

Acknowledgement

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Research context

This thesis is situated in the domain of musculoskeletal rehabilitation and the topic of this thesis deals with non-specific chronic low back pain (NSCLBP). Chronic low back pain (CLBP), which can be defined as low back pain lasting for more than three months, is a widespread problem with a high socio-economic burden leading to a high rate of work-related disability. Finding an effective treatment for this condition would have a great impact on society, socially as well as economically. A patho-anatomical cause for low back pain (LBP) cannot be found in 85% of the patients, in which case it is referred to as NSCLBP. However, there are indications that there is a link between CLBP and muscle atrophy of the m. multifidus, which is considered as an important stabilising muscle of the spine. So, far it is not clear whether the micro-anatomy of the muscle is affected by the presence of NSCLBP. Moreover there could be an alteration in muscle fibre type size and/or distribution when comparing healthy controls with LBP patients within the m. erector spinae and/or the m. multifidus.

This document is part of the master thesis of the second master year in rehabilitation sciences and physiotherapy at the University of Hasselt. This study took place at REVAL rehabilitation research centre at campus Diepenbeek of the University of Hasselt under the supervision of Drs. Sjoerd Stevens, Dr. Anouk Agten and Prof. Dr. Frank Vandenabeele. It is part of an already existing project, investigating the structural and functional effects of HIT programs in patients with NSCLBP. This project is under the supervision of Dr. Anouk Agten, Drs. Jonas Verbrugghe, Drs. Sjoerd Stevens, Prof. Dr. Frank Vandenabeele, Prof. Dr. Annick Timmermans and Prof. Dr. Bert Op 't Eijnde.

This thesis was written as a duo master thesis. The research design and research method was determined by Drs. Sjoerd Stevens, Dr. Anouk Agten and Prof. Dr. Frank Vandenabeele. The research question was drawn up in collaboration with Drs. Sjoerd Stevens and Dr. Anouk Agten. Patients were already recruited for the existing project, namely the LBP-HIT trial, investigating the structural and functional effects of HIT programs in patients with NSCLBP. For this thesis, a subgroup of this larger study was used.

For the data-acquisition the following procedure was followed:

- The correct location for the biopsies as well as for the ultrasound images has been verified through dissections of the back muscles, which was performed by us in the first master year.
- The determination of the biopsy location by ultrasound imaging was performed by a radiologist.
- The biopsy was performed by Prof. Dr. Frank Vandenabeele with assistance of Dr. Anouk Agten. We were allowed to follow this procedure with several participants.
- The colouring of the coupes was done by Dr. Anouk Agten and Drs. Sjoerd Stevens. We were allowed to assist during some of these colourings.
- The CSA of the different muscle fibre types were determined using AxioVision® SE64 from Carl Zeiss. We analysed 24 of a total of 62 coupes. The remaining coupes were analysed by Drs. Sjoerd Stevens and Dr. Anouk Agten.

The determination of the correct statistical analysis, as well as the analysis itself was done independently for the one-way ANOVA and the spearman's correlation. For the repeated measures test, a brief instruction was provided by Drs. Sjoerd Stevens and Dr. Anouk Agten. The academic writing process was performed independently. However, feedback was provided by Drs. Sjoerd Stevens and Dr. Anouk Agten.

Table of content

1. Abstract.....	5
2. Introduction.....	6
3. Method	8
3.1. Study design and participants	8
3.2. Measurements	9
3.2.1. Muscle biopsy technique.....	9
3.2.2. Histological analysis.....	10
3.2.2.1. Measurement of muscle fibre characteristics.....	10
3.3. Outcome measures	11
3.3.1. Primary outcome measures	11
3.3.2. Secondary outcome measures	11
3.4. Medical ethics.....	11
3.5. Statistical analysis.....	11
4. Results.....	13
4.1. Comparison between m. erector spinae and m. multifidus.....	13
4.2. Comparison between NSCLBP group and healthy controls	13
4.3. Correlation between muscle CSA and muscle fibre type CSA.....	14
4.4. Comparison between NSCLBP group and healthy controls of CSA of m. multifidus and m. erector spinae.....	14
5. Discussion.....	15
5.1. Reflection results.....	15
5.1.1. Comparing m. multifidus with m. erector spinae.....	15
5.1.2. Comparison of healthy controls with NSCLBP patients.....	16
5.1.3. Correlation between muscle CSA and muscle fibre CSA	19
5.2. Reflection of strengths and weaknesses	19
5.3. Recommendation for further research	20
6. Conclusion	22
7. List of references.....	24
8. Appendix	27

1. ABSTRACT

Background: NSCLBP is a widespread socio-economic burden. Little is known about the pathophysiology. There might be a possible correlation between NSCLBP and alterations in muscle fibre type and/or muscle fibre size of the paraspinal muscles.

Objectives: Investigating: 1) the difference in muscle fibre type percentage and/or size when comparing both research groups for both the m. multifidus and the m. erector spinae. 2) the difference in muscle fibre type percentage and/or size when comparing the m. multifidus with the m. erector spinae in both research groups. 3) a correlation between the CSA of the m. erector spinae/m. multifidus and muscle fibre type CSA in both research groups.

Participants: This research consisted of 19 NSCLBP patients and 13 controls.

Measurements: Primary outcome measures were CSA, percentage and proportional area of different muscle fibre types. The secondary outcome measure was the total muscle CSA.

Results: There was a significant difference in the CSA of muscle fibre type I, this for both research groups. The percentage of muscle fibre type I was significantly larger in the NSCLBP patients when compared with the controls, this for both muscles. In the m. multifidus the proportional area of muscle fibre type I was significantly larger for the NSCLBP group. Percentages of muscle fibre type IIx were significantly larger for both muscles. The proportional area of muscle fibre type IIx was significantly larger in the m. erector spinae. There was a correlation between the CSA of muscle fibre type IIa and total muscle area of the m. multifidus in healthy subjects.

Conclusion: No significant atrophy of the m. multifidus could be found in NSCLBP patients. Results suggest that muscle fibre type II is most susceptible for atrophy. Fear avoidance behaviour of NSCLBP patients could explain an increase in muscle fibre type I. Further research is necessary.

2. INTRODUCTION

Low back pain, with a lifetime prevalence up to 84%, can be considered as a worldwide problem, causing more global disability than any other musculoskeletal condition (Airaksinen et al., 2006; Hoy et al., 2012; Hoy et al., 2014). Low back is defined as pain below the costal margin and above the superior gluteal folds, with or without radiation to the lower limbs (European guidelines for the treatment of non-specific chronic low back pain). Seventy-five percent of patients with LBP will not have recovered fully and without disability, one year after their first consultation with a physician (Croft, Macfarlane, Papageorgiou, Thomas, & Silman, 1998) in this case low back pain can become a chronic problem. CLBP can be defined as low back pain lasting for at least 12 weeks (Airaksinen et al., 2006). The cause and pathophysiology of LBP is not always clear in which case it is referred to as non-specific low back pain. Only in 15% of patients with low back pain a clear patho-anatomical cause can be identified (Airaksinen et al., 2006; Deyo & Weinstein, 2001).

