon these in light of human back pain literature and the canine EMG research available.
2 Review of the literature

2.1. Functional anatomy of epaxial muscles in dogs

2.1.1. Anatomy of epaxial muscles
The canine epaxial muscles are described as three longitudinal muscular systems, each with multiple fascicles overlapping several vertebrae (Evans 1993). Moving in the direction medial to lateral, these are as follows: 1) the transversospinalis muscle system, including *M. spinalis*, *M. semispinalis*, *Mm. multifidi*, *Mm. rotatores* and *Mm. intertransversari*, 2) the longissimus system, including *M. longissimus capitis*, *M. longissimus cervisis* and *M. longissimus thoracis et lumborum*, and 3) the iliocostalis system, including *M. iliocostalis thoracis* and *M. iliocostalis lumborum* (Evans 1993, Figure 1).

![Schematic illustration of the transversospinalis (light blue), the longissimus (green) and the iliocostalis muscle systems (yellow). Modified from Evans and De Lahunta (2010).](image-url)
In the cervical spine, the *M. splenius* lies most superficially, with *M. semispinalis complexus* and *M. biventer* underneath (Figure 3). The *M. multifidus cervicis* is one of the deepest neck muscles, located medially to the spinous processes and dorsolaterally to the articular facets. It has a large mass relative to other epaxial muscles in the neck and contains six completely inseparable divisions further divided into superficial and deep portions (Sharir et al., 2006) (Figure 2). In the caudal cervical region, the most medial muscle is the *M. spinalis et semispinalis cervicis*, and the *M. longissimus cervicis* lies on top of this, spanning the cervicothoracic junction (Evans 1993; Evans and De Lahunta 2010).

![Figure 2. The Mm. multifidi in the cervical, thoracic and lumbar regions (Evans 1993). Reprinted with permission.](image)

In the thoracic and lumbar spine, the *Mm. multifidi* is the most medial muscle to the spinous processes (Figure 2). It is a segmental muscle with individual parts originating from articular, transverse and mamillary processes of one vertebra, it overlaps two vertebrae and it inserts onto the spinous process on the cranial vertebrae (Ritter et al. 2001). Lateral to *Mm. multifidi* lays the *M. longissimus*, with multiple fibres of variable lengths spanning several segments (Ritter et al., 2001). The *M. spinalis et semispinalis thoracis* is the most medial muscle next to the spinous processes and dorsomedially to the *M. longissimus* (Figure 3). It extends from the spinous processes of the first few lumbar vertebrae to the last few cervical vertebrae (Evans 1993). The most lateral epaxial muscle, the *M. iliocostalis thoracis*, originates from the cranial aspect of the vertebral ends to the ribs. Fibres form a muscle belly from which thin tendons arise, attaching on the caudal aspects of the ribs and transverse processes of the seventh vertebra (Evans 1993; Ritter et al., 2001).

In the lumbar spine, the *M. multifidus lumborum* (Figure 1 and 2) is the most medial muscle next to the spinous processes, followed by *M. longissimus lumborum* and *M. iliocostalis lumborum* laterally. The fascicles of the *M. multifidus lumborum* are attached tightly to one another, forming a virtually homogeneous muscle belly (Evans 1993) (Figure 4). The *M. longissimus lumborum* continues caudally from the *M. longissimus thoracis* and attaches to the lumbar vertebrae, the ileum, the thoracolumbar fascia and the *M. iliocostalis lumborum*. The latter muscle overlaps several spinal segments and originates from the ileum, inserting onto the tenth to thirteenth ribs. The muscle bellies of the *M. longissimus* and *M. iliocostalis lumborum* are fused tightly together (Evans 1993; Webster et al., 2014).
Most of the epaxial muscles are multisegmentally innervated (Evans 1993). The Mm. multifidi are innervated by the medial branches of the rami dorsales in the cervical, thoracic and lumbar regions (Evans 1993). M. longissimus and the M. iliocostalis are innervated by the dorsal branches of cervical, thoracic and lumbar nerves (Evans 1993).

Based on the anatomical location of the epaxial muscles, their function is to produce movements of the spine (Evans 1993; Ritter et al., 2001). The localization dorsally to the transverse processes provides them with the anatomical features suitable for extension and lateral bending of the spine, but also restricts and control flexion of the spine in the sagittal plane (Evans 1993; Ritter et al. 2001).

2.1.2. Muscle function based on muscle architecture
Muscle architecture is defined as the arrangement of muscle fibres within the muscle relative to the force generation axis. Muscle architecture represents the following five parameters: muscle mass, muscle belly and tendon length, muscle fibre length, muscle
physiological cross-sectional area (PCSA) and muscle pennation angle (Myatt et al., 2011; Lieber and Ward 2011; Webster et al., 2014).

Muscle mass is the weight of the muscles in grams or kilograms (Webster et al., 2014). Muscle belly length represents the whole line of action for that muscle (Webster et al., 2014). Muscle fibre length reflects the number of sarcomeres in series and determines the range of lengths over which a muscle can generate active force (Zajac 1992; Sharir et al., 2006). The PCSA reflects the number of sarcomeres in parallel, and thus, the potential for force generation (Sharir et al., 2006).

Muscle architecture can be utilized in mathematical calculations to estimate muscle function (Webster et al., 2014). Muscle volume can be estimated by dividing muscle mass by a muscle density of 1.06 g/cm$^3$ (Mendez and Keys, 1960). Muscle volume is used to calculate PCSA: muscle volume/mean fascicle length. The size (mass or volume) and also the PCSA of a muscle are directly related to the muscle’s ability to generate force (Sharir et al., 2006). The maximum isometric force ($F_{\text{max}}$, unit N) is defined in physics as the interaction that causes an object of a certain mass to change its velocity (Force = Mass x Acceleration) (Blandpied and Neumann 2017). $F_{\text{max}}$ can be approximated by multiplying PCSA with 0.3 MPa, which is the estimated value of the maximum stress a muscle can produce, the reported maximum isometric stress of vertebrate skeletal muscle cells (Wells 1965). The $F_{\text{max}}$ is directly related to PCSA such that a muscle with large PCSA will also have a higher $F_{\text{max}}$ (Myatt et al., 2011; Webster et al., 2014). The power production of a muscle is the product of the $F_{\text{max}}$ and the shortening velocity of that muscle (unit P) (Hunter et al., 2017). The architectural index (AI) normalizes fascicle length for muscle belly length (AI=muscle fascicle length/muscle belly length). The AI reflects the number of sarcomeres in series in a muscle, and thus, the potential velocity of a muscle contraction (Sharir et al., 2006). This allows for comparison of AI between muscles, species and breeds.

Muscle architecture helps to determine the functional role of a muscle (Sharir et al., 2006; Webster et al., 2014). The principle is that a muscle with long fascicles and/or high AI works contributes in large movements. A muscle with short fascicles and high PCSA has high capacity for force production (Webster et al., 2014). A muscle with long fascicles and high PCSA is able to work over a wide range of motion with high velocity and high force, and therefore, has high propensity for power generation (Webster et al., 2014). In dogs, muscular architectural data have been used to predict a muscle’s maximal functional capacity, force production and power generation in order to describe work performance and stress on tissues (Williams 2008a; 2008b; Webster et al., 2014), while in human medicine muscle architecture additionally is used in the planning of surgical procedures (Ward et al., 2009a).

### 2.1.3. Differences in muscular architecture between dog breeds

In our domestic animals, selective breeding for a certain purpose or appearance has widely influenced animals’ structure and function (Pasi and Carrier 2003; Williams 2008a; 2008b; Webster et al., 2014). Differences between species and breeds can be studied using allometry (Webster et al., 2014). Allometry investigates 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 (Schmidt-Nielsen 1984; Myatt et al., 2011). Allometric scaling is used in biology when comparing two animals of different sizes or the same individual during growth (Schmidt-Nielsen 1984; Myatt et al., 2011).
An extreme example of differences in locomotion anatomy between dog breeds is the Greyhound (selectively bred for sprint speed and anaerobic stamina) and the Pit bull terrier (selectively bred for fighting). The Greyhound has less muscle mass in their distal limbs, stronger hind limbs and greater capacity to store elastic energy in the extensor muscle-tendon system of their tarsal joints. The fighting Pit bull terrier has great muscle mass in the distal front limbs and low capacity for elastic energy storage (Pasi and Carrier 2003). These structural differences are what make the Greyhound a sprinter and the Pit bull terrier more suitable for physical combat (Pasi and Carrier 2003).

Differences indicating specialization for sprinting or fighting are found also in the epaxial muscles of Greyhounds and Staffordshire bull terriers (Webster et al., 2014). The *M thoracis et lumborum* is the largest of the epaxial muscles in these two breeds, with the muscle mass significantly greater in the Greyhound. The Greyhound has longer fascicle lengths in several muscles and a higher AI especially in the *M. longissimus capitis* and in the combined *M. longissimus* and *M. iliocostalis* functional unit. This indicates that these muscles work over a large range of motion in the Greyhound compared with the Staffordshire bull terrier (Webster et al., 2014). The PCSA is greater in the neck muscles of Staffordshire bull terriers than Greyhounds and also in the *M. iliocostalis lumborum*. This suggests that these muscles have a higher capacity for force production and stability in the Staffordshire bull terrier than in the Greyhound. On the other hand, the PCSA is greater in the *M. longissimus muscle* and in the combined *M. longissimus* and *M. iliocostalis* functional unit in the Greyhound. This means that the Greyhound has higher propensity for power generation (high force over a wide range of motion) in these muscles in particular. Therefore, the Greyhounds’ epaxial muscles have the capacity to supplement power produced by the hind limbs (Webster et al., 2014). The epaxial muscles in the Staffordshire bull terrier are more prone to produce force and provide greater stabilization (Webster et al., 2014).

The selective breeding for anatomical and conformation features has resulted in chondrodystrophy (Packer et al., 2013). In chondrodystrophic breeds, such as the Dachshund, Pekingese, Shih tzu, Lhasa apso and Welsh corgi, there are alterations in the epiphyseal chondroblastic growth resulting in dwarfism, with long backs and short legs in these breeds of dogs (Packer et al., 2013). Reports regarding muscle architecture and function between chondrodystrophic and non-chondrodystrophic breeds are lacking. On the other hand, the biomechanics of the spine is similar between chondrodystrophic and non-chondrodystrophic dogs despite the longer backs of the former (Smolders et al., 2012). However, it is not known whether the physical demands on the chondrodystrophic spine differs from the demands on the non-chondrodystrophic spine. Only a few reports exist on epaxial muscle architecture in dogs, and this literature focuses on breed differences (Sharir et al., 2006; Webster et al., 2014). These studies have not investigated the influence of spinal pathology or chondrodystrophy on muscular force production capacity or considered the effects of ageing, exercise or pain on muscular architecture.

### 2.1.4. Muscle function based on electrical activity

Muscle function can also be evaluated by measuring its electrical activity using electromyography (EMG). The timing of muscle activation and specific muscle function depend on the gait of the animal and the vertebral level at which the EMG is recorded (Schilling and Carrier 2010). The activity of *Mm. multifidi* and *M. longissimus* has been investigated at the Th13, L3 and L6 vertebral levels in dogs during walking, trotting and galloping (Schilling and Carrier 2010). Muscle recruitment was higher during trotting than
walking, but similar during trotting and galloping (Schilling and Carrier 2010). At the walk, the *Mm. multifidi* and *M. longissimus* produced lateral flexion of the spine and counteracted the long-axis torsion of the trunk and forces exerted on the trunk by the hind limbs. During trotting these muscles stabilized the spine in the sagittal plane against inertia at the centre of mass (Schilling and Carrier 2010). Both *Mm. multifidi* and *M. longissimus* showed bilateral activity during trotting (Ritter et al. 2001), while the *M. iliocostalis thoracis* activity was uniphasic. The lumbar part of *M. iliocostalis* presented very short bursts of biphasic activation during trotting, indicating that the lumbar *M. iliocostalis* in dogs has very little bilateral activity (Ritter et al., 2001). During gallop the epaxial muscle activity appears to produce sagittal extension. Epaxial muscle activity in dogs is bilateral in symmetrical gaits (walking, trotting) and provides further spinal stability in the sagittal plane (Schilling and Carrier 2010).

Electromyographic studies show that epaxial muscles are active during the second half of the stance phase in walk and trot. This means that the epaxial muscles *Mm. multifidi* and *M. longissimus* are active also during spinal flexion, a movement that they per se cannot produce due to their anatomical location (Ritter et al., 2001). These muscles should therefore be considered as counteracting spinal flexion (Ritter et al., 2001) in addition to producing spinal extension (Evans 1993).

2.2. Comparison between epaxial muscles in quadrupeds and paraspinal muscles in humans

Multiple studies have highlighted the similarities in vertebrae and muscle morphology, muscle function and forces exerted on the spine between humans and quadrupeds (Smit 2002; Hodges et al., 2006; Stubbs et al., 2006; Valentin et al., 2015c). The quadrupeds are represented by a variety of different species shaped by evolution, environmental demands and selective breeding for certain locomotory behaviours (Biewener 2003; Williams et al., 2008a; Hudson et al., 2011; Webster et al., 2014).

When looking at the integrity of the human spine, the theoretical model of spinal stability has been applied (Panjabi 1992). According to this theory spinal stability consists of three subsystems: the active system (muscles), the passive system (joints and soft tissue) and the neural system (neural conduction) (Panjabi 1992). Later, this model has been developed to consider human spinal movement as a continuous interaction between both stability and mobility systems (Hoffman and Gabel 2013). This model can also be applied to quadrupeds, because despite the different positions of the vertical spine in humans and the horizontal spine in dogs, the load on the vertebrae is axially compressive in both species and remarkably similar (Smit 2002). However, the load of gravity is not compressive in the horizontal spine of the dog as it is in humans. Therefore, the muscles and ligaments in the dog spine have to be responsible for the axially compressive forces (Sharir et al., 2006). To prevent sagging of the trunk in the sagittal plane and to reduce muscle energy required for this, support is provided by passive ligamentous structures such as the *lig. Nuchae* dorsally and the linea alba ventrally (Smit 2002). The vertebral body is stronger and has higher bone density in quadrupeds than in humans, likely as a
consequence of the higher axial compression in quadrupeds (Smit 2002). From a muscle function point of view, this thesis considers spinal stability in the dog to include local/static stability, (maintaining the integrity of the vertebral column) and dynamic stability (controlling and resisting movements of the spine) (Ritter et al., 2001; Webster et al., 2014).

The human equivalent to the epaxial muscles in the dog are the paraspinal muscles (Moore and Dalley 2006). The paraspinal muscles consist of two muscle groups: the Mm. multifidi and the M. erector spinae (M. spinalis lies next to the spinous processes, followed by the M. longissimus and M. iliocostalis laterally). The function of Mm. multifidi is stabilization and control of spinal segments during local movements and extension of the spine (MacDonald et al., 2006; Moore and Dalley, 2006). The function of the M. erector spinae during bilateral action is to extend the spine and head and to control the flexion of the spine (Moore and Dalley 2006). In terms of their anatomy and main functions, the human paraspinal muscles are very similar to the epaxial muscles in dogs (Moore and Dalley 2006; Evans 1993). However, there are a few dissimilarities in function. In most quadrupeds, the centre of mass of the head and neck is located in front of the vertebral column (Smit 2002; Sharir et al., 2006). This requires activation of the cervical epaxial muscles to maintain this position (Sharir et al., 2006). In humans, the head is carried vertically on top of the vertebral column and much of the weight is carried passively (Sharir et al., 2006; Richmond et al., 2001). The position of the head and neck is important for visual and auditory orientation in both species, but in dogs, feeding, grooming, hunting and fighting tasks require large movements of the spine, sometimes combined with high force production (Sharir et al., 2006; Webster et al., 2014). Muscles involved in maintaining the position of the head and neck in dogs, such as the M. splenius, have higher PCSA than the equivalent muscles in humans, suggesting higher force production (Sharir et al., 2006).

