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FACULTEIT GENEESKUNDE EN LEVENSWETENSCHAPPEN

master in de revalidatiewetenschappen en de kinesitherapie

Masterproef

Morphological characteristics of the lumbar paraspinal muscles in individuals with non-specific chronic low back pain and healthy controls: a fine needle biopsy study

Promotor :
Prof. dr. Frank VANDENABEELE

Copromotor :
dr. Anouk AGTEN

Sjoerd Stevens

Scriptie ingediend tot het behalen van de graad van master in de revalidatiewetenschappen en de kinesitherapie

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Master thesis part II:

Morphological characteristics of the lumbar paraspinal muscles in individuals with non-specific chronic low back pain and healthy controls: a fine needle biopsy study

Student: Sjoerd Stevens, BSc

Promotor: Prof. dr. Frank Vandenabeele

Copromotor: dr. Anouk Agten

Preface

This Master of Science thesis was done at Hasselt University together with the Rehabilitation Research Center (REVAL) and Biomedical Research Institute (BIOMED).

I would like to express my gratitude to my supervisors. My promotor Prof. dr. Frank Vandenabeele and co-promotor dr. Anouk Agten. Second I would like to thank Dennis Mathijsen MSc, for his professional accompaniment during the dissection sessions.

My family and friends deserve my thanks for their great support. Finally, I would thank everyone who has contributed to the process of writhing this thesis.

Lozenweg 65 Hamont-Achel, 12.04.2017 S.Stevens, BSc

Background

This research was done within the domain of musculoskeletal rehabilitation sciences. This research was part of a larger study that investigates the effect of different training modalities on back muscle morphology in a population with non-specific chronic low back pain (NSCLBP). This research is a preliminary study to explore the data that are already available in this phase of the study. Both data from subjects with NSCLBP (n= 9) and healthy controls (n= 10) were used. These data came from biopsy samples that were collected from the multifidus (MF) and erector spinae (ES) muscle. Data that were extracted from these samples were fiber cross-sectional area (CSA), relative area, and percentage from a given fiber type. Both differences between muscles were compared within one group, as were differences between groups within one muscle.

Low back pain (LBP) is one of the most prevalent musculoskeletal disorders these days, and frequently leads to disability. Most cases of low back pain are considered as NSCLBP, because there is no apparent cause of pain. Pain and disability should persist for more than twelve weeks to make the diagnosis. People with NSCLBP often present with different comorbidities, which increases healthcare and socioeconomic costs. These are just a few reasons of why it is important to address NSCLBP. But before tackling the problem of NSCLBP, we need to know if there are specific alteration on a morphological level. If there are morphological changes, they could might be reversed with exercise training. If these changes can be reversed with training, could this make the pain of NSCLBP decrease or disappear.

As mentioned before, this study was done within a larger research project that investigates the effect of different high intensity training programs in persons with NSCLBP. This research is done by dr. Anouk Agten, Jonas Verbrugghe, Prof. dr. Frank Vandenabeele, Prof. dr. Annick Timmermans and Prof. dr. Bert Op 't Eijnde. This master thesis was a mono-thesis. The design of the research was already available at the beginning of this master thesis. The research question was already available, but was further specified by the student. The data acquisition was already on going when the student started participating in this study. However, after biopsies were taken, these samples were further analyzed by the student. Data regarding muscle fibers were all gathered by the student. Next together with the co-promotor we tried to statistically analyze the data, but because of the complexity this had to be done in conjunction with an external agency (CENSTAT). With regards to the biopsy technique the

student participated in the further development of this technique. Therefore, a dissection of human cadavers was done by the student. Writhing of the thesis was done exclusively by the student, and afterwards was checked with the co-promotor. When necessary changes were implemented.

Morphological characteristics of the lumbar paraspinal muscles in individuals with non-specific chronic low back pain and healthy controls: a fine needle biopsy study

Stevens. S BSc, Prof. dr. Vandenabeele. F, dr. Agten. A

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1. Abstract

Background: Low back pain (LBP) is the second leading cause of disability worldwide. A possible explanation for this pain could be an alteration in skeletal muscle fiber morphology. Human skeletal muscles consist of different fiber types with different morphological and functional characteristics. Anatomical and functional differences between the multifidus (MF) and erector spinae (ES) have been described in literature. Are these differences also present for muscle morphology? Individuals with LBP often show abnormalities in muscle/ fiber cross-sectional area (CSA) and fiber type composition. However, there is no consensus on these abnormalities in the literature.

Objectives: The first objective of this research was to investigate the differences in fiber CSA, relative fiber area and fiber type distribution between the MF and ES within both the non-specific chronic low back pain (NSCLBP) and healthy control group. The second objective was to compere these differences between the healthy controls and people with NSCLBP for both the MF and ES.

Participants: Patients were recruited to participate in a large randomized controlled trail (RCT) comparing different training modalities on morphological muscle characteristics. From this large RCT the first available data were used to conduct this research. In total nine NSCLBP patients and ten healthy control subjects were used in this article.

Measurements: A fine needle biopsy technique was used to take samples from the MF and the ES. These muscle samples were than used to measure fiber type percentage and fiber CSA. From these data, the relative CSA's were calculated per fiber type. These data were statistically analyzed using a repeated measurements ANOVA.

Results: The type I CSA was significant (p= 0.0012) larger in de MF compared to the ES in the healthy control group. Type IIAX CSA in the ES was significant (p= 0.0420) larger compared to the MF. A significant (p= 0.0136) difference for type IIX CSA was found comparing the ES and MF in the NSCLBP group. When comparing the NSCLBP group with the control group, no significant differences were found regarding CSA. The ES of the NSCLBP group contained a significant higher % type I fibers (p= 0.0114) and type I relative area (p= 0.0172). The ES of the control group contained a significant higher % type IIX (p= 0.0109) and type IIX relative area (p= 0.0339). The MF contained a significant (p= 0.0156) higher % type I fibers compared with the ES in the NSCLBP group.

Conclusion: Results could indicate that the MF has a stabilizing role in the healthy lumbar spinal column. It looks like individuals with NSCLBP are trying to “over” stabilize their spinal column with the thought that “pain induces damage” thereby increasing the neural signal that induces a change in fiber type composition.

2. Introduction

Low back pain (LBP) is one of the most prevalent musculoskeletal disorders and the second leading cause of disability worldwide (Woolf and Pfleger. 2003, Allegri et al. 2016). The prevalence of chronic low back pain (CLBP) between the age of 20 and 65 years old is 13.1%. CLBP is characterized by pain below the costal margin and above the inferior gluteal fold persisting more than twelve weeks. In less than 15% of this population there is a specific cause of pain, therefore in most of the cases the diagnosis of non-specific chronic low back pain (NSCLBP) is indicated. Low education, low income, depression, sleep disturbances and multiple comorbidities are strongly associated with CLBP. A high economic burden is also associated with CLBP (Shmagel, Foley, Ibrahim. 2016, Airaksinen et al. 2006, Gore, Sadosky, Stacey, Tai, Leslie. 2012). This explains why it is important to address NSCLBP. We were interested in seeing morphological muscle differences between people with NSCLBP and healthy controls. When there are differences present, could they contribute to pain experienced by NSCLBP?

