Skeletal Muscle Fibre Characteristics of the Lumbar Multifidus Muscle in Patients Undergoing Microdiscectomy for Unilateral Lumbar Disc Herniation

ORIGINAL ARTICLE

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Skeletal Muscle Fibre Characteristics of the Lumbar Multifidus Muscle in Patients Undergoing Microdiscectomy for Unilateral Lumbar Disc Herniation

S. Stevens[1,3#], A. Agten[1,4#], T. Snijders[3], M. Plazier[2], S. Bamps[2], T. Assieker[3], M. W. Betz[3], A. Timmermans[1], L. J. C. van Loon[3], F. Vandenabeele[1]

[1] *Rehabilitation Research Center, Faculty of Rehabilitation Sciences, Hasselt University, Diepenbeek, Belgium*
[2] *Department of Neurosurgery, Jessa Hospital, Hasselt, Belgium*
[3] *Department of Human Biology, NUTRIM School of Nutrition and Translational Research in Metabolism, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands*
[4] *Department of Anatomy and Embryology, NUTRIM School of Nutrition and Translational Research in Metabolism, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands*
[#] *Shared first author*

CORRESPONDING AUTHOR:
Anouk Agten
Rehabilitation Research Center
Faculty of Rehabilitation Sciences
Hasselt University
Agoralaan 3590
Diepenbeek, Belgium
E-mail: anouk.agten@uhasselt.be

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SUMMARY

Background. Lumbar disc herniation (LDH) is the most common diagnosed degenerative pathology in the lumbar spine. Because of its role in spinal stability there is an increased interest in the role of the *Lumbar Multifidus* muscle in low back pain research. Despite surgical treatment long-term, disability and pain remain a persistent problem. The aim of the study is to compare side-to-side *Lumbar Multifidus* muscle fibre characteristics in unilateral LDH patients, and compare both sides to a healthy control group.

Methods. Thirty patients (n = 17 men and n = 13 women) scheduled for microdiscectomy for unilateral disc herniation and ten healthy controls (n = 5 men and n = 5 women) were included in this study. Biopsies of the *Lumbar Multifidus* muscle were analysed by means of immunohistochemistry combined with immunofluorescence microscopy to determine type I and type II muscle fibre type distribution, cross-sectional area, myonuclear- and satellite cell content, inflammation and various indices of muscle fibre capillarisation.

Results. The proportion of muscle fibres with centrally located myonuclei, various indicis of muscle fibre capillarisation and pro-and anti-inflammatory cell content were higher in the patients compared with the healthy controls. No differences were observed in type I and type II muscle fibre characteristics between the injured and uninjured side within the LDH patients.

Conclusions. This study shows clear differences in *Lumbar Multifidus* muscle fibre characteristics between LDH patients, irrespective of injured or uninjured side, and healthy controls. Additional studies are warranted to establish the clinical significance of these differences in muscle fibre morphology in LDH compared with healthy controls.

Study registration. This trial was registered on ClinicalTrials.gov under the identification number NCT03753711.

KEY WORDS

Biopsy; lumbar disc herniation; multifidus; satellite cells; skeletal muscle fibres.

INTRODUCTION

Low back pain is one of the most frequent and debilitating musculoskeletal conditions globally causing high socioeconomic burden (1). The most common diagnosed degenerative pathology in the lumbar spine is lumbar disc herniation (LDH) (2). It is characterised by localised or focal displacement of disc material beyond the limits of the intervertebral disc space, most often in the posterolateral region (3). Nowadays it is not clear which loading conditions are responsible for lumbar intervertebral disc failure. Due to similar mechanical behaviour of the ovine and human intervertebral disc, the ovine model can be used to investigate the mechanical loading on the intervertebral disc itself (4).

In a selected group of patients experiencing progressive neurological deficits, red flags, or failure of conservative management, surgery may be required (5). Although surgery provides short time relief of leg pain when compared to conservative management, no significant long-term differences in clinical outcomes have been observed (6, 7). Despite surgical treatment up to 36% of patients keep experiencing persistent low back pain symptoms (8). This could indicate that surrounding tissues outside the epidural space contribute to pain persistence and long-term disability. The *Lumbar Multifidus* muscle (LMM) is an increasing subject of interest in low back pain research, especially because of its functional role in spinal stability and control of spinal motion (9, 10). Recent human research has shown that changes in muscle mass and muscle quality of the paraspinal muscles (*e.g.*, fat infiltration, fibrosis) are often associated with unilateral LDH, especially at the side of radiculopathy (11). Moreover, degenerative changes of the LMM such as atrophy, fat infiltration and fibrosis have been observed in different animal intervertebral disc injury models (12-16). Inflammatory dysregulation has been proposed as a potential mechanism of LMM degeneration, stepping away from simple denervation atrophy or reflex inhibition paradigm (17). This mechanism is supported by *in vivo* studies showing increased numbers of inflammatory, fibro-adipogenic, and satellite cells in skeletal muscle of spinal pathologies (18-21). These observations indicate that a more complex process than just simple denervation or disuse atrophy is involved. It should be noticed that these studies (18-21) are all lacking an age-matched healthy control group, which makes it difficult to draw firm conclusions.

