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Faculteit Revalidatiewetenschappen

master in de revalidatiewetenschappen en de kinesitherapie

Masterthesis

The effects of unilateral lumbar disc herniation on the vascular properties of the multifidus muscle

Antje Grosemans
Mike Taffin

Scriptie ingediend tot het behalen van de graad van master in de revalidatiewetenschappen en de kinesitherapie, afstudeerrichting revalidatiewetenschappen en kinesitherapie bij neurologische aandoeningen

PROMOTOR :
Prof. dr. Frank VANDENABEELE

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The effects of unilateral lumbar disc herniation on the vascular properties of the multifidus muscle.

"What are the effects of unilateral lumbar disc herniation on the vascular properties of the multifidus muscle?"

Antje Grosemans (1644335)

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Promotor: Prof. dr. Vandenabeele Frank

Co-promotor: dr. Agten Anouk

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Hasselt University, Belgium

"Intervertebral disc herniation is a frequent problem of the lumbar spine which often results in low back pain."

"The paraspinal muscles play a crucial biomechanical role in limiting excessive intervertebral movement and spinal stability."

"An adequate function and density of the capillary bed is necessary for maintaining a healthy functioning muscle."

"First preliminary study to suggest an increased vascularization in the multifidus muscle of persons with a unilateral lumbar disc herniation."

ACKNOWLEDGEMENTS

We would like to thank our supervisors prof. dr. Vandenabeele Frank, dr. Agten Anouk, and drs. Stevens Sjoerd for their assistance at every stage of this research project. Additionally, we would like to give a special mention and thanks to Stevens S. for his invaluable feedback and for providing the necessary data that formed the basis for our thesis.

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Borgloon, 7th June 2021 A.G.
Houthalen-Helchteren, 7th June 2021 M.T.

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RESEARCH CONTEXT

This thesis is written as part of the Master of Rehabilitation sciences and physiotherapy program at University Hasselt, within the domain of musculoskeletal rehabilitation. It will focus on the vascular properties of the multifidus muscle in patients with unilateral lumbar disc herniation (UDH). UDH is a frequent problem of the lumbar spine which often results in compression of the spinal nerve, generating diverse clinical symptoms, such as sciatica and low back pain (LBP)[1]. According to the Global Burden of Disease study (2010), Low back pain was ranked highest out of 261 pathologies concerning the years lost to disability and overall burden[2,3]. Given the high prevalence and socio-economic burden, insight in the pathophysiology of UDH could be beneficial.

Due to the current COVID-19 pandemic, the recruitment of patients for scientific objectives was deemed infeasible and therefore ceased. The research question was established in conjunction with Prof. dr. Vandenabeele Frank, dr. Agten Anouk and drs. Stevens Sjoerd. The used study design and datasets were provided by our mentor drs. Stevens Sjoerd. This thesis, which is part of an ongoing project by drs. Stevens Sjoerd., was written by two Master students, Grosemans Antje and Taffin Mike. In accordance with the guidelines set by the faculty, this thesis is written in a central format. The data-processing and statistical analysis were performed independently. Disagreements were resolved by achieving a consensus through discussion.

[1] Andrade, P., Hoogland, G., Garcia, M. A., Steinbusch, H. W., Daemen, M. A., & Visser-Vandewalle, V. (2013). Elevated IL-1β and IL-6 levels in lumbar herniated discs in patients with sciatic pain. Eur Spine J, 22(4), 714-720. https://doi.org/10.1007/s00586-012-2502-x

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ABSTRACT

Background: Intervertebral disc (IVD) herniation is a frequent problem of the lumbar spine which often results in low back pain. Structural and functional alterations of paraspinal muscles are a ubiquitous finding in IVD herniation. An adequate function and density of the capillary bed is necessary for maintaining a healthy functioning muscle.

Objectives: This preliminary, cross-sectional study was conducted to investigate the vascularization of the multifidus in patients with a lumbar unilateral disc herniation (UDH).

Participants: Thirty patients, between 18 and 55 years old, scheduled for minimally invasive surgery (MIS) to resolve symptoms of UDH, were recruited from July 2018 through December 2019 at the Jessa Hospital in Hasselt, Belgium. Additionally, ten healthy controls, without acute or chronic low back pain (>3 months), between 25 and 60 years old, were included.

Measurements: Multifidus muscle biopsies were obtained during MIS discectomy. An average of 45±20 fibres, per fibre type, were used in the quantification of capillaries. Vascular properties of the multifidus were quantified by measuring capillary contact (CC), capillary-to-fibre ratio (C/Fi), Capillary-to-fibre Perimeter Exchange Index (CFPE index), and capillary density (CD).