Human skeletal muscles consist of different fiber types with different morphological and functional characteristics. Using analyses of antibodies against myosin heavy chains (MyHC) three singular and two hybrid fiber types can be classified. A difference can be made between type I slow twitch oxidative (MyHC1), type IIA fast twitch oxidative/ glycolytic (MyHC2A), and type IIX fast twitch glycolytic fibers (MyHC2X). Hybrid fibers either consist out of type I and IIA or type IIA and IIX MyHC composition (Bloemberg and Quadrilatero. 2012, Schiaffino. 2010). Type I muscle fibers contain high concentrations of hemoglobin owing to a strong vascularization. This contributes to the red color of the fibers. Energy supply is mainly provided through aerobic processes. Therefore, type I fibers contain high numbers of mitochondria and aerobic enzymes. These adaptations make it possible to sustain low contraction forces for a long period (tonic activity). Type II fibers contain less blood supply mainly because high contraction forces restrict blood flow to the muscle. Energy supply is derived from anaerobic metabolism using glucose as a substrate. Type II muscle fibers contain high densities of myofibrils and show strong ATPase activity, this indicates fast twitch contractions. These characteristics make it possible to generate fast contractions for a short period (phasic activity) (Burgerhout, Mook, Morree, and Zijlstra. 2006).

Knowing these histological and metabolic differences in fiber type exist, it would be interesting to see a connection between fiber composition and functional differences between muscles. For instance, the multifidus (MF) muscle is often mentioned a stabilizing muscle in its relation to the vertebral column (Dickx et al. 2010, Ward et al. 2009, Moseley, Hodges, Gandevia. 2002). In contradiction, the erector spinae (ES) is mentioned a mobilizing muscle (Stark, Fröber, Schilling. 2013). It would be interesting to see these functional differences reflect in fiber type composition between the ES and MF. Until now, there is no consensus on fiber type composition of both muscles in the literature (Cagnie et al. 2015, Hesse, Fröber, Fischer, Schilling. 2013, Macdonald, Moseley, Hodges. 2006). What has been known, is that morphological alterations occur in individuals with LBP compared to healthy individuals.

A common problem seen in individuals with LBP is a decrease in total muscle cross-sectional area (CSA) of the ES, MF or both (Wan, Lin, Li, Zeng, Ma. 2015, Kamaz, Kıreşi, Oğuz, Emlik, Levendoğlu. 2007, Danneels, Vanderstraeten, Cambier, Witvrouw, Cuypers. 2000, Wallwork, Stanton, Frenke, Hides. 2009, Hides, Gilmore, Stanton, Bohlscheid. 2008, Lee et al. 2006, Goubert, Oosterwijck, Meeus, Danneels. 2016). Other studies indicate that there is no decrease in total CSA of the paraspinal muscles (Fortin, Videman, Gibbons, Battié. 2014, D'Hooge et al. 2012, Paalanne et al. 2011, Hultman, Nordin, Saraste, Ohlsèn. 1993). Three other studies that looked at individual fibers failed to show a decrease in fiber CSA (Mannion. 1999, Mannion, Weber, Dvorak, Grob, Müntener. 1997, Crossmann, Mahon, Watson, Oldham, Cooper. 2004).

Differences in fiber type composition have been reported comparing individuals experiencing LBP with healthy individuals (Mannion. 1999, Mannion et al. 1997, Mazis et al. 2009. Demoulin, Crielaard, Vanderthommen. 2007). In contradiction, the study of Crossmann et al. 2004 indicates there were no abnormalities in fiber type composition.

In conclusion, there is no consensus regarding skeletal muscle/ fiber atrophy and fiber composition in individuals with LBP. The first objective of this research was to investigate the differences in fiber CSA, relative fiber area and fiber type distribution between the ES and MF within the NSCLBP group and healthy control group. The second objective was to compare these differences between both groups for the ES and MF. Muscle samples were taken from both MF and ES muscle using ultrasound guided fine needle biopsies.

3. Method

3.1 Participants

Participants were recruited by means of free recruitment. Participant who were interested and met the in- and exclusion criteria were informed and investigated by a general practitioner at REVAL Hasselt. During this intake session, an information brochure was explained to the participant. Participant who were interested received an informed consent. When they returned the signed informed consent within one week, they were included in the study. Nine of these participants were used in this article. Ten healthy controls were recruited through convenience sampling. In total 19 biopsy samples were taken from both populations together. Anthropometric data for both groups are displayed in table 1. There were no significant differences between the two groups for weight (p = 0.979), length (p = 0.958), BMI (p = 0.839), age (p = 0.095), and gender (p = 0.053).

Table 1. Anthropometric data included subjects

NSCLBP (N = 9)					**Healthy (N = 10)**				
Weight (kg)	**Length (cm)**	**BMI (kg/m²)**	**Gender**	**Age (years)**	**Weight (kg)**	**Length (cm)**	**BMI (kg/m²)**	**Gender**	**Age (years)**
97	174.2	32.0	M	46	71.9	179.6	22.3	F	38
64.6	168.7	22.7	F	48	73.7	171.6	25.0	F	32
80.4	182.3	24.2	M	53	70.4	166.6	25.4	F	46
69.4	169.4	24.2	F	49	74.1	173.3	24.7	M	25
93.9	165.5	34.3	F	38	79.5	181.2	24.2	M	25
68.5	172.7	23.0	F	48	67	166.9	24.1	F	41
70.2	174.6	23.0	F	54	79.8	185	23.3	M	47
72.8	186.8	20.9	M	46	107.5	191.7	29.3	M	37
66.3	171	22.7	M	29	72.9	164.7	26.9	M	51
					60.7	160.6	23.5	F	46

Weight, Length, BMI, Age significant at 0.05 (T-test). Weight p= 0.979, length p= 0.958, BMI p= 0.839, age p= 0.095. Gender significant at 0.05 (Chi-square). Gender p= 0.053.

The following inclusion criteria were used. The most important complaint had to be NSCLBP, this was defined as pain between the lower ribs and upper fold of the buttock, with or without radiation to the leg (European guideline for the treatment of NSCLBP). Pain had to persist for more than twelve weeks, to be considered chronic. Pain had to be non-specific, there couldn't be any objective sign of pathology. Participants had to be between the age of 25-60 years old and Dutch speaking.