Currently, the mechanism of LMM degeneration and atrophy *in vivo* in humans remains largely unknown. The aim of the present study is to compare side-to-side LMM fibre characteristics and muscle cell type content in unilateral LDH patients, and compare both sides to a healthy control group. This study provides insight into the LMM characteristics of hernia patients and the potential importance of specific cell types in the development of LMM degeneration and atrophy. Subsequently this may help to improve existing and/or develop new intervention strategies to more effectively prevent or reverse the deconditioning of paraspinal muscles after LDH.

MATERIALS AND METHODS

Subjects

Thirty adults with symptoms of unilateral LDH, scheduled for minimally invasive discectomy, were recruited from July 2018 until December 2019 at the Jessa Hospital, Hasselt, Belgium. Participants were informed about the opportunity to participate in the study by their neurosurgeon during their preoperative consultation if they met the following criteria: unilateral LDH diagnosed using medical imaging, age between 18 and 55 years old, and fluent in Dutch (both spoken and written). Participants were excluded when they had undergone surgery within the last year, had degenerative or other spinal pathologies, or had other known pathologies that could interfere with muscle biology. Healthy participants were recruited by convenience sampling using local advertisements. Participants, between 25 and 60 years of age and able to understand the Dutch language, were included if they either had no acute or chronic low back pain (> 3 months). Healthy subjects were excluded if they had been in rehabilitation or exercise therapy for an acute condition within the last 3 months. All subjects were informed about all the aspects of the study and were included in the study after providing their informed consent. Ethical approval was given by the Medical Ethics Committee from the Jessa Hospital and Hasselt University of Belgium (B243201836859 - Date of approval June 27, 2018).

Muscle samples and clinical data

LMM samples were taken during minimally invasive microdiscectomy for unilateral LDH. After patients were anaesthetised and placed in the genu-pectoral position a midline incision was made over the spinous process of the involved segment. A fine needle biopsy technique was used to obtain a muscle sample from the contralateral (non-herniated side) LMM, using the 12G semi-automated Bard® Mission® Core Biopsy Instrument. The ipsilateral biopsy sample (herniated side) was taken directly from the LMM when surgically preparing the access to the posterior lamina at the level of surgery. LMM samples in the healthy control group were taken at the right side of the body at the spinal level of the spinous process of vertebra L4, as described previously (22). All samples were placed on cork and embedded in optimum cutting compound. Samples were immediately

frozen using isopentane cooled in liquid nitrogen. Frozen samples were stored at - 80 °C in the clinical biobank until further analyses.
Demographic data (*e.g.*, age, gender, body mass index (kg/m²)) were obtained from all participants. Leg and back pain duration and intensity were assessed using the Visual Analogue Scale (VAS) using a 10-point Likert scale. The presence and duration of motor deficits was assessed by manual muscle testing (23). Disability was assessed using the Oswestry Disability Index (24).

Immunohistochemistry

Frozen muscle biopsies were cut into 5 µm thick cryosections using a cryostat at - 20 °C and thaw mounted on uncoated pre-cleaned glass slides. Samples from the ipsilateral and contralateral sides of the LMM were mounted on the same slide. Samples were stained with antibodies against CD31 (1:50, endothelial cell mouse IgGg1 Dako M0823), CD68 (1:100, monoclonal mouse Anti-Human IgG1, Dako M0718), CD206 (1:200, Rabbit polyclonal to Mannose Receptor IgG, Abcam 64693), PAX-7 (1:1, cell supernatant from cells obtained from the DSHB, USA), myosin heavy chain I (1:25 mouse IgM A4.840 (slow isoform), DSHB, USA), laminin (1:50, sigma-aldrich, |L9393 USA). For immunofluorescent detection, secondary antibodies used were as follows: Pax7 (biotin-vector BA-2000, 1:200, streptavidin 488 1:200, Invitrogen, Molecular Probes, Carlsbad, CA, USA); myosin heavy chain type I (clone A4.480) (goat anti-mouse IgM Alexa Fluor 488, 1:500, Invitrogen); laminin (goat anti-rabbit IgG Alexa Fluor 647, 1:400, Invitrogen); CD68 (1:200, goat anti-mouse IgG 488, Invitrogen); CD206 (1:200, goat anti-rabbit 555, Invitrogen); CD31 (biotin-vector BA-2000, 1:200, Avidin Texas red 555 1:400, Invitrogen, Molecular Probes, Carlsbad, CA, USA). Nuclei were labelled with DAPI (4',6-diamidino-2-phenylindole) (1:20000, Sigma-Aldrich, Oakville, ON, Canada). Prior to cover slipping slides with fluorescent mounting media (DAKO, Burlington, ON, Canada). Slides were viewed with the Nikon Eclipse Ti Microscope (Nikon Instruments Inc., USA), equipped with a high-resolution Photometrics CoolSNAP HQ2 fluorescent camera (Nikon Instruments, Melville, NY, USA). Images were captured and analysed using the Nikon NIS Elements AR 3.2 software (Nikon Instruments Inc., USA).
For muscle fibre size an average of 218 ± 204 (range 52-1324) fibres were analysed per fibre type in each biopsy sample to determine muscle fibre type distribution, muscle fibre size, percentage of fibres with central nuclei, and myonuclear content and domain size. To quantify capillaries an average of 45 ± 20 (range 18-119) fibres were analysed per fibre type/cross-section based on the work of Hepple *et al.* (25). Quantification was made of (I) capillary contacts (CC), (II) the capillary-to-fibre ratio (C/Fi), (III) capillary-to-fibre perimeter exchange (CFPE) index and (IV) capillary density, (CD). To quantify satellite cell content an average of 218±130 (range 67-685) fibres were analysed per fibre type/cross-section, and for macrophages an average of 210 ± 83 (range 78-438) fibres/cross-section were analysed.