Several studies using CT or ultrasound imaging have shown that in CLBP patients the multifidus muscle has a significantly smaller CSA at the L5 level, indicating that there might be a relationship between CLBP and muscle atrophy of the m. multifidus (Beneck & Kulig, 2012; Danneels, Vanderstraeten, Cambier, Witvrouw, & De Cuyper, 2000; T. A. Ranger et al., 2017; Wallwork, Stanton, Freke, & Hides, 2009). However, a few studies failed to find evidence of muscle atrophy (Lee et al., 2006; Scott, Vaughan, & Hall, 2015).

When regarding the m. erector spinae or the paraspinal muscles, which is considered as the combination of the m. erector spinae and the m. multifidus, evidence is inconclusive about whether in CLBP patients this muscle group is affected by atrophy (Goubert, Oosterwijk, Meeus, & Danneels, 2016).

So far, the literature is inconclusive as to what causes muscle atrophy in CLBP patients. One hypothesis suggests that there might be a possible correlation between low back pain and an alteration in the microanatomy of the paraspinal muscles. More specifically, there might be an alteration in muscle fibre typing and/or muscle fibre size of the paraspinal musculature (Cagnie et al., 2015; A. F. Mannion, 1999).

When looking at muscle fibre type percentage in healthy subjects, there is conflicting evidence concerning differences in muscle fibre type percentage when comparing healthy subjects with

LBP patients (Cagnie et al., 2015). Regarding a difference in muscle fibre type percentage between the m. erector spinae and the m. multifidus, Cagnie et al. (2016) concluded that there is no evidence in the literature for a significant difference in muscle fibre type percentage in LBP.

Combining both muscle fibre size and type percentage, the proportional area can be calculated, giving an indication of the relative area occupied by each muscle fibre type. The available literature provides conflicting evidence regarding this parameter (Cagnie et al., 2015; Crossman, Mahon, Watson, Oldham, & Cooper, 2004; A. F. Mannion, 1999).

Another hypothesis suggests that atrophy is caused by pain related nerve inhibition. Since the m. multifidus has an important stabilising function on the spine and is responsible for two thirds of the stiffness of the lumbar spine, dysfunction of this muscle could possibly lead to LBP (Danneels et al., 2000; Freeman, Woodham, & Woodham, 2010; Wilke, Wolf, Claes, Arand, & Wiesend, 1995). Danneels et al. (2000) suggests that atrophy of the multifidus is due to reflex inhibition and changes in the coordination of the trunk muscles as a response to pain. Reflex inhibition disrupts the activity of the alpha motor neurons which leads to an inhibition of the accurate activity of the multifidus and to eventually muscle atrophy (Danneels et al., 2000; Freeman et al., 2010).

So far, a possible correlation between the CSA of the lumbar paraspinal muscles and muscle fibre type percentage and/or size has not yet been investigated. Verdijk et al. (2010) however, has looked for a correlation between the CSA of the m. Quadriceps and muscle fibre size in elderly men. It was concluded that a greater thigh muscle area was correlated with greater muscle fibre CSA.

In this study we will investigate: 1) if a difference in muscle fibre type percentage and/or size can be found when comparing CLBP with healthy controls for both the m. multifidus and the m. erector spinae, 2) if a difference in muscle fibre type percentage and/or size can be found when comparing the m. multifidus with the m. erector spinae in both the LBP group and the control group, 3) if a correlation exists between the CSA of the lumbar m. erector spinae and muscle fibre type CSA in both the LBP group and the control group and 4) if a correlation exists between the CSA of the lumbar m. multifidus and muscle fibre type CSA in both the LBP group and the control group.

3. METHOD

3.1. Study design and participants

This research will be a case-control trial, it will consist of 32 participants divided into a NSCLBP group (n=19) and a healthy control group (n=13). For this study a subgroup of a larger study, namely the LBP-HIT trial, was used (n=125). Patients were recruited through free recruitment (classical and social media, flyers). The healthy subjects were recruited through convenience sampling. Since only a sample of these 125 participants underwent a biopsy at baseline, inclusion to this case-control trial was based on the available biopsies. The baseline characteristics of the included participants consisted of following anthropometric characteristics: length, weight, BMI, age and gender (see table 1).

Inclusion criteria for the NSCLBP group:

- Most important complaint: non-specific chronic low back pain
 - Low back is defined as pain below the costal margin and above the superior gluteal folds, with or without radiation to the lower limbs (European guidelines for the treatment of non-specific chronic low back pain)
 - Chronic: episode lasting for more than 12 weeks
 - Non-specific: pain cannot be attributed to a known cause or pathology
- Age: 25-60 years
- Dutch speaking (written and spoken)

Inclusion criteria for healthy controls:

- No chronic low back pain (lasting for more than three months)
- No acute low back pain with a VAS-score >8/10 in the last 24 hours
- Age: 25-60 years
- Dutch speaking (written and spoken)

Exclusion criteria for the NSCLBP groups:

- Invasive surgery to the spinal column in the last 18 months (arthrodesis will always be excluded, microsurgery is permitted)
- Radiculopathy (uni- or bilateral)

- Comorbidities: paresis and sensory disorders from neurological origin, diabetes mellitus, Rheumatoid arthritis, pain intensification > 3/10 and pain >8/10 in the last 48 hours.
- Current compensation complaints and/or work related disability > 6 months

Exclusion criteria for healthy controls:

- Rehabilitation or exercise therapy for any acute condition

3.2. Measurements

The measurement procedure consisted of a biopsy, for which the exact location was determined by ultrasound. During this ultrasound the CSA of the m. multifidus and m. erector spinae were also measured (see figure 1).

3.2.1. Muscle biopsy technique

First, the participants were seen by a radiologist who determined the exact location of the muscle biopsy by using ultrasound imaging. The correct location for the biopsies as well as for the ultrasound images has been verified through dissections of the back muscles. During these dissections, the m. multifidus was located and it was verified that at the level of L4 it is possible to reach the m. multifidus and the m. erector spinae with the biopsy needle through one skin puncture (see figure 2). Therefore, the radiologist marked at the right side of the body, the point between the m. erector spinae and the m. multifidus at level L4-L5. For 25 participants, the CSA of the m. multifidus and m. erector spinae were also determined using ultrasound.