The following exclusion criteria were used. Participants could not have undergone invasive spinal surgery within the last eighteen months. No radiculopathy (uni-and bilateral). No comorbidities: paresis and sensory deficits with a neurological cause, diabetes mellitus, rheumatoid arthritis, pain augmentation >3/10 and pain >8/10 within the last 24 hours. They couldn't have compensatory complaints and/or absence of work for more than six months. No rehabilitation or exercise therapy with regards to LBP within the last six months.

Inclusion criteria healthy controls: No chronic back pain (>3 months), no acute back pain with VAS > 8/10 within the last 24 hours, they had to be between 25-60 years of age and Dutch speaking. Exclusion criteria healthy controls: Rehabilitation or exercise therapy for acute condition.

3.2 Procedure

3.2.1 Cadaveric dissection

This study was confirmed to the declaration of Helsinki by the world medical association and approved by the local ethics committee (file number 15.142/REVA15.14). The first part of the study consisted of a cadaveric dissection to improve the biopsy technique. This technique was used to collect biopsy samples from the MF and ES in the second part of the study. Two cadavers were used. The first cadaver (male) was used to dissect the complete back musculature. First the skin was removed from the occiput to the inferior gluteal folds. Next the fat and connective tissues were removed to expose the underlying musculature. The first muscle covering the lumbar spine was the latissimus dorsi, this muscle was cut craniocaudally along its medial insertion. Next the deep back musculature was exposed. This was covered by the thoracolumbar faciae and the serratus posterior inferior muscle. These were both cut craniocaudally at their medial insertion and folded back to expose the ES. Medial and slightly deeper from the ES, the MF was located. These two muscles were transversally cut to expose the intermuscular septum between the ES an MF. The second cadaver (female) was used to cut out a transversal piece at the L4 spinal level, to expose a transverse surface of the paravertebral musculature. Next measurements of distance, depth and angle were taken with regards to the biopsy technique. Pictures from the biopsy are displayed in the results section.

3.2.2 Muscle biopsy technique

Participant were lying prone with a small amount of lumbar flexion. The exact location of the muscle biopsy was predetermined using ultrasound imaging by a radiologist. A sterile field was created at the side of biopsy. This location was in-between the processes transversus L4 and L5 at the level of the L4 processes spinosus at the right side of the body. Subjects were administered a local anesthetic (Xylocaine® 5 cc superficial and 1 cc deep). A three millimeter incision through the skin was made at the predetermined spot. Next a coaxial needle was inserted until a bouncy resistance was felt, this was determined the muscular fasciae. The coaxial needle was pushed just through the fasciae. Next the biopsy needle was inserted in the biopsy gun system (Bard®Magnum® Biopsy System). The biopsy needle was inserted through the coaxial needle. The ES was biopsied at an angle of approximately 30 degrees lateral from the vertical axis, the biopsy angle of the MF muscle was predetermined by the radiologist to avoid risk of damaging other structures (figure 1). The biopsy gun was fired 22 mm deep into the muscle. The muscle samples were laid on a piece of cork and threated with tissue-tek®. Next the biopsy samples were frozen in isopentane (Prolabo®) cooled in liquid nitrogen. When frozen the biopsy samples were stored in a freezer at -80 degrees until cutting in cryosections.

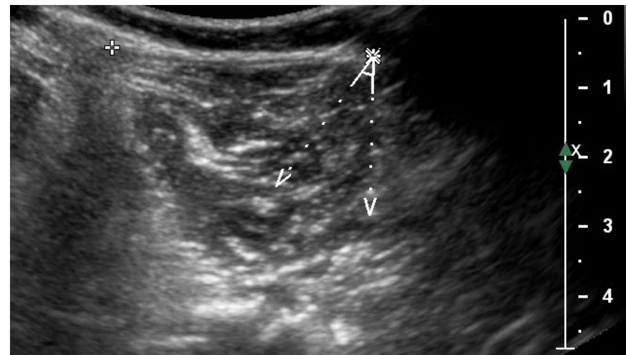


Figure 1: Determination of biopsy angle and depth with ultrasound imaging

3.2.3 Histological analysis

A hematoxylin and eosin staining (H&E) was performed to determine if the biopsy samples were cut transversely (figure 2). Muscle samples that were cut correctly got stained with primary and secondary antibodies against different MHC's. Samples were first treated with 10% normal goat serum as a blocking buffer. After this procedure samples were stained with primary antibodies: cell membrane (polyclonal rabbit anti-laminin, Abcam), type I muscle fibers (monoclonal mouse anti-type I MHC (IgG2b) dshb, BA-F8), type IIa muscle fibers (monoclonal mouse anti-type IIa MHC (IgG1) dshb, SC-71), type IIX fibers (monoclonal mouse anti-type IIX MHC (IgM) dshb, 6H1). Next the samples were stained with a secondary fluorescent antibody: cell membrane (Alexa Fluor 532 goat anti-rabbit IgG (RED) Invitrogen), type I muscle fibers (Alexa Fluor 350 goat anti-mouse IgG2b (BLUE) Invitrogen), type IIA muscle fibers (Alex Fluor 488 goat anti-mouse IgG1 (GREEN) Invitrogen), type IIX muscle fibers (Alexa Fluor 555 goat ant-mouse IgM (RED) Invitrogen). The muscle samples were covered with coverslips with ProLong® Gold antifade and let to dry. After drying the samples were stored at -20° until fluorescence microscopy (figure 3).