Results: No significant differences were found in CC, CD, C/Fi, and CFPE of the lumbar multifidus between the affected and unaffected side of persons with UDH. Furthermore, only a significant difference ($p<0.001$) for C/Fi between persons with UDH and healthy controls was found. Post hoc tests showed a significantly higher C/Fi in the affected and unaffected side compared to controls in both type I ($p<.0001$; $p<.0001$) and type II fibres ($p<.0001$; $p<.0001$).

Conclusion: This is the first preliminary study that observes an increased vascularization in the multifidus muscle of persons with a unilateral lumbar disc herniation, regardless of the affected or non-affected side, compared to healthy controls.

Keywords: Intervertebral disc herniation; low back pain; multifidus muscle; lumbar spine; vascularization; capillarization; angiogenesis

INTRODUCTION

Intervertebral disc (IVD) herniation is a frequent problem of the lumbar spine which often results in compression of the spinal nerve, generating diverse clinical symptoms, such as sciatica and low back pain (LBP) (Andrade et al., 2013). LBP is defined as pain, muscle tension, or stiffness localized on the posterior aspect of the body below the 12th rib and above the lower gluteal folds, with or without leg pain (Kalichman et al., 2017). As reported by the Global Burden of Disease study (2010), Low back pain was ranked highest out of 261 pathologies concerning the years lost to disability and overall burden (Hoy et al., 2014; Vos et al., 2012). With a lifetime prevalence of 38.9% most people will suffer from back pain at one point during their life (Hoy et al., 2012). Only a small percentage of LBP is specific in nature resulting from an identifiable cause (Koes et al., 2006), such as a herniated disc.

According to the literature the paraspinal muscles, as for example the multifidus and erector spinae, play a crucial biomechanical role in limiting excessive intervertebral movement and spinal stability (Goel et al., 1993; Panjabi et al., 1989). Especially the multifidus, which is the most medial paraspinal muscle, is designed for support. The lumbosacral part consists of five fasciculi originating from the dorsal aspect of the sacrum, the posterior sacroiliac ligaments and the mammillary processes, passing in a craniomedial direction towards the insertions on the spinous processes of L1−L5 (Gray & Lewis, 1918, p. 400). Structural and functional alterations of the paraspinal muscles are a ubiquitous finding in IVD herniation (Danneels et al., 2000; Hodges et al., 2014). Nerve root compression leads to denervation and associated multifidus fatty infiltration, fibrosis and atrophy (Battié et al., 2012; Hodges et al., 2006; Park et al., 2018). Recent meta-analyses by Cooley et al. (2018) and Stevens et al. (2020) reported significant side-to-side differences. Both type I and II muscle fibre cross sectional area (CSA) decreased, and the number of type I fibres increased on the side of IVD herniation. This in contrast to descriptive analysis where total muscle CSA did not significantly differ between sides. These structural changes are often accompanied by muscle dysfunction and can even predict LBP (Hildebrandt et al., 2017; Ranger et al., 2017). However, this does not exclude muscle dysfunction as a possible mediator in the development of lumbar IVD herniation.

Almost 40% of the human body is made up of skeletal muscle (Janssen et al., 2000). These muscles contain a dense capillary network that provides the cells of oxygenated blood, carrying growth factors and nutrients and removes waste and excessive heat. An adequate function and density of the capillary bed is necessary for maintaining a healthy functioning muscle. (Hendrickse & Degens, 2019; Joanisse et al., 2017). Furthermore, these capillaries have the capacity to remodel dynamically in response to altered functional demands. For example, highly oxidative muscles are expected to have a denser capillary network than glycolytic muscles due to their higher oxygen requirement (Hendrickse & Degens, 2019). Differences in capillary density can even be found within a single muscle, which was observed in the plantaris muscle of rats by Degens et al. (1992). Only a few studies have investigated the vascularity of the multifidus in humans (Jørgensen et al., 1993; Shahidi et al., 2017). The study of Shahidi et al. (2017) showed a decrease of vascularity in the multifidus muscle in patients with degenerative lumbar spine diseases. In both rodent (Wüst et al., 2009) and human (Ahmed et al., 1997; Bosutti et al., 2015) muscles a positive correlation between the fibre size and the number of capillaries was found. This prompted Hendrickse and Degens (2019) to suggest that angiogenesis and fibre growth are coupled. Without angiogenesis, an increase in fibre CSA enlarges the diffusion distance which results in a theoretical diffusion limitation (Degens, 2012; Verdijk et al., 2016).

The often multifaceted aetiology of back pain creates difficulties in understanding the pathophysiology of multifidus degeneration. Currently, little is known about the mechanisms underlying the structural and functional changes. And in particular to what extent the vascularization is influenced by or plays a role in these changes.

Therefore, this preliminary, cross-sectional study was conducted with the aim to investigate the vascular properties of the multifidus in patients with a lumbar unilateral disc herniation.

