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Faculteit Revalidatiewetenschappen

master in de revalidatiewetenschappen en de kinesitherapie

Masterthesis

Muscle characteristics in ambulatory patients with Multiple Sclerosis and the effect of exercise training

Lena Fonteyn
Jonas Ruyters

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

PROMOTOR :
Prof. dr. Bert OP 'T EIJNDE

COPROMOTOR :
De heer Jan SPAAS

2019
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www.uhasselt.be
Universiteit Hasselt
Campus Hasselt:
Martelarenlaan 42 | 3500 Hasselt
Campus Diepenbeek:
Agoralaan Gebouw D | 3590 Diepenbeek

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Muscle characteristics in ambulatory patients with Multiple Sclerosis and the effect of exercise training.

The primary objective of the research was to find possible changes in muscle characteristics of persons with MS. The secondary objective was to examine the effects of a HICT exercise intervention of muscle characteristics and clinical outcomes of MS patients.

Master thesis part two

Lena Fonteyn (1541749) & Jonas Ruyters (1539877)

Promotor: Prof Dr. Op ’t Eijnde Bert

Copromotor: Drs. Spaas Jan

Hasselt University

Highlights:

1. This research found a significant difference in the capillary density, as well in VO_{2max} and W_{max} of mildly affected patients compared to healthy, but sedentary subjects.
2. There were no significant correlations found between MS and fiber CSA, minimal Feret diameter, fiber distribution, oxidative capacity or capillarity.
3. The VO_{2max} and W_{max} of MS patients improved significantly after a HICT, but no significant changes in human muscle characteristics were found. Perhaps other determinants, not studied in this research, could explain these improvements.

Acknowledgement

First and foremost, we want to give our gratitude to our promotor Prof. Dr. Op ‘t Eijnde Bert and copromotor Drs. Spaas Jan for the guidance during this project. We would like to thank Prof. Dr. Op ’t Eijnde Bert for his acknowledgments and support. Thanks to Drs. Spaas Jan for his critical eye and follow up. Aided by their guidelines**,** patience and support we were able to deliver our master thesis. Furthermore, we like to thank all of the MS patients, as well as the healthy controls, for participating in this study by donating muscle samples.

Next, we like to thank our parents for reading our thesis and giving their support.

Peperstraat 83, 3080 Tervuren, 29/05/2020 L.F.

Ketelbutersstraat 16, 3990 Peer, 29/05/2020 J.R.

Research context

MS is a chronic autoimmune disease of the central nervous system (CNS) (Iwanowski et al., 2019) and has an impact on all aspects of the patients' daily life (Compston & Coles, 2008). Not only disorders of the sensorimotor, visual, cognitive and autonomous systems are common in MS, but also secondary complaints such as reduced physical activity levels, loss of muscle mass and force (Keegan, & Noseworthy., 2002). While the effects on the neurologic system have been extensively studied, the effects on the muscle characteristics have not. Recently, more attention was brought to possible effects of exercise on the course of the disease**.** Multiple studies have found positive results after exercise interventions on contractile characteristics and muscle fiber size. (Dalgas et al. 2010, Keytsman et al. 2019, Wens et al. 2015a). However, all effects on muscles have to be clearly described to improve exercise therapy. Specific exercise interventions have to be set up, with a higher quality of life as one of many goals.

This master thesis fits in the domains of cardiorespiratory and neurological rehabilitation. It is a duo thesis of Lena Fonteyn and Jonas Ruyters and is executed under supervision of promotor Prof. Dr. Op 't Eijnde Bert and copromotor Drs. Spaas Jan. Being a part of the research project "Carnosine in neuroinflammation and demyelination: a versatile dipeptide to halt damage and boost repair in Multiple Sclerosis? ", this study examined the effects of MS on human muscle characteristics (project code R-10493, Hasselt University).

The research question was determined in cooperation with the promotor and copromotor. Muscle samples used in this research were received from the studies of Keytsman et al. (2017) and Keytsman et al. (2019). Samples were already available and coloured. The data analysis, the statistics and the data description were performed by the two students. The central format was applied in the thesis.