As discussed before, in the thoracic and lumbar spine, the epaxial muscles in the quadruped work constantly to maintain gravity-resisting posture (Ritter et al., 2001; Webster et al., 2014) and to produce and counteract movements of the spine during different gaits (Ritter et al 2001; Webster et al., 2014). The vertical position of the human spine requires postural control and stability of the trunk muscles (Simoneau and Heiderscheit 2017). The need to resist gravity is different than in quadrupeds (Smit 2002), as most of the activities of human daily living, such as sitting and walking, are performed with the spine in vertical position (Simoneau and Heiderscheit 2017). In the human lumbar spine, anatomical and biomechanical research supports that both the superficial and deep Mm. multifidi and M. erectors spinae control movements and contribute to the dynamic stability of the spine (MacDonald et al., 2006; Rosatelli et al., 2008; Ward et al., 2009a). Mm. multifidi and M. erector spinae are active in any posture or movement related to lumbar spine extension (MacDonald et al. 2006). The superficial and deep Mm. multifidi are differently active during loading tasks of the upper limbs (Moseley et al., 2002; 2003); their activity is not limited to simply tonic activity (MacDonald et al., 2006). The superficial Mm. multifidi counteracts flexion movements of the spine to maintain a vertical position, while the deep Mm. multifidi refines and controls this movement without generating torque itself (MacDonald et al., 2006). Also the Mm. multifidi architecture, high PCSA and short muscle fibres indicate that this muscle is designed for stabilization tasks and high force production (Ward et al., 2009a; 2009b). Also in quadrupeds such as
horses and pigs (Hodges et al., 2006; Stubbs et al., 2006; 2011), the Mm. multifidi is considered similar to the human one, providing segmental stabilisation to intervertebral joints, while the M. longissimus provides dynamic stabilization over several motion segments (Stubbs et al., 2011). Detailed information on specific functions of the different epaxial muscles is sparse in dogs.

2.3. Evaluation of muscle structure with diagnostic imaging in humans and dogs

2.3.1. Evaluation of muscle size
Muscle size can be objectively quantified using cross-sectional area (CSA) and volume measurements (Valentin et al., 2015a). The CSA of a muscle is determined by the total quantity of muscle fibres, hence a measure of muscle size area (cm²) (Kalichman et al., 2010). Muscle volume is the quantity of space a muscle occupies in three-dimensional space (cm³) and can be calculated by multiplying the CSA with muscle length (Boom et al., 2008). These measurements are related to the muscle’s ability to generate force (Webster et al., 2014).

2.3.1.1. Magnetic resonance imaging
The majority of the studies on paraspinal muscle CSA in humans and epaxial muscle transverse area in dogs uses manual tracing of the muscle borders from MR images (Henderson et al., 2015; Ranger et al. 2017). The CSA and transverse area indicate the same measurement and will from here onwards be referred to simply as CSA. The CSA of epaxial muscles is measured manually by drawing a region of interest, tracing the muscular margins using some imaging software such as “Osirix” or “Image J” (Fortin and Battié 2012; Henderson et al., 2015) (Figure 5). Muscular measurements have been done in humans at the level of the centre of the intervertebral disc, the middle of the lamina and the superior and inferior endplate (Kang et al., 2007; D’Hooge et al., 2012; Fortin et al., 2015), and in dogs at the level of the vertebral endplate (Henderson et al., 2015) or the intervertebral disc space (Lerer et al., 2015).

Whereas the CSA includes the entire muscle and fat tissue within the specific muscle borders of that muscle, the functional cross-sectional area (FCSA) represent the fat-free area (Fortin et al., 2014). The FCSA can be obtained using different threshold techniques that separate the signal intensity between fat and muscle (Fortin et al., 2014). This has been used in humans, but not yet in dogs.

In dogs, the CSA of M. multifidus lumborum and the M. sacrocaudalis dorsalis lateralis was measured from MR images of a 1.0 Tesla, T2 sequences in patients with and without lumbosacral stenosis (Henderson et al., 2015). The results were presented as asymmetry indices between left and right sides (Henderson et al., 2015). The results from CSA measurement can also be reported as a percentage difference between left and right sides (Niemeläinen et al., 2011) or as the larger and smaller side (Fortin et al., 2014).

Considering the large variety of breeds in the canine population, it is important to standardize the vertebral level and slice orientation of the measurements when the CSA is evaluated (Cain et al., 2016; Henderson et al., 2015). Further, it is important to account for possible differences in body size and conformation (Cain et al., 2016). Therefore, the CSA of the intervertebral disc, the vertebral endplate or the vertebral body can be measured and
used to calculate a muscle ratio. This will standardize the muscular size to another structure, and comparison between different breeds and different sizes is possible (Henderson et al., 2015).

Figure 5. Low-field transverse image from a healthy Dachshund at the Th9-10 segment presenting the CSA measurements at the Mm. multifidi (M), M. longissimus (LD) and M. spinalis et semispinalis thoracis (S).

2.3.1.2. Computed tomography
Muscle size can be measured on CT using the same manually drawing techniques as on MR images (Danneels et al., 2000; Bouche et al., 2011). The only available report on this topic in dogs used multiplanar reformatted CT images and anatomical dissections to clarify the anatomy of the lumbosacral region in the transverse plane (Cain et al., 2016). The transverse area measurements of lumbosacral epaxial and hypaxial muscles were conducted in a soft tissue window display setting (350 width, 40 window level). Regions of interest (ROI) from the vertebral body in the same image as the muscular ROIs were used to correct for variations in size of dogs. A standardized bone window (1500 width, 300 level) was used for vertebral body measurements; transverse area ratios and side-to-side asymmetry were used to detect signs of muscle atrophy in dogs with and without lumbosacral pain (Cain et al., 2016). Repeatability was tested, but not inter-rater reliability, and a mixture of hard copy images and digital images was used, compromising standardization (Cain et al., 2016). The image analysis, slice thickness and window width and level settings were standardized (Cain et al. 2016), but also settings during image acquisition, different spinal levels and slice orientations need to be addressed and standardized (Kalichman et al., 2017). Only then is it possible to reliably assess the sensitivity and reliability of this method and whether it can detect diseased dogs from non-diseased dogs.

2.3.2. Evaluation of muscle composition
Fat infiltration is a sign of muscle degeneration and occurs when muscle fibres are replaced by non-contractile fat tissue, decreasing the contractile ability of that muscle, resulting in altered muscle function (D’Hooge et al., 2012). Quantification of fat infiltration provides information about the level of degeneration and possible functional capacity of the muscle investigated (D’Hooge et al., 2012; Kalichman et al., 2017).
2.3.2.1. Magnetic resonance imaging

In human back pain research, different visual grading systems or pixel intensity techniques have been used to quantify fat infiltration of the paraspinal muscles (Ranger et al., 2017). A recent systematic review suggests that the most common technique appears to be utilizing pixel intensity to estimate fat infiltration (Ranger et al., 2017). On MRI, the T1 relaxation time for fat tissue is short and the signal intensity is high compared with skeletal muscle on the T1-weighted sequence. Therefore, fat appears hyperintense (lighter in colour) relative to muscle, which is hypointense (darker in colour) (Elliott et al., 2005; D’Hooge et al., 2012). However, not all signal alterations in muscular MRI are fat infiltration (Trampus et al., 2018). In dogs, it seems that acute spinal cord compression results in a hyperintense signal in the epaxial muscles on T2-weighted sequences, suggesting denervation oedema, whereas more chronic spinal cord compression introduces hyperintense signals on T1-weighted sequences, suggesting fatty infiltration (Trampus et al., 2018; Kamath et al., 2008).

In humans, intramuscular fat is reliably quantified using the signal intensity obtained by MRI (Elliott et al., 2005; 2006; Valentin et al., 2015a). Fat infiltration can be estimated using the signal intensity from the entire ROI and dividing this value with an area of subcutaneous or intermuscular fat (Elliott et al., 2005; 2006) (Figure 6). Another way to quantify fat infiltration that has recently been applied in dogs is the use of a standardized area from the middle of the muscle belly (Lerer et al. 2015) (Figure 7). This method reduces the risk of including intermuscular fat in the measurement; however, this measurement does not represent the situation in the entire area. The choice of the fat area has been made in different ways; Elliott et al. (2005 and 2006) used a fat ROI from one selected spinal level to calculate fat indices in several spinal levels of human neck muscles. In dogs, the fat area was chosen adjacent to the muscle of interest as this would account for possible darkening in the images as they progress from superficial to deep (Lerer et al. 2015). This also meant that each muscle ROI would have a corresponding fat ROI. This allows for comparison of the magnitude of fat infiltration between muscles (Lerer et al., 2015). Another reported quantification of muscle composition or fat infiltration used in human research is the FCSA/total CSA ratio (Fortin et al., 2014).

Comparing results between studies is not always straightforward. A recent systematic review in humans pointed out that studies use both T1 and T2 sequences in the estimation of fat infiltration, and many studies do not report the sequence used at all. Additionally, numerous studies fail to report the magnet field strength (Ranger et al., 2017).
Figure 6. Low-field transverse MR image from a healthy Dachshund at the Th13-L1 segment showing the CSA for Mm. multifidi (M) and M. longissimus (LD). The black oval represents the fat ROI. The signal intensity mean is provided by the software in the ROI information box.

Figure 7. Low-field MR image from a healthy Dachshund at the Th13-L1 segment. The large grey ovals represent the muscular measurements from the M. longissimus (LD), and the small grey ovals represent the muscular measurement from Mm. multifidi (MF). The black ovals represent the fat measurement drawn adjacent to each muscle.

2.3.2.2. Computed tomography
Muscle density is a measure of muscle composition and can be estimated by the CT muscle attenuation value (Hounsfield units, HU) (Anderson et al., 2012). Muscle density reflects the number of muscle fibres, the area of the individual muscle fibre and the contractile ability of each muscle fibre (Kalichman et al., 2010). Fat displays attenuation values in the negative range and muscle tissue in the positive range relative to water. This means that fat tissue appears dark on the CT image and muscle brighter, i.e. a darker muscle with a lower attenuation value has a greater fat content (Goodpaster et al., 2000). Density can be reported according to the density provided by the software in the entire muscle ROI or from a small, standardized area in the centre of the muscle with the most preserved muscle mass (Figure 8). Variation in reported scanners, imaging parameters,
slice orientation and within and between patients and muscles has made it difficult to establish normal values for density in individual muscle or patient groups (Kalichman et al., 2017).

Figure 8. Transverse CT image from a healthy Dachshund at the Th13-L1 segment showing how the muscle attenuation value can be obtained in two different ways. On the left side, the ROI is drawn from the total CSA, and on the right side the small standard size ovals represent the muscle attenuation value.

2.4. Psychometrics in muscle evaluation

2.4.1. Validation of muscle structure evaluation methods
When muscle structure is evaluated, it is crucial to use standardized and validated methods, otherwise interpretation of the results is not trustworthy and comparison between studies is difficult (Valentin et al., 2015a; Crawford et al., 2017; Kalichman et al., 2017). Various imaging modalities, imaging processing software and measurement protocols may contribute to conflicting results (Fortin and Battié 2012). Current research emphasizes validation of muscular measurements against an anatomical reference in both humans (Crawford et al., 2017) and dogs (Cain et al., 2016) and the importance of carefully reporting imaging and software parameters, the measurement protocol and intra- and inter-rater reliability (Valentin et al., 2015a; Valentin et al., 2015c; Mhuiris et al., 2016; Crawford et al., 2017). Also variations in anatomy must be considered; recent studies have reported intra- and inter-rater reliability in several spinal segments and on the left and right sides (Battié et al., 2012). A recent review found that the CSA evaluation method used to quantify paraspinal muscle atrophy in humans with low back pain (LBP) remained unclear to the reader in 7 out of 22 studies (Ranger et al., 2017). Another systematic review investigated reliability and validity of M. multifidus measurements in older adults (<50 years of age). It found consistent evidence that muscular CSAs from MRI and density and attenuation from CTs could be done with moderate to substantial reliability (Cuellar et al., 2017).

Researchers tend to use different scales to report reliability such as the Fleiss guidelines (Hyun et al., 2016), Kappa scores (Abbot et al., 2017) or the scale presented by Landis and Koch (1977), which also makes it more difficult to make direct comparisons between studies. The large variety in imaging modalities, imaging parameters and
different software used to quantify muscular fat infiltration also hinder comparison of intra- and inter-rater reliability results between studies in humans (Valentin et al., 2015a; Crawford et al., 2017).

### 2.4.2. Intra-rater reliability

The intra-rater reliability is the consistency between the same observer’s measurements on two or more separate occasions, and it is also often called repeatability or test-retest (Bartko and Carpenter 1976). The intra-rater reliability has more often been reported in studies quantifying spinal muscle size and composition than inter-rater reliability (Niemeläinen et al., 2011; Battie et al., 2012), but information regarding the experience of the observers or the amount of practice that they had is often lacking (Valentin et al., 2015a). Further, the intra-class correlation (ICC) may have been reported only for combined muscular measurements and not for individual muscles or for only one side (Meakin et al., 2013; Niemeläinen et al., 2011).

Still, the intra-rater reliability reported in spinal muscle CSA, fat infiltration and muscle density measurements is generally high (Niemeläinen et al., 2011; Valentin et al., 2015c; Paalanne et al., 2011; Kalichman et al., 2010; Battie et al., 2012). In humans with posterolateral disc herniation, the intra-rater reliability for the CSA of the paraspinal muscles ranged from 0.90 to 0.99 for both left and right sides (Battie et al., 2012). The intra-rater reliability was almost perfect for 1.5 Tesla MRI volume measurements in *M. psoas major* (ICC), *M. erector spinae* (ICC) and *M. multifidus* (ICC) in humans and sheep (Valentin et al., 2015c). However, the intra-rater reliability of *M. multifidus* volume measurements was somewhat lower (ICC 0.86-0.94) and the 95% CI greater (0.23-0.84) than for other larger paraspinal muscles, such as the *M. longissimus dorsi* (ICC 0.99, 95% CI 0.98-0.99), in both humans and sheep (Valentin et al., 2015b; Valentin et al., 2015c). Valentin et al. (2015a) reported that assessing *M. multifidus* in humans might require greater experience to achieve acceptable repeatability relative to assessment of *M. erector spinae*. Another study found the intra-rater reliability to be clearly higher in L5 than in L1, suggesting that certain spinal segments could be more difficult to measure than others (Mhuiris et al., 2016).

Muscle density assessed with Kappa statistics showed high intra-rater reliability for both *M. multifidus* (0.94) and *M. erector spinae* (0.99) (Kalichman et al., 2010). Muscle density has not been evaluated in dogs, but the intra-rater reliability for the CSA in *M. multifidus, M. sacrocaudalis dorsali, M. quadratus lumborum* and *M. psoas* was high (Cain et al., 2016).

### 2.4.3. Inter-rater reliability

The inter-rater reliability is the consistency between two or more different observers using the same measurement at the same time (Bartko and Carpenter 1976). The inter-rater reliability of muscular structure quantification is not reported as frequently (Valentin et al., 2015a; Hu et al., 2011) and is often slightly lower than the intra-rater reliability for muscular CSA, fat infiltration and muscle density (Kalichman et al., 2010; Mhuiris et al., 2016). One study reported almost perfect inter-rater reliability for 120 different paraspinal muscle measurements (ICC 0.91, 95% CI 0.89-0.93), but the experience of observers was not reported, nor was the ICC for separate muscles (Urrutia et al., 2018). High inter-rater reliability (ICC 0.85-0.87) was also reported for fat infiltration in *M. multifidus* and *M. erector spinae* measured on opposed-phased MRI (Paalanne et al., 2011), which is in agreement with the results of Valentin et al. (2015a) (*M. multifidus* left ICC 0.83, *M.
**multifidus** right ICC 0.88, *M. erector spinae* left and right ICC 0.96), although Paalanne et al. did not report ICCs for separate muscles. The inter-rater reliability for muscle density evaluated from CT images obtained at several spinal levels ranged from 0.70-0.97 (Kalichman et al., 2010).