METHOD

Participant recruitment

Thirty patients who were scheduled for minimally invasive surgery (MIS) to resolve symptoms of unilateral disc herniation (UDH), were recruited from July 2018 through December 2019 at the Jessa Hospital in Hasselt, Belgium. A neurosurgeon asked the patients to participate during their preoperative consultation if they met the following inclusion criteria: unilateral lumbar disc herniation diagnosed by medical imaging, age between 18 and 55 years old, and comprehend both written and spoken Dutch. The exclusion criteria consisted of surgery within the last 12 months, degenerative, spinal or other known pathologies that could influence muscle biology. Furthermore, ten healthy controls, without acute or chronic low back pain (>3 months), aged between 25 and 60 years old, and able to understand written and spoken Dutch, were included. Healthy subjects were excluded if they underwent rehabilitation or exercise therapy for an acute condition within the last 3 months. All subjects were informed about the purpose of the study and were presented with an informed consent. This trial was registered on ClinicalTrials.gov under the identification number NCT03753711. Ethical approval was provided by the Jessa Hospital and Hasselt University, Belgium, Medical Ethics committee.

Histology and immunofluorescence multifidus muscle biopsy

Multifidus muscle biopsies were obtained during MIS discectomy: after the incision was made, the skin was moved slightly lateral exposing the contralateral paraspinal musculature. A fine needle biopsy was taken from the exposed multifidus muscle, using the 12G semi-automated Bard® Mission® Core Biopsy Instrument. The ipsilateral biopsy, at the herniated side, was taken directly while surgically preparing the access to the posterior lamina. According to the protocol of Agten et al. (2018) biopsies in the healthy controls were taken from the multifidus on the right side at the level of the L4 spinous process. The collected samples were placed on a piece of cork, then covered with optimum cutting compound and immediately frozen using isopentane cooled in liquid nitrogen. Waiting further analysis, frozen samples were stored at -80°C in the clinical biobank.

Serial transverse sections (5µm) were cut with a microtome (Leica CM1900 Cryostat; Leica) and placed on uncoated pre-cleaned glass slides. To identify myosin heavy chain isoforms, immunofluorescent staining was performed according to Groen et al. (2014). First, muscle slides were air dried for 30 minutes at room temperature (RT), then fixated with acetone for 5min followed by another air-drying step of 15 minutes and marked with a DAKO pen (DAKO S2002; Agilent). Second, slides were incubated for 45 minutes using primary antibodies against CD 31 (1:50, endothelial cell mouse IGg1 Dako) diluted in 0.05% Tween-phosphate-buffered saline (Tw/PBS), then washed 3x for 5 minutes in phosphate-buffered saline (PBS). Thereafter, they were incubated for 45 minutes with anti-mouse IgG (dilution 1:200; Vector Laboratories) in 0.05% Tw/PBS, followed by a washing step in PBS. Subsequently, incubated again for 45 minutes, using Avidine Texas Red (1:400; Vector Laboratories, CA, USA), Myosine Slow Fibers (1:25 Mouse IgM A4840; Development Studies Hybridoma Bank, IA, USA) and Anti-laminin (1:50 Rabbit IgG; Sigma-Aldrich, MO, USA) in 0.05% Tw/PBS and again washed in in PBS. Then, incubated a last time for 30 minutes with the appropriate conjugated secondary antibodies (300 nM DAPI; Invitrogen, CA, USA, 1:200 Alexa Fluor 488, 1:400 Alexa Fluor 647; Life Technologies, CA, USA) and finally washed again in PBS. In the last step they were mounted using Mowiol (Calbiochem, Amsterdam, the Netherlands). Stained samples were viewed with a fluorescent microscope (Leica AL6000; Leica)