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Table of Contents

1. Abstract

Background: Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) that results in a diminished physical fitness and quality of life (QoL). Effects of MS on muscle characteristics have not been extensively studied. Understanding consequences of MS on muscles may help creating specific interventions with an improvement in clinical outcomes and QoL as a goal.

Objectives: Examining differences in fiber properties, oxidative capacity and capillarity, through comparing MyHC, SDH and CD31 stained samples between MS and healthy controls (HC). Correlations between clinical outcomes, subject characteristics and muscle characteristics were investigated. Furthermore, effects of a High Intensity Concurrent Training (HICT) intervention on these properties were examined in MS patients.

Participants: Muscle samples of sixteen MS patients and fifteen HC were analysed. Afterwards, muscle samples of six MS patients who participated in an exercise intervention were examined.

Measurements: Primary outcome measures were fiber type distribution, fiber CSA, minimal Feret diameter, oxidative capacity, capillary density and capillary/fiber ratio. Furthermore, maximal oxygen uptake (VO_{2max}) and the maximal cycling resistance (W_{max}) were investigated during a maximal graded exercise test.

Results: A significant difference in capillary density, VO_{2max} and W_{max} was found between HC and MS patients ($p<0.05$). Type I fibers had a significantly higher CSA, min. Feret diameter and fiber type distribution than type IIa and IIa+x fibers ($p<0.05$) in both MS patients and HC. No correlation between EDSS score and muscle properties was found. Secondly, VO_{2max} and W_{max} improved significantly ($p<0.05$) after the intervention. However, no improvements in muscle characteristics were observed.

Conclusion: The capillary density, VO_{2max} and W_{max} seemed significantly affected by MS. No further effects were found. However, correlations between BMI - which is influenced by the disease - and muscle properties were observed. HICT improved VO_{2max} and W_{max} significantly, while no changes were observed in muscles.

2. Introduction

Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system (CNS). It is characterized by an inflammatory reaction, where lymphocytes migrate from the lymphatic nodes into the blood circulation and target myelin antigens of the CNS. This causes demyelination, axonal degeneration and leads to neurodegeneration. (Pelletier & Hafler. 2012) MS is presumed to be the most occurring neurodegenerative disease (Wildner, Stasiołek, & Matysiak. 2019) and predominantly affects young to middle-aged adults. Impairments of the sensorimotor, visual, cognitive and autonomous systems are common (Keegan, & Noseworthy. 2002). A decreased muscle strength, fatigue, pain and fear of movement are some of the numerous factors that contribute in a decline of quality of life and physical activity (van den Akker et al. 2018; Ellis & Motl. 2013). It can lead to muscular atrophy in combination with the reduced neural drive (Kent-Braun et al. 1997*)*.

In other pathologies, such as Chronic Obstructive Pulmonary Disease (COPD), effects of the disease on muscle properties and possible effects of exercise training have been extensively investigated. Dysfunctions such as muscle atrophy, a shift in fiber type, a poor oxidative capacity, mitochondrial dysfunction and a decrease in muscle force were concluded in the review of Maltais et al. (2013) when investigating the lower limbs. Possible consequences of these dysfunctions are difficulties engaging in physical activity, low exercise tolerance, poor quality of life and higher morbidity. Interestingly, such dysfunctions can be targeted with specific physical rehabilitation programs. In MS however, multiple aspects of muscle (dys)function as well as their relation with overall physical function remain not yet fully understood, which hinders the development of such rehabilitation strategies (Kent-Braun et al. 1997; Wens et al. 2014; Wens et al. 2015a). Pathology specific exercise programs have to be created to improve muscle force, cardiorespiratory fitness and physical activity. These programs may improve the quality of life, reduce cardiovascular risk and mortality (Wens, Dalgas, Stenager, & Eijnde. 2013).