Some studies rely on measurements from a single segment (Hides et al., 2007; Meakin et al., 2013), or the ICC is not reported for all measured segments (Kalichman et al., 2010), although regional variation in anatomy and fat infiltration is evident in the lumbar spine in asymptomatic humans (Moore and Dalley 2006; Urrutia et al., 2018) and in the thoracolumbar spine in the dog (Webster et al., 2014). Such regional variations in morphology may interfere with interpretation of results (Crawford et al., 2017), as the inter-rater reliability in manually drawn ROIs has been found to be lower at L1 than at L5 in humans (Mhuiris et al., 2016). Further, the inter-rater reliability of individual muscles may not be reported, but only that of combined muscles (Valentin et al., 2015a; Meakin et al., 2013). In dogs, the epaxial muscle CSA and fat infiltration has been investigated, but only one study of three investigated intra-rater reliability of their measurements (Cain et al., 2016) and none examined the inter-rater reliability (Henderson et al., 2015; Lerer et al., 2015; Cain et al., 2016).

### 2.4.4. Agreement between MRI and CT in evaluation of muscle structure

The CSA measurements of paraspinal muscles and vertebral body obtained from 1.5 Tesla MR images have high intra-rater (ICC 0.91) and inter-rater (ICC 0.89) reliability in comparison with the same measurements obtained from CT images, where intra-rater (ICC 0.78) and inter-rater (ICC 0.65) reliability has been clearly lower (Hyun et al., 2016). Hu et al. (2011) compared the intra- and inter-rater reliability FCSA and fat infiltration in *Mm. multifidi* and *M. erector spinae* between MRI (1.5 Tesla) and CT and concluded the reliability to be acceptable using both methods, but nevertheless found MRI to be the superior modality.

Veterinary studies have compared the intra- and inter-rater reliability and the inter-method agreement between low-field MRI (0.24 tesla) and CT in the evaluation of vertebral canal and vertebral body morphology (De Decker et al., 2010). The results suggested high repeatability and good agreement for most cervical vertebral measurements with both MRI and CT (De Decker et al., 2010). However, there are no studies in dogs comparing muscle structure between MRI and CT.

### 2.5. Confounding factors for evaluation of muscle structure

Increased age along with possibly reduced physical activity have clear weakening effects on trunk muscle morphology (Cuellar et al., 2017), often leading to sarcopenia (Narici and Maffulli 2003). Decreased muscle mass is accompanied by a decrease in muscle fibre length and an increase in fat infiltration, physiological changes that occur normally in aged tissue (Narici and Maffulli 2003). Also muscle density decreases with higher age and increased BMI (Kalichman et al., 2010; Hicks et al., 2005).

In humans, paraspinal muscle CSA is known to be larger and muscle density greater in men than in women (Kalichman et al., 2010; Kalichman et al., 2017). Women have greater
fat infiltration in their lower paraspinal muscles than men (Kalichman et al., 2017; Niemeläinen et al., 2011).

Muscles are very plastic and respond to load or disuse (Hunter et al., 2017). Disuse decreases muscle mass and volume, resulting in reduced muscle strength (Narici and Maffulli 2003; de Boer et al., 2007). Exercise, on the other hand, and especially consistent strength training, will increase muscle mass and volume (Hunter et al. 2017). In dogs, it is not known which factors influence the evaluation of epaxial muscles and it is important to account for possible confounding factors.

2.6. Muscle structure and low back pain in humans

2.6.1. Structural changes in relation to low back pain in humans

In addition to altered function, structural changes, such as decreased CSA and increased fat infiltration, have been reported in the paraspinal muscles in relation to pain or pathology in humans (Hides et al., 1994; 1996; Danneeels et al., 2000; Battié et al., 2012; D’Hooge et al., 2012; Gildea et al., 2013; Fortin et al., 2014). The *Mm. multifidi* CSA has been reported decreased in patients with acute and chronic LBP, with atrophy presenting locally on the side of the symptoms (Hides et al., 1994; Hides et al., 2008a; Fortin et al., 2013). Decreased CSA in *Mm. multifidi* and *M. psoas* was found on the symptomatic side relative to the asymptomatic side in patients with unilateral back pain (Barker et al., 2004), and decreased *Mm. multifidi* CSA has also been associated with LBP and hip pain in classic ballet dancers (Gildea et al., 2013). A systematic review of 15 case-control studies presented moderate evidence that the *Mm. multifidi* CSA is smaller in chronic LBP patients than in healthy people (Goubert et al., 2016). However, there is little evidence that the CSA would be decreased in patients with recurrent or acute LBP compared with healthy controls (Goubert et al., 2016).

Although decreased *M. multifidus* CSA has been considered an indicator of back pathology (Ranger et al., 2017), several studies detected no changes in CSA between diseased and normal sides (Battié et al., 2012; Fortin et al., 2016) or between symptomatic and healthy subjects (Goubert et al., 2016). This has been explained by the muscle itself being enlarged by the fat infiltration and increased extracellular fluid caused by denervation (Battié et al., 2012; Teichtal et al., 2015). Interestingly, asymmetry in the lumbar *Mm. multifidi* CSA has been reported also in healthy subjects (Niemeläinen et al., 2011) and the CSA may not always be decreased in lumbar disc herniation (Battié et al., 2012).

Increased fat infiltration in the *Mm. multifidi* and *M. erector spinae* has been frequently reported both on T1 and T2-weighted MRI sequences in humans with LBP (Battié et al., 2012; D’Hooge et al., 2012; Fortin et al., 2016). Fat infiltration in the *Mm. multifidi* and *M. erector spinae* is clearly associated with pain and disability in these patients (Teichtal et al., 2015). Patients with posterolateral disc herniation in their lumbar spine, with symptoms lasting less than six weeks showed an increase in MRI signal intensity on T2-weighted sequences ipsilateral to and at the level below the herniation, indicating increased fat infiltration (Battié et al., 2012). Fortin et al. (2016) reported similar findings, also on T2-weighted MRI sequences in the *Mm. multifidi* and *M. longissimus* muscles in patients with lumbar disc herniation. Also Kang et al. (2013) found the most severe *Mm. multifidi* atrophy at the level below the involved nerve root in patients with unilateral lumbar radiculopathy on T1-weighted MRI. Such localized
atrophy of Mm. multifidi on the side of and below the level of the disc herniation has been discussed to relate to denervation and possible polysegmental innervation of the Mm. multifidi. However, according to anatomy literature, the Mm. multifidi is innervated by the medial branch of the dorsal rami (Moore and Dalley 2006).

The fat infiltration in the lumbar paraspinal muscles is higher in patients with continuous LBP than in patients with only recurrent LBP (Goubert et al., 2017). Generalized fat infiltration may occur also in symptomatic LPB patients (D’Hooge et al., 2012). However, there is no consensus regarding how muscle degeneration characteristics (CSA, fat infiltration and asymmetry) are related to LBP or to muscle strength and endurance (Suri et al., 2015). A systematic review found decreased CSA of the Mm. multifidi to be both associated with LBP and important for the prediction of LBP (Ranger et al., 2017). The same study found conflicting evidence for an association between Mm. multifidi fat infiltration and LBP and fat infiltration as a predictor of LBP (Ranger et al., 2017). Another systematic review of six observational cohort studies considered it unlikely that lumbar muscle characteristics detected on MRI or CT would predict future LBP or physical dysfunction (Suri et al., 2015).

2.6.2. Reasons for paraspinal muscle atrophy in humans

Reported reasons for the structural changes in human paraspinal muscles have been disuse or denervation atrophy (Hides et al., 2007; Battié et al., 2012), reflex inhibition (Danneels et al., 2000; Hodges et al., 2006), inflammatory mechanisms, vasoconstriction, changes in metabolism (Hodges et al., 2006), hypertrophy caused by muscle spasms (Barker et al., 2004) and changes in mechanical properties of muscles such as increased muscle fibre stiffness (Brown et al., 2011). Several studies use translational animal models such as pigs, sheep and rabbits to explain paraspinal muscle atrophy in humans and several mechanisms with different time courses have been considered (Hodges et al., 2006; 2014; 2015; Dulor et al., 1998; Brown et al., 2011)

The Mm. multifidi CSA has been reported to decrease locally at one side and at a single spinal level within 3 days after intervertebral disc lesion in pigs (Hodges et al., 2006). The reason for this acute change is suggested to be disuse as a consequence of reflex inhibition (Hodges et al., 2006). The same study found also enlarged adipocytes and reduced water and lactate content in the multifidus muscle after both disc and nerve lesions, suggesting these to be precursors for chronic fat infiltration changes (Hodges et al., 2006). In an experimental rabbit model, the multifidus muscle was significantly stiffer 12 weeks after a disc injury compared to controls (Brown et al., 2011). In disc herniation (Goubert et al., 2017) and LBP (Wan et al., 2015) of more chronic nature, the muscular changes are more general and explanatory mechanisms relate to injury of the nerve root involved (Goubert et al., 2017; Kader et al., 2000; Wan et al., 2015). However, also localized bilateral multifidus atrophy has been found in humans with unilateral LBP lasting > 1 year (Beneck et al., 2012). A human study investigated humans with LBP in remission and found increased fat infiltration in Mm. multifidi, M. erector spinae and M. psoas on T1-weighted MRI compared to healthy controls, but no difference in muscular CSA was detected (D’Hooge et al., 2012). Here, disuse was considered the most likely explanation.

Disuse atrophy occurs in muscles during immobilization or inactivity due to spinal cord injury, immobilization, bed rest and chronic denervation (Ciciliot et al., 2013; Hides et al., 2007). A known consequence of the lost neural influence and decreased mechanical load in disuse is a shift from slow to fast muscle fibre types (Suetta et al., 2012; Ciciliot et
al., 2013; Wall et al., 2014), decreased muscle mass, such as CSA and volume, and increased fat infiltration (Wall et al., 2014; Suetta et al., 2012). Disuse atrophy occurs already 5-14 days after immobilization (Wall et al., 2014). But disuse atrophy is also part of the normal muscular degeneration in sarcopenia (Narici and Maffulli 2003).

A denervated muscle is characterized by an increase in ‘moth-eaten’ muscle fibres and enlarged adipocytes (Mattila et al., 1986; Hodges et al., 2006; Kamath et al., 2008) as well as reduced water and lactate concentrations (Hodges et al., 2006). Lumbar disc herniation in humans causes muscle fibre type grouping and small, angulated fibres and a moth-eaten appearance in the *Mm. multifidi* on the diseased side (Zhao et al., 2000). Acute denervation (<1 month) cause oedema like changes in muscle tissue, normal T1-weighted signals and increased T2-weighted signals on MRI (Kamath et al., 2008). Subacute to chronic denervation (1 month - 6 months) display further increase in extracellular water and progressive fat infiltration with increased signals both on T1- and T2 weighted MRI (Dulor et al., 1998; Kamath et al., 2008). Increased fat infiltration in the *Mm. multifidi* of patients with unilateral disc herniation, symptoms lasting less than 6 weeks was explained by denervation or injury to nerve root (Battié et al., 2012).

In sheep, 6 months after experimental intervertebral disc lesion, a reduction in slow muscle fibres and an increase in fast fibres were found, but also an increase in TNF-alpha expression (Hodges et al., 2014). The TNF-alpha is involved in regulation of muscle function, muscle fibre degradation and myoblast differentiation. This observation could therefore explain the muscle fibre alterations seen in muscle atrophy (Hodges et al., 2014). Recent research found no multifidus atrophy at 3 months after an experimental disc lesion, but transformation of slow muscle fibres to fast as well as adipose and connective tissue proliferation at 6 months after injury, suggesting structural remodelling of the multifidus muscle (Hodges et al., 2015).

### 2.6.3. Muscle function in relation to low back pain in humans

In humans, LBP is a widespread problem defined as localized pain in the lower back between the lowest rib and buttock line, and it is one of the most investigated spinal problems in human medicine (Hartvigsen et al., 2018). Common causes of LBP are disc bulge, disc herniation, lumbar spinal stenosis and pain originating from muscles or articular facets (Brinjikji et al., 2015; Hartvigsen et al., 2018), although the nociceptive source often remains unidentified (Hartvigsen et al., 2018). Low back pain is known to alter muscle function (D’Hooge et al., 2013), decrease functional ability (Suri et al., 2015) and increase sick leave and early retirement (Hoy et al., 2014).

There are several theories that attempt to explain the changes in sensorimotor control and movement that are related to pain. Firstly, the vicious cycle or the ‘pain-spasm-pain’ cycle can be simplified as follows: a person with back pain will have increased activity in the paraspinal muscles, a spasm, to protect the injured area and the spasm introduces further pain through chemical and mechanical reactions in the muscle tissue (Roland 1986). The ‘pain adaption model’ (Lund et al., 1991) states that the dysfunction observed in chronic pain is a normal adaptation to pain, but not the cause of the pain. The force produced during muscle contraction is decreased, not increased, as would be the case with muscle spasm (Lund et al., 1991). Years later, Hodges and Tucker (2011) presented a new refined model on movement’s adaptation to pain, emphasizing the variability between individuals. They suggested that adaptation to pain comprises redistribution of activity...
within and between muscles, change in muscular mechanical behaviour, protection from further pain or injury and involvement of changes at several levels of the motor system and that this adaptation offered short-term benefits, but may have detrimental long-term consequences (Hodges and Tucker 2011).

Even anticipation of pain redistributes activity within and between paraspinal muscles (Moseley et al., 2004). Asymptomatic LBP patients show higher co-contraction activity of trunk flexor and extensor muscles than healthy subjects during a trunk flexion task (D’Hooge et al., 2013). Patients with recurrent chronic LBP have lower EMG activity in both deep and superficial Mm. multifidi during both predictable and unpredictable loading (MacDonald et al. 2010). Patients with chronic LBP caused by disc herniation have impaired lumbar postural control and proprioception (Leinonen et al., 2003), and when performing an unexpected perturbation task there is inefficiency of the postural recovery of the spine (Mok et al., 2011). Further, when hypertonic saline solution was injected unilaterally into the M. longissimus at the L4 level to induce pain, the Mm. multifidi recruitment was decreased bilaterally and at two spinal levels, L3-4 (Dickx et al., 2010).

It is clear that pain alters movement strategies, but when pain subsides and the injury heals, the motor unit recruitment may recover, while the redistribution of activity within the muscle might not (Hodges and Tucker 2011; D’Hooge 2013a; 2013b). These motor control changes that can persist in LBP patients during symptom remission are considered to increase the risk for recurrence of LBP episodes (Hodges and Tucker 2011).

2.6.4. Management of back pain and muscular dysfunction in humans

It is suggested that muscle dysfunction in many humans with back pain does not resolve automatically, but requires targeted exercise interventions (Hides et al., 1996; Danneels et al., 2001; Falla and Hodges 2017). Dysfunction of the lumbar trunk muscles may contribute to the occurrence and persistence of LBP and limit function and physical performance (Suri et al., 2015). Greater muscle strength, endurance and neuromuscular control in the lumbar trunk muscles are beneficial in the prevention and treatment of back pain (MacDonald et al., 2006; Suri et al., 2015). Stabilization training of the paraspinal muscles increases M. multifidus CSA in women with chronic LBP (Klizieni et al., 2015) and in elite athletes (Hides et al., 2008b), but also general exercise has provided pain relief and improved functional ability in patients with LBP (Koumantakis et al., 2005).

Strategies in human physiotherapy have gradually moved from immobilization and rest to exercise therapy (Richardson and Jull 1995). One approach is specific and individually adapted sensorimotor control training (Hall et al., 2009; Falla and Hodges 2017). The following paragraph gives a brief overview of the sensorimotor approach (Danneels L. personal communication; Hodges 2013).

This motor control training has the objective of optimizing spinal loading, reducing irritation, encouraging normal movement and providing appropriate sensory input (MacDonald et al., 2006; Hodges 2013). This involves training of controlled compliance of the spine rather than training of rigidity or stiffness, and the former is achieved by correction of identified changes in deep muscle activation, changes in superficial muscle control and optimization of posture and movement (Macedo et al., 2012). This training includes specific localized exercises aimed at restoring the control function of the deep segmental muscles (Hall et al., 2009). These exercises are designed specifically to re-activate and facilitate the crucial function of the deep muscles, including the Mm. multifidi (Hodges 2013). Specific motor control training targeting the core stabilizing muscles will
initially be taught in non-weight-bearing positions. This will then progress to functional exercises relevant to each participant's work and daily activities with the aim of restoring function and normal motor control during these tasks (Hodges 2013).