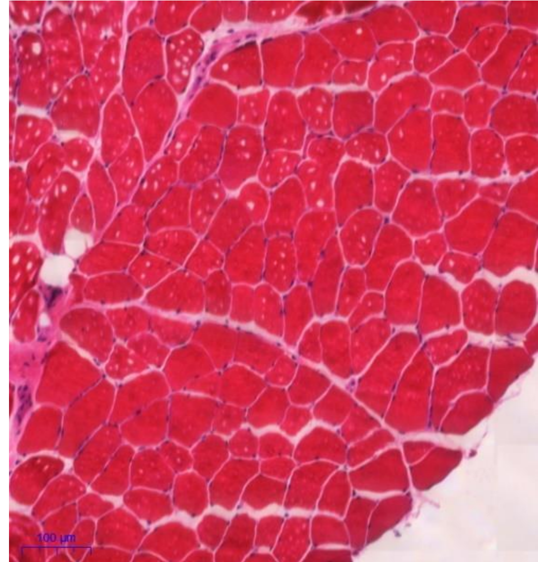


Figure 3: Hematoxylin and eosin stained picture

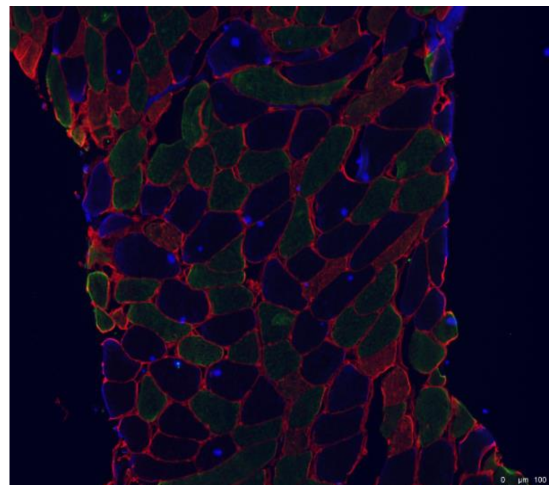


Figure 2: Picture from fluorescence microscopy

Slides were observed with a fluorescence microscope. A picture was taken and each fiber type was measured and counted using AxioVision® SE64 from Carl Zeiss. Blue fibers were classified as type I, green fibers as type IIA, and red fibers as type IIX. When fibers were strong green and intermediate red they were classified as type IIA/X hybrid fibers. Type I/IIA hybrid fibers could not be classified based on the intensity of the blue coloring. First fibers were encircled so their surface area could be measured, afterwards each fiber type was counted. With this

information mean surface areas were calculated by fiber type, as was the percentage of each fiber type within one sample. The relative areas were calculated by multiplying CSA times percentage type, this was than divided by the sum of all CSA's times type percentages.

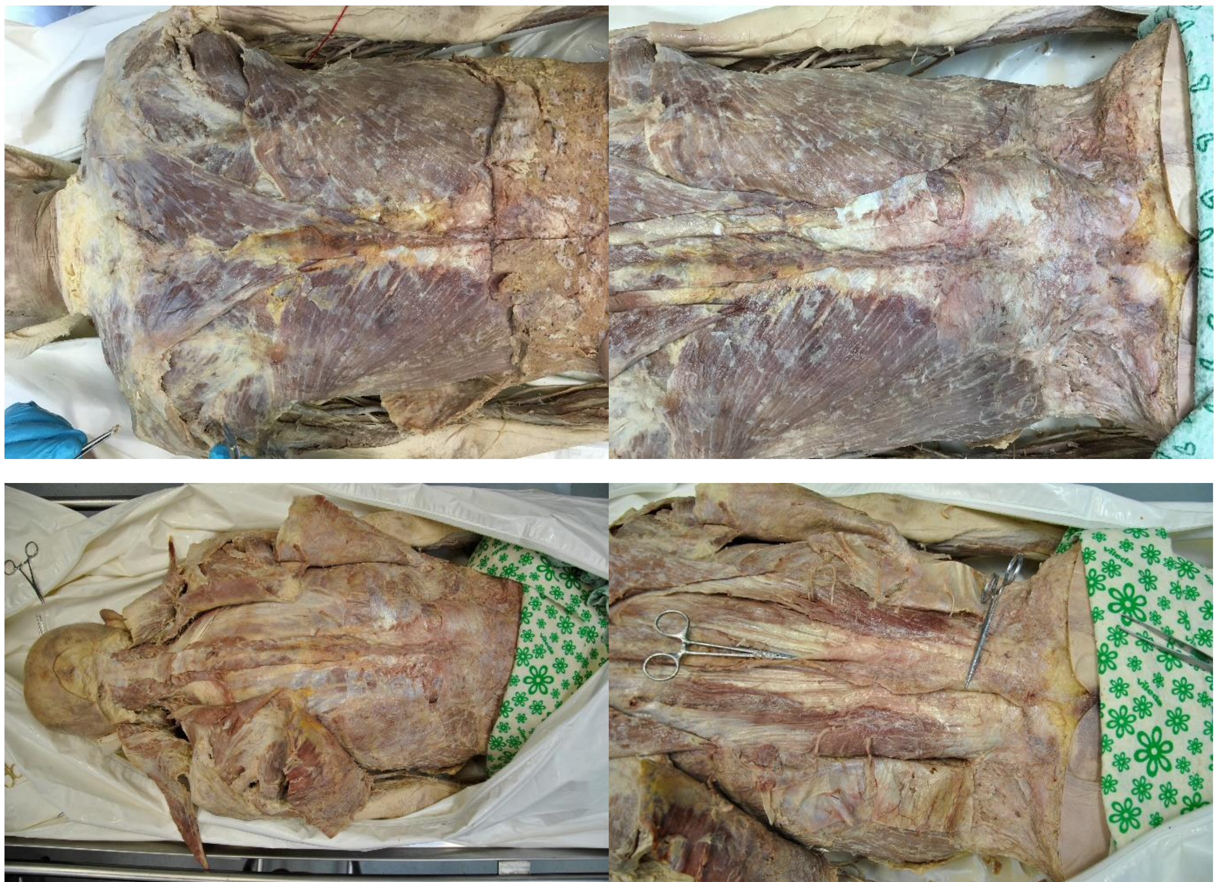
3.3 Data analyses

Data were analyzed using JMP® Pro 12.2.0 from SAS. A repeated measurements ANOVA was used. Significance was set at the 5% point with a confidence interval of 95%. Normality of the data was checked using a normal quartile plot calculated from the conditional residuals. When a significant interaction was found, post-hoc analyses were performed. To perform the post-hoc analyses an all pairwise Tukey HSD was used.

4. Results

4.1 Results cadaveric dissection and biopsy technique

Pictures from the cadaveric dissection are displayed in figure 4. When the MF and ES were cut transversely at the L4 spinal level a clear distinction could be made between both muscles. No differentiation could be made between the different parts of the lumbar ES. The intermuscular septum between both muscles was visible about 2-3 cm lateral from the L4 processes spinosus. Biopsy samples had to be taken about 2-3cm lateral from the processus spinosus. The biopsy angle from a vertical axis had to be 30-35° for the ES and 15-20° for the MF, both with a depth of 22mm (figure 5). To avoid the risk of damaging other structures, and to ensure the exact muscle was biopsied every participant had to be examined by an experienced radiologist.