After the determination of the puncture site for the muscle biopsy, the participants underwent the actual biopsy in REVAL rehabilitation research centre at campus Diepenbeek of the University of Hasselt. Participants were placed in a prone position with a small amount of lumbar flexion. The biopsy procedure was performed by a MD connected to the study. A sterile field was created at the side of biopsy. Subjects were administered a local anesthetic (Xylocaine® 5 cc superficial and 1 cc deep). A three mm incision through the skin was made at the predetermined location. Next a coaxial needle was inserted until a bouncy resistance was felt, this indicated that the muscular fasciae was reached. 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 m. erector spinae was biopsied at an angle of approximately 30 degrees lateral from the

vertical axis, the biopsy angle of the m. multifidus was predetermined by the radiologist to avoid risk of damaging other structures. The biopsy gun was fired 22 mm deep into the muscle. The muscle samples were laid on a piece of cork and treated 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.

3.2.2. Histological analysis

A hematoxylin and eosin staining (H&E) was performed to determine if the biopsy samples were cut transversely. 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 fibres (monoclonal mouse anti-type I MHC (IgG2b) dshb, BA-F8), type IIA muscle fibres (monoclonal mouse anti-type IIA MHC (IgG1), dshb, SC-71), type IIX muscle fibres (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 fibre (Alexa Fluor 350 goat anti-mouse IgG2B (BLUE) Invitrogen), type IIA muscle fibres (Alexa Fluor 488 goat anti-mouse IgG1 (GREEN) Invitrogen), type IIX muscle fibres (Alexa Fluor 555 goat anti-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°. Slides were observed with a fluorescence microscope during which a picture was taken (see figure 3).

3.2.2.1. Measurement of muscle fibre characteristics

Each muscle fibre type was measured and counted using AxioVision® SE64 from Carl Zeiss. These measurements were performed by four independent assessors, blinded for pathology and muscle type. Blue muscle fibres were classified as type I, green muscle fibres as type IIA and red muscle fibres as type IIX. When muscle fibres coloured strong green and intermediate red they were classified as type IIA hybrid muscle fibres. Type I/IIA hybrid muscle fibres could not be classified, based on the intensity of the blue colouring. First muscle fibres were encircled so their surface area could be measured. Afterwards, each muscle fibre type was counted. With this information, mean surface areas were calculated by muscle fibre type. The

percentage of each muscle fibre type within one sample was also determined. The relative areas were calculated by multiplying CSA times percentage type. This was then divided by the sum of all CSA's times type percentages. A minimum number of 100 cells needed to be counted in order to ensure the quality of the coupe. If this number was not reached, the coupe was not taken into account.

3.3. Outcome measures

3.3.1. Primary outcome measures

Primary outcome measures were: 1) the CSA of the muscle fibre types I, IIa, IIx and IIax, 2) the percentage of the muscle fibre types I, IIa, IIx and IIax and 3) the proportional area of the muscle fibre types I, IIa, IIx and IIax. These measurements were obtained from both the right m. multifidus and the right m. erector spinae. On the one hand, this study investigated whether a difference could be found between the CSA of the muscle fibres when comparing the m. erector spinae with the m. multifidus within the NSCLBP group and the control group. On the other hand it was also investigated whether a difference in muscle fibres could be found when comparing the NSCLBP group with the control group.

3.3.2. Secondary outcome measures

Secondary outcome measures were the CSA of the right m. multifidus and m. erector spinae, measured using ultrasound imaging. In this study, a possible correlation between the CSA of the different muscle fibres and the CSA of the actual muscle was investigated.

3.4. Medical ethics

This study was approved by the Medical Ethics Committee of the University of Hasselt. The identification number for this study is 15.142/REVA15.14.

3.5. Statistical analysis

When comparing the baseline characteristics, it was first determined whether the data were distributed normally. This was only the case for length and was therefore analysed using an unpaired t-test. The other anthropometric characteristics, namely weight, BMI and age were analysed using a non-parametric test (Wilcoxon Rank Sum Test). Gender was analysed using a χ^2 test.

For the primary outcome measure, normality was first checked using a Shapiro-Wilk W test on the residuals of the measurements. Since the data were distributed normally, a repeated measurements ANOVA was used to analyse the primary outcome measures. Multiple comparisons were performed using an all pairwise Tukey HSD after the determination of the interactions. The secondary outcome measure was analysed using a Pearson's correlation since the data was distributed normally. The analysis of the ultrasound measurements was performed using a unpaired t-test, with the data being distributed normally. In order to perform the statistical analysis JMP® Pro 13 from SAS was used. The significance was set at a P-value of 0.05 with a confidence interval of 95%.

4. RESULTS

There were no significant differences in age, height, weight, BMI or gender when comparing the NSCLBP group with the healthy control group. (Table 1)

4.1. Comparison between m. erector spinae and m. multifidus

The results show that in healthy controls, only a significant difference ($P=0.0017$) in the CSA of type I muscle fibres can be found when comparing the m. erector spinae with the m. multifidus in the healthy control group. The mean CSA of type I muscle fibres in the m. multifidus ($7619.2581\mu\text{m} \pm 2218.4661$) is 17.32% larger than the mean CSA of type I muscle fibres in the m. erector spinae ($6299.5343\mu\text{m} \pm 1640.7513$). A similar result is found in the NSCLBP group, with a significant difference ($P=0.0451$) for only the CSA of type I muscle fibres. In this group the mean CSA of type I muscle fibres of the m. multifidus ($7517.0194\mu\text{m} \pm 2586.6472$) is 15.48% larger than the mean CSA of type I muscle fibres of the m. erector spinae ($6353.157\mu\text{m} \pm 1643.3334$). (Table 2 and 3)

4.2. Comparison between NSCLBP group and healthy controls

When comparing the NSCLBP group with healthy controls, a significant difference was found for the mean percentage of type I ($P<0.0001$) and type IIx muscle fibres ($P=0.0022$) in the m. erector spinae. The NSCLBP group ($65.107042\% \pm 9.8426495$) had 11.09% more type I muscle fibres than the control group ($54.013968\% \pm 13.717734$). When looking at the type IIx muscle fibres, the healthy control group ($14.57963\% \pm 11.163603$) had 8.82% more muscle fibres for this type than the NSCLBP group ($5.7570356\% \pm 4.9862494$). (Table 4)

The proportional area was significantly different for both type I ($P<0.0001$) and type IIx muscle fibres ($P=0.0088$) in the m. erector spinae when comparing both groups. The proportional area of type I muscle fibres was 9.88% higher in the NSCLBP group ($71.149097\% \pm 8.3484784$) than in the control group ($61.273215\% \pm 12.612844$). For the type IIx muscle fibres, the healthy control group ($10.97149\% \pm 9.6180521$) had a proportional area that was 6.69% higher than that of the NSCLBP group ($4.2864834\% \pm 3.5838353$). (Table 4)

Considering the m. multifidus, a significant difference could be found for the mean percentage of type I ($P=0.0158$) and type IIx muscle fibres ($P=0.0406$). The mean percentage of type I muscle fibres was with a difference of 7.85%, significantly higher in the NSCLBP group