In recent years, human physiotherapy has progressed towards an active and biopsychosocial approach where the aims are, through advice and exercise, to encourage patients to move their spine normally, with normal breathing and without bracing or fear, and to improve control of the spine, maintaining a balance between stiffness and movement (Tsao et al., 2010; Falla and Hodges 2017). Based on careful assessment and clinical reasoning, the right patients are selected to receive a tailored treatment approach at the right time (Falla and Hodges 2017).

2.7. Muscle structure and spinal pathology in dogs

2.7.1. Intervertebral disc disease (IVDD)
Intervertebral disc disease (IVDD) is common in dogs and is often a consequence of intervertebral disc degeneration (Bergknut et al., 2013; Langerhus and Miles 2017). Intervertebral disc degeneration occurs naturally in older dogs of most breeds, but in chondrodystrophic breeds, and particularly in Dachshunds, the degeneration occurs rapidly and at an early age (Bergknut et al., 2013; Smolders et al., 2013). Studies have suggested that intervertebral disc degeneration in chondrodystrophic dogs involves chondroid metaplasia of the nucleus pulposus, whereas in non-chondrodystrophic dogs fibrous metaplasia takes place, usually more slowly and at an older age (Bergknut et al., 2013). However, recent studies show chondroid metaplasia of the nucleus pulposus in both chondrodystrophic and non-chondrodystrophic dogs (Hansen et al., 2017). In chondrodystrophic breeds, the intervertebral discs are rich in chondrocyte cells, whereas the discs in non-chondrodystrophic dogs are rich in notochordal cells (Smolders et al., 2012). In disc degeneration, there is a decrease in proteoglycan, hyaluronic acid and water content and an increase of collagen in the nucleus pulposus, causing structural and functional failure of the intervertebral discs. Structural and functional failure may result in various structural changes of the intervertebral discs and adjacent vertebral bodies such as annular tears of the annulus fibrosus (Bergknut et al., 2013). Abnormal biomechanical loading and genetic predisposition may affect or accelerate this degenerative process, leading to Hansen type I (disc extrusion/disc herniation (IVDH)) and Hansen type II (disc protrusion) IVDD (Hansen 1952).

In Hansen type I, common in young to middle-aged chondrodystrophic breeds, there are degenerative changes in the annulus fibrosus and partial or complete rupture and separation of the annular lamellae. These changes are most often encountered in the dorsal part of the annulus fibrosus and may thus result in dorsal extrusion of the degenerated nucleus pulposus into the vertebral canal, causing spinal cord compression mostly in the thoracolumbar region (Hansen 1952; Smolders et al., 2013). These acute thoracolumbar disc extrusions occur most frequently in Dachshunds (Smolders et al., 2013). In Hansen type II, common in older, large breed dogs, there is partial rupture and disorganization of the annulus fibrosus. The nucleus pulposus protrudes towards the annulus fibrosus, causing a disc bulge and spinal cord compression, often in the cervical or thoracolumbar region (Hansen 1952; Smolders et al., 2013).

A process associated with intervertebral disc degeneration is calcification of the intervertebral discs, which is frequently observed in chondrodystrophic dogs, but rarely in
non-chondrodystrophic dogs. Although most frequently present in the thoracic spine, intervertebral disc calcification can be found at all spinal levels and can be seen macroscopically as early as 9 months of age (Smolders et al., 2013; Lappalainen et al., 2014). Dachshunds are considered to be genetically predisposed to disc calcifications (Mogensen et al., 2011; Jensen and Christensen 2000), and studies suggest that the calcifications are associated with type I disc extrusions (Lappalainen et al., 2014; Rohdin et al., 2010). Other factors predisposing to intervertebral disc extrusion are miniature size, long back, obesity (Packer et al., 2013) and increasing age (Bergknut et al., 2013).

2.7.1.1. Diagnosis of Hansen type I disc disease
Symptoms of Hansen type I disc disease involving intervertebral disc herniation (IVDH) are often acute and range from back pain only to different grades of paresis or plegia without deep pain perception (Besalti et al., 2006; Henke et al., 2013). The severity of the neurological deficits is categorized into five neurological grades: 1) pain only, no neurological deficits; 2) ambulatory paraparesis; 3) non-ambulatory paraparesis; 4) paraplegia with intact deep pain perception; and 5) paraplegia with absent deep pain perception (van Wie et al., 2013). Grading helps in choosing the best treatment option and in stating prognosis for recovery.

The diagnosis is made based on history, clinical signs, neurological examination and diagnostic imaging findings (Moore et al., 2016). MRI has become a standard imaging modality in diagnosis of IVDH in small animals (Cooper et al., 2014; Moore et al., 2016). MRI allows visualization of location and characteristics of the disc extrusion and assessment of possible changes in the spinal cord itself (Besalti et al., 2006; Cooper et al., 2014), although CT can also be used (Cooper et al., 2014; Moore et al., 2016).

2.7.1.2. Diagnostic imaging of intervertebral disc degeneration and IVDD
Degeneration of intervertebral discs can be reliably classified from MR images using Pfirrmans grades: 1) the disc structure is homogeneous and bright white; 2) the disc structure is non-homogeneous without horizontal bands; 3) the disc structure is non-homogeneous and grey; 4) the disc structure is non-homogeneous and grey to black; and 5) the disc structure is non-homogeneous and black (Bergknut et al., 2011). The intervertebral disc disease on MRI can be classified as follows: 1) normal or disc degeneration only; 2) bulging of intervertebral disc (symmetric uniform extension of 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 (complete definition in Besalti et al., 2006).

2.7.1.3. Treatment for Hansen type I disc disease
The recommended treatment is surgery for IVDH with neurological grades 2-5 (Moore et al., 2016; Langerhus and Miles 2017). Techniques used are hemilaminectomy, minihemilaminectomy and fenestration, with hemilaminectomy being the most frequently used technique (Moore et al., 2016; Langerhuus and Miles 2017). Surgery should take place as soon as possible after diagnosis, especially in dogs with absent deep pain perception (Moore et al., 2016). Recovery rates after hemilaminectomy are 93% for grades 3 and 4 and 61% for grade 5 (Langerhuus and Miles 2017). Conservative treatment is recommended for grade 1 and sometimes grade 2 patients and consists of rest, analgesia and medication with anti-inflammatory and sometimes prednisone medication (Moore et
al., 2016; Langerhus and Miles 2017). Recovery after conservative treatment is 79% for grade 3, 62% for grade 4 and 10% for grade 5 (Langerhus and Miles 2017).

Although the recovery rates for IVDH are high, there is individual variation in time to ambulation after surgery (Olby et al., 2004). It is well recognized that the neurological grade prior to and immediately after surgery affects the prognosis (Ruddle et al., 2006). Further, 24% of dogs that have had a disc extrusion once may get a second disc extrusion (Mayhew et al., 2004).

Physiotherapy is nowadays routinely recommended postoperatively for dogs recovering from thoracolumbar IVDH (Sims et al., 2015; Moore et al., 2016; Hodgson et al., 2017; Zidan et al., 2018), although only one randomized, blinded, prospective clinical trial on the effects of postoperative physiotherapy in dogs with acute thoracolumbar IVDH exists. Postoperative physiotherapy is planned and carried out based on the neurological grade and the surgeons’ recommendations (Sims et al., 2015; Hodgson et al., 2017; Zidan et al., 2018). Physiotherapy focuses on restoring hind limb motor function with active therapeutic exercise and on preventing contractures and compensatory muscle stiffness through passive range of motion exercises and manual therapy (Sims et al., 2015; Hodgson et al., 2017). Nerve regeneration may be enhanced with therapeutic low-level laser; muscle activation is stimulated and atrophy prevented with electrical muscular stimulation (EMS) and sometimes acupuncture as an additional complement (Sims et al., 2015; Zidan et al., 2018; Joaquim et al., 2010). Conservatively treated dogs are less frequently than surgically treated dogs referred to physiotherapy (Moore et al., 2016). The potential of targeting epaxial muscles with exercise to increase strength and control in the spine of dogs recovering from IVDH has not been examined.

2.7.2. Fibrocartilaginous embolism
Fibrocartilaginous embolism (FCE) is the most frequent cause of ischaemic myelopathy in dogs (De Risio 2015). It is a vascular spinal cord injury caused by fibrocartilaginous material originating from the intervertebral disc clogging the spinal cord vessels and resulting in ischaemic necrosis of the spinal cord (De Risio 2015). FCE most often occurs in large, non-chondrodystrophic dogs (Gandini et al., 2003; De Risio 2015), but has also been reported in small dogs (Nakamoto et al., 2009). FCE may occur in both young and old dogs and in any spinal cord segment (De Risio 2015).

The onset of clinical signs is acute; after the first 24-48 hours, FCE is non-progressive, and it is often related to strenuous physical activity or exercise (Gandini et al., 2003; De Risio 2015). Clinical signs are often severe paresis or plegia on one side and only mild deficits on the contralateral side with an absence of spinal hyperesthesia (Gandini et al., 2003, Nakamoto et al., 2009; De Risio 2015). Diagnosis is best confirmed with MRI showing a non-compressive, sometimes lateralized spinal cord lesion that is hyperintense on T2-weighted images (De Risio 2015). The treatment for FCE is conservative and includes controlled exercise and physiotherapy (Gandini et al., 2003). Evidence suggests that intensive active physiotherapy, including hydrotherapy, can enhance the return of motor function in dogs with FCE (Gandini et al., 2003)

The reported successful outcomes of FCE refer to complete or partial recovery of neurological functions, allowing the dogs to be functional during activities of daily living in the home environment (De Risio 2015; Mari et al., 2017). Dogs with FCE regain various levels of walking ability (De Risio 2015; Mari et al., 2017). Time to recovery has been reported to last 6 days, 11 days or up to 3.7 months (De Risio 2015). The loss of deep pain perception indicates a poor prognosis (Gandini et al., 2003; De Risio 2015).
2.7.3. Muscular function in relation to spinal pathology in quadrupeds

Spinal motion and muscle function have been shown to be altered by pain or disease also in horses (Jeffcott et al., 1982; Wennerstrand et al., 2004; 2009) and dogs (Sutton et al., 2016). Standardbred trotters with experimentally induced back pain in the *M. longissimus dorsi* show stiffness in their thoracolumbar spine and inability to perform at higher speeds (Jeffcott et al., 1982). When back pain was experimentally induced unilaterally in the *M. longissimus* muscle in horses, increased extension of the spine and compensatory lateral movements of the lumbar spine towards the painful side were observed (Wennerstrand et al., 2009). Naturally occurring chronic back pain in horses caused a decreased range of motion in the caudal thoracic spine and thoracolumbar junction (Wennerstrand et al., 2004).

Dachshunds recovering from disc extrusion and decompressive hemilaminectomy showed abnormal pelvic movements and asymmetric pelvic limb weight bearing compared with neurologically normal Dachshunds six months postoperatively (Sutton et al., 2016). In large breed dogs, mild asymmetric pelvic limb lameness caused greater thoracolumbar lateral motion towards the side with less weight bearing (Hicks et al., 2014). Lameness in dogs may increase long axis rotation of the spine towards the asymptomatic side, and to compensate for this, the activity in *M. longissimus* measured by surface EMG was higher on the side contralateral to the lame limb (Fischer et al., 2013). Decompressive surgery at the L2-3 segment resulted in spinal instability in flexion and extension, lateral bending and axial rotation in both chondrodystrophic and non-chondrodystrophic dogs (Smolders et al., 2012). It is logical to think that the epaxial muscle function in dogs would be altered by changes in spinal movement caused by lameness, decompressive surgery or pain, although this topic has not been investigated in veterinary medicine.

2.7.4. Epaxial muscle structure in dogs with spinal pathology

At the time of commencing this thesis, no reports existed on epaxial muscle structure in relation to spinal disease in dogs. Now, however, there are four reports on this topic in dogs (Lerer et al., 2015; Henderson et al., 2015; Cain et al. 2016; Trampus et al., 2018). The findings are in line with the previously mentioned human research (Hides et al., 2007; Danneels et al., 2000; D’Hooge et al., 2012; Battié et al., 2012). It has been shown that Belgian Shepherds with lumbosacral pain presented with significantly smaller CSA in the *M. psoas* at L5-6 and L6-7 levels, *Mm. multifidi* at the L6-7 level and the *M. sacrocaudalis dorsalis lateralis* at the L6-7 and 7-S1 levels than Belgian Shepherds with no lumbosacral pain (Cain et al., 2016). However, the sample size used was small (11 dogs with pain and 6 dogs without pain) and the patients had been selected into pain and non-pain groups based on retrospectively retrieved information from medical records.

Another retrospective high-field MRI study detected significantly decreased CSA in the *M. multifidus lumborum* and *M. sacrocaudalis dorsalis lateralis* in German Shepherds with lumbosacral stenosis compared with a control group of different breeds without lumbosacral stenosis (Henderson et al., 2015). The German Shepherds with lumbosacral stenosis had greater asymmetry in their epaxial muscles than the control dogs. However, the disease group consisted of one breed, while the control group comprised dogs of different breeds. Therefore, one cannot say whether the detected differences were related to the disease or simply to the breed. On the other hand, considering that the breed is a confounding factor, the identification of breed-specific differences in the epaxial muscles
may improve knowledge about pathogenesis of spinal disease. Both of these studies (Henderson et al., 2015; Cain et al., 2016) provided limited information regarding activity level and duration of clinical signs. In addition, the pelvic limb position was not standardized during scanning, introducing further limitations when interpreting the results.

A study of chondrodystrophic and non-chondrodystrophic dogs quantified the location and magnitude of fat infiltration in the *Mm. multifidi, M. erector spinae* and *M. psoas* from high-field T2-weighted MR images (Lerer et al., 2015). This study found higher fat infiltration in the *M. psoas* and *Mm. multifidi* in non-chondrodystrophic dogs than in chondrodystrophic dogs. Higher fat infiltration was detected also in dogs with non-IVDH spinal pathology than in dogs with IVDH. Non-chondrodystrophic dogs usually develop IVDD gradually over a longer period (Bergknut et al., 2013; Smolders et al., 2013). This is often the case also with non-IVDH spinal pathology (Lerer et al., 2015). Therefore, the authors suggest that the chronicity or severity of general spinal pathology may be related more strongly to muscular fat infiltration in *Mm. multifidi* and *M. psoas* than to the IVDH itself because of the prolonged discomfort and disuse associated with a general spinal pathology (Lerer et al., 2015).

The *M. erector spinae* had higher fat infiltration than *Mm. multifidi* and *M. psoas* (Lerer et al., 2015). This level of fat infiltration was not considered related to breed or pathology, but the authors suggested that the *M. erector spinae* is targeted first with the fat infiltration in comparison with *Mm. multifidi* (Lerer et al., 2015).

Contrary to Lerer et al. (2015), another study found that epaxial muscles in dogs with acute spinal cord injury show oedema-like signals, such as hyperintense signals in T2-weighted and iso- or hypointense signals in T1-weighted MR images (Trampus et al., 2018). More chronic spinal cord injuries or pathologies would show hyperintense signals on T1-weighted images, suggesting increased fatty infiltration (Lerer et al., 2015; Trampus et al., 2018). Non-compressive spinal cord injuries seem not to alter the MRI signals in the epaxial muscles and the spinal cord compression and the severity of neurological deficits are likely to be associated with signal alterations on MRI (Trampus et al., 2018).
3 AIMS

The overall aims of this thesis were to investigate a method to quantify muscle size and composition using MRI and CT and to evaluate the influence of intervertebral disc disease on epaxial muscle size, composition and internal architecture in dogs.

Detailed objectives were as follows:

1. To investigate the influence of intervertebral disc herniation (IVDH) and non-compressive fibrocartilaginous embolism (FCE) on cross-sectional area (CSA) and fat infiltration in the thoracolumbar *Mm. multifidi* and *M. longissimus* using MRI (I).