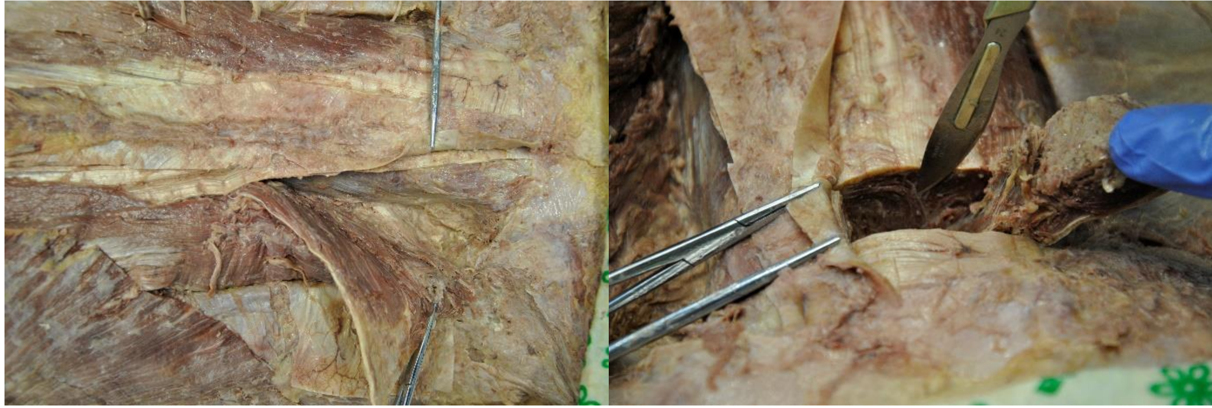


Figure 4: Cadaveric dissection of the paravertebral muscles. Picture 1. fat and connective tissue covering the lumbar spine, Picture 2. latissmus dorsi and thoracolumbar faciae, Picture 3. cutting away the latissmus dorsi/ exposing the serratus posterior inferior and thoracolumbar faciae, Picture 4. Cutting away the thoracolumbar faciae/ exposing the deep paravertebral musculature, Picture 5. Cutting away the ES along its origin on the cristae iliaca/ exposing the MF, Picture 6. The intermuscular septum between ES and MF.

Before biopsy samples were taken, the biopsy location was predetermined by a radiologist using ultrasound. Ultrasound imaging was used to measure the distance between the processus spinosus of the L4 vertebrae and the intramuscular septum. Next the biopsy angle and depth were checked especially for the MF. This was to avoid damaging other structures than the muscle itself (figure 1). Markers were applied on the skin so it would be clear where to biopsy had to be taken. In this study it can be said with certainty that the correct muscle was biopsied. However, no differentiation could be made between the different parts of both the MF and the ES muscle. This was the same case for both the cadaveric dissection as for the ultrasound imaging.

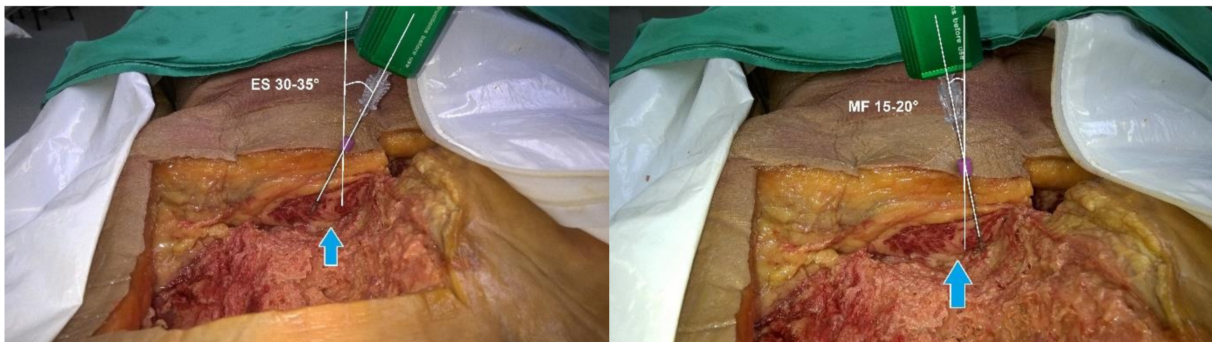


Figure 5: Cadaveric biopsy of the MF and ES at the processes spinosis L4. Blue arrow indicates the intermuscular septum between the ES and MF. Angle of biopsy MF 15-20°, ES 30-35° measured from the vertical axis.

4.2 Results biopsy samples

Results regarding the biopsy samples are displayed in table 2. When comparing the ES and MF within the group of healthy controls, the type I CSA of the MF (7701.14µm) was significant (p= 0.0012) larger compared to the ES (6041.18 µm). The CSA of type IIAX in the ES (4653.54 µm)

was significant (p= 0.0420) larger compared to the MF (3639.53 µm). No significant differences were found for fiber type percentage nor for relative fiber area. Next ES and MF were compared within the NSCLBP group. No significant differences were found except for type IIX CSA. The CSA of type IIX fibers was significantly (p= 0.0136) larger in the ES (5538.84 µm) compared with the MF (3748.15 µm).

Looking at the differences between the healthy controls and the NSCLBP population for the ES, there are no significant differences related to CSA. But the ES of the NSCLBP group does contain a significant (p= 0.0114) higher percentage of type I fibers (65.53%) compared to the healthy control group (54.95%). The ES of the healthy controls contained a significant (p= 0.0172) higher percentage of type IIX fibers compared to the NSCLBP population. The relative area for type I was also significantly (p= 0.0109) higher in the NSCLBP group (69.81%) compared to the healthy controls (60.08%). The relative area for type IIX was significant (p= 0.0339) higher in the healthy control group (11.82%) compared to the NSCLBP group (3.76%). When comparing the MF in both groups, type I fiber percentage was significant (p= 0.0156) higher in the NSCLBP group (63.78%) compared to the healthy controls (52.77%).

Table 2: Results repeated measurements ANOVA

Healthy ES versus MF

Outcome	Type	ES	MF	Difference	P-value
CSA	I	6041.18	7701.14	1659.96	**P= 0.0012***
CSA	IIA	5161.24	5148.22	13.02	P= 0.9788
CSA	IIAX	4653.54	3639.53	1014.01	**P= 0.0420***
CSA	IIX	4315.73	3906.56	409.18	P= 0.4296
%	I	54.95	52.77	2.17	P= 0.6699
%	IIA	21.68	20.36	1.32	P= 0.7949
%	IIAX	9.05	11.04	1.98	P= 0.6974
%	IIX	14.31	15.83	1.52	P= 0.7661
R%	I	60.08	65.81	5.73	P= 0.1942
R%	IIA	19.98	16.60	3.39	P= 0.4409
R%	IIAX	8.12	6.89	1.24	P= 0.7781
R%	IIX	11.82	10.71	1.10	P= 0.8012

ES healthy versus NSCLBP

Outcome	Type	Healthy	NSCLBP	Difference	P-value
CSA	I	6041.18	5945.47	95.71	P= 0.9033
CSA	IIA	5161.24	4836.67	324.57	P= 0.6811
CSA	IIAX	4653.54	4981.17	327.62	P= 0.6917
CSA	IIX	4308.78	5452.39	1143.60	P= 0.1831
%	I	54.95	65.53	10.59	**P= 0.0114***
%	IIA	21.68	21.68	0.00	P= 0.9999
%	IIAX	9.05	8.40	0.65	P= 0.8734
%	IIX	14.31	4.38	9.94	**P= 0.0172***
R%	I	60.08	69.81	9.73	**P= 0.0109***
R%	IIA	19.98	19.01	0.97	P= 0.7953
R%	IIAX	8.12	7.43	0.70	P= 0.8527
R%	IIX	11.82	3.76	8.06	**P= 0.0339***