Statistical analysis

Anthropometric and clinical data are displayed as mean ± SD. Main outcome variables (fibre distribution and size, myonuclear content, satellite cell and macrophage content) were analysed using JMP Pro 14.1.0 software (SAS Institute Inc, Cary, NC, USA, 1989 –2007). A mixed model was performed with fibre type and group (injured *vs* uninjured *vs* healthy) as within subject factors. For secondary measurements, the patients in the LDH group were subdivided into two groups based on the duration of radicular pain (acute < 12weeks, chronic > 12weeks). For secondary subgroup analysis a mixed model was performed with fibre type and side (injured *vs* uninjured) and fibre type and duration (acute *vs* chronic) as within subject factors. Normality of the data was checked using the normal quartile plots calculated from the conditional residuals. When a normal distribution was not assumed, the data set was transformed using a square root transformation. Significance was set at the 5% point with a confidence interval of 95%. When a significant interaction or main group effect (whenever 3 groups were used) was found, a *post-hoc* Tukey-HSD was used to locate the differences between groups. For the secondary outcome measures, a *post-hoc* t-test was performed when there was a significant interaction effect. Results are reported as mean ± SE.

RESULTS

Participants' characteristics

All LDH patients

Twenty-nine patients diagnosed with a unilateral LDH (age: 40 ± 9 y) and ten healthy controls (age: 42 ± 8 y) were included in the final analyses. From one patient the biopsy sample was deemed not usable because of insufficient quality. Participants' characteristics are displayed in **table I**. No significant differences were observed in age, weight, height, gender or BMI between groups. Fifteen patients underwent surgery at the spinal level L4-L5, and 14 patients at the level L5-S1. Based on VAS-scores low back pain was present in 87% of all unilateral LDH patients, with an average pain severity score of 4.7 ± 2.9 on a 10-point Likert scale. All

patients experienced leg pain with an average VAS score of 5.8 ± 2.1 on a 10-point Likert scale. Average duration of low back and leg pain was 23 ± 34 and 6 ± 11 months, respectively. Motor deficits (*e.g.*, reduced strength or paralysis) were present in 60% of the unilateral LDH patients with an average duration of 7 ± 11 months. Patients experienced severe disability with an average score of 41 ± 17% on the Oswestry Disability Index.

Acute vs chronic LDH patients

Subgroup analyses were performed to assess lateralisation between the injured and uninjured side between patients experiencing acute radicular pain (< 12weeks, n = 14) or patients experiencing more chronic radicular pain (> 12 weeks, n = 15). No between group differences except for the duration of radicular pain ($p < 0.05$) could be observed, indicating no different patient characteristics between groups (**table I**).

Muscle fibre type composition and size

All LDH patient's vs controls

In both the LDH patients and control subjects type I muscle fibres were significantly greater compared with type II muscle fibres (main effect of fibre type $p < 0.0001$, **figure 1**). When comparing side-to-side differences within the LDH group, no significant differences were observed in type I and type II muscle fibre size or fibre type composition (**figure 1**). When comparing the heathy control group to the LDH group (injured and uninjured side) no significant differences were found for muscle fibre size and type composition (**figure 1**). Detailed values are displayed in **table II**.

Table I. Participants' characteristics of healthy controls and all included lumbar disc hernia patients, and subdivided in an acute (< 12weeks) and chronic (> 12 weeks) group.

	Control subjects	LDH patients		
	All (n = 10)	**All (n = 29)**	**Acute (n = 14)**	**Chronic (n = 15)**
Age (yr)	42 ± 8	40 ± 9	40 ± 9	40 ± 9
Length (m)	1.79 ± 0.07	1.76 ± 0.10	1.77 ± 0.11	1.75 ± 0.10
Weight (kg)	83.9 ± 17.4	81.8 ± 19.5	81.9 ± 19.3	81.7 ± 20.4
BMI (kg/m²)	26.0 ± 4.0	26.5 ± 4.8	26.8 ± 4.7	26.2 ± 5.0
Gender (male:female)	5:5	17:12	9:5	8:7
LBP (months)	-	17.3 ± 27.1	9.0 ± 13.9	24.7 ± 34.1
Leg pain (months)	-	6.01 ± 9.13	1.20 ± 0.75	10.4 ± 11.07*
Motor (months)	-	4.30 ± 9.51	0.68 ± 0.69	7.53 ± 12.52
Back (VAS)	-	4.74 ± 2.97	4.64 ± 3.34	4.90 ± 2.73
Leg (VAS)	-	5.88 ± 2.17	5.08 ± 2.43	6.75 ± 1.56
Level (L4-L5:L5-S1)	-	15:14	8:6	7:8
ODI (%)	-	39.8 ± 17.2	37.4 ± 18.6	43.1 ± 16.2

BMI: body mass index; LBP: low back pain; VAS: visual analogue scale; ODI: Oswestry disability index; LDH: lumbar disc hernia; *Indicating a significant difference compared to the acute subgroup, $p < 0.05$. Data represent mean ± SD.