(61.621801% $\pm$ 11.874334) compared to the healthy control group (53.775547% $\pm$ 14.71721). For type IIx muscle fibres, the healthy control group (13.86307% $\pm$ 13.4726) had a significantly higher percentage than the NSCLBP group (7.2304115% $\pm$ 7.5549766). The difference between both groups was 6.63%. (Table 5)

4.3. Correlation between muscle CSA and muscle fibre type CSA

A correlation could only be found between the CSA of the m. multifidus and the CSA of type IIax muscle fibres in healthy controls ($P=0.0117$). A trend towards a possible correlation was visible between the CSA of the m. multifidus and the CSA of type IIa muscle fibres in healthy controls ($P=0.0551$). In the NSCLBP group a trend for a possible correlation was noticed between the CSA of the m. multifidus and the CSA of type IIx ($P=0.0681$) (Table 6). No correlations were found between the CSA of the m. erector spinae and the CSA of the different muscle fibre types (Table 7).

4.4. Comparison between NSCLBP group and healthy controls of CSA of m. multifidus and m. erector spinae

When comparing the NSCLBP group with healthy controls there is no significant difference in CSA for either the m. multifidus or the m. erector spinae. (Table 8 and 9)

5. DISCUSSION

5.1. Reflection results

In this research, primary outcome measures were about the detection of possible differences in muscle fibre type percentage and size. On the one hand, the possible differences between the m. erector spinae and the m. multifidus were investigated. On the other hand the possible differences between the patients with NSCLBP and the healthy controls were investigated. Furthermore, a possible correlation between muscle CSA and muscle fibre CSA was investigated.

5.1.1. Comparing m. multifidus with m. erector spinae

When comparing the muscle fibre type percentage and size between the m. multifidus and the m. erector spinae, differences were expected because of a different function. The literature states that the m. multifidus is the most important stabiliser of the lumbar spine whereas the m. erector spinae has a stabilising as well as a mobilising function (Danneels et al., 2000; Freeman et al., 2010; Kay, 2000; Nitz & Peck, 1986; Panjabi, 2003). This difference in functions is also reflected in different muscle fibre types. Muscle fibre type I consists of oxidative slow twitch muscle fibres which are associated with stabilising muscles, whereas muscle fibre type IIx and muscle fibre type IIa consist of glycolytic fast twitch muscle fibres, which are associated with mobilising muscles. All three muscle fibre types express one myosin heavy chain (MHC) isoform (A. F. Mannion, 1999). Furthermore, there are three hybrid types (IC, IIC and IIax), that express two MHC isoforms. The 'C fibres' consist of a combination of MHCI (slow twitch) and MHCIIa (fast twitch), which have both fast and slow twitch characteristics. The 'C fibres' can be divided into IC, which has a predominance of MHCI. The other type of 'C fibres' is IIC, which has a predominance of MHCIIa. The third hybrid type is a combination of MHCIIa and MHCIIx, called IIax muscle fibres. However, due to the procedure of our muscle fibre type colouring, only the hybrid muscle fibre type IIax could be distinguished (Staron, Herman, & Schuenke, 2012).

Based on this information a majority of muscle fibre type I and/or a larger muscle fibre size would be expected in favour of the m. multifidus when compared to the m. erector spinae in the healthy controls (A. F. Mannion, 1999). Results of this current study confirmed this

hypothesis with larger muscle fibre type I in favour of the m. multifidus in comparison with the m. erector spinae in healthy controls.

However, also in the patients with NSCLBP, a significant difference of muscle fibre type I CSA was found in favour of the m. multifidus when compared to the m. erector spinae. These findings are contradictory with the hypothesis of reflex inhibition. This hypothesis states that atrophy arises in the m. multifidus due to nerve inhibition. This would lead to a decrease in type I muscle fibres since its main function is stabilisation, which correlates with slow twitch muscle fibres. (Danneels et al., 2000; Freeman et al., 2010).

When looking at the proportional area and the muscle fibre type percentage, no significant differences were found in the current study between the m. erector spinae and the m. multifidus, neither in the patients with NSCLBP, nor in the healthy controls. In literature, the results about differences between the m. erector spinae and the m. multifidus are inconclusive. Jorgensen et al. (1993) found a significant difference in muscle fibre type percentage when comparing the m. erector spinae with the m. multifidus in cadavers of men, without a known history of LBP, as well as in healthy well trained men. In this study, more muscle fibre type I were found in the m. longissimus in comparison with the m. multifidus (Jorgensen, Nicholaisen, & Kato, 1993). However, no significant difference could be found in muscle fibre CSA when comparing the muscle fibre CSA of the m. multifidus and the m. longissimus. Another study investigated the difference between the m. multifidus and the m. longissimus in healthy subjects but failed to find any evidence of a difference between these two muscles (Thorstensson & Carlson, 1987) The results of Rantanen et al. (1994) were similar. This study investigated the difference between the m. multifidus and the m. iliocostalis in cadavers but also failed to find any evidence (Rantanen, Rissanen, & Kalimo, 1994).

5.1.2. Comparison of healthy controls with NSCLBP patients

When comparing the healthy controls with the NSCLBP group, significant differences were found in muscle fibre type percentage and proportional muscle fibre type of the m. erector spinae for muscle fibre type I and muscle fibre type IIx. When looking at these outcome measures in the m. multifidus, only a significant difference was found in muscle fibre type percentage, for muscle fibre type I and muscle fibre type IIx. The proportional area of the muscle fibre type was, in contrast with the m. erector spinae, not significantly different in the

m. multifidus. Remarkable is that this larger proportional area and percentage of muscle fibre type I were in favour of the NSCLBP group, this for the m. erector spinae as well as for the m. multifidus. In contrast, the CSA of muscle fibre type I for the m. multifidus was larger in favour of the healthy controls, although not significant. Given that the proportional area is a combination of the muscle fibre CSA and the muscle fibre type percentage, this can explain why the proportional area was not significantly different for the m. multifidus when comparing healthy controls with the NSCLBP group.

When comparing these results with the available literature, current findings are in the same line as Bajek et al. (2000) which investigated the m. multifidus in patients who underwent disc surgery in comparison with healthy subjects. In this research, a larger percentage of muscle fibre type I was found in favour of the LBP group (Bajek et al., 2000). In contrast with the current research and the latter, Mazis et al. (2009) and Mannion et al. (1997) found significantly more type I muscle fibres in healthy controls than in the LBP group (Mazis et al., 2009). Furthermore, Mannion et al. (1997) found significantly more type IIx muscle fibres in the LBP group whereas in the current research, the significant difference was found in favour of the healthy controls (A. F. Mannion, Weber, Dvorak, Grob, & Muntener, 1997). Crossman et al. (2004) could not find any significant alterations in muscle fibre type proportion or size of the paraspinal muscles between healthy men and men with LBP.