NSCLBP ES versus MF

Outcome	Type	ES	MF	Difference	P-value
CSA	I	5945.47	6520.91	575.43	P= 0.3135
CSA	IIA	4836.67	5226.45	389.78	P= 0.4947
CSA	IIAX	4930.98	4160.88	770.10	P= 0.2266
CSA	IIX	5538.84	3748.15	1790.69	**P= 0.0136***
%	I	65.53	63.78	1.75	P= 0.7057
%	IIA	21.68	23.89	2.20	P= 0.6344
%	IIAX	8.40	5.24	3.16	P= 0.4952
%	IIX	4.38	7.09	2.71	P= 0.5590
R%	I	69.81	70.48	0.67	P= 0.8786
R%	IIA	19.01	22.22	3.21	P= 0.4630
R%	IIAX	7.43	3.44	3.99	P= 0.3629
R%	IIX	3.76	3.86	0.11	P= 0.9805

MF healthy versus NSCLBP

Outcome	Type	Healthy	NSCLBP	Difference	P-value
CSA	I	7701.14	6520.91	1180.24	P= 0.1486
CSA	IIA	5148.24	5226.45	78.20	P= 0.9214
CSA	IIAX	3639.53	4279.76	640.24	P= 0.4318
CSA	IIX	3936.39	3782.57	153.81	P= 0.8623
%	I	52.77	63.78	11.01	**P= 0.0156***
%	IIA	20.36	23.89	3.53	P= 0.4262
%	IIAX	11.04	5.24	5.79	P= 0.1937
%	IIX	15.83	7.09	8.74	P= 0.0522
R%	I	65.81	70.48	4.67	P= 0.2281
R%	IIA	16.60	22.22	5.63	P= 0.1476
R%	IIAX	6.89	3.44	3.45	P= 0.3723
R%	IIX	10.71	3.86	6.85	P= 0.0791

* significant at the level 0.05. ES: erector spinae, MF: multifidus, CSA: cross sectional area in µm, R%: relative area. Relative area was calculated (CSA I x % type I) / ((CSA I x % type I) + (CSA IIA x % type IIA) + (CSA IIX x % type IIX) + (CSA IIAX x % type IIAX) x 100

5. Discussion

In heathy individuals, the CSA of type I fibers was larger in the MF compared to the ES. When looking at the literature the MF is often mentioned as a stabilizing muscle (Dickx et al. 2010, Ward et al. 2009, Moseley et al. 2002) and the ES a mobilizing muscle in their relation to the spinal column (Stark et al. 2013). Logically the MF should contain a larger relative area of type I muscle fibers to maintain its stabilizing function. This can be realized by increasing the number of fibers (hyperplasia) or increasing the individual fiber size (hypertrophy). In this study there is a larger CSA of type I fibers, however there is no significant difference in relative area. A possible reason that there is no difference in relative area is that the sample sizes were too small. Other studies also failed to show differences between both muscles (Crossmann et al. 2004) or stated that there is no consensus related to fiber type composition (Cagnie et al. 2015, Hesse et al. 2013, Macdonald et al. 2006).

Type IIAX CSA in the healthy control group and type IIX CSA in the NSCLBP group were both significant larger in the ES compared to the MF. No other differences were detected. These results should be interpreted with care because differentiation between type IIX and type IIAX were made based on color intensities rather than clear color differences. However, if these differences are not due to a faulty interpretation of color intensities. The higher CSA's of type IIX and type IIAX could be indicative of the mobilizing function of the ES. In the next phase of this study (the larger RCT), we expect a better statement can be made by increasing the study population. By increasing the sample size it could be possible that the relative areas of certain fiber types reach significance. This would make the connection between muscle function and morphological characteristics more evident.

Although there were little differences comparing the ES and MF within the healthy control group or the NSCLBP group. More differences were found comparing the ES of the healthy control group with the ES of the NSCLBP group. There are only four studies that looked at individual fiber CSA. The review of Demoulin et al. 2007 showed marked atrophy of type II muscle fibers. In contrast with Demoulin et al. 2007, in this study no difference in fiber CSA were found. This was in line with three other studies including two biopsy studies, who failed to show any difference in muscle fiber size (Mannion. 1999, Mannion et al. 1997, Crossmann

et al. 2004). This study is in line with the last three researches on individual fiber CSA. If we assume total muscle CSA is predominantly determined by individual fiber CSA, these studies could be used to compare our research with. However, when looking at studies regarding total muscle CSA there is no consensus. Different studies indicate there is a reduction of the total muscle CSA. The study of Wan et al. 2015 showed a significant decrease in CSA of the MF and ES in individuals with LBP using magnetic resonance imaging (MRI). Two studies using computed tomography (CT) imaging showed a significant decrease in CSA of the MF (Kamaz et al. 2007, Danneels et al. 2000). Three studies using ultrasound imaging showed a significant decrease in CSA of the MF muscle at either L4, L5 or both (Wallwork et al. 2009, Hides et al. 2008, Lee et al. 2006). The review of Goubert et al. 2016 showed moderate evidence for atrophy of the MF, with respect to the ES the evidence was inconclusive. Other studies indicate there is no reduction of total muscle CSA when LBP is present. Decrease in muscle size and mass of the MF and ES has been seen with an increase in age or BMI, this was shown in a 15-year long MRI study by Fortin et al. 2014. There was no association between LBP and the changes in muscle morphology in this study. This evidence was further supported by two MRI studies, that both showed no change in CSA of the paravertebral muscles in individuals with LBP (D'Hooge et al. 2012, Paalanne et al. 2011). The study of Hultman et al. 1993 could not find a decrease in CSA between CLBP and controls for the ES using CT imaging.

When looking at fiber type percentages the ES of the NSCLBP group contained a significant larger percentage of type I fibers, and a significant lower percentage of type IIX fibers. This was the same case for the relative areas. When looking at the MF there was a significant higher percentage of type I fibers. Although not significant, there was also a trend to a decrease in percentage and relative area of type IIX fibers in the MF. If the amount of type IIX fibers was decreased and the amount of type I was increased in the ES, it could mean it is enlarging its aerobic capacity. This could make the ES more suitable to carry out a stabilizing function instead of a mobilizing function. These results are in contradiction with the current literature. Mannion. 1999 states there is a shift towards a higher proportion of type II fibers, at the expense of type I fibers. Mannion et al. 1997 found that the proportion of type I fibers was lower in LBP compared to healthy controls. Mazis et al. 2009 also found a higher proportion of type II fibers, compared to type I using a biopsy study. Demoulin et al. 2007 stated in their review, there was a fiber type conversion from type I to type II. All these studies indicate that

whenever LBP is present there is either and increase in type II fibers, a decrease in type I fibers or both. In this study we found the opposite, a possible explanation could be increased muscular activity mediated by the central nerve system.