Table II. Muscle fibre characteristics in healthy controls and lumbar disc herniation patients, both at the injured and uninjured side.

		Controls (n = 10)	LHD injured (n = 29)	LDH uninjured (n = 29)
Muscle fibre type composition and size				
Fibre size (µm²)	I	5824 ± 419	5819 ± 299	5965 ± 312
	II	3932 ± 459*	3341 ± 305*	3625 ± 239*
Fibre type composition (%)	I	63 ± 4	56 ± 3	61 ± 3

		Controls (n = 10)	LHD injured (n = 29)	LDH uninjured (n = 29)
	II	36 ± 4*	44 ± 3*	39 ± 3*
Muscle fibre myonuclear and satellite cell content				
Myonuclear content (number / fibre)	I	4.01 ± 0.28	4.78 ± 0.19†	4.95 ± 0.2†
	II	2.61 ± 0.31*	3.21 ± 0.19*†	3.43 ± 0.21*†
Myonuclear domain (µm²)	I	1453 ± 71	1208 ± 47†	1196 ± 50†
	II	1483 ± 79*	1011 ± 48*†	1060 ± 53*†
Central nuclei (%)	I	1.93 ± 1.95	7.58 ± 1.2	8.57 ± 1.25
	II	2.8 ± 2.33	4.74 ± 1.25	3.11 ± 1.4
Satellite cell content (number / 100 fibres)	I	8.79 ± 1.45	12.61 ± 0.8	10.46 ± 0.8
	II	4.01 ± 1.45*	3.71 ± 0.8*	4.24 ± 0.85*
Muscle fibre capillarization				
CC	I	2.83 ± 0.24	3.76 ± 0.16†	3.72 ± 0.17†
	II	2.66 ± 0.27*	3.03 ± 0.16*	2.96 ± 0.17*
C/Fi	I	0.77 ± 0.12	1.82 ± 0.08†	1.76 ± 0.08†
	II	0.27 ± 0.13*	1.03 ± 0.08*†	0.98 ± 0.08*†
CFPE (capillaries / 1000 µm)	I	4.64 ± 0.26	5.84 ± 0.18†	5.74 ± 0.19†
	II	3.5 ± 0.29*	4.28 ± 0.18*†	4.29 ± 0.19*†
CD (capillaries / mm²)	I	286 ± 30	361 ± 19†	356 ± 20†
	II	291 ± 34	370 ± 19†	360 ± 20†
Muscle fibre inflammatory cells				
M1 (number / mm²)	MIX	0.97 ± 0.47	2.01 ± 0.27	2.76 ± 0.28†
M2 (number / mm²)	MIX	8.87 ± 2.23	15.09 ± 1.26†	13.59 ± 1.31

Data represent mean ± SE. CC: capillary contacts; C/Fi: capillary-to-fibre ratio; CFPE index: capillary-to-fibre perimeter exchange; CD: capillary density; LDH: lumbar disc hernia; Type I: type I muscle fibres; Type II: type II muscle fibres; MIX: mixed muscle fibre types; M1: cells positive for CD68 and DAPI; M2: cells positive for CD68, CD206 and DAPI. *Significant different compared with type I muscle fibres, $p < 0.05$; †*Post-hoc* significantly different compared to healthy control group, $p < 0.05$.

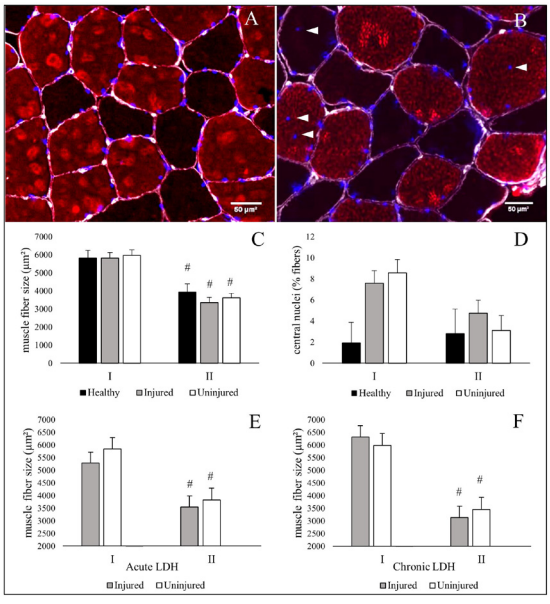

Figure 1. A, B: Representative image of immunohistochemical analyses of the lumbar multifidus muscle cross-section in healthy control **(A)** and lumbar disc herniation (LDH) patients **(B)**: myosin heavy chain I (red), laminin (white), dapi (blue), with white arrows indicating central nuclei. **C-D**: Type I and type II muscle fibre size **(C)** and central myonuclei **(D)** in healthy controls and LDH patient (injured and uninjured side). **E, F**: Type I and type II fibre size in the acute **(E)** (n = 14) and chronic **(F)** (n = 15) subgroups of the LDH patients for both injured and uninjured side.

Data are expressed as mean ± SE. #Indicating a significance with type I muscle fibres $p > 0.05$.

Acute vs chronic LDH patients

Although no significant differences were observed for muscle fibre size for the whole LDH group, the acute patient subgroup showed smaller type I (- 11%) and type II (- 8%) muscle fibre sizes when comparing the injured to the uninjured side (main effect of side p = 0.0735, **figure 1**). Fibre type distribution was not different between the acute and chronic subgroup, or between the injured and uninjured side within the groups. Detailed values are displayed in supplementary **appendix 1**.