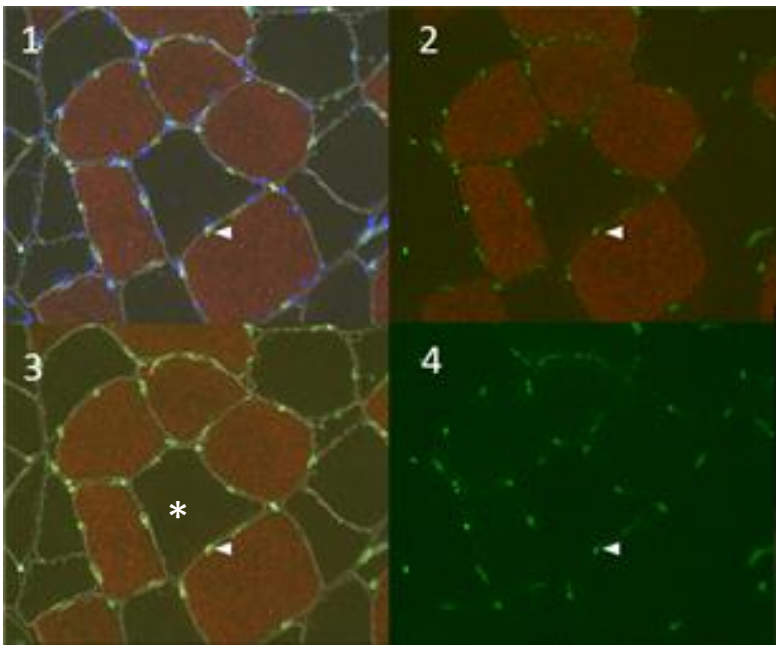


Figure 1 *Immunofluorescence staining*

This figure shows the final result of immunofluorescence staining. Type I fibres are coloured red, and type II are coloured black. The white arrow is indicating a capillary vessel (CD31+).

Quantification and outcome measures

Patient characteristics of interest are length, weight, body mass index (BMI), intensity and duration of back pain, disability and fear of movement. Pain intensity was measured using a visual analogue scale (VAS), disability was measured using the Oswestry Disability Index (ODI) and fear of movement using the Tampa scale for Kinesiophobia (TSK). Fibre characteristics were quantified using ImageJ, analysis software (ImageJ 1.52a; ImageJ, 2018). In accordance with the work of Hepple in 1997 an average of 45±20 (range 18-119) fibres, per fibre type, were used in the quantification of capillaries. Vascular properties of the multifidus were quantified by measuring capillary contact (CC), capillary-to-fibre ratio (C/Fi), Capillary-to-fibre Perimeter Exchange Index (CFPE index), and capillary density (CD). CC is defined as the number of capillary contacts for a single fibre. Next, the sharing factor (SF) for each of the capillaries around the fibre is determined. By taking the sum of the fractional contribution of each CC, the C/Fi for that individual fibre is obtained (Hepple, 1997). For example, the C/Fi for the fibre indicated with an asterisk in Figure 1 is calculated. Ten capillaries are surrounding the fibre (CC=10), six are in contact with three fibres (i.e., the sharing factor for each of these capillaries is 3), and four are in contact with two fibres (i.e., the sharing factor for each of these capillaries is 2). By taking the sum of these two proportions, the C/Fi for that fibre can be derived: C/Fi = (6 x ⅓) + (4 x ½) = 4. CFPE index is the quotient of the individual Capillary-to-fibre ratio (C/Fi) and the fibre perimeter, CD is the ratio of the C/Fi to the fibre area.

Statistical analysis

Anthropometric and baseline data were analysed using “excel Microsoft 365”. Results for age, length, weight, BMI, VAS, duration of symptoms, ODI, and TSK are displayed as mean and standard deviation (mean ± SD). Main outcome variables were processed using JMP Pro 15.2.0 software (SAS Institute Inc, Cary, NC, USA, 1989 −2020). Mixed models was used to analyse vascular differences within patients and between patients and healthy controls. Fibre type and group (affected, unaffected, controls) were set as fixed effects and subject ID as a random effect. When analysis showed a significant effect, pairwise comparisons-Tukey HSD was used. Results for CC, CD, C/Fi, and CFPE are displayed as mean ± standard error (SE), and p-value. Statistical significance was set at 5% ($p<.05$) with a confidence interval of 95%. Power analysis was not implemented since there was no preliminary data available.

RESULTS

Anthropometric characteristics

A total of 29 patients diagnosed with UDH and ten healthy controls were included in this study. The distribution of gender, age and other anthropometric characteristics are described in Table 1. The mean age of persons with UDH from which multifidus muscle was collected was 40 ± 9 years and 42 ± 8 years in the healthy controls. The UDH group consisted of 17 males and 12 females. The control group was made up of 5 males and 5 females. No significant differences were found between both groups regarding anthropometric data.

Table 1

Anthropometric characteristics

	UDH	Controls	p-value
Gender (male:female)	17:12	5:5	0.6355
Age (years)	40 ± 9	42 ± 8	0.4555
Length (m)	1.76 ± 0.10	1.79 ± 0.07	0.2955
Weight (kg)	81.8 ± 19.2	83.9 ± 17.4	0.7546
BMI (kg/m²)	26.5 ± 4.7	26.0 ± 4.0	0.7583

Values are depicted as ratio or mean ± standard deviation

Baseline data

In the UDH group, 25 persons (86%) reported low back pain, with a mean VAS back score of 4.78 ± 2.94 and a VAS leg score of 5.88 ± 2.15 (Table 2). The mean duration of back pain was 19.12 ± 27.45 weeks. All participants experienced a sensory deficit, which lasted on average for 5.96 ± 8.98 weeks. Whereas 18 out of 29 (62%) experienced motor deficits with a mean duration of 6.81 ± 11.12 weeks. Psychometric results for disability (ODI) and kinesiophobia (TSK) were 40.35 ± 17.37 and 42.75 ± 8.39 respectively. At the time of the study, 6 out of 29 (21%) participants were not working. The nature of unemployment was not questioned.