Pakzad, Fung, Preuss. 2016 found some evidence for increased EMG activity in certain back muscles in people with LBP. Pain catastrophizing increased this activity even more. They even suggest that these changes may alter normal phasic muscle activity. If phasic activity is converted to a more tonic activity, this could stimulate an increase in type I muscle fibers. The fiber composition of a muscle is primary determined by different neural activity patterns. These patterns can be appointed as impulse rate and total impulses per day (Schiaffino and Reggiani. 2011). To sum, an alteration in neural stimulation due to pain or pain catastrophizing could possibly cause a fiber type shift in patients with NSCLBP. This could explain the results seen in this study.

Still some questions remain unanswered. Differences are present between the ES and MF within the healthy control group regarding type I CSA. This could indicate the MF is more suitable to fulfill a stabilizing function compared to the ES. When NSCLBP is present, differences between the ES and MF regarding type I CSA are not present. This could indicate the MF is losing its capability to stabilize the spinal column. This decrease in CSA could be indicative of fiber atrophy. This decrease from 7701µm to 6520µm was not significant (p= 0.1486). Logically we expected a further decrease in CSA when comparing the healthy control group with the NSCLBP group for both muscles. However, the opposite was happening on to fronts. First there was no atrophy/ hypertrophy present, but a shift in fiber type percentage was existing. This shift in fiber type caused the relative areas to shift as well, without change in fiber CSA. Second, type I fibers increased whilst type II fibers decreased in the NSCLBP group. This would make the NSCLBP group more capable of stabilizing their spine. It is not clear if this is a true shift in fiber type, or hyperplasia were new fibers are created (which is a rare given). All this would mean that individuals with NSCLBP increase their "aerobic area", their tonic muscle activity. The next question should be if these changes are the cause of NSCLBP or are these changes an adaptation to another underlying problem? Based on the results found in this study and the available literature, a neural mechanism such as an upregulated pain matrix (Kregel et al. 2015) by for instance pain catastrophizing (Pakzad et al. 2016) could lead to long

persisting morphological adaptations of the paravertebral muscles. But what induces these alterations in the central nerve system. For instance, a temporary injury could lead to central sensitization which on the long term induces an increase in neural signaling to the paravertebral muscles. This increased signaling could than induce morphological adaptations. This would make the morphological changes seen in this study a sequel and not the cause of pain experienced by individuals with NSCLBP.

An important disadvantaged in this study was the small population, this could have led to a lower statistical power. A cardiopulmonary exercise test (CPET) was assessed at baseline in both the healthy control and NSCLBP group. This could have attracted athletic people who had personal benefit of doing a CPET. This could have caused a selection bias by which the control group was not representative for the general population. Moreover, a muscle sample needs to contain 150 individual muscle fibers to represent the entire muscle. In a minority of the samples this number was not reached. The counting of these fibers was done by one individual, this could have led to an observer bias. Differentiation between type IIX and hybrid type IIAX was done based on color intensity. This could have led to wrong classification of fiber types.

This study was the first to use a fine needle biopsy technique to obtain muscle samples from the ES and MF. This technique is minimally invasive and does not damage the biopsy sample. Biopsy samples were taken with a high level of accuracy by using ultrasound to determine the correct sampling site. This study is one of a few that uses a healthy control group and people with true NSCLBP, because most of the biopsy studies used either cadaveric specimens or intraoperative biopsy samples. Another advantage of this study is the use of antibody coloring instead of ATPase techniques. This made it possible to differentiate between the different type II fibers. Also, H&E staining allowed to see if biopsy samples were cut transversally, this makes the CSA measurements more reliable.

In conclusion, this study could indicate that the MF has a stabilizing role in the lumbar spinal column according to its fiber type composition. This because it contains a larger CSA of type I fibers compared to the ES in healthy individuals. Differences in relative areas probably could not reach significance because of low statistical power. The ES of individuals with NSCLBP

shows more characteristics of a stabilizing muscle compared to the healthy controls. Both type I percentage and relative area were increased, whereas type IIX areas were decreased. These changes could also be the case for the MF. It looks like individuals with NSCLBP are trying to "over" stabilize their spinal column with the thought that "pain induces damage" thereby increasing the neural signal that induces a change in fiber type composition. Future studies should increase the study population to find differences that this study was unable to show. They should also focus on the principles of hypertrophy, hyperplasia and fiber shift. Research to investigate which of these principles are responsible for the changes that occur with NSCLBP is necessary. Another important question that needs to be answered is if training can influence these changes, and in which direction. If we assume augmented neural activity induces a fiber shift or hyperplasia, which increases the stabilizing capacity of the paraspinal muscles by enlarging their type I fiber area. Should we go for the popular stabilization straining, or for a more mobilizing approach?

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7. Appendix

Protocol: H&E

Protocol: MHC fiber typing human muscles

Information brochure

Protocol H&E kleuring

- Coupes goed labelen met potlood
- Laat de cryocoupes 30’ drogen
- 10’ in koude aceton
    - Staat in de diepvries
    - Als het nog proper is → terug in frigo, anders in waste onder de trekkast (niet-gehalogeneerd afval)
- 30’ drogen
- 5’ PBS 1x
- 5-10’ Haematoxyline
- 10’ tap water (geen gedestilleerd, dit stopt de reactie)
    - Laten lopen in bakje
- 1’ AD water
- 3’ eosine
- Vooraf in bakje AD (om te zorgen dat eosine niet te fel in de ethanol komt. Reeks 70% → xyleen 2
    - Ethanol kort < 1’
    - Xyleen 5’ als je een 10-tal glaasjes hebt
        - Op voorhand checken of alles proper is
- Monteren met DPX verdund
- Overnacht laten staan onder trekkast

Protocol: MHC fiber typing human muscles

Staining of the cell membrane (laminin; red), type I (blue), Type IIa green and type IIx (red) muscle fibers.