2. To assess intra- and inter-rater reliability of epaxial muscle CSA and fat content measurements evaluated on MRI and CT images in clinically healthy Dachshunds and to compare the CSA measurement between the two modalities (II).

3. To estimate and compare the functional role in terms of force and power-generating capacity of epaxial muscles in chondrodystrophic Dachshunds and in non-chondrodystrophic Border terriers, based on muscle architecture (III).

4. To investigate the predicted epaxial muscle force and power-generating capacity in Dachshunds with and without IVDD using muscle architectural data (III).
4 MATERIALS AND METHODS

4.1. Study design

Study I was a retrospective study using MRI images obtained from patient records. Study II was a research methodology study, using retrospectively obtained MRI and CT images. Study III was an anatomical study investigating cadavers. The required sample size was estimated in all studies by performing a power analysis using the appropriate main outcome variables from similar human (Hides et al., 2008a; Valentin et al., 2015b) (Studies I and II) and canine studies (Webster et al., 2014) (Study III).

4.2. Ethical approval of study protocols

The Royal Veterinary College Ethics and Welfare Committee reviewed the protocol for Study I, and no ethics approval was required due to the retrospective nature of the study. The Finnish National Animal Experiment Board (ESAVI/5794/04.10.03/2011) approved the protocol for Study II, and written consent was obtained from dog owners. The protocol for Study III was approved by the Viikki Campus Research Ethics Committee, University of Helsinki (7/2013), and dog owners provided written consent.

4.3. Animals

Study I included 52 client-owned Dachshunds with IVDH causing thoracolumbar spinal cord compression requiring surgery and 12 control dogs of different breeds with non-compressive FCE diagnosed by MRI at the Royal Veterinary College (RVC) between 2003 and 2010. Study II included 10 client-owned Dachshunds that were considered healthy by their owners. They had no history of back pain and no evidence of IVDH or spinal cord compression on either MRI or CT. Study III investigated cadaver muscles from 17 client-owned Dachshunds and 7 Border terriers euthanized for various reasons between fall 2013 and spring 2016 at the Veterinary Teaching Hospital of the University of Helsinki. All dogs were donated by their owners to the hospital for research and teaching purposes.

4.4. Background data of dogs

In all three studies, the following data were retrieved from patient records: breed, age, gender and body weight. In Study I, the following additional information was collected from the records: duration of neurological deficits, duration of pain reported by the owner, neurological grade at presentation and side and site of the spinal cord lesion. Depending on the location of the lesion, the lesions were classified as ‘left-sided,’ ‘midline’ or ‘right-sided.’ The neurological grade at presentation was retrieved from patient records. The
dogs were categorized into three groups based on the nature of the spinal cord lesion: Dachshunds with acute compressive lesions (duration of neurological signs < 7 days prior to presentation, n=30), Dachshunds with chronic compressive lesions (> 7 days, n=22) and other dogs of different breeds with acute non-compressive lesions (FCE, n=12).

In Study II, dogs’ level of physical exercise was quantified using an owner questionnaire. In Study III, background data were collected after euthanasia. The reason for euthanasia was obtained from patient records. The pain history of dogs was collected using an owner questionnaire (Lappalainen et al., 2014). The responses were used to calculate a pain sum where 0 indicated ‘no pain’ and 6 indicated the worse possible pain.’ Information about the duration of walks was obtained with the owner questionnaires used in Study II. The dogs were divided into groups based on MRI findings: Dachshunds with (n=8) and without IVDH (n=8). The group of Border terriers (n=7) acted as the control breed.

4.5. Diagnostic imaging

The MRI and CT parameters used in all studies are presented in Table 1. In Study I, MR images from the tenth thoracic (Th10) to the third lumbar vertebrae (L3) were obtained with high-field MRI with the dogs in dorsal recumbency.

In Study II, the area from the ninth thoracic (Th9) to the first lumbar vertebrae (L1) was scanned in the sagittal and transverse planes using both MRI and CT. Both scanners were calibrated according to the hospital’s daily procedure. The restricted space in the MRI scanner required the dogs being positioned according to normal hospital practice, in right lateral recumbency. The same coil (9101819001 Esaote S.p.A) was used for all dogs. MRI was performed first, followed immediately by CT. The spinal CT images were obtained using a soft tissue algorithm (B 50), with the dogs positioned in dorsal recumbency, according to normal hospital practice.

In Study III, MR images were obtained from the first thoracic vertebrae to the sacrum using high-field MRI. The cadavers were placed in dorsal recumbency in a foam cradle to ensure straightness of the spine. All images were stored in the hospital’s Picture Archive and Communication System (PACS).
Table 1. Imaging parameters for MRI and CT used in Studies I-III. NA= not available.

<table>
<thead>
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<th>MRI PARAMETER</th>
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<th>II</th>
<th>III</th>
</tr>
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<td>T1-weighted</td>
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<td>Time to repeat (ms)</td>
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<td>830-970</td>
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<table>
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</table>
4.6. Measurement technique for muscle structure

A dedicated DICOM viewer (Osirix, version 6.5.2, Pixmeo, Bernex, Switzerland) was used in the image evaluations in Studies I and II. In Study I, the muscular measurements were made in random order at the level of the disc at each segment.

In Study II, an ECVN Diplomate (TSJ) not involved in the muscular measurements performed the selection of the corresponding MRI and CT transverse images for every segment (Th9-L1). Firstly, a transverse MR image was chosen based on visible nerve root canals at each intervertebral level. With help of additional landmarks and their shape, the corresponding transverse CT image was then selected. The additional landmarks were articular facets, spinous processes, dorsal arches of the vertebrae, transverse processes, os costae and intervertebral discs (Figures 9A and B). The MRI measurements were performed using a full dynamic window (window level (WL) 1647, window width (WW) 3294, image size of 256 x 256). The measurements for the CT were made in the muscle window (WL 50, WW 400) and further adjusted in the bone window (WL 300, WW 1500) with an image size of 512 x 512.

![Image](image_url)

**Figure 9.** Low-field T1-weighted transverse MR image (A) from the Th13-L1 segment in a healthy Dachshund and the corresponding transverse CT image (B) (Study II).

4.6.1. Muscular cross-sectional area (CSA) (I, II)

In Studies I and II, the muscle size (CSA cm²) of the muscles were measured bilaterally by tracing the muscle margins visible on the MRI to create a ROI around each muscle (Figure 10). The software’s pencil tool was used and possible intermuscular fat was excluded from the ROI. When the boundary between the fat and muscle was unclear, the ROI was defined through the middle of this region to allow a reasonable approximation of the muscle’s anticipated boundary (Fortin and Battié 2012). The same routine was executed when the boundaries between *M. longissimus* and *Mm. levatores costarum* were indistinct. When the boundary between *M. longissimus* and *M. iliocostalis* was not evident, the *M. iliocostalis* was included in the *M. longissimus* measurement.

In Study I, CSA measurements were performed at the level of the intervertebral disc in *Mm. multifidi*, *M. longissimus* and *M. iliocostalis* as well as in *Mm. multifidi*, *M. longissimus* and *M. iliocostalis* combined. The *Mm. multifidi* muscle was measured alone, whereas the *M. longissimus* and the *M. iliocostalis* were measured together forming the ‘epaxial muscle’ measurement, as it was difficult to distinguish the borders between these muscles. The CSA of the intervertebral disc was measured in the same image as the muscular measurements (Figure 11).
In Study II, the thoracolumbar *Mm. multifidi, M. spinalis et semispinalis thoracis* and *M. longissimus thoracis et lumborum* were measured bilaterally on both MRI and CT images (Figure 10). The *M. spinalis et semispinalis* muscle belly was not clearly visible caudally to the eleventh thoracic vertebrae (Th11). Therefore, this muscle was measured at only the first two segments (Th9-Th11).

**Figure 10.** Low-field *T1*-weighted transverse MR image from the Th9-10 segment in a healthy Dachshund (A) and a transverse CT image from the Th9-10 segment in another healthy Dachshund (B) illustrating the CSA measurements of *M. semispinalis et spinalis (S), M. longissimus (LD) and Mm. multifidi (M)* (Study II).

**Figure 11.** Sagittal (A) and transverse (B) *T1*-weighted MR images from the Th12-13 segment in a FCE dog. The sagittal image (A) illustrates the measurement sites (Th12-13 and Th13-L1 green arrows) and the anatomical landmarks Th10, L1 and sacrum. The transverse image (B) illustrates the *Mm. multifidi CSA, the epaxial muscular CSA and the intermuscular fat CSA* (Study I). *T= Thoracic vertebrae (Th).*
Figure 12. A presents the CSAs for the Mm. multifidi (M), M. longissimus (LD) and fat (black oval) on a T1-weighted transverse MR image from the Th12-T13 segment in a healthy Dachshund. B presents the muscle attenuation values for the M. semispinalis et spinalis CSA (light green), the M. longissimus CSA (yellow) and the Mm. multifidi CSA (blue) at the Th9-10 segment (Study II).

4.6.2. Muscular fat infiltration (I-II)
In Studies I and II, the muscle fat content on MRI was calculated using the software-provided signal intensity from the muscle CSA ROI and from a small standard size area of intermuscular fat (Elliott et al., 2005). The fat ROI was drawn in the same image as the muscular measurements to account for possible variations in the MRI acquisitions. This fat ROI was drawn at the visually whitest (highest signal intensity) spot in the images in Study I (Figure 11B), while in Study II the possible decrease in signal intensity with depth from the coil was considered. Therefore, and to further ensure objectivity, the fat ROI was drawn in a standardized spot at the same depth as the midpoint of the M. longissimus (Figure 12A).

4.6.3. Muscle attenuation (II)
The muscle fat content on CT investigated in Study II was the software-provided muscle attenuation value (Hounsfield units, HU) obtained from individual muscle CSA ROIs (Figure 12B).

4.6.4. Validation of muscular measurements (I, II)
In Study I, the intra-rater reliability of the observer was tested. Dachshunds with IVDH (n=6), Cavalier king Charles spaniels with syringomyelia (n=2), a Bishon frise with FCE and a Pekingese with inflammatory disease of the spinal cord were retrieved from patient records of the RVC. The selection was made based on availability to evaluate the epaxial muscles in at least four segments in the Th10-L3 range. The CSA of the intervertebral disc, Mm. multifidi, epaxial muscle and combined Mm. multifidi and epaxial muscles from Th10-L3 on both sides was measured in random order by the same assessor (AFB). Validating the repeatability, the muscles were measured twice at an interval of three weeks and the assessor was blinded to the earlier measurements and to the background data of the dogs at the time of the measurements.

In Study II, one physiotherapist (AFB, observer 1) and one veterinary radiologist (AKL, observer 2) performed the measurements at five spinal levels (Th9-10, Th10-11, Th11-12, Th12-13 and Th13-L1) in each of the 10 dogs, twice each, resulting in 200 sets
of measured images. The MRI images were measured first, followed by the CT images. The images were mixed randomly between dogs, and measurements were performed in a random order. The randomization list was computer-generated. The observers were blinded to each other and to the background data of the dogs, and review of measured images was not permitted. AFB had some experience with the measurement method from Study I, while AKL had no previous experience. AFB instructed AKL in the measurement technique and both practiced independently until comfortable with the technique.

4.7. Morphometric measurements of dog conformation

In Studies II and III, gross anatomy measurements (cm) were taken with a flexible tape measure before further investigation procedures by the same researcher (Figure 13). The measurements were girth circumference, distance from occipital protuberance to base of tail (spine length), distance from midpoint between dorsal border of scapula to base of tail (back length), girth circumference (girth) and height at the withers (height). Body weight was obtained using commercial scales accurate to 20 g (Study II: Slim-Line, Eickemeyer, UK, Study III: Soehnle S20 2763, Soehnle Professionals, Germany). The body mass index (BMI, kg/cm²) was calculated for each dog as follows: Body weight (BW) kg / (height at the withers cm x length from occipital protuberance to base of tail cm) (Mawby et al., 2004; Figure 13).

![Figure 13. Morphometric measurements of dog conformation in a Dachshund: spine length (A), back length (B), height at withers (C) and girth circumference (dotted oval).](image)

4.8. Anatomical dissections

4.8.1. Preparations and dissection procedure (III)
After euthanasia, the cadavers were immediately frozen at -20°C with a straight spine until further procedures. Before the dissections, the cadavers were defrosted at 4°C for a maximum of 48 hours and scanned with MRI to rule out or confirm IVDH diagnosis. The dissection procedure was identical for all cadavers. The cadavers were skinned and the front limbs removed. The dissection started on the right-hand side. The epaxial muscles in
the cervical, thoracic and lumbar spine were identified and systematically isolated, removing external tendons carefully. 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 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).

4.8.2. Muscle architectural measurements (III)
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 balances were calibrated and repeatability tested at the start of each data collection session using certified adjustment weights.

The muscle belly length was measured from origin to insertion with a flexible plastic tape measure (Figure 14B) accurate to 1 mm. If a muscle had multiple insertions, the length was measured to the insertion point farthest from the origin. This allowed the muscle belly length to represent the whole line of action for that particular muscle.

To determine muscle fascicle length, an incision was made through the muscle belly, longitudinally to the muscle fibres. A minimum of 5 and a maximum of 10 fascicles were selected randomly and measured using a digital caliper (Alpha Tools, Germany) (Figure 14C). Where the muscle overlapped several spinal segments, fascicle lengths were sampled throughout the entire length of the muscle belly.

The resting pennation angle was defined as the angle between the internal tendon and the muscle fibres and was recorded using a clear plastic protractor (Figure 14A) to an accuracy of 1°.

The Architectural Index (AI) normalizes the muscle fascicle length to muscle belly length and was calculated by dividing muscle fascicle length with muscle belly length. AI = muscle fascicle length (mm) / muscle belly length (mm).

Muscle volume was estimated by dividing muscle mass with muscle density of 1.06 g/cm³. Muscle volume = muscle mass (g) / 1.06 g/cm³ (Mendez and Keys, 1960). The PCSA was calculated for each muscle as muscle volume divided by mean fascicle length. PCSA = muscle volume / mean fascicle length (Webster et al., 2014).
4.8.3. Scaling of body size and shape (III)
In the comparison of Dachshunds and Border terriers, allometric scaling was used to account for differences in body shape and size. Parameters were scaled to dog mass: muscle mass scaled as \((\text{muscle mass} / \text{dog mass in grams})\); muscle belly length as \((\text{muscle length} / \text{dog mass}^{0.33})\); muscle fascicle length as \((\text{fascicle length} / \text{dog mass}^{0.33})\) and PCSA as \((\text{PCSA} / \text{dog mass}^{0.66})\) (Webster et al., 2014).

The Dachshund and Border terrier had different height at the withers and back length so allometric scaling of length measures based on body mass may not have been the most appropriate method of scaling. Therefore, muscle belly and fascicle lengths were additionally scaled to back length to normalize these muscle characteristics across dogs with differing body forms.

4.9. Evaluation of intervertebral discs
In Study III, an ECVN diplomate (TSJ) evaluated all intervertebral discs from the first thoracic vertebra to the sacrum. The images were reviewed in a random and blinded manner. A Pfirrmann grade (1-5) was given for each intervertebral disc from the first thoracic vertebra to the sacrum (Th1-S1) based on evaluation of sagittal T2-weighted images (complete definition in Bergknut et al., 2011). Averages of the Pfirrmann grades were calculated for the whole spine. The type of intervertebral disc disease was determined based on evaluation of T1- and T2-weighted transverse images 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 (Besalti et al., 2006). The dogs were 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.
4.10. Formulae and statistical methods applied

In Studies I and II, SPSS IBM statistics, versions 19, 22 and 24 (IBM statistics, New York, NY, USA) were used in the analysis. In Study III, the SAS® System for Windows, version 9.3 (SAS Institute Inc., Cary, NC, USA) and SPSS IBM statistics, version 24 were used. The level of significance was set at <0.05 in all analyses.

Normality of the data was assessed for normal distribution using the Kolmogorov-Smirnov and Shapiro-Wilk tests (I, II, III), normal QQ-plots (III) and in Study I the data was additionally assessed graphically using histograms, and logarithm transformation was calculated where needed.