An explanation for these varying results can be found in the method used for counting muscle fibres. There are a few differences in the method followed by these studies (Bajek et al., 2000; A. F. Mannion et al., 1997; Mazis et al., 2009). First of all, the level of biopsy differed between these studies, ranging between L3 and S1. Secondly, there was a difference in the type of muscle fibres that were analysed. A third difference was the average amount of muscle fibres that were counted per sample. Bajek et al. (2000) counted between 400 and 1100 muscle fibres per sample, which was the highest amount of these three studies. Mazis et al. (2009) had an average of 100 muscle fibres that were counted per sample, which was the lowest number. In Mannion et al. (1997) an average of 170 muscle fibres were counted per sample, which was similar to the current study where an average of 182 muscle fibres per sample were counted. These variations could be explained by differences in the quality of the coupes.

Another study stated that there is atrophy of muscle fibre type I when comparing the healthy controls with the LBP group (F. A. Mannion, Dumas, Stevenson, & Cooper, 1998). Their

explanation for these results is that muscles with less muscle fibre type I would be less fatigue resistant. However, a few studies stated that it is mostly muscle fibre type II that gets effected in LBP patients (M. Mattila et al., 1986) (Mikko Mattila et al., 1986; Zhu, Parnianpour, Nordin, & Kahanovitz, 1989). This is in the same line as the results of the current study, where the percentage of muscle fibre type IIx was significantly lower in the NSCLBP group in comparison with the healthy controls for the m. multifidus. Furthermore, in the m. erector spinae, also a significant difference was found for proportional area and percentage of type IIx with less muscle fibres in the NSCLBP group when compared with the healthy controls.

This current study found only a significant change in muscle fibre type I and IIx percentage when comparing NSCLBP patients with healthy controls, with a higher amount of type I muscle fibres and a lower amount of type IIx muscle fibres in NSCLBP patients. A possible hypothesis is that patients with low back pain have a larger percentage type I because of a constant muscular contraction. Low back pain patients often suffer from mal-adaptive coping strategies such as fear avoidance and high levels of anxiety regarding their pain. These mal-adaptive coping strategies are associated with muscle guarding, keeping the muscle in a constant contraction (Main & Watson, 1996; O'Sullivan, 2005).

Another possible hypothesis is that of the muscle fibre shift. Three studies state that muscular atrophy starts with atrophy of fast-twitch muscle fibre size due to loss of motor units after which denervation occurs and in the last phase of this atrophic process the fast-twitch muscle fibre disappears. Thereafter, re-innervation of slow-twitch muscle fibres occurs with new motor units due to nerve plasticity. So the fast-twitch muscle fibre type percentage decreases and a muscle fibre type shift occurs in favour of the slow-twitch muscle fibres (Aoyagi & Shephard, 1992; Campbell, McComas, & Petito, 1973; Lexell, 1995). However, conflicting results can be found in the literature concerning this muscle fibre shift. Hence, it is not yet clear if immobilization leads to a transition of slow motor units to fast motor units or vice versa (Canepari, Pellegrino, D'Antona, & Bottinelli, 2010).

So far, it is not clear whether the observed shift in muscle fibre type is a result of the presence of low back pain or could be seen as the cause of it. Therefore, a third possible hypothesis is that patients with low back pain are born with a lower percentage type II muscle fibres and are thus more susceptible to develop low back pain. However, the literature is inconclusive whether atrophy is the result or cause of CLBP (Goubert et al., 2016).

5.1.3. Correlation between muscle CSA and muscle fibre CSA

When looking at the secondary outcome measures, there was only a correlation found in the healthy controls for the m. multifidus between the CSA of muscle fibre type IIax and the total CSA of the m. multifidus. However, when looking at the difference in total CSA of the m. erector spinae or the m. multifidus between the healthy controls and NSCLBP group, no significant differences were found. Undermining the hypothesis of muscle atrophy occurring in CLBP patients. These findings were in contrast with a recent review which concluded that there is atrophy of the m. multifidus in LBP (Tom A. Ranger et al., 2017). In the literature there is very little information about a possible correlation between the total muscle CSA and the muscle fibre CSA. However, Verdijk et al. (2010) investigated the m. vastus lateralis in older men and found a significant proportionate correlation between the total muscle CSA and the muscle fibre CSA of type I and the muscle fibre CSA of type II (Verdijk et al., 2010).

5.2. Reflection of strengths and weaknesses

There were a few limitations in the current research that may have affected the results. The sample size of the population consisted of 32 participants (NSCLBP: n= 19, healthy: n=13) and when investigating a possible correlation, there were only 25 participants (NSCLBP: n=13, healthy: n=12) taken into account. Due to the small sample size, the minimal detectable change value increases. Moreover, Cagnie et al (2010) concluded that there is evidence for differences in muscle fibre type depending on gender. Although baseline characteristics were comparable, when men have other muscle fibre characteristics than women, this could again affect the minimal detectable change value.

Furthermore, the duration of NSCLBP was not taken into account. Inclusion criteria specified NSCLBP complaints for longer than 12 weeks. However there might be a difference in muscle morphology between patients with NSCLBP for 12 weeks and patients with NSCLBP for several years. For example, there is moderate evidence for muscular atrophy in LBP patients in the m. multifidus (Tom A. Ranger et al., 2017). However it is not yet known if this muscular atrophy is the cause or the result of LBP (Goubert et al., 2016).

The measurements of the total muscle CSA were determined with ultrasound. Studies have shown that ultrasound is a reliable method for measuring the CSA of the m. multifidus in CLBP patients as well as in healthy subjects (Huang et al., 2014; Nabavi, Mosallanezhad,

Haghighatkah, & Mohseni Bandpeid, 2014; Sions, Velasco, Teyhen, & Hicks, 2015; Teyhen et al., 2011; Wallwork, Hides, & Stanton, 2007) Hides et al. (1995) showed that ultrasound measurements were equally accurate as MRI in determining the CSA of the m. multifidus. According to the study by Belavy et al. (2015) there is a moderate correlation between MRI and ultrasound measurements (Belavy, Armbrecht, & Felsenberg, 2015). However, due to low resolution, ultrasound doesn't take fat infiltration into account (Hides, Richardson, & Jull, 1995). This could be an explanation why in this study, no atrophy of the m. multifidus could be detected.

When looking at the results of the primary outcome measures, it must be taken into account that the quality of the coupes differed. This was due to errors in the cutting of the biopsies or errors in the colouring procedure. Therefore, the minimal count was lowered to 100 cells per coupe instead of the primary benchmark of 150 cells. Also the colour brightness differed per coupe, which made it harder to differentiate between muscle fibre type Iax and muscle fibre type Ix. The latter could also cause a problem in inter-rater reliability. Additionally, the measurements were followed by a short explanation. No training period was taken into account. Therefore a learning effect cannot be excluded and the intra-rater reliability could also be affected.