Muscle fibre myonuclear and satellite cell content

All LDH patient's vs controls

No significant side-to-side differences were found for myonuclear content and satellite cell content within the LDH group (**table II**). Type I and II muscle fibre myonuclear content of the uninjured and injured side LDH patient were significantly greater when compared with healthy control subjects (main effect of group $p < 0.05$, **table II**). Type I and type II myonuclear domain size was significantly smaller in the LDH compared with the healthy control group (main effect of group, $p < 0.0001$, **table II**). In the LDH patients, the percentage of muscle fibres containing one or more central myonuclei was greater in type I compared to type II fibres (main effect of fibre type p= 0.0597, **figure 1**). In addition, the percentage of muscle fibres containing one or more central myonuclei was greater in LDH patients (both sides) compared with healthy controls (main effect of group p = 0.0951, **figure 1**). In both the LDH patient (injured and uninjured side) and control subjects type I muscle fibre satellite cell content was significantly greater compared with type II muscle fibres (main effect of fibre type $p < 0.0001$, **figure 2**). No significant differences were observed in type I and type II muscle fibre satellite cell content between LDH patients and healthy controls (**figure 2**). Detailed values are displayed in **table II**.

Acute vs chronic LDH patients

The injured side showed a significant smaller myonuclear domain size compared to the uninjured side within the acute subgroup (main effect of side $p < 0.05$, supplementary **appendix 1**). Type I myonuclear domain was significant larger in the chronic group compared to the acute group at the injured side (main effect of duration $p < 0.05$, supplementary **appendix 1**). No differences in muscle fibre myonuclear or satellite cell content were observed between the acute and chronic group, or between the injured and uninjured side (supplementary **appendix 1**).

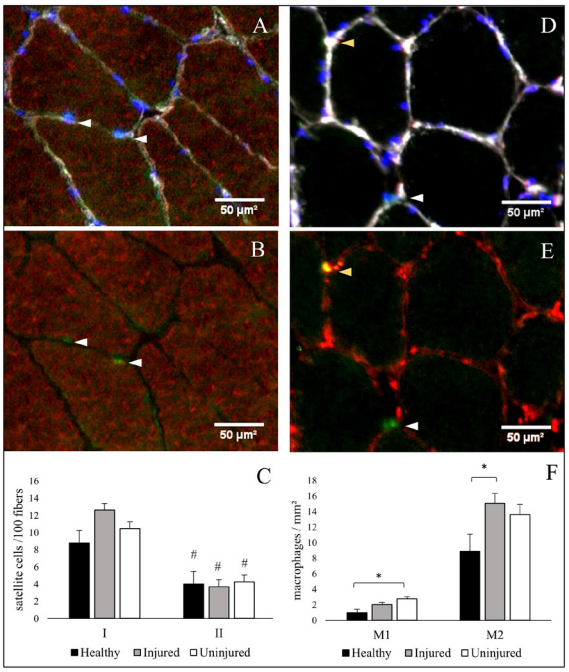

Figure 2. A, B: Representative image of immunohistochemical analyses of the lumbar multifidus muscle cross-section stained for satellite cells. **(A)**: myosin heavy chain I (red), laminin (white), dapi (blue), white arrows indicating satellite cells. **(B)**: myosin heavy chain I (red), pax-7$^+$ cells (green), white arrows indicating satellite cells. **(C)**: type I and type II muscle fibre satellite cells/100 muscle fibres in healthy controls and LDH patient (injured and uninjured side). **D, E**: representative image of immunohistochemical analyses of the lumbar multifidus muscle cross-section stained for macrophages. **(D)**: laminin (white), dapi (blue), white arrow indicating CD68$^+$ M1 macrophage, yellow arrow indicating CD206$^+$ M2 macrophage costained with CD68$^+$. **(E)**: CD68$^+$ cells (green), CD206$^+$ cells (red), white arrow indicating CD68$^+$ M1 macrophage, yellow arrow indicating CD206$^+$ M2 macrophage costained with CD68$^+$. **(F)**: number of M1 and M2 macrophages/mm^2 in healthy controls and LDH patient (injured and uninjured side).

Data are expressed as mean ± SE. *Indicating a significant between group difference $p < 0.05$. #Iindicating a significance with type I muscle fibres $p > 0.05$.

Muscle fibre capillarisation

All LDH patient's vs controls

No significant side-to-side differences were found for any of the muscle fibre capillarisation indices in the LDH group. In all groups, type I muscle fibre capillarisation was significantly greater compared with type II muscle fibres (main effect of fibre type $p < 0.0001$, **figure 3**), except for CD (**table II**).