Mean and standard deviation (SD) were used to report age and body weight (I, II, III), BMI (II, III), duration of neurological deficits and duration of pain reported by the owner (I). Mean and SD were also used to summarize back length, height and girth circumference as well as Pfirrmann grade, type of intervertebral disc disease, pain score, duration of walks and muscle variables (III) of the dogs.

4.10.1. Study I

To compensate for possible discrepancy in body weight and body conformation between IVDH Dachshunds and FCE dogs, a muscle-to-disc ratio (Muscle:Disc) (Kang et al., 2007) was calculated for all muscular measurements, generating the following variables: MM:Disc, EPAX:Disc and MMEPAX:Disc.

\[
\text{Muscle:Disc ratio} = \frac{\text{Muscle CSA}}{\text{Disc CSA}}
\]

The Muscle-to-fat ratio (Muscle:Fat) was calculated for all muscle measurements in all dogs for all individual segments, generating the following variables: MM:Fat, EPAX:Fat and MMEPAX:Fat.

\[
\text{Muscle:Fat Ratio} = \frac{\text{Muscle Mean Hyperintensity}}{\text{Subcutaneous Fat Mean Hyperintensity}}
\]

The difference in gender, age, body weight and disc CSA between the two groups was compared with Chi-Square test and independent samples t-tests. The difference between Muscle:Disc ratio and Muscle:Fat ratio measurements on the lesion and non-lesion side in the IVDH Dachshunds was analysed using linear mixed models to account for multiple segments from the same dog. Age, body weight, sex, breed, site of lesion, segment, neurological status at presentation, duration of neurological deficits and duration of pain reported by the owner were used as covariates to investigate their effects on the difference between sides in the muscular variables.

Most of the lesions occurred at the Th12-13 segment, and therefore, this segment was used in the analysis between groups. Independent samples t-tests were performed to test the difference between lesion and the non-lesion sides at the Th12-13 segment in acute and chronic Dachshunds. In the comparison between IVDH Dachshunds and FCE dogs, the average of the left- and right-side measurements was calculated for all variables (Muscle:Disc ratio and Muscle:Fat ratio). This was done to maintain sample size, as several of the FCE dogs had midline lesions affecting the spinal cord and categorizing
them into lesion and non-lesion sides was not possible. The following equation was used to calculate the average between the left and right sides:

\[
\text{Muscle: Disc ratio average} = \frac{\text{Multifidus: Disc ratio Left} + \text{Multifidus: Disc ratio Right}}{2}
\]

The differences between acute compressive, chronic compressive and acute non-compressive lesions were analysed using one-way analysis of variance (ANOVA) and Fisher’s LSD post-hoc comparisons in lesion and non-lesion sides and in the average variables at the Th12-13 segment.

4.10.2. Study II
The MRI and CT CSA, the MRI fat content value and CT muscle attenuation were investigated for each muscle bilaterally. Intra-rater reliability was investigated by comparing the measurements from two occasions. The inter-rater reliability was investigated by comparing the measurements from all spinal segments on the first measurement occasion between the two observers. The agreement for the CSA was assessed between the two imaging modalities by comparing the measurements obtained from the first occasion. The intra-rater agreements between the two measurement occasions and the inter-rater agreement between the two observers were analysed using a two-way mixed model and absolute agreement intra-class correlation coefficient (ICC), using a confidence interval of 95% (95% CI). The analysis was performed both for the mean of all segments and for individual segments.

The CSA measurements between MRI and CT were compared using computed mean variables for the two observers to account for differences in observers’ measurements. The agreement was tested with a two-way mixed model and absolute agreement intra-class correlation coefficient at 95% CI and reported according to Landis and Koch (1977), thus: ‘slight agreement’ 0.01–0.20, ‘fair agreement’ 0.21–0.40, ‘moderate agreement’ 0.41–0.60, ‘substantial agreement’ 0.61–0.80, ‘almost perfect’ agreement 0.81–1.00. The correlations between the MRI- and CT-acquired CSA mean variables were analysed with the two-tailed Pearson correlation test, for each muscle on left and right sides.

Considering the different scanning positions in MRI (lateral recumbency) and CT (dorsal recumbency), we tested the difference between left and right sides in the two scanning positions. The mean muscle variables between the left and right sides for individual segments were compared using the paired t-test, and when data were not normally distributed, they were compared using the Wilcoxon signed-rank test.

4.10.3. Study III
Independent samples and Student’s t-tests were used to compare the means of the descriptive variables between affected and non-affected Dachshunds and between Dachshunds and Border terriers.

All included Dachshunds were compared with all included Border terriers, and all investigated muscles were analysed. An average was calculated for each architectural variable, using the data from both the left and right sides, and 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. An
Analysis of Covariance (ANCOVA) model, with breed as the only fixed factor and dog age as a covariate, was used.

In the analysis between affected and non-affected Dachshunds, unscaled data from only seven muscles in the thoracic and lumbar spine (\textit{M. longissimus cervicis, thoracis, M. longissimus thoracis et lumborum, M. spinalis et semispinalis thoracis, M. iliocostalis thoracis, M. multifidus lumborum, M. iliocostalis lumborum}) were included, as MR images were available only from the first thoracic vertebrae to the sacrum. Differences in the muscle variables between groups were investigated using ANCOVA models. Body weight, back length, Pfirrman grade, age, pain score and duration of walks were used as possible covariates.

Two different statistical modelling strategies were used: 1) the full set of dogs excluded pain score and duration of walks from the covariate list and 2) for dogs with no missing covariate information, all possible covariates were used, i.e. body weight, Pfirrman grade, age, back length, pain score and duration of walks. The statistical modelling was started in both strategies by including all possible covariates in the ANCOVA model. As all of the models had significant multicollinearity present, they all had to be simplified from the full model. The multicollinearity was caused by 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. Exclusion of the correlating variable-pairs (back length and age), and duration of walks from the analysis reduced the multicollinearity.

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, the modelling was terminated and the results not shown. In all of the fitted models, multicollinearity of the model was assessed based on the tolerance values of the model. Heteroscedasticity of the models was investigated using White’s test.
5 RESULTS

5.1. Animals (I-III)

The signalment of the dogs included in Studies I-III is presented in Table 2. In Study III, one Dachshund was excluded from the analysis on postmortem evaluation due to muscle deterioration caused by severe diabetes. Of the Border terriers, three were affected with IVDH (Table 2).

Table 2. Descriptive data of the dogs included in Studies I-III. *The FCE group consisted of several different breeds: Wales terrier, Border terrier, Bishon frisé, Dachshund, Staffordshire bull terrier, Whippet, Cocker spaniel, Chihuahua, Lhasa apso, German spitz (all n=1 per breed) and Mixed breed (n=2). Intervertebral disc herniation (IVDH), Fibrocartilaginous embolism (FCE). NA = not available.

<table>
<thead>
<tr>
<th>Breed/group</th>
<th>n</th>
<th>Gender</th>
<th>n</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>BMI (kg/cm²)</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>13</td>
<td>7.1± 2.1</td>
<td>7.2 ± 2.4</td>
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</tr>
<tr>
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<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>9</td>
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<td>7.2 ± 2.7</td>
<td>NA</td>
</tr>
<tr>
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<td></td>
<td>Female</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Different breeds*</td>
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<td>7</td>
<td>5.6 ± 2.4</td>
<td>10.1 ± 3.9</td>
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</tr>
<tr>
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<td>5</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Dachshund healthy total n = 10</td>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Dachshund</td>
<td>8</td>
<td>Male</td>
<td>1</td>
<td>13.9± 3.4</td>
<td>9.06 ± 3.3</td>
<td>16.18 ± 6.5</td>
</tr>
<tr>
<td>affected</td>
<td></td>
<td>Female</td>
<td>7</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Dachshund</td>
<td>8</td>
<td>Male</td>
<td>3</td>
<td>8.9 ± 4.9</td>
<td>8.4 ± 1.9</td>
<td>14.5 ± 4.5</td>
</tr>
<tr>
<td>non-affected</td>
<td></td>
<td>Female</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Border terrier</td>
<td>3</td>
<td>Male</td>
<td>1</td>
<td>14.0±0.4</td>
<td>5.0±3.4</td>
<td>6.7±4.6</td>
</tr>
<tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Border terrier</td>
<td>4</td>
<td>Male</td>
<td>1</td>
<td>10.9±2.7</td>
<td>7.3±0.7</td>
<td>9.4±1.0</td>
</tr>
<tr>
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<td></td>
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<tr>
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<td></td>
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</table>

In Study I, Dachshunds were significantly older than FCE dogs (P=0.016). In Study III, Dachshunds had significantly longer back lengths (39.9 ± 3.4 cm), lower heights (28.4 ± 3.6 cm) and higher BMI (17.7 ± 5.3 kg/cm²) than Border terriers (34.0 ± 1.3 cm, P<0.0001; 37.2 ± 2.1 cm, P<0.0001; 10.0 ± 1.8 kg/cm², P=0.002, respectively). In the comparison 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=0.014) (Table 3). A comparison of affected and non-affected Border terriers was not made due to the low number of dogs per group.
5.2. Background data (I-III)

In Study I, mean duration of neurological deficits was significantly longer in Dachshunds (8.4 ± 16.0 days) than in FCE dogs (2.4 ± 3.9, P=0.019). Mean duration of pain reported by the owner was longer in Dachshunds (28.7 ± 99.1 days) than in FCE dogs (1.4 ± 4.0 days), but was not statistically significant (P=0.075).

In Study II, the dogs had no history of back pain or other illnesses. They all had moderate exercise, defined as brisk walks for at least 20 minutes 2-4 times daily. At least one of the walks lasted > 1 hour. All dogs were allowed to run and play free on a weekly basis, and they exercised on various terrain.

In Study III, clinical signs of the dogs are presented in Tables 3 and 4. Three owners did not return the pain and exercise questionnaires. Compared with Border terriers, Dachshunds had significantly longer back lengths (34.0 ± 1.3 cm vs. 39.9 ± 3.4 cm, P<0.0001), lower height (37.2 ± 2.1 cm vs. 28.4 ± 3.6 cm, P<0.0001) and higher BMI (10.0 ± 1.8 vs. 17.7 ± 5.3, P=0.002). There were no significant differences in the other descriptive variables between the two breeds. Affected Dachshunds had higher Pfirrmann grade than non-affected Dachshunds, but this difference was not significant. Pain score and duration of walks were similar for both affected and non-affected dogs (Tables 3 and 4). The reason for euthanasia was old age in most dogs. Only in three affected dogs was the reason for euthanasia hind limb paralysis; intervertebral disc extrusion was confirmed in these cases with post-mortem MRI.
Table 3. Background data of affected and non-affected Dachshunds are presented for each individual dog, and the mean and standard deviation (SD) are also provided.

<table>
<thead>
<tr>
<th></th>
<th>Height (cm)</th>
<th>Back length (cm)</th>
<th>Pain score</th>
<th>Duration of walks (min)</th>
<th>Pfirrmann grade</th>
<th>IVDD type</th>
<th>Lesion site</th>
</tr>
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<tbody>
<tr>
<td>Affected Dachshunds</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td>27.8</td>
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<td>27.2</td>
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<td></td>
<td>3.0</td>
<td>3</td>
<td>L5-6</td>
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<td></td>
<td>2.3</td>
<td>3</td>
<td>L7-S1</td>
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<td>42.5</td>
<td>0</td>
<td>45.0</td>
<td>3.0</td>
<td>4</td>
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<td>2.78 ± 1.8</td>
<td>31.6 ± 13.5</td>
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<td>Mean ± SD</td>
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<td>32.5 ± 15.3</td>
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<td>1.17 ± 0.5</td>
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Table 4. Background data of affected and non-affected Border terriers are presented for each individual dog, and the mean and standard deviation (SD) are also provided.

<table>
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<th></th>
<th>Height (cm)</th>
<th>Back length (cm)</th>
<th>Pain score</th>
<th>Duration of walks (min)</th>
<th>Pfirrmann grade</th>
<th>IVDD type</th>
<th>Lesion site</th>
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<td>T13-L1</td>
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<td><strong>Non-affected Border terriers</strong></td>
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<tr>
<td><strong>Mean ± SD</strong></td>
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<td>33.8 ± 7.5</td>
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<td>35.3 ± 14.1</td>
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</tbody>
</table>

5.3. Validation of muscular measurements

5.3.1 Intra-rater reliability (I, II)
In Study I, the reliability was ‘almost perfect’ with an intra-class correlation ranging between 0.923 and 0.991 for the muscular measurements and 0.931 for the Disc CSA.

In Study II, observer 1 demonstrated ‘almost perfect’ intra-rater reliability for all measurements (ICC 0.828–0.993). Observer 2 showed ‘almost perfect’ intra-rater reliability for both MRI and CT measurements (ICC 0.844–0.998), except for the MRI fat value of the *M. spinalis et semispinalis* left, *M. spinalis et semispinalis* right and the *M. longissimus* left, for which the agreements were classified as ‘substantial’ (ICC 0.650–0.773).

Limiting the analysis to individual spinal segments caused the intra-rater agreement for both observers to decrease for the *Mm. multifidi* MRI CSA and MRI fat value (0.289–0.483). The intra-rater agreement decreased for the *M. spinalis et semispinalis* MRI fat value (0.436) and CT CSA (0.055–0.495) at the T9-10 and T10-11 segment.

5.3.2. Inter-rater reliability (II)
The inter-rater reliability was ‘almost perfect’ between the two observers for the CSA measurements (ICC 0.866–0.948) for both MRI and CT. The inter-rater reliability values for the MRI fat content varied between ‘substantial’ and ‘almost perfect’ for all muscles (0.685–0.854). The agreement for the CT muscle attenuation was ‘almost perfect’ for all measurements (0.959–0.987). Analysis of individual spinal segments showed ‘moderate’ inter-rater agreement for the *Mm. multifidi* MRI CSA and MRI fat value at the Th10-11
segment (0.411–0.543) and ‘slight’ agreement for *M. longissimus* left MRI fat value at the Th13-L1 segment (0.213).

5.3.3. Agreement between MRI and CT (II)

The agreement between the MRI and CT for CSA was ‘almost perfect’ (0.824–0.894). There was a high positive correlation (r = 0.719–0.841) between MRI CSA and CT CSA (all P-values <0.001). The *Mm. multifidi* MRI-acquired CSA on the right side was significantly larger than on the left side for the Th11-12 spinal segment (P=0.028). The *Mm. multifidi* MRI fat content on the right side was significantly higher at the Th10-11 segment than on the left side (P=0.005). The MRI fat content for the *M. longissimus* on the right side was significantly higher for all segments relative to the left side: Th9-10 (P=0.009), Th10-11 (P=0.005), Th11-12 (P=0.005), Th12-13 (P=0.005) and Th13-L1 (P=0.007). The CT muscle attenuation for *Mm. multifidi* was higher on the left (P=0.017) and the CT muscle attenuation for *M. spinalis et semispinalis* was higher on the right at the Th10-11 segment (P=0.047).

5.4. Comparison between lesion and non-lesion sides in IVDH Dachshunds (I)

Study I found no significant difference between lesion and non-lesion sides in Dachshunds for any of the Muscle:Disc ratio variables, with no P-value <0.656. The covariate ‘duration of neurological deficits’ had a significant effect on the EPAX:Disc variable (P=0.029). There was no further effect of any of the covariates on the difference between lesion and non-lesion sides in Dachshunds.

No significant difference emerged between lesion and non-lesion sides for any of the Muscle:Fat variables, with no P-value <0.298. Of the covariates, ‘neurological grade at presentation’ had a significant effect on the MM:Fat variable (P=0.040) and ‘duration of pain reported by the owner’ had a significant effect on the difference between sides in the EPAX:Fat variable (P<0.001).