However, there are also several strengths of the current research. Measurements were taken by four independent and blinded assessors so no bias occurred due to expectations for a particular group or muscle. Furthermore the current study is the first one to take into account a hybrid muscle fibre type, namely muscle fibre type Iax. Also, the current research is the first to investigate a possible correlation between muscle fibre CSA and the total muscle CSA.

5.3. Recommendation for further research

Similar research with larger sample sizes is recommended. Since the available literature is inconclusive whether muscle fibre type I or muscle fibre type II is most affected by inactivity. Further research with more subjects could clarify which muscle fibre type is most affected in subjects with low back pain. Moreover, Appel et al (1990) stated that muscle fibre type I is most susceptible for atrophy (Appell, 1990). This is in contrast with the results of Mattilla et al (1986), where it is stated that muscle fibre type II is smaller due to a sedentary lifestyle and thus more affected by inactivity. Therefore it would also be interesting to do research with

inclusion of a training component to see if changes in muscle fibre types and symptoms could be reversed.

Since the current research could be influenced by measurement errors in the differentiation of muscle fibre type IIa, IIax and IIx, it could be interesting to look at muscle fibre type II as a whole or to work with an alternate colouring method. The latter could also be interesting to do research for alterations in the remaining hybrid muscle fibre types IC and IIC.

Since Cagnie et al. (2015) concluded that muscle fibre type significantly differs between men and women, it would be interesting to analyse results as a whole as well as in men and women separately. When looking at the current research, atrophy of the m. multifidus could not be proven. However, previous literature stated that there is moderate evidence for atrophy of the m. multifidus in patients with LBP when comparing with healthy subjects (Beneck & Kulig, 2012; Danneels et al., 2000; Wallwork et al., 2009). Therefore, further research with MRI could be interesting, not only since structure differentiation between muscle mass and fat infiltration is not possible with ultrasound but also because the literature so far is inconclusive about whether or not atrophy occurs. (Hides et al., 1995; T. A. Ranger et al., 2017).

6. CONCLUSION

This case control study was the first to take a hybrid muscle fibre type, namely muscle fibre type IIax, into account. Also correlations in back muscles between muscle fibre size and total muscle area were not measured before. Results of this case control study suggest that there's a significant difference in the CSA of muscle fibre type I, with a larger CSA of muscle fibre type I in the m. multifidus when comparing with the m. erector spinae. This was the case in the healthy controls as well as in the NSCLBP group.

Furthermore results suggest that NSCLBP patients have significantly more type I muscle fibres in comparison with healthy subjects. This in the m. multifidus as well as in the m. erector spinae. In the latter also the proportional area of muscle fibre type I was significantly larger for the NSCLBP group. Also percentages of muscle fibre type IIx were significantly larger in both muscles. However, this time in favour of the healthy subjects. The proportional area of muscle fibre type IIx was also significantly larger in the m. erector spinae in favour of the NSCLBP group when comparing with the healthy subjects.

However the colouring procedure could have had an influence on the results of the differences between muscle fibre type IIa, muscle fibre type IIx and muscle fibre type IIax. Therefore further research is recommended with a different colouring which could differentiate in all hybrid muscle fibre types or with the same colouring but without differentiation in variations of muscle fibre type II.

When looking at a possible correlation between muscle fibre size and total muscle area, there was only a correlation in the healthy subjects for muscle fibre type IIax. However this could again be due to measurement errors. Moreover, in this research there were no significant differences in total muscle area between the healthy controls and the NSCLBP group, neither in the m. multifidus, nor in the m. erector spinae. However previous literature stated that there is atrophy in the m. multifidus of LBP patients. These results suggest that further research needs larger sample sizes or more sophisticated measurement techniques like MRI to also be able to differentiate between muscle mass and fat infiltrations.

With current results two hypotheses could be formed about the differences between muscle fibre types when comparing healthy subjects with NSCLBP patients. The first hypothesis is that muscle fibre type I has larger muscle fibre sizes due to fear avoidance in low back pain patients,

since muscle fibre type I consists of slow and oxidative muscle fibre twitches, intended for long sustained contractions (A. F. Mannion, 1999).

The second hypothesis is that subjects with NSCLBP already start with a lower percentage of muscle fibre type II. This could be an explanation for the normal values in muscle fibre size of the muscle fibre type II. Since the process of atrophy normally begins with a decrease in muscle fibre size and this was not the case in the current study. However there are also studies suggesting that muscle fibre type II is more susceptible for atrophy after immobilization or due to sedentary lifestyles (Mikko Mattila et al., 1986). However, there are three studies arguing the opposite, namely that the muscle fibre type I is more susceptible for atrophy (F. A. Mannion et al., 1998). Therefore further research is recommended whether or not with the inclusion of training to see if this possible ongoing atrophic process is also reversible.

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8. APPENDIX

TABLES

Table 1 – Comparison of baseline characteristics

Table 2 – Comparison of m. multifidus versus m. erector spinae in healthy controls

Table 3 – Comparison of m. multifidus versus m. erector spinae in NSCLBP group

Table 4 – Comparison of Healthy group with NSCLBP group for the m. erector spinae

Table 5 – Comparison of healthy group with NSCLBP group for the m. multifidus

Table 6 – Correlation between CSA of m. multifidus and mean CSA of fibre type in healthy controls and NSCLBP group

Table 7 – Correlation between CSA of m. erector spinae and mean CSA of fibre type in healthy controls and NSCLBP group

Table 8 – Comparison between NSCLBP group and healthy controls of the CSA of m. multifidus

Table 9 – Comparison between NSCLBP group and healthy controls of the CSA of m. erector spinae

Table 10 – List of abbreviations

FIGURES

Figure 1 – Determination of the CSA of m. erector spinae via ultrasound imaging

Figure 2 – Verification of biopsy location through dissection

Figure 1 – Picture of fluorescence microscopy

TABLES

Table 1: Comparison of baseline characteristics

	Healthy (n=13)	NSCLBP (n=19)	P-value
Length (m)	1.7489231 ±0.0880601	1.7587 ±0.0839085	0.7505
Weight* (kg)	77.246154 ±13.752795	75.665 ±14.086435	0.4611
BMI* (kg/m²)	25.018308 ±3.0817527	24.4551 ±4.2423884	0.4725
Age* (Yr)	40.90328 ±8.5127427	44.712115 ±8.1003227	0.1787
Gender(f)**	7	10	0.7189

*= Wilcoxon rank sum test

**= χ^2 test

Table 2: Comparison of m. multifidus versus m. erector spinae in healthy controls