Table 2

Baseline data- participants

	UDH
Working n(%)	23 (79)
Back pain n(%)	25 (86)
Duration (weeks)	19.12 ± 27.45
Sensory deficit n(%)	29 (100)
Duration (weeks)	5.96 ± 8.98
Motor deficit n(%)	18 (62)
Duration (weeks)	6.81 ± 11.12
Pain intesity	
VAS (Back)	4.78 ± 2.94
VAS (Leg)	5.88 ± 2.15
ODI	40.35 ± 17.37
TSK	42.75 ± 8.39

Values are depicted as mean ± standard deviation or number(%); *VAS,* Visual Analoge Scale; *ODI,* Oswestry Disability Index; *TSK,* Tampa Scale of Kinesiphobia

Outcome measures

The primary outcome measures (Table 3) included CC, CD, C/Fi and CFPE. No significant differences were found in CC, CD, C/Fi, and CFPE of the lumbar multifidus between the affected and unaffected side of persons with UDH. Furthermore, no significant differences were found between persons with UDH and healthy controls for CC, CD, and CFPE in the multifidus muscle except for C/Fi, which showed a significant ($p<.0001$) effect of group (Figure 2c). Post hoc tests showed a significantly higher C/Fi in the affected and unaffected side compared to the controls in both type I ($p<.0001$; $p<.0001$) and type II fibres ($p<.0001$; $p<.0001$). The estimated means were 0.96 ± 0.11 (95% CI, 0.73-1.19) for type I C/Fi and 0.40 ± 0.11 (95% CI, 0.18-0.63) for type II C/Fi in the control group. In the UDH group the C/Fi estimated means for type I and II were 1.86 ± 0.07 (95% CI, 1.72-2.00) and 1.07 ± 0.07 (95% CI, 0.93-1.21) in the affected side and 1.83 ± 0.07 (95% CI, 1.68-1.97) and 1.09 ± 0.07 (95% CI, 0.94-1.23) in the unaffected side, respectively.

Table 3

Outcome measures

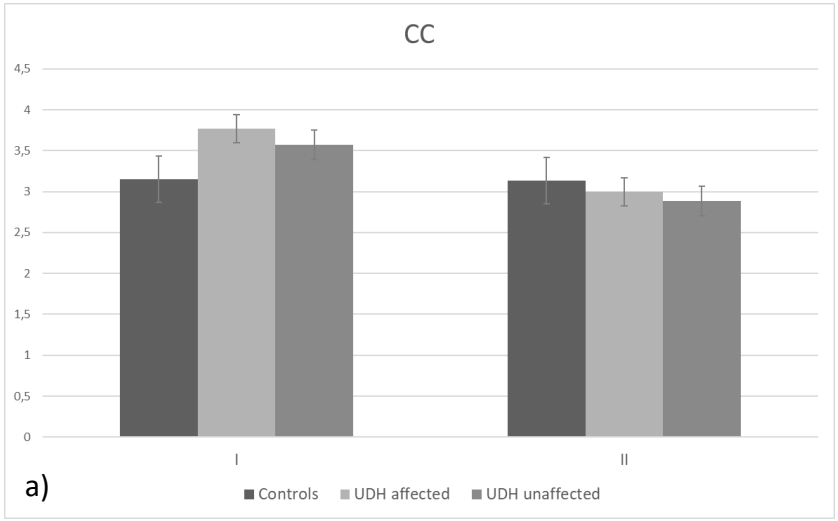
		UDH affected	UDH unaffected	Controls
CC	I	3.77 ± 0.17	3.57 ± 0.18	3.15 ± 0.28
	II	3.00 ± 0.17†	2.89 ± 0.18†	3.14 ± 0.28
CD	I	351.99 ± 19.21	361.47 ± 20.76	286.39 ± 31.47
	II	372.75 ± 19.21	366.06 ± 20.76	307.17 ± 31.47
C/Fi	I	1.86 ± 0.07*	1.83 ± 0.07*	0.96 ± 0.11
	II	1.07 ± 0.07*†	1.09 ± 0.07*†	0.40 ± 0.11†
CFPE index	I	5.75 ± 0.19	5.73 ± 0.20	4.87 ± 0.31
	II	4.26 ± 0.19†	4.29 ± 0.20†	3.93 ± 0.31†