<u>Reagents:</u>

1x PBS Add 100ml 10X PBS (Invitrogen) to 900ml distilled H_2O
Blocking Buffer 10% normal goat serum (Invitrogen)

<u>Antibodies:</u>

1) Cell membrane (extracellular matrix, laminin):
    a. 1° AB: polyclonal rabbit anti-laminin (Abcam, ab11575)
        i. Store at -20°C (in aliquots)
        ii. Dilution: 1/200
    b. 2° AB: Alexa Fluor 532 goat anti-rabbit IgG (Invitrogen, A-11009) RED
        i. Store at -20°C (in aliquots)
        ii. Dilution: 1/500
2) Type I muscle fibers:
    a. 1° AB: Monoclonal mous anti-type I MHC (IgG2b) (dshb, BA-F8)
        i. Store at -20°C (in aliquots)
        ii. Dilution: 1/50
    b. 2° AB: Alexa Fluor 350 goat anti-mouse IgG2b (Invitrogen, A-21140) BLUE
        i. Store at -20°C (in aliquots)
        ii. Dilution: 1/500
3) Type IIa muscle fibers:
    a. 1°AB: Monoclonal mouse anti-type IIa MHC IgG1 (dshb, SC-71)
        i. Store at -20°C (in aliquots)
        ii. Dilution: 1/500
    b. 2° AB: Alexa Fluor 488 goat anti-mouse IgG1 (invitrogen, A-21121) GREEN
        i. Store at -20°C (in aliquots)
        ii. Dilution: 1/500
4) Type IIx muscle fibers:
    a. 1° AB: Monoclonal mouse anti-type IIx MHC IgM (dshb, 6H1)
        i. Store at -20°C (in aliquots)
        ii. Dilution: 1/50
    b. 2° AB: Alexa Fluor 555 goat anti-mouse IgM (Invitrogen, A-21426) RED
        i. Store at -20°C (in aliquots)
        ii. Dilution: 1/500

<u>Equipment</u>:

Dapi pen
Histochemistry jars (wash steps)
Pippettes and tips
Filter paper and tissues

cover slips, ProLong® Gold antifade reagent
fluorescence microscope

Procedure for Fiber Typing:

1) Cut muscles into cross-sections and store at -80°C (Marc Jans)
2) Air dry sections for 20min at RT (in closed box)
3) Circle sections with DAPI Pen
4) Blocking: Block sections for **1hour** in blocking buffer (in closed box with wet filterpaper)
5) Add 1° AB cocktail (diluted in blocking buffer) to each slide (per slide, 1 blanco: only blocking buffer) and incubate for **2hours** at RT (in closed box with wet filter paper)
6) Wash slides in histo jar 3x5min with 1x PBS

WORK IN DARK

7) Add 2° AB cocktail (diluted in blocking buffer) to slides and incubate for **1hour** at RT in the dark (in closed box with wet filterpaper)
8) Wash slides in histo jar 3x5min with 1x PBS
9) Mount coverslips with ProLong® Gold antifade reagent and let dry
10) Observe slides under fluorescence microscope and take pictures
11) Store stained slides at -20°C

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IN EEN NOTENDOP

Wat houdt het in?

Een Onderzoek naar het meest effectieve revalidatieprogramma voor personen met aspecifieke chronische lage rugpijn.

⇒ 4 revalidatieprogramma's aan hoge intensiteit van elk 12 weken worden met elkaar vergeleken

Wat wordt van u verwacht?

U zal willekeurig worden ingedeeld in één van deze groepen

⇒ Volgen van een revalidatieprogramma van 12 weken (2sessies van 2u per week) in het onderzoekscentrum REVAL van de Universiteit Hasselt

⇒ Studiemetingen vóór, tijdens en na 12 weken therapie in het onderzoekscentrum REVAL van de Universiteit Hasselt

Wat is de waarde?

Deze studie levert een bijdrage aan de optimalisatie van de behandeling voor chronische lage rugpijn.

Waarde voor de deelnemers:

Volledige sportmedische screening en het kosteloos volgen van een volledig revalidatie-programma onder begeleiding van ervaren therapeuten

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ONDERZOEK NAAR OEFENTHERAPIE BIJ PERSONEN MET LAGE RUGPIJN

Klinische Studie

Anouk Agten
Jonas Verbrugghe

Doel van de studie

Lage rugpijn is een veelvoorkomende aandoening met belangrijke impact op het dagelijks leven en functioneren. Ter behandeling van deze aandoening zijn verschillende vormen van revalidatie mogelijk. De effectiviteit hiervan is echter vaak nog onduidelijk. Deze studie wil onderzoeken welk revalidatieprogramma aan hoge intensiteit het meest effectief is ter behandeling van langdurig aanhoudende lage rugpijn.

Wie kan deelnemen?

- Personen tussen 25 en 60 jaar
- Langdurige lage rugpijn (> 12weken)
- Geen revalidatie voor lage rugpijn gevolgd in de laatste 6 maanden

Trainingszaal

Studieverloop

In deze studie worden de proefpersonen willekeurig ingedeeld in 4 groepen. Deze 4 groepen volgen elk een specifiek oefen-programma van 12 weken waarin er 2 maal per week getraind wordt onder begeleiding van ervaren therapeuten. De trainingen gaan steeds door in het onderzoekscentrum REVAL van de Universiteit Hasselt in Diepenbeek. Het volledige trainingsprogramma is kosteloos voor de deelnemers.

Voor de start van het trainingsprogramma en na 12 weken worden enkele studiemetingen afgenomen. U krijgt een evaluatie van uw resultaten mee naar huis.

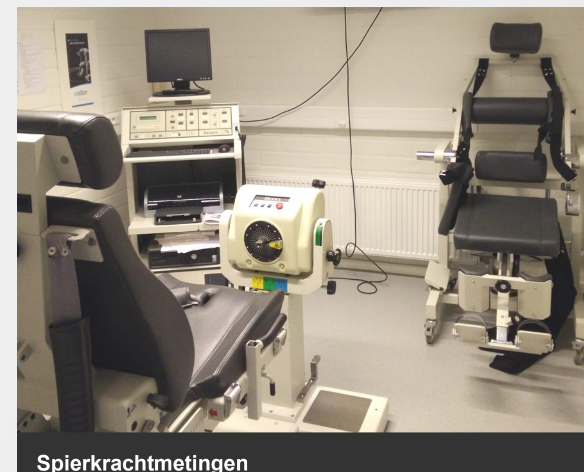
Studiemetingen

Sportmedische screening:

- Hartcontrole (ECG)
- Maximale inspanningstest
- Spierkrachtmetingen
- Lichaamssamenstelling

Bijkomende onderzoeken:

- Spierbiopt
- bloedafname
- Invullen van enkele vragenlijsten
- Activiteitenmonitoring



Spierkrachtmetingen

Wat is de waarde voor u

Volledige sportmedische screening en het kosteloos volgen van een volledig revalidatieprogramma onder begeleiding van ervaren therapeuten.

Wanneer

Geïnteresseerden zullen eerst gescreend worden door een arts. Deze screening zal starten vanaf **oktober 2016**.

Hierna bekijken we samen met elke participant de mogelijkheden voor de eerste testen en de start van het oefenprogramma.

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**Morphological characteristics of the lumbar paraspinal muscles in individuals with non-specific chronic low back pain and healthy controls:
a fine needle biopsy study**

Richting: **master in de revalidatiewetenschappen en de kinesitherapie-revalidatiewetenschappen en kinesitherapie bij inwendige aandoeningen**
Jaar: **2017**

in alle mogelijke mediaformaten, - bestaande en in de toekomst te ontwikkelen - , aan de Universiteit Hasselt.

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