Healthy	Mean ES	SD	Mean MF	SD	P-value
I CSA*	6299.5343	±1640.7513	7619.2581	±2218.4661	0.0017
Ila CSA	5004.0126	±1307.2646	4989.16	±1878.3922	0.9718
Ilx CSA	4059.4764	±1622.8786	4000.8948	±1250.3277	0.7005
Ilax CSA	4485.4811	±1512.4308	3706.1902	±2165.7135	0.0637
% I	54.013968	±13.717734	53.775547	±14.71721	0.9562
% Ila	21.83564	±6.6340695	21.670068	±8.7190978	0.9696
% Ilx	14.57963	±11.163603	13.86307	±13.4726	0.8688
% Ilax	9.5707631	±9.4409035	10.691315	±7.2957348	0.7962
R% I**	61.273215	±12.612844	66.298851	±13.771462	0.2152
R% Ila	19.641764	±5.6952993	17.61714	±7.56652	0.6162
R% Ilx	10.97149	±9.6180521	9.2583185	±10.143708	0.6714
R% Ilax	8.1135307	±8.7253868	7.1273267	±5.1942333	0.8070

* CSA: cross sectional area, measured in μm .

** R%: proportional area

Table 3: Comparison of m. multifidus versus m. erector spinae in NSCLBP group

(NSCLBP)	Mean ES	SD	Mean MF	SD	P-value
I CSA*	6353.157	±1643.3334	7517.0194	±2586.6472	0.0451
Ila CSA	5396.6474	±3037.1499	5478.2403	±1779.7969	0.8883
Ilx CSA	4631.8919	±2303.2932	4027.1677	±2252.5024	0.3696
Ilax CSA	5137.2985	±2059.1486	4523.1778	±2032.7634	0.4053
% I	65.107042	±9.8426495	61.621801	±11.874334	0.2180
% Ila	20.675106	±8.3752404	22.90623	±10.743185	0.4296
% Ilx	5.7570356	±4.9862494	7.2304115	±7.5549766	0.6017
% Ilax	8.460816	±7.6334532	8.2415574	±6.2694305	0.9381
R% I**	71.149097	±8.3484785	70.060173	±12.205414	0.6750
R% Ila	17.690384	±7.5494163	19.497129	±10.788564	0.4869
R% Ilx	4.2864834	±3.5838353	4.6157777	±6.1689807	0.8991
R% Ilax	6.8745616	±6.2195523	5.8269204	±5.2022417	0.6867

* CSA: cross sectional area, measured in μm .

** R%: proportional area

Table 4: Comparison of Healthy group with NSCLBP group for the m. erector spinae

(M. Erector spinae)	Mean Healthy	SD	Mean NSCLBP	SD	P-value
I CSA*	6299.5343	±1640.7513	6353.157	±1643.3334	0.9421
Ila CSA	5004.0126	±1307.2646	5396.6474	±3037.1499	0.5956
Ilx CSA	4059.4764	±1622.8786	4631.8919	±2303.2932	0.4247
Ilax CSA	4485.4811	±1512.4308	5137.2985	±2059.1486	0.5048
% I	54.013968	±13.717734	65.107042	±9.8426495	<0.0001
% Ila	21.83564	±6.6340695	20.675106	±8.3752404	0.6819
% Ilx	14.57963	±11.163603	5.7570356	±4.9862494	0.0022
% Ilax	9.5707631	±9.4409035	8.460816	±7.6334532	0.6951
R% I**	61.273215	±12.612844	71.149097	±8.3484784	<0.0001
R% Ila	19.641764	±5.6952993	17.690384	±7.5494163	0.4387
R% Ilx	10.97149	±9.6180521	4.2864834	±3.5838353	0.0088
R% Ilax	8.1135307	±8.7253868	6.8745616	±6.2195523	0.6227

* CSA: cross sectional area, measured in μm .

** R%: proportional area

Table 5: Comparison of healthy group with NSCLBP group for the m. multifidus

(M. Multifidus)	Mean Healthy	SD	Mean NSCLBP	SD	P-value
I CSA*	7619.2581	±2218.4661	7517.0194	±2586.6472	0.8880
Ila CSA	4989.16	±1878.3922	5478.2403	±1779.7969	0.5019
Ilx CSA	4000.8948	±1250.3277	4027.1677	±2252.5024	0.8555
IlaX CSA	3706.1902	±1265.7135	4523.1778	±2032.7634	0.2897
% I	53.775547	±14.71721	61.621801	±11.874334	0.0158
% Ila	21.670068	±8.7190978	22.90623	±10.74185	0.7003
% Ilx	13.86307	±13.4726	7.2304115	±7.5549766	0.0406
% IlaX	10.691315	±7.2957348	8.2415574	±6.2694305	0.4460
R% I**	66.298851	±13.771462	70.060173	±12.205414	0.2721
R% Ila	17.61714	±7.56652	19.497129	±10.788565	0.5802
R% Ilx	9.2583185	±10.143708	4.6157777	±6.1689807	0.1775
R% IlaX	7.1273267	±5.1942333	5.8269204	±5.2022417	0.7016

* CSA: cross sectional area, measured in μm .

** R%: proportional area

Table 6: Correlation between m. multifidus CSA and mean CSA of muscle fibre type in healthy controls and NSCLBP group

Healthy controls	P-value	ρ	NSCLBP	P-value	ρ
CSA type I	0.4138	0.2604	CSA type I	0.2973	0.3132
CSA type IIA	0.0551	0.5659	CSA type 2A	0.1848	0.3923
CSA type IIX	0.0117	0.6973	CSA type 2AX	0.3857	0.2757
CSA type IIX	0.2550	0.3977	CSA type 2X	0.0681	0.6315

Table 7: Correlation between m. erector spinae CSA and mean CSA of muscle fibre type in healthy controls and NSCLBP group

Healthy controls	P-value	ρ	NSCLBP	P-value	ρ
CSA type I	0.8313	0.0690	CSA type I	0.2583	0.3383
CSA type IIA	0.6454	0.1484	CSA type 2A	0.8366	0.0636
CSA type IIX	0.9360	0.0260	CSA type 2AX	0.5684	0.1936
CSA type IIX	0.8702	-0.0559	CSA type 2X	0.5827	0.1866

Table 8: Comparison between NSCLBP group and healthy controls of the CSA of m. multifidus

	NSCLBP	Healthy	P-value
Mean	6.8069231	6.9833333	0.8172
SD	1.6946946	2.0726414	

Table 9: Comparison between NSCLBP group and healthy controls of the CSA of m. erector spinae

	NSCLBP	Healthy	P-value
Mean	15.723077	16.533333	0.6798
SD	3.4871559	5.9792571	

Table 10: List of abbreviations

Abbreviation	Full term
BMI	Biomass index
CT	Computed tomography
CSA	Cross-sectional area
LBP	Low back pain
L	Lumbar level
MRI	Magnetic resonance imaging
M	Musculus
NSCLBP	Non-specific chronic low back pain