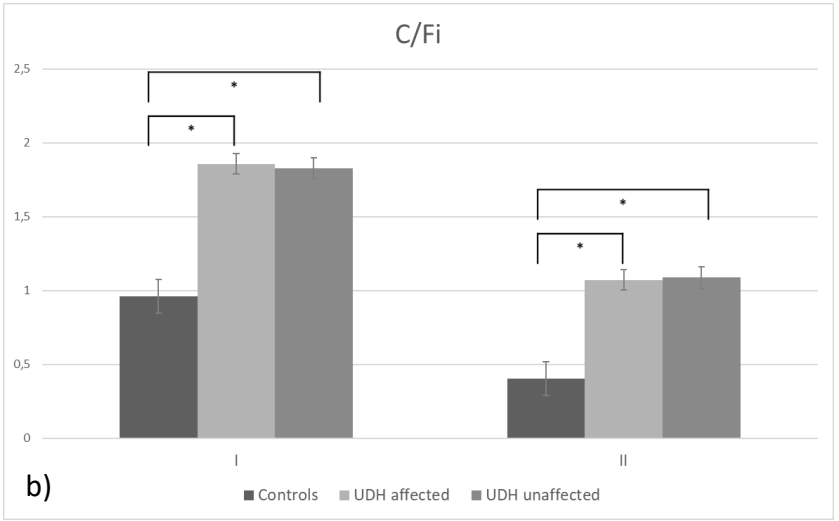
Values are depicted as estiimated mean ± standard error ; *UDH,* unilateral disc herniation; *CC,* capillary contact; C/Fi, capillary to fibre ratio; *CFPE index,* capillary to fibre perimeter exchange index; *CD,* capillary density

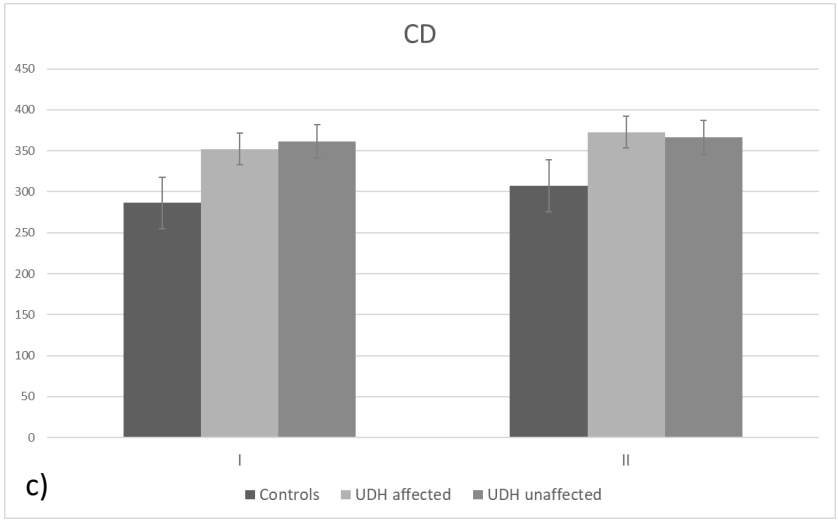
* significant difference with controls (p<0.05)

† significant difference between fibre type I or II within goup (p<0.05)

Regarding the secondary outcomes, there was an overall significant effect of fibre type in CC (p=0.0002), C/Fi (p<.0001) and CFPE (p<.0001) but not in CD (p=0.3430). Post hoc tests showed for CC a significant difference in fibre type in the affected (p=0.0002) and unaffected (p=0.0042) side but not in the controls (p=1.0000). Estimated means for CC type I and type II were, respectively, 3.77 ± 0.17 (95% CI, 3.42-4.11) and 3.00 ± 0.17 (95% CI, 2.66-3.34) in the affected side and 3.57 ± 0.18 (95% CI, 3.20-3.94) and 2.89 ± 0.18 (95% CI, 2.55-2.25) in the unaffected side. For C/Fi post hoc tests revealed a significantly higher ratio in type I fibres compared to type II in the affected side (p<.0001), unaffected side (p<.0001) and controls (p<.0001). The CFPE-index was significantly higher in type I compared to type II for all groups: affected side (p<.0001), unaffected side (p<.0001) and controls (p=0.0122). Estimated means for the affected side were 5.75 ± 0.1 (95% CI, 5.38-6.13) and 4.26 ± 0.19 (95% CI, 3.89-4.63), in the unaffected side 5.73 ± 0.20 (95% CI, 5.34-6.12) and 4.29 ± 0.20 (95% CI, 3.89-4.68) and in control group the 4.87 ± 0.31 (95% CI, 4.25-5.48) and 3.93 ± 0.31 (95% CI, 3.31-4.55) for type I and type II respectively. The detailed estimated means can be found in Table 3.