5.5. Difference between IVDH Dachshunds and FCE dogs (I)

No significant difference in the Disc CSA existed between Dachshunds and FCE dogs (P=0.150). The average Multifidus:Disc ratio was significantly lower in Dachshunds (P=0.036) than in FCE dogs (Figures 15A). When the analysis was limited to include only dogs with lesion at the Th12-13 segment, the average Multifidus:Fat ratio was significantly higher in the Dachshunds compared to FCE dogs (P=0.022) (Figures 15B). The Epaxial:Disc ratio was significantly lower (P<0.001) and the Epaxial:Fat ratio was significantly higher (P=0.017) in Dachshunds than in FCE dogs (Figure 16A and B).
5.5. Difference between compressive and non-compressive spinal cord lesions (I)

The comparison of acute and chronic compressive lesions with non-compressive lesions revealed that the average Multifidus:Disc ratio was significantly greater (P<0.001) and the average Multifidus:fat ratio was significantly lower in dogs with non-compressive lesions (P<0.001) (Figures 17A and B). The Epax:Disc ratio was significantly higher in the non-compressive lesions than in the compressive lesions (P<0.001). The Epax:Fat ratio was lower in the non-compressive lesions than in the compressive lesions (P<0.030) (Figure 18).
Figure 17. Boxplot A presents the difference in the Multifidus:Disc ratio and boxplot B the difference in the Multifidus:fat ratio between acute compressive, chronic compressive (IVDH Dachshunds) and non-compressive spinal cord lesions (FCE dogs) at the Th12-13 segment.

Figure 18. Boxplot A presents the difference in the Epax:Disc ratio and boxplot B the difference in the Epax:fat ratio between acute compressive, chronic compressive (IVDH Dachshunds) and non-compressive spinal cord lesions (FCE dogs) at the Th12-13 segment.

5.6. Muscular architecture (III)

5.6.1. Estimated functional roles of epaxial muscles in Dachshunds and Border terriers

The estimated functional roles of the 13 investigated muscles are illustrated in the scatter plot below (Figure 19). For both Dachshunds and Border terriers, the M. longissimus thoracis et lumborum, the M. iliocostalis lumborum and the M. multifidus lumborum have high capacity for force generation. The M. splenius, M. semispinalis biventer and M. longissimus capitis are suitable for production of large movements.
Figure 19. 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 muscle to the top left of the plot have high capacity for generating force.

SP= splenius, SB= semispinalis biventer, SC= semispinalis complexus, LCa= longissimus capitis, SSc= spinalis et semispinalis cervicis, SSt= spinalis et semispinalis 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.

5.6.2. Difference in muscular architecture between Dachshunds and Border terriers

Our results showed that Dachshunds have significantly greater muscle mass in M. splenius (P=0.044), M. longissimus capitis (P=0.049) and M. iliocostalis thoracis (P=0.001), longer muscle belly in M. ilocostalis thoracis (P=0.0412) and higher PCSA in M. semispinalis complexus (P=0.005) and M. ilocostalis lumborum (P=0.017) (Figure 19). Border terriers had significantly longer muscle fascicles in M. semispinalis complexus (P=0.004) and M. ilocostalis lumborum (P=0.003) and higher PCSA in M. spinalis et semispinalis thoracis (P=0.032) than Dachshunds (Figures 20 and 21). The AI was higher in several muscles in the Border terrier compared to the Dachshunds, but the difference was not significant (Figure 22).
Figure 20. Bar charts showing the differences in muscle parameters of muscle mass (A), PCSA (B), muscle belly length (C) and muscle fascicle length (D) between the studied breeds, scaled to body mass. The bars represent the mean with 95% confidence interval for Dachshunds and Border terriers. Significant differences (P<0.05) are indicated with an asterisk.
Figure 21. Differences in muscle parameters of muscle belly length (A) and muscle fibre length (B) between the two breeds, scaled to back length. The bars represent the mean with 95% confidence interval for Dachshunds and Border terriers. Significant differences (P<0.05) are indicated with an asterisk.

Figure 22. Architectural index (AI; fascicle length/muscle belly length) for Dachshunds and Border terriers. Bars represent the mean with 95% confidence interval.
5.6.3. Difference in muscular architecture between affected and non-affected Dachshunds

The difference between affected and non-affected Dachshunds in seven muscles is illustrated in Figure 23. The analysis, which included pain score and exercise variables as covariates, showed that affected Dachshunds (n=6) had significantly longer fascicle lengths than non-affected Dachshunds (n=7) in the *M. longissimus cervicis* (P=0.012). In the analysis using the complete set of data, the affected dogs had longer fascicle lengths than non-affected dogs in the *M. longissimus thoracis et lumborum* (P=0.005) (Figure 23 D). No other significant differences were detected.

![Figure 23](image_url)

**Figure 23.** Bar charts presenting unscaled data for muscle mass (A), PCSA (B), muscle belly length (C) and muscle fascicle length (D) for each of the seven investigated muscles in affected and non-affected Dachshunds. Asterisks indicate significant differences.

The analysis of the full set of data with only body weight and Pfirrmann grade as covariates showed 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<0.05), and on muscle mass for *M. multifidus thoracis* (P=0.026).

In the analysis with all covariates included, the effect of body weight remained significant on muscle mass for *M. iliocostalis lumborum* (P=0.058), *M. iliocostalis thoracis* (P=0.005), *M. longissimus cervicis* (P=0.010), *M. longissimus thoracis et
lumborum (\(P=0.042\)), M. multifidus lumborum (\(P=0.010\)), M. spinalis et semispinalis (\(P=0.006\)) and M. multifidus thoracis (\(P=0.032\)). The effect on fascicle length was significant for M. iliocostalis lumborum (\(P=0.021\)), M. longissimus thoracis et lumborum (\(P=0.047\)) and M. spinalis et semispinalis (\(P=0.030\)) and on PCSA for M. longissimus cervicis (\(P=0.021\)) and M. longissimus thoracis et lumborum (\(P=0.046\)).

In the sensitivity analysis, with back length and age as covariates, a significant effect of back length on all response variables existed 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 \(P<0.05\)).
6 DISCUSSION

Extensive research has been conducted on the human paraspinal muscles in relation to different spinal pathologies, providing the basis for different strategies in back pain management. While spinal diseases are very common also in dogs, this topic has received little attention in veterinary medicine. This thesis presents a validated measurement method for evaluating epaxial muscle size and composition and elucidates the influence of IVDH on epaxial muscle CSA, fat infiltration and internal architecture.

6.1. Validation of techniques for muscle structure evaluation

We found that thoracolumbar epaxial muscle structure measured as CSA and fat content can be reliably and repeatedly measured from both MRI and CT. The intra-rater reliability of the CSA measured on high-field MRI (Study I) and low-field MRI (Study II) was almost perfect (ICC 0.923-0.991 and 0.888-0.984, respectively). The intra-rater reliability for MRI fat content, CT CSA and CT muscle attenuation ranged from ‘substantial’ to ‘almost perfect’ (ICC 0.650-0.998) (Study II). This is in concordance with previous research in humans, where the more experienced observer usually had higher intra-rater reliability than a novice observer (Niemeläinen et al., 2011; Valentin et al., 2015a; Fortin et al., 2018). Appropriate training is required to produce consistent muscular morphological measurements and decrease the variation in the confidence interval (Valentin et al., 2015a). This was noted also in our study, as the more experienced observer was more consistent, independent of educational background, although both observers were classified as novices.

The inter-rater reliability was not tested in Study I. In Study II, inter-rater reliability was ‘substantial’ to ‘almost perfect’ for most MRI and CT variables, but there was large variation in the confidence intervals in the MRI CSA for M. longissimus and in the MRI fat content for Mm. multifidi left, M. spinalis et semispinalis left and right and M. longissimus left. One explanation for this may have been that the poor spatial resolution on low-field MRI was not sufficient for observers to distinguish between the muscle borders. Another explanation may have been inadequate observer training. The agreement between the observers decreased further when the analysis was restricted to individual segments. This indicates that some spinal segments, in this case Th9-10 and Th10-11 are more difficult to measure than others, hence requiring more training. Additionally, a novice observer is known to have a higher standard error in the inter-rater reliability than an experienced observer (Fortin et al., 2018). Human studies have also reported some spinal segments to be more difficult than others to measure (Valentin et al., 2015a; Mhuiris et al., 2016). This finding would not have been seen without a separate analysis for each segment. In human studies, the intra- and inter-rater reliability is often investigated in only one segment (Teichtal et al., 2015), the ICCs are reported as a mean for all measurements across segments (Urrutia et al., 2018) or the analysis is insufficiently described (Paalanne et al., 2011; Ranger et al., 2017). In addition to intra-rater reliability, the inter-rater reliability should always be investigated when a novel method is tested because certain segments or muscles may be more difficult to measure than others.
The choice of the small fat ROI that is used to calculate the muscle:fat ratio may influence the inter-rater reliability. In Study I, the visually whitest spot of subcutaneous fat was chosen for the drawing of the fat ROI. This certainly introduced some subjectivity to the method. Investigating also inter-rater reliability in Study I would have accounted for this subjectivity. A standardized spot at the middle of the *M. longissimus* was used in Study II. Similar methods have been used in humans (Elliott et al., 2005; 2006; Valentin et al., 2015a; 2015b). However, Lerer et al. (2015) drew the fat ROI adjacent to the muscle of interest, resulting in one corresponding fat ROI for each muscle. This method may be more accurate, as it accounts for possible various distances from the coil caused by different scanning positions, scanners or parameters used in retrospective samples (Lerer et al., 2015).

We found that the CT CSA and CT muscle attenuation showed high intra- and inter-rater reliability and consistent CIs compared with the MRI variables (Study II). This suggests that the helical dual-slice CT is a more reliable modality to use for measuring the epaxial muscle size and composition than low-field MRI. On the other hand, drawing muscles on CT is more approximate than on MRI due to the poorer soft tissue resolution. Also the partial volume effect (De Decker et al., 2010) may have affected the measurements because CT slices were thinner than the MRI slices, resulting in higher agreement on CT.

There was no difference between left and right sides in the CSA measurement on MRI or CT, suggesting that the two different positions, lateral recumbency in MRI and dorsal recumbency in CT, did not affect the CSA measurement. The MRI fat content for the *M. longissimus* was significantly higher on the right side in all segments. It is possible that the positioning of the Dachshunds on their right side may have caused asymmetrical loading of the MRI coil resulting in signal intensity artefacts (Jones and Witte 2000). This finding highlights the importance of reporting the ICC and CIs from both sides if identical scanning positions are not possible.

By using a sample of young and healthy Dachshunds and reporting the results from all available segments on both left and right sides, we accounted for the confounding factors reported in human research. When measuring muscle structure from MRI or CT, it is extremely important to use already validated methods or to thoroughly test and report the novel methods as well as the training and educational background of the observers. Measurements averaged over multiple segments seem to have better reliability. This need to be considered carefully, as the method may lack sensitivity to detect changes at specific levels. Further, it is vital to standardize the measurement for discrepancy in body size and imaging acquisition variability. Otherwise the methods used may appear more reliable than they actually are.

### 6.2. Functional roles of epaxial muscles in Dachshunds and Border terriers

Our estimation of functional roles based on architectural features of epaxial muscles was in concordance with previous research (Sharir et al., 2006; Webster et al., 2014). We found long muscle fascicles in the *M. splenius*, *M. semispinalis complexus* and *biventer, M. semispinalis et spinalis cervicis* and *M. longissimus capitis*, hence, suggesting involvement in production of large movements. The *M. multifidus lumborum, M.*
longissimus thoracis et lumborum and M. iliocostalis lumborum seem to be force producers in both breeds because of their relatively high PCSA and short muscle fascicles. But in relation to other lumbar epaxial muscles, the M. multifidus lumborum in both Dachshunds and Border terriers had less capacity to generate force.

The Dachshunds had significantly higher PCSA in the M. semispinalis complexus (P=0.005) and in the M. iliocostalis lumborum (P=0.017) than did the Border terriers. The Border terrier, in turn, had longer muscle fascicles in the M. semispinalis complexus (P=0.004) and in the M. iliocostalis lumborum (P=0.003). In the Border terriers, this indicates that these muscles work over a wide range of motion in addition to providing stability, whereas in the Dachshund they possess greater capacity for force production and through that stabilization. The stabilizing function of the intervertebral disc on the spine decreases with increased degeneration (Bergknut et al., 2013). Bearing this in mind, our results suggest that the lumbar epaxial muscles might work harder to compensate for this lost stability. Our theory is that the chondrodystrophic Dachshund, which is susceptible to disc degeneration, could benefit from the higher spinal stability provided by force-producing muscles. However, increasing spinal stiffness by using more muscle force may not be the most appropriate strategy for a dog with spinal disease, and the role of sensorimuscular control should be considered when implementing these results in clinical practice. This motor control training should have the intention of optimizing spinal loading, reducing irritation and encouraging normal movement (Hodges 2013). In doing so, a healthy environment is created, facilitating tissue recovery in case of injury or maintaining the uninjured spine in good condition (preventive strategy) (Hodges 2013).

The chondrodystrophic conformation in Dachshunds offers one logic explanation for the longer muscle fascicles in M. semispinalis complexus and M. iliocostalis lumborum in Border terriers. This finding indicates movement over a greater range in the Border terrier cervical and lumbar spine. Longer legs in an animal, hence greater leverage might facilitate more movement through the spine from the hind limbs during running (Williams et al., 2008a; Hudson et al., 2011). This may apply to the long-legged Border terriers in our study. There is liability to greater bending moments in a long back (Verheijen and Bouw 1982) and the epaxial muscles in the long spine of the Dachshund might need to work harder to counteract the pull of gravity and managing daily tasks than the short spine of the Border terrier, and hence, the greater PCSA in the Dachshund M. iliocostalis lumborum.

The Dachshund and the Border terrier have been originally bred and used for the same purpose: hunting underground in burrows, digging and crawling, and above ground, running (Border Terrier Club; Suomen Mäyräkoiraliitto). Therefore, it was not a surprise that the investigated architectural parameters were very similar in the M. multifidus lumborum and M. longissimus thoracis et lumborum between the two breeds. The selective breeding in Dachshunds and Border terriers may not have been as extreme as it has been in the sprinting Greyhound and the fighting Staffordshire bull terrier, where the M. longissimus had significantly higher mass and PCSA in the former than in the latter (Webster et al., 2014). The combination of M. longissimus and M. iliocostalis lumborum increased the propensity for power production in the Greyhound (Webster et al., 2014). It must be noted, that our results are predictions based on muscle internal architecture and need to be further tested with empirical data, kinematic and EMG studies. 
6.3. Implications of IVDH on epaxial muscle structure

6.3.1. Epaxial muscle structure between lesion and non-lesion sides in Dachshunds

Contrary to human studies on disc herniation and epaxial muscle structure (Hyun et al., 2007; Battié et al., 2012) and to animal back pain studies (Hodges et al., 2006; Stubbs et al., 2010), we found no difference in the CSA between lesion and non-lesion sides in Dachshunds with IVDH (Study I). Similarly, we found no difference in fat infiltration between lesion and non-lesion sides. Still, in humans, fat infiltration occurs in the \textit{M. multifidus} on the affected side and at the level below the lesion in patients’ lumbar disc or nerve root pathology (Battié et al., 2012). This can be explained by the different spinal anatomy and characteristics of the IVDH between humans and dogs. In dogs, the spinal cord extends into the caudal lumbar region (Evans, 1993b), while in humans the spinal cord ends in the caudal thoracic spine (Moore and Dalley, 2006). In humans, the lower lumbar segments are most commonly affected by disc lesions, and therefore, the disc herniation more likely compresses individual nerve roots and not the spinal cord, resulting in unilateral signs (Bogduk 2005b). In dogs, the spinal canal is smaller than in humans, resulting in IVDH affecting the spinal cord itself, often ventrally (Besalti et al., 2006; Trampus et al., 2018), leading to bilateral deficits (Coates 2010). Because of this, it is possible that the epaxial muscles could be bilaterally affected, hence no difference between sides were detectable in our sample of Dachshunds.