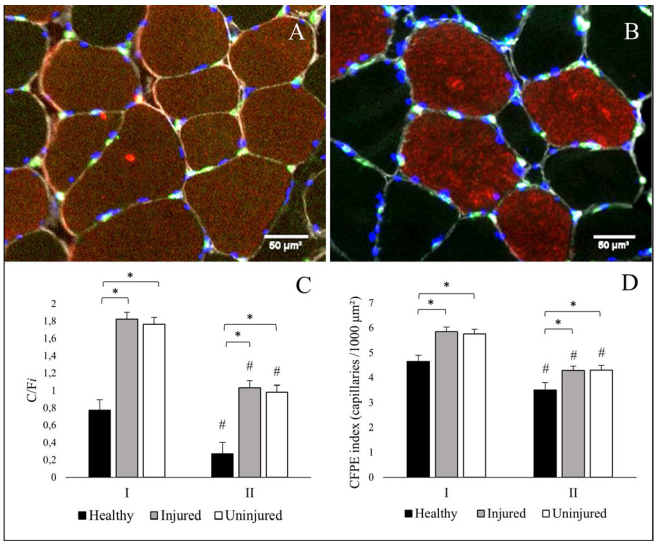

Figure 3. A, B: Representative image of immunohistochemical analyses of the lumbar multifidu muscle cross-section in healthy control **(A)** and lumbar disc hernia (LDH) patients **(B)**: myosin heavy chain I (red), laminin (white), dapi (blue), CD31 (green). **C, D**: Type I and type II muscle fibre capillary to fibre ratio (C/Fi) (C) and capillary to fibre perimeter exchange (CFPE) index (D) in healthy controls, and LDH patient (injured and uninjured side).

Data are expressed as mean ± SE. *Indicating a significant between group difference $p < 0.05$. #Indicating a significance with type I muscle fibres $p > 0.05$.

Type I and II muscle fibre CC, C/F*i*, CD as well as CFPE-index were greater in LDH patients compared with healthy controls (main effect of group $p < 0.05$, **figure 3**). Detailed values are displayed in **table II**.

Acute vs chronic LDH patients

Type I and type II muscle fibre capillarisation were not different between the acute and chronic group, or between the injured and uninjured side within the groups (supplemental **appendix 1**).

Muscle fibre inflammatory cells

All LDH patient's vs controls

No significant side-to-side differences were found for muscle fibre inflammatory cell density in LDH patients (**figure 2**). A significant greater number of pro-inflammatory M1 cells was found between the LDH group and healthy controls (main effect of group $p > 0.05$, **figure 2**), while for the anti-inflammatory M2 cells a borderline significant difference was found (main effect of group $p = 0.0595$, **figure 2**). Detailed values are displayed in **table II**.

Acute vs chronic patients

No differences in muscle fibre inflammatory cells were observed between the acute and chronic group, or between the injured and uninjured side (supplemental **appendix 1**).

DISCUSSION

The present study is the first to compare LMM fibre characteristics both at the injured and uninjured side between unilateral LDH patients and age-matched healthy controls. Although muscle fibre size did not differ between groups, the proportion of muscle fibres with centrally located myonuclei, as well as muscle fibre inflammatory cell content, was greater in the LMM of unilateral LDH patients when compared with healthy controls. Interestingly, type I and II muscle fibre capillarisation were considerably greater in unilateral LDH patients compared with healthy controls. Finally, no significant differences were observed in satellite or inflammatory cell content between the injured and uninjured sides or between groups.

Previous studies suggested type I and II muscle fibres to be larger in patients with LDH compared to healthy control subjects (26, 27). However, these studies used post-mortem biopsies to serve as controls, which may have influenced their findings as cellular breakdown, autolytic activity, and structural alterations of muscle tissue cannot be excluded (28). In the present study, *in vivo* LMM samples were obtained from age-matched healthy controls, providing a more accurate evaluation of the true differences in LMM morphology between healthy individuals and people suffering from LDH. In contrast to our expectations, no significant differences were observed in type I and II muscle fibre size between LDH patients and healthy controls. As lateralisation has been observed following lumbar disc herniation, we also aimed to assess differences in LMM fibre characteristics between the injured and uninjured side in patients with LDH. Previous studies have reported significantly smaller LMM muscle fibres (type I and II) at the injured compared to the uninjured side in LDH patient (29-31). In our study, we observed no differences in muscle fibre size (or fibre type distribution) between the injured and non-injured side of LDH patients. The lack of differences may, in part, be explained by the timing of the muscle biopsy sampling in relation to the onset of low back pain. Previous animal studies report profound muscle atrophy in the acute (3-6 days) phase, that was no longer present following a more prolonged period (3-6 months) (12, 14). Hence, we additionally (retrospectively) evaluated differences in muscle fibre characteristics between patients having an acute (< 12 weeks, n = 14) or chronic radiculopathy (> 12 weeks, n = 15) at the time of muscle biopsy sampling. Although not significant, patients

in the acute subgroup tended to show a greater difference in type I (~ - 11%) and type II (~ - 8%) muscle fibre size between the injured and uninjured side, compared with the chronic subgroup (type I ~ + 5% and type II ~ - 10%). The current study is the first to show potential evidence for acute, but not chronic, atrophy (type I) in human LMM samples in patients suffering from LDH. As the LMM is the primary stabiliser of the lumbar spine (32), this muscle relies especially on the type I muscle fibres for their stabilising function (33). In previous research, we have already reported a greater proportion of type I muscle fibres in patients with low back pain compared to healthy controls, without differences in type I muscle fibre size (34). We speculate that patients with acute LDH have decreased motor neuron activity (*e.g.*, inhibition) of the LMM, (35) resulting in a decrease in type I muscle fibre size. In chronic low back pain patients, LMM motor neuron activity is likely to be higher (36) leading the recovery of muscle fibre size compared to the more acute phase LDH. However, clearly more *in vivo* human research is warranted to confirm these speculations.