FIGURES

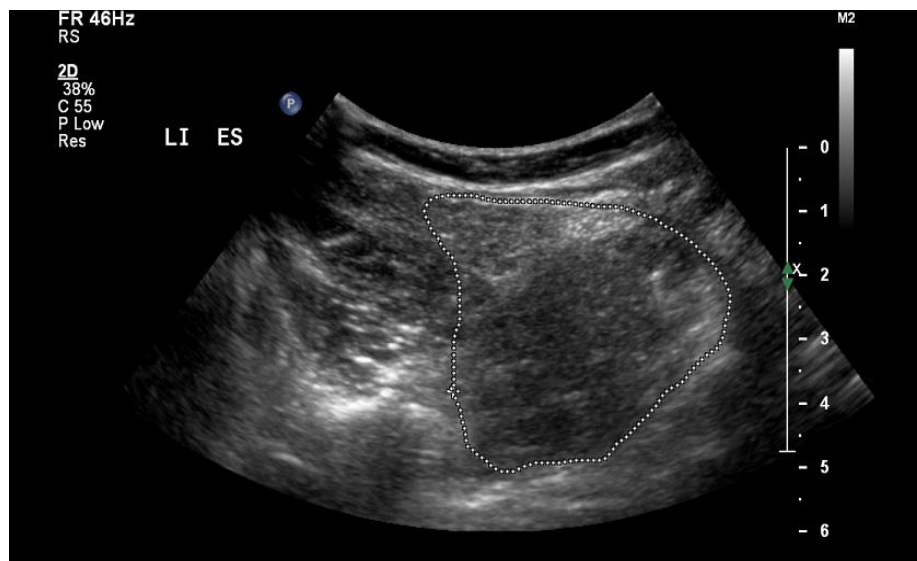


Figure 2: Determination of the CSA of m. erector spinae via ultrasound imaging

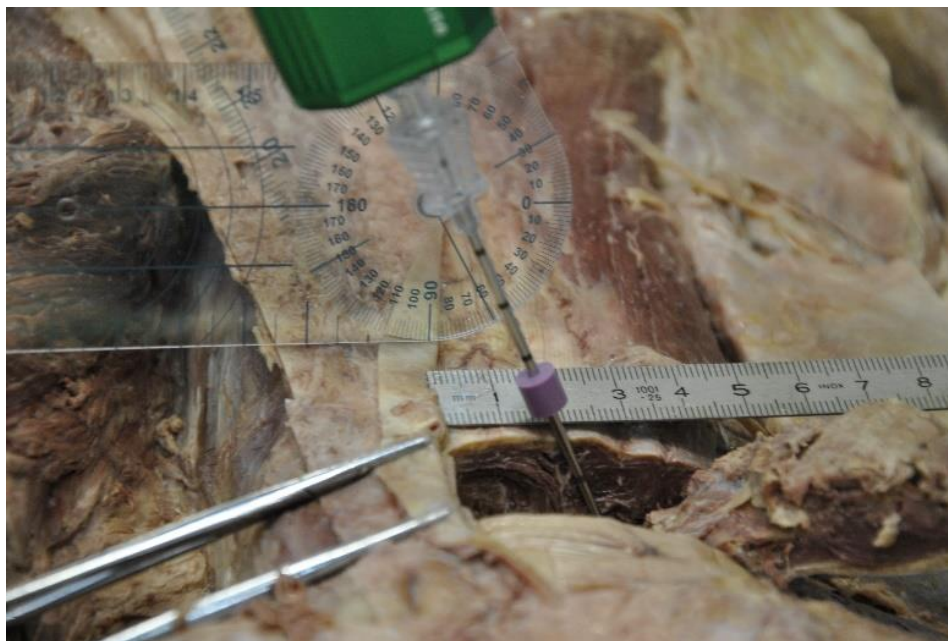


Figure 3: Verification of biopsy location through dissection

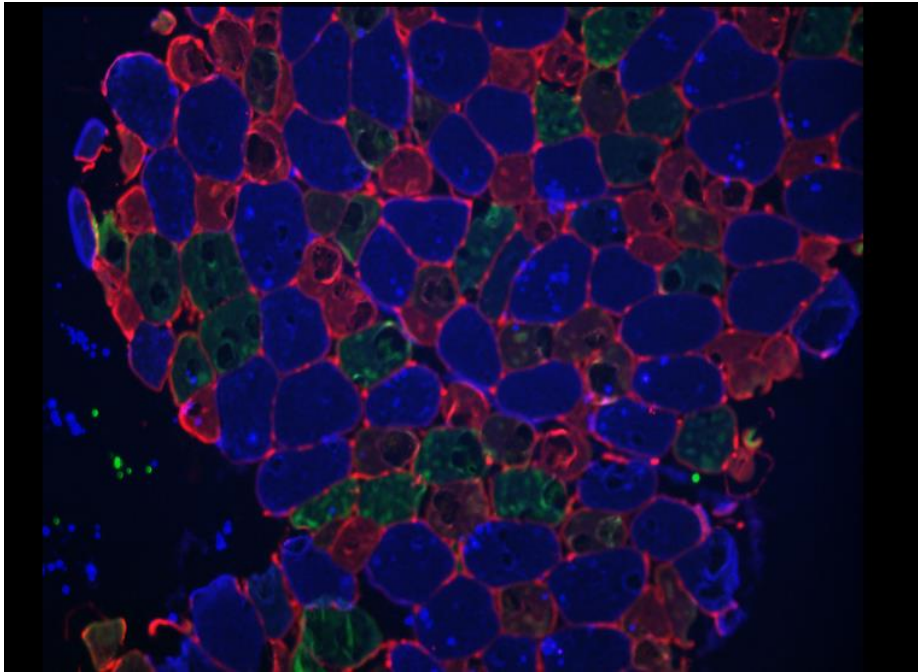


Figure 4: Picture of fluorescence microscopy

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DATUM	INHOUD OVERLEG	HANDTEKENINGEN
14/12/17	Overleg doel en opbouw thesist	Promotor: Copromotor: Student(e): Student(e):
21/12/17	Overleg thesist tellen met Axiovision	Promotor: Copromotor: Student(e): Student(e):
13/2/18	Kleuringen thesist (Tne)	Promotor: Copromotor: Student(e): Student(e):
27/2/18	Overleg statistiek	Promotor: Copromotor: Student(e): Student(e):
6/3/18	Overleg statistiek	Promotor: Copromotor: Student(e): Student(e):
7/3/18	Meervolgen Bloppen (Teile en Tne)	Promotor: Copromotor: Student(e): Student(e):
20/3/18	Kleuringen thesist (Teile)	Promotor: Copromotor: Student(e): Student(e):
		Promotor: Copromotor: Student(e): Student(e):
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