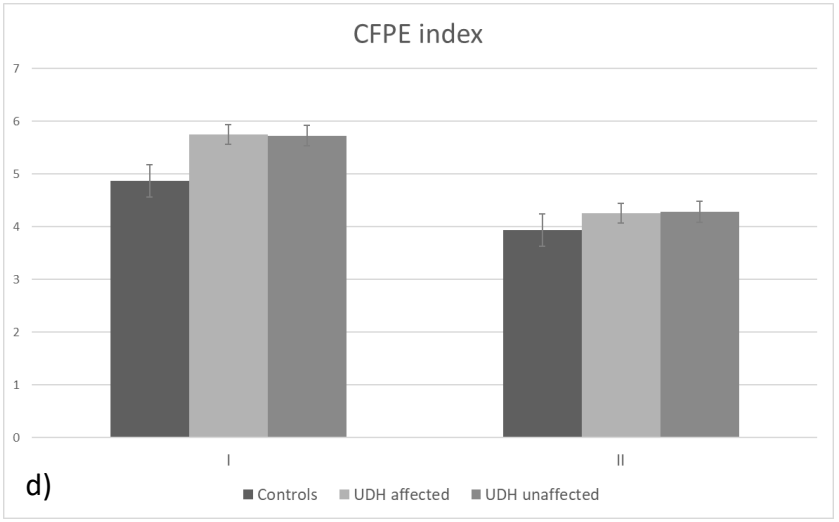


Figure 2 *Primary outcome measures*

DISCUSSION

To our knowledge this preliminary study is the first to investigate the vascular properties of the multifidus muscle in humans with UDH that included healthy controls. In contrast to the recent findings in the systematic review and meta-analysis of Stevens et al. (2020), which reported side-to-side differences regarding degeneration of the multifidus e.g., fibre type CSA atrophy, we could not find significant side-to-side differences in capillarization. However, patients with UDH showed a higher level of capillarization in comparison to healthy controls. This in contrast to the decline in vascularization in patients with degenerative lumbar spine pathologies that Shahidi et al. observed in 2017. It's important to point out that they did not use a control group. Concurrently, colleague students working with the same dataset but focusing on different aspects within the same research project observed a higher amount of myonuclei, a smaller myonuclear domain, higher amounts of pro-inflammatory M1 cells and anti-inflammatory M2 cells and an acute but no chronic muscle atrophy in patients with UDH compared to healthy controls. No significant differences were found for satellite cell content and central nuclei between UDH and controls. There were no side-to-side differences found within UDH group for all forementioned variables.

These findings give rise to two possible mechanisms to explain the increase in capillarization in UDH patients. The first mechanism assumes that the increase in the number of, anti-inflammatory, M2 macrophages induces angiogenesis through the release of growth factors (Latroche et al., 2017; Varin & Gordon, 2009). Alternatively, the second mechanism suggests that an increased intramuscular pressure (IMP) leads to hypoxia and stimulates angiogenesis (Konno et al., 1994; Nagahisa & Miyata, 2018). According to the physiological model of the chronic functional compartment syndrome, a muscle contraction may lead to an increase in IMP (Kramer et al., 2005). When the IMP rises above the 30-40 mmHg threshold, perfusion and oxygenation in the paravertebral muscles is significantly reduced. The effect of a continued hypoxia may be compensated by extracellular matrix remodelling and angiogenesis (Jensen et al., 1999), regulated by gene expression biomarkers such as vascular endothelial growth factor and neuronal Nitric-Oxide synthase (Nagahisa & Miyata, 2018; Nielsen et al., 2020). In various, but not all, positions an increased IMP has been observed in patients with LBP, this suggests that a change of spinal alignment may play a role (Konno & Kikuchi, 1995; Konno et al., 1994). However, the study of Kramer et al. (2005), with healthy

subjects, reported that this increase did not occur in all subjects. The same was found in animal models where induced muscle damage in mice lead to an increased fibre capillarization, that was maintained up to six months post-injury (Hardy et al., 2016).

In muscle regeneration and degeneration studies, remodelling of the vascular network is often neglected. Considering the increased vascularization, myonuclei, macrophages, and the resolved atrophy found in this research project, the multifidus of UDH patients seems to undergo a regenerative rather than a degenerative process. This shows the importance of the capillary network in skeletal muscle regeneration as it influences the distribution of inflammatory cells, cytokines, chemokines, and growth factors (Hardy et al., 2016). A sustained cycle of inflammation and vascularization however, may give rise to fatty infiltration, fibrosis and atrophy (DiPietro, 2016). These degenerative features are often found in UDH patients (Stevens et al., 2020) and can cause pain and disability (Stanuszek et al., 2021). Even though some animal models suggest inflammatory dysregulation as a mediating factor in multifidus muscle degeneration (Hodges et al., 2015; James et al., 2019). It seems that the regenerative capacity, rather than inflammation, can predict postoperative disability and pain (Chen et al., 2021). Therefore, when putting together a rehabilitation programme, to minimise long term pain and disability, the generative capacity of the multifidus should be kept in mind.