6.3.2. Epaxial muscle size in compressive and non-compressive spinal cord lesions

Dachshunds with compressive IVDH had smaller CSA in \textit{Mm. multifidi} and \textit{M. longissimus} than dogs with non-compressive FCE. When the Dachshunds were further divided into acute compressive lesions and chronic compressive lesions, the CSA was still significantly smaller and the fat infiltration significantly greater between the compressive and non-compressive lesions. This agrees with human reports, where the CSA of paraspinal muscles has been decreased in LBP patients or discectomy patients relative to asymptomatic subjects (Hides et al., 2008a; Danneels et al., 2000; Bouche et al., 2011). Considering the acute onset of IVDH in Dachshunds, a likely explanation for our findings could be as previously reported in pigs, muscle disuse due to reflex inhibition (Hodges et al., 2006). In dogs, the epaxial muscle CSA has been shown to be decreased in also dogs with lumbosacral pain compared with controls (Henderson et al., 2015; Cain et al., 2016). Lumbosacral problems in the dog are chronic in nature (Cain et al., 2016) and the reason for decreased CSA in these dogs could be general disuse due to prolonged symptoms. The FCE dogs in our study were not asymptomatic. However, they were considered a suitable control group because of the acute non-compressive and non-painful spinal cord lesion caused by the FCE (Gandini et al., 2003, Nakamoto et al., 2009). The clinical signs in acute IVDH are similar to those of FCE, and it is sometimes difficult to distinguish between the two conditions (De Risio 2015), further justifying this control group.

It is also possible that the smaller CSA in Dachshunds was a sign of denervation atrophy because of the painful and compressive character of the IVDH (Coates 2010; Ruddle et al., 2006). The Dachshunds were acute emergency cases, presented with symptoms lasting for less than seven days. An experimental study in pigs showed decreased CSA only three days after a disc and nerve root lesion (Hodges et al., 2006). Previous studies have discussed denervation and disuse as possible reasons for \textit{Mm.
multifidi atrophy (Mattila et al. 1986; Hodges et al., 2006). In humans with LBP, local Mm. multifidi atrophy is considered neurogenic (Beneck and Kulig, 2012). Also, in pigs with a nerve lesion, the M. multifidus CSA was reduced over several segments, while a disc lesion caused a local decrease in CSA (Hodges et al., 2006). Denervation affects muscles innervated by the nerve at the affected level. The nerve root innervating muscle fibres arises from the spinal segment of the same number as the nerve root (Hodges et al., 2006). The major bulk of fibres arising from that segment are adjacent to the spinous process of the segment below the lesion, hence denervation atrophy would be expected in segments caudal to the lesion (Hodges et al., 2006). Analysing the difference between groups in several segments, particularly caudal to the lesion, could have confirmed the denervation theory in Study I.

6.3.3 Epaxial muscle composition in compressive and non-compressive lesions

The Dachshunds with compressive spinal cord lesions had greater fat infiltration in the Mm. multifidi and M. longissimus than the non-compressive FCE dogs. In humans with IVDH, symptoms lasting for less than 6 weeks, the fat infiltration was increased in the Mm. multifidi ipsilateral and distal to the disc lesion (Battiè et al. 2012). In comparison, patients with LBP and associated leg pain showed increased fat infiltration in the Mm. multifidi bilaterally and at multiple levels (Kader et al. 2000). Fat infiltration in human paraspinal muscles has also been related to denervation (Kamath et al., 2008; Hyun et al., 2007), and chronic denervation causes high signal intensity on T1-weighted MR images (Kamath et al., 2008). In Study I, the durations of neurological deficits and pain reported by owners were longer in the Dachshunds than in the FCE dogs. It would be logical to think that the increased fat infiltration in the Dachshunds was primarily due to chronic denervation or disuse caused by spinal cord compression. However, the IVDH in Dachshunds is still a very acute condition and fat infiltration as a result of denervation is known to be visible on MRI a few weeks after insult (Kamath et al., 2008). Therefore, we could not completely confirm our findings to be fat infiltration.

Interestingly, a recent study using a 1.0 Tesla MRI in dogs with acute thoracolumbar disc extrusion found a hyperintense signal on T2-weighted and an isointense signal on T1-weighted MR images in epaxial muscles, consistent with denervation oedema. The altered signal was observed ipsilateral to the compression or bilateral despite a unilateral spinal cord compression (Trampus et al., 2018). Unfortunately, this study was not able to confirm the signal alterations with histology although attempts were made. The higher signal intensity on T1-weighted MRI images in our study could suggest fat infiltration in epaxial muscles of the Dachshunds. However, analysing also T2-weighted images would be useful to determine whether our signal intensity findings were related to oedema or to true fat infiltration. In addition confirming MRI signal alterations to histology would be important to address in future studies. Trampus et al. (2018) reported signal alterations in dogs that had a higher neurological grade or were non-ambulatory. This suggests that the signal alterations were associated with the compressive character of the disease and also supports our findings of a signal intensity change in Dachshunds with compressive spinal cord lesions but not in non-compressive FCE dogs’ lesions.

Contrary to our results, another recent MRI study on epaxial muscle fat infiltration in chondrodystrophic and non-chondrodystrophic dogs noted that dogs with higher disc degeneration grades demonstrated less fat infiltration in Mm. multifidi and M. psoas (Lerer et al., 2015). Non-chondrodystrophic dogs with non-IVDH pathology showed higher fat
infiltration in their muscles than chondrodystrophic dogs with IVDH pathology (Lerer et al., 2015). A logical explanation for this finding would be the acute onset of the potentially paralysed IVDH Dachshund requiring immediate diagnostic work-up and surgery (Moore et al., 2016; Langerhus and Mils 2017), hence, the time from onset of clinical signs to MRI is short. A non-chondrodystrophic patient with non-IVDH spinal pathology would possibly have suffered from symptoms causing disuse atrophy for a longer time before having an MRI.

Research has also shown that fat infiltration in human paraspinous muscles increases with age (Fortin et al., 2014). Fat infiltration of the *Mm. multifidi* has been associated with LBP only in adults, not in adolescents (Kjaer et al. 2007). In our population, the age of the Dachshunds was significantly greater (7.3 ± 2.3 years) than the age of FCE dogs (5.6 ± 2.4 years, P=0.016), and it is possible that the fat infiltration identified is related to age or general disuse rather than to IVDH.

### 6.3.4. Epaxial muscle architecture in Dachshunds with and without IVDD

We had expected to find signs of disuse atrophy in the muscle architecture of affected Dachshunds relative to non-affected Dachshunds. This could have been reflected as decreased muscle mass or volume and shortened muscle fascicles (de Boer et al., 2007) because of possible pain and reluctance to move in IVDD. Interestingly, we detected no difference between affected and non-affected Dachshunds that would have confirmed disuse atrophy, and our results indicated that Pfirrmann grade or IVDD did not affect the muscle architecture in thoracolumbar epaxial muscles.

Affected Dachshunds were older and had higher degeneration of their intervertebral discs than non-affected Dachshunds. This agrees with the consensus that disc degeneration increases with age (Bergknut et al., 2011). However, it was only possible to assess the current situation from MRI, and we could not establish how long the disc degeneration or IVDD had been present. Further, the severity of spinal cord compression on MRI does not always correspond to clinical signs (Besalti et al., 2006). Several of our affected Dachshunds had only mild spinal cord compressions and/or multiple affected intervertebral discs. Muscular properties were measured for the entire muscle and this would unlikely have enough sensitivity to detect differences at certain spinal levels or regions.

The non-affected Dachshunds had shorter fascicle lengths in the *M. longissimus cervicis* and *M. longissimus thoracis et lumborum* than affected Dachshunds, with no differences in PCSA or volume. Decreased PCSA and volume in addition to decreased fascicle length are seen in disuse atrophy (de Boer et al., 2007; Narici and Maffulli 2003), and also in sarcopenia (Narici and Maffulli 2003). This suggests that fascicle length in the *M. longissimus* muscle was decreased independently of muscle size. It is therefore unlikely that disuse atrophy due to IVDH would be the major cause of these differences. Many of the dogs in both the affected and non-affected groups were older than 13 years. The dogs may have been showing signs of sarcopenia, masking the difference between groups.

We do not have an explanation for why the affected Dachshunds had longer muscle fascicles in the *M. longissimus cervicis* and *M. longissimus thoracis et lumborum*. One theory is that the muscles in the affected Dachshunds are compensating to maintain a pain-free posture of the spine to be functional despite the IVDD. Dogs with IVDD may have kyphosis of their spine (Coates 2010). Muscles adapt to load and eccentric work increases
fibres length (Franchi et al., 2014; Narici and Maffulli 2003), resulting in the eccentrically working epaxial muscles in a kyphotic spine increasing their fibre length. Another theory is that the affected, older dogs with more disc degeneration have a less stable spine, causing the *M. longissimus* muscle to work over a greater range of motion, with longer muscle fibres as a result of adaptation to the abnormal load.

### 6.4. Limitations

This study had several limitations regarding the selection of cases. In Study I, it was not possible to have a control group of healthy Dachshunds due to the retrospective nature of the study and ethical and financial reasons. The FCE group consisted of different breeds of various conformations compared with the homogeneous Dachshund group. This variety in muscle size between groups was standardized by using the Muscle:Disc ratio previously applied in human studies (Kang et al. 2007). However, it is not known whether the relationship between the size of muscle and intervertebral disc is constant across species. Considering this and the breed differences that we found in epaxial muscle architecture between Dachshunds and Border terriers in Study III, it cannot be completely excluded that the discrepancy in body conformation related to breed in Study I influenced the results. In Study II, although accounting for confounding factors, the inclusion of only healthy dogs may have introduced a selection bias. The methodology may appear more reliable than had atrophied muscles of actual IVDD dogs been measured. The data collection in Study III relied entirely on dog owners donating their pets to research after euthanasia. This resulted in a small sample size with very old dogs and dogs with possible underlying diseases. Because of this, also three of the seven Border terriers included were classified as affected. Analysis of affected and non-affected Border terriers was not conducted due to difficulties with fitting ANCOVA models to such small sample sizes.

Further limitations occurred regarding the background data of the dogs. Study I relied on the background information of duration of pain and neurological deficits as they were written in patient records. Study I compared the CSA and fat infiltration between compressive and non-compressive spinal cord lesions at only one spinal segment (Th12-13). This choice was made because most lesions in our sample occurred here and we wanted to maintain an adequate sample size for the analysis. Running the analysis at the segments caudal to the lesion would perhaps have confirmed or contradicted the previous results regarding denervation atrophy ( Hodges et al., 2006; Stubbs et al., 2010; Battie et al., 2012). In Study III, 21 of the 24 owners completed the questionnaires, and although the questionnaires were not validated 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, which may have decreased the reliability of the replies. Another limitation was that 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 IVDD. The high age and IVDD in the study dogs may have influenced our results, and 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.

There are some limitations related to the measurement method. Study I did not test the inter-rater reliability or report ICC or CIs from left and right sides. The observer was blinded to the background data and diagnosis of the dogs at the time of the measurements, but the observer was not blinded to the side of the disc lesion on the MR images. In Study II, the restriction of the analysis to individual segments caused a decrease in sample size, which should be borne in mind when interpreting the results.

6.5. Future research

Future research should focus on confirming the findings of decreased CSA and increased fat infiltration in the epaxial muscles of dogs using a prospective methodology to collect data. The Dachshunds with IVDH should be compared to a control group of healthy Dachshunds. A validated measurement method should be used; the observer should be appropriately trained and blinded to the disc lesion and to the side in the MR or CT images. Both the CSA and fat infiltration should be evaluated because these two combined provide more information about atrophy than CSA alone (Crawford et al., 2017). Evaluations should take place at different points in time to better clarify the reasons for potential findings (Hodges et al., 2015).

There is also a need to consider evaluating T2-weighted images because of the detected oedematous-like signals in epaxial muscles in these sequences in dogs with acute IVDH (Trampus et al., 2018). The use of high-field MRI should be favoured and new technologies, such as pixel separation and fat suppression techniques, might introduce further possibilities to measure muscle structure also in dogs (Mhuiris et al., 2016; Crawford et al., 2017). The potential of muscle atrophy as a sign of spinal pathology should be investigated in dogs with mild protrusions since evaluating muscles may help in determining whether the lesion is clinically relevant. When quantifying atrophy, the CSA and fat infiltration should be evaluated together since fat can alter CSA appearance and CSA alone does not give information about internal structure of the muscle (Teichtal et al., 2015; Crawford et al., 2017).

In humans, the detected fat infiltration in Mm. multifidi on MRI has been confirmed with histology (Smith et al., 2014). Trampus et al. (2018) attempted to do the same in dogs, but they did not get consistent results due to the small sample of biopsies. Future research looking prospectively into epaxial muscle structure in dogs with IVDH should aim to confirm the increased signal intensity on MRI with intraoperative biopsies at different recovery stages. This would provide important information about what is actually going on in the muscles of acute IVDH dogs.

Based on the results of this thesis, the predicted force production capacity of M. multifidus lumborum compared with other lumbar epaxial muscles was relatively small. It seems from a force production perspective that both M. longissimus thoracis et lumborum and M. iliocostalis lumborum in our dogs provide sufficient stability to the spine, and M. multifidus lumborum may not be as important. However, this thesis did not investigate the sensorimotor control capacity or the metabolic profiles of Mm. multifidi or other epaxial muscles. In other quadrupeds such as horses and pigs, the Mm. multifidi is considered a local stabilizer of intervertebral joints (Hodges et al., 2006; Stubbs et al., 2006), while the
longissimus muscle is more of a dynamic stabilizer working over several motion segments (Stubbs et al., 2006). It may be that the *Mm. multifidi* is a provider of segmental stability also in dogs, but bearing in mind that the spine in the dog is more flexible than the spine in the horse (Evans 1993; Stubbs et al., 2006), further studies on this topic is warranted. Spinal control is required during all movements, and this control might be challenged during force production in the body (Tsao et al., 2010). In humans, the quality of movement and force produced by *M. erector spinae* depend on the ability of *Mm. multifidi* to segmentally control the spine (Moore and Dalley 2006; Neumann 2017). Without sufficient control of *Mm. multifidi*, the spinal movements might become unconstrained, leading to irritation or pain and in the long term to degeneration in the lumbar spine (Tsao et al., 2010; Neumann 2017). Managing this lack of control by increasing stability with greater muscle force (created by the more superficial polysegmental muscles) can perhaps create a stable, but rigid spine, which is not desirable (Hodges 2013).

In rehabilitation of dogs with spinal disease, we must consider the insights obtained in humans and in the animal models used. However, we should bear in mind that the human spine is vertically oriented and transfer this information to the horizontal quadruped spine, also being mindful of the different gaits in dogs (Schilling and Carrier, 2010). There is a need to review current rehabilitation routines in veterinary medicine and investigate targeted, controlled, breed-specific retraining exercises that stimulate force production and facilitate sensorimotor control in the epaxial musculature of dogs during different phases of recovery from spinal disease.
7 CONCLUSIONS

1. Dachshunds with compressive IVDH had significantly smaller CSA and greater fat infiltration in terms of increased signal intensity on MRI in the Mm. multifidi and M. longissimus than dogs with non-compressive FCE. These changes may be related to the compressive character of the intervertebral disc extrusion and/or the duration of neurological deficits or pain.

2. Muscle structure, measured as CSA and fat content, in Dachshunds can be reliably evaluated using either MRI or CT. Appropriate observer training is recommended to maintain high intra- and inter-rater reliability since evaluation of small muscles in specific spinal regions did show a lower degree of reliability. The CSA measurement is comparable between MRI and CT.

3. The functional roles in epaxial muscles of Dachshunds and Border terriers were predicted using calculations based on muscle architecture. The neck muscles produced large movements and lumbar epaxial muscles provided dynamic stabilization of the spine in both breeds. The M. iliocostalis lumborum in the Dachshund was more suitable for generating force and providing stabilization, while in the Border terrier it was adapted more for large movements. This may suggest a greater requirement for spinal stability provided by the epaxial muscles in the Dachshund.

4. The estimated force and power production capacity in epaxial muscles of IVDH-affected and non-affected Dachshunds were similar based on muscle architecture. Affected Dachshunds had longer fascicle lengths in the M. longissimus cervicis and thoracis et lumborum than non-affected Dachshunds, but the reason for this difference between groups remains unknown.
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