Skeletal muscle fibres are multinucleated cells, with each muscle fibre containing hundreds to thousands of myonuclei. These myonuclei provide the transcriptional activity required to regulate myocellular homeostasis, and support muscle reconditioning (37). In the present study, we observed a significantly greater myonuclear content in LDH patients when compared to controls. This may indicate that muscle fibres in LDH patients require increased transcriptional capacity to support muscle protein synthesis to recover from the acute phase of atrophy or are in need of increased transcriptional capacity due to higher protein turnover rates following increased muscular activity as seen in low back pain (38). On other hand, the greater myonuclear content could also represent a reduced efficiency of the existing myonuclei in these patients, a phenomenon that has been suggested to occur in age-related muscle fibre atrophy (39). Interestingly, the percentage of muscle fibres with centrally located myonuclei was three times higher in muscle tissue collected from LDH patients when compared with healthy controls. Centrally located myonuclei are one of the hallmarks of muscle fibre regeneration/repair following injury and, as such, have been suggested to be a proxy for muscle fibre damage (40). Hence, our findings suggest that muscle tissue of LDH patients is in a state of muscle fibre repair/regeneration when compared with healthy controls. As myonuclei are post-mitotic, provision of additional or replacing damaged myonuclei to support fibre homeostasis depends on a pool of myogenic precursor cells, also known as satellite cells (41). Muscle satellite cells are essential in skeletal muscle fibre regeneration and repair (42, 43). In addition, a decline in satellite cell content has been hypothesised to be an important contributing factor in the development of skeletal muscle fibre atrophy in aging as well as various other myopathies (44, 45). Although the muscle fibres of LDH patients clearly show more muscle fibre damage based on centrally located myonuclei, satellite cell content was not different compared with healthy controls. In addition, satellite cell content did not differ between the injured and uninjured side or between acute and chronic LDH patients. As satellite cell content does not seem to be reduced in muscle tissue of the LDH patients, these data suggest that muscle fibre repair/regeneration and reconditioning is not limited by satellite cell number in the LMM of these patients.

The delivery of oxygen and metabolic substrates, as well as removal of waste products, are of critical importance for muscle fibre homeostasis and reconditioning. Capillary rarefaction has been shown to be associated with muscle fibre atrophy and may limit muscle fibre size recovery during exercise training/rehabilitation (45, 46). The present study shows that various indices of muscle fibre capillarisation were substantially higher (~ 22 to 281%) in muscle tissue of LDH patients (both on the injured as well as uninjured side) when compared with the control group. These results appear to be in line with the study by Strobel *et al.* who reported increased oxygen tension with a concomitant increase in muscle tension in patients with low back pain, suggesting a high capillary content to be related to increased muscle tension (47). Previous studies have reported an average of 276 ± 69 capillaries/mm^2 in patients with degenerative spinal pathologies, (18) which is in line with our findings in healthy controls (268 ± 30 capillaries/mm^2). We observed a significantly higher capillary density within our patient sample, suggesting a difference in capillary content between patients in this study and elderly patients with advanced degenerative spinal disorders (18). Although the increase in muscle capillary contents may be in line with the increased muscle tension hypothesis leading to hypoxia, stimulating angiogenesis, there may also be alternative explanation for this observation. (48, 49). A different mechanism that could cause the increase in capillary content is inflammatory dysregulation of the LMM, which has been suggested as in important mechanism of LMM degeneration (35). Macrophages play an essential role in the regulation of vascularisation in skeletal muscle tissue (50). A relative high density of especially anti-inflammatory macrophages (M2) has been associated with increased neovascularisation (51). Although we observed a greater number of anti-inflammatory macrophages within the LMM muscle tissue collected in our LDH patients, no correlations were observed between the number of anti-inflammatory macrophages and various indices of muscle fibre capillarisation. This may suggest that the number of macrophages is not likely the primary reason for the higher muscle fibre capillary density observed in LDH

patients. Hence, further investigations are needed to unravel underlying mechanisms.

Clinical significance and limitations

The present study is the first extensive evaluation of *in vivo* muscle fibre characteristics in a large group of LDH patients (injured and uninjured side) and age-matched healthy controls. Although these data provide novel insights in the potential underlying mechanisms on the morphological changes in muscle fibre characteristic as a result of LDH, the cross-sectional nature does not allow us to establish causality. Hence, we cannot exclude the possibility that alterations in muscle fibre characteristics were already present on beforehand, and may even have contributed to the development of disc injury itself (*e.g.*, increased spinal loading). Insight in skeletal muscle fibre morphological changes following LDH is of clinical importance as it provide insight to optimise existing and develop new exercise rehabilitation intervention strategies in these patients. Thus far, exercise rehabilitation programs have been mainly based on morphological animal studies by Hodges and colleagues (12-14). These studies show muscle size reduction (by muscle inhibition) and early fatty infiltration, based upon which they correctly recommend that acute low back pain should be treated using motor control training to overcome initial muscle inhibition. In addition, they recommend to progressively add resistance and endurance exercise training when evolving to a chronic phase of low back pain to prevent atrophy, fibrosis, fatty infiltration and promote anti-inflammatory effects (35). Furthermore, Dohnert *et al*. showed that exercise therapy in patients with disc protrusion reduced pain and improved function (52). Based on the present study results (*i.e.*, type I fibre atrophy in acute, and type II muscle fibre atrophy in both acute and chronic LDH patients), it could be suggested to incorporate both motor control as well as progressive resistance exercise training during the initial phase of rehabilitation. Including resistance exercise training may be of particular importance as it is likely vital to overcome, or maybe even prevent, type II fibre atrophy following LDH. Clearly, more long-term human follow-up studies are warranted to determine if the differences observed in this study may have an impact on the rehabilitation strategy of the LMM.