There were several limitations to this study that need to be recognized. Firstly, the recruitment was based on convenience sampling from a surgical population in one hospital. Secondly, a small sample size was used, and the control group was considerably smaller than the UDH group. This may affect power to detect differences in markers of vascularization. Thirdly, despite the fact that muscle biopsy locations were standardized, the histologic samples represent only a small part of the multifidus muscle and may not reflect whole muscle biology. Despite these limitations, the quantification and comparison with healthy controls of vascular properties provides novel information on the pathological multifidus muscles. This information may provide insight and direct future research on possible degeneration and regeneration of the paraspinal musculature, and essentially provide information on how to minimize this degenerative process and how to stimulate regeneration. Future studies about vascular properties of the multifidus muscle with a larger sample size and control group and recruited in multiple hospital or outpatient settings are required.

CONCLUSION

This is the first preliminary study that observes an increased vascularization in the multifidus muscle of persons with a unilateral lumbar disc herniation, regardless of the affected or non-affected side, compared to healthy controls.

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APPENDIX

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KNOWLEDGE IN ACTION

INVENTARISATIEFORMULIER WETENSCHAPPELIJKE STAGE DEEL 2

DATUM	INHOUD OVERLEG	HANDTEKENINGEN
1/10/2020 8/10/2020	Meeting via Google meet. Uitleg onderwerpen masterproef Meeting in persoon met sjoerd. Uitleg onderwerp masterproef	Promotor: Copromotor/Begeleider: Student(e): Student(e):
11/11/2020 30/11/2020	Mail: Vragen MP2 missing data & protocol Meeting via Google meet. Missing data - statistiek uitleg ivm mixed models in JMP	Promotor: Copromotor/Begeleider: Student(e): Student(e):
8/02/2021	Meeting via Google meet. voortgang	Promotor: Copromotor/Begeleider: Student(e): Student(e):
7/03/2021	Update Introductie en methode +17/03 mail: stainingprotocol ontvangen +27/03 Statistiek gepost op drive	Promotor: Copromotor/Begeleider: Student(e): Student(e):
20/04/2021	Meeting via Google meet ivm healthy controls + 25/04 drive: update methode incl.staining + 10/05 data healthy controls ontvangen	Promotor: Copromotor/Begeleider: Student(e): Student(e):
16/05/2021	Eerste versie MP2 doorgestuurd	Promotor: Copromotor/Begeleider: Student(e): Student(e):
		Promotor: Copromotor/Begeleider: Student(e): Student(e):
		Promotor: Copromotor/Begeleider: Student(e): Student(e):
		Promotor: Copromotor/Begeleider: Student(e): Student(e):
		Promotor: Copromotor/Begeleider: Student(e): Student(e):

In te vullen door de promotor(en) en eventuele copromotor aan het einde van MP2:

Naam Student(e): ..... **Datum:**.....

Titel Masterproef: .....

1) Geef aan in hoeverre de student(e) onderstaande competenties zelfstandig uitvoerde:
   - NVT: De student(e) leverde hierin geen bijdrage, aangezien hij/zij in een reeds lopende studie meewerkte.
   - 1: De student(e) was niet zelfstandig en sterk afhankelijk van medestudent(e) of promotor en teamleden bij de uitwerking en uitvoering.
   - 2: De student(e) had veel hulp en ondersteuning nodig bij de uitwerking en uitvoering.
   - 3: De student(e) was redelijk zelfstandig bij de uitwerking en uitvoering
   - 4: De student(e) had weinig tot geringe hulp nodig bij de uitwerking en uitvoering.
   - 5: De student(e) werkte zeer zelfstandig en had slechts zeer sporadisch hulp en bijsturing nodig van de promotor of zijn team bij de uitwerking en uitvoering.

Competenties	NVT	1	2	3	4	5
Opstelling onderzoeksvraag	O	O	O	O	O	O
Methodologische uitwerking	O	O	O	O	O	O
Data acquisitie	O	O	O	O	O	O
Data management	O	O	O	O	O	O
Dataverwerking/Statistiek	O	O	O	O	O	O
Rapportage	O	O	O	O	O	O

2) Niet-bindend advies: Student(e) krijgt toelating/geen toelating (schrappen wat niet past) om bovenvermelde Wetenschappelijke stage/masterproef deel 2 te verdedigen in bovenvermelde periode. Deze eventuele toelating houdt geen garantie in dat de student geslaagd is voor dit opleidingsonderdeel.

3) Deze wetenschappelijke stage/masterproef deel 2 mag wel/niet (schrappen wat niet past) openbaar verdedigd worden.

4) Deze wetenschappelijke stage/masterproef deel 2 mag wel/niet (schrappen wat niet past) opgenomen worden in de bibliotheek en docserver van de UHasselt.

Datum en handtekening Student(e)	Datum en handtekening promotor(en)	Datum en handtekening Co-promotor(en)