CONCLUSIONS

Clear differences in LMM fibre characteristics are apparent between LDH patients, irrespectively of the injured or uninjured side, and healthy control subjects. With the exception of acute muscle atrophy in the initial stage after LDH, bilateral involvement of various muscle fibre associated structures/cells are evident. Although this study provides further insight into the potential underlying mechanisms of changes in muscle fibre characteristics in the LMM, future studies should investigate whether exercise interventions can change LMM muscle fibre characteristics and improve clinical outcomes.

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DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

SS, FV, AA., LvL, TS, MP, AT: research conceptualization and design. SS, FV, AA, MP, SB, TA, MB: experiments performance. SS, FV, AA, TS, TA, MB, SB: data analysis. SS, FV, AA, LvL, TS, AT: results interpretation. SS, FV, AA: manuscript drafting. SS, AA, FV: figures preparation. SS, FV, AA, TS, MP, SB, TA, MB, AT, LvL: manuscript editing and revision. All authors approved the final version of the manuscript.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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SUPPLEMENTS

Appendix 1. Muscle fibre characteristics for acute and chronic lumbar disc herniation patients in both the injured *vs* uninjured side.

		Acute LDH (n = 14)		Chronic LDH (n = 15)	
		Injured	**Uninjured**	**Injured**	**Uninjured**
Muscle fibre size and type composition					
Fibre size (µm²)	I	5283 ± 430	5847 ± 441	6313 ± 450	5987 ± 467
	II	3543 ± 435*	3825 ± 466*	3127 ± 457*	3452 ± 477*
Fibre type composition	I	56.23 ± 4.25	61.60 ± 4.50	56.82 ± 3.79	62.77 ± 4.00
(%)	II	45.27 ± 4.25*	39.92 ± 4.64*	43.18 ± 3.79*	37.24 ± 4.00*
Muscle fibre myonuclear and satellite cell content					
Myonuclear content (number/fibre)	I	4.75 ± 0.24	5.02 ± 0.26	4.75 ± 0.30	4.77 ± 0.32
	II	340 ± 0.25*	3.39 ± 0.29*	2.96 ± 0.31*	3.35 ± 0.33*
Myonuclear domain size (µm²)	I	1108 ± 76	1172 ± 78†	1324 ± 56#	1254 ± 60
	II	1020 ± 77	1140 ± 82†	1020 ± 58*	1052 ± 62*
Central nuclei (%)	I	7.91 ± 2.03	8.49 ± 2.21	7.16 ± 1.62	8.17 ± 1.62
	II	5.27 ± 2.11	2.00 ± 2.80	4.06 ± 1.70*	3.37 ± 1.70*
Satellite cell content (number/100 fibres)	I	11.94 ± 1.03	10.40 ± 1.03	13.28 ± 1.28	10.55 ± 1.28
	II	4.93 ± 1.03*	4.43 ± 1.12*	2.61 ± 1.28*	4.06 ± 1.32*
Muscle fibre capillarization					
CC	I	3.63 ± 0.20	3.67 ± 0.22	3.82 ± 0.25	3.63 ± 0.26
	II	3.17 ± 0.21*	2.97 ± 0.22*	2.83 ± 0.25*	2.83 ± 0.26*
C/Fi	I	1.81 ± 0.11	1.76 ± 0.12	1.80 ± 0.12	1.71 ± 0.13
	II	1.05 ± 0.11*	1.01 ± 0.12*	0.98 ± 0.12*	0.91 ± 0.13*
CFPE (capillaries/1000 µm)	I	6.01 ± 0.25	5.74 ± 0.27	5.64 ± 0.29	5.63 ± 0.31
	II	4.41 ± 0.26*	4.19 ± 0.27*	4.13 ± 0.29*	4.27 ± 0.31*
CD (capillaries/mm²)	I	390 ± 29	359 ± 32	333 ± 28	351 ± 30
	II	374 ± 30	339 ± 32	366 ± 28	379 ± 30
Muscle fibre inflammatory cells					
M1 (number/mm²)	Mix	1.76 ± 0.43	2.71 ± 0.43	2.23 ± 0.38	2.80 ± 0.41
M2 (number/mm²)	Mix	13.46 ± 2.14	13.66 ± 2.14	16.53 ± 4.56	13.36 ± 1.69

Data represent mean ± SE. CC: capillary contacts; C/Fi: capillary-to-fibre ratio; CFPE: capillary-to-fibre perimeter exchange; CD: capillary density; LDH: lumbar disc herniation; Type I: type I muscle fibres; Type II: type II muscle fibres; Mix: mixed muscle fibre types; M1: cells positive for CD68 and DAPI; M2: cells positive for CD68, CD206 and DAPI. *Significant effect of type; †Significant effect of side. #Significant effect of duration (acute *vs* chronic).