Skeletal muscle characteristics were previously investigated in the animal model of MS, namely Experimental Autoimmune Encephalomyelitis (EAE). The previously executed literature study concluded a shift in muscle fiber types and a decrease of fiber quality. This may have an impact on the muscle function. In addition, a decrease in muscle fiber cross sectional area (CSA) and weight may explain the diminished force in EAE animals. Wens et al. (2015a) and Dalgas et al. (2010) conducted two of the few studies of the possible effects on human muscle properties. Both studies investigated the effect of exercise on fiber CSA, fiber distribution and muscle strength of MS patients. Positive results in favor of exercise were found on contractile characteristics, muscle fiber size and endurance capacity. However, these studies used different exercise therapies and modalities thus no consensus can be reached.

The first aim of this study was to find possible changes in muscle characteristics of persons with MS. Therefore, this study investigated the muscle fiber type composition, fiber CSA, oxidative activity and capillarity in the vastus lateralis muscle in MS patients and healthy controls. Furthermore, correlations between clinical outcomes (exercise capacity, disease severity) and these muscle properties were investigated.

The second aim was to examine the effects of a High Intensity Concurrent Training (HICT) on muscle properties and clinical outcomes of MS patients.

We hypothesized that a shift in fiber type distribution, from type I to type II fibers, was present. Namely, a higher type II fiber distribution would be observed in MS patients. Subsequently, a decrease in fiber CSA and minimal feret diameter would be present.
For the secondary research question, positive effects on muscle properties and clinical outcomes of MS patients would be observed.

3. Methods

3.1 Design

3.1.1 Part 1

Appertaining to the first research question, muscle samples of persons with MS were compared to samples of healthy controls (HC). Following outcomes were compared: 1) muscle fiber type, 2) muscle fiber CSA, 3) muscle fiber minimal Feret diameter, 4) muscle fiber oxidative capacity, 5) capillary density and 6) capillary/fiber ratio. Furthermore, following clinical outcomes were investigated during a maximal graded exercise test: 1) maximal oxygen uptake (VO_{2max}) and 2) maximal cycling resistance (W_{max}). In addition, correlations between above mentioned cardiovascular parameters, muscle characteristics and patient characteristics were examined.

3.1.1 Part 2

After the baseline measurements, six persons with MS conducted an exercise program from whom another muscle biopsy was extracted. The same muscle characteristics as above were compared before and after the intervention.

The studies who supplied the muscle samples were approved by the local Ethical Committee of the Jessa hospital and Hasselt University, and were performed in accordance with the Declaration of Helsinki. The studies were registered at ClinicalTrials.gov (NCT02466165).

3.2 Subjects

3.2.1 Part 1. Subjects

Muscle samples were obtained from MS patients (n=16) and HC (n=15). Both groups were matched for age, height, weight, smoking behavior and body mass index (BMI).

Participants were excluded if they participated in another study, had (in case of MS) an acute exacerbation six months prior to the start of the study or had an EDSS score >6 (Expanded Disability Status Scale). Furthermore, the Physical Activity Scale for Individuals with Physical Disabilities (PASIPD) was used to measure physical activity of all participants (Washburn et al. 2002). It is a 13-item questionnaire about recreational, occupational and household activities over the past seven days. Scores are the average hours of participation daily multiplied with a metabolic equivalent value.

3.2.2 Part 2. Subjects

Afterwards, six persons of the MS group were withheld to participate in an exercise program intervention.

3.3 Muscle sampling

Vastus lateralis muscle samples were directly obtained after baseline measurements and after the exercise program of twelve weeks. Muscle biopsies from MS patients were obtained from the weakest leg, biopsies from HC were obtained from a random leg. One muscle sample was taken per participant. Tissue sampling was performed by an experienced biomedical doctor. First, fibers were aligned before the samples were embedded in Tissue Tek OCT. After the embedment, samples were frozen in liquid N_2-cooled isopentane to ensure the conservation of the muscle structures and stored at -80°C. Afterwards, samples were prepared for immunostaining by cutting cryosections of 10 µm with the cryostat. Sample preparation and staining was performed by an experienced researcher, who was not blinded.

3.4 Analysis of muscle properties

Similar immunofluorescence analyses of muscle samples were performed in part 1 and 2.

3.4.1 Muscle fiber type composition and size (MyHC staining)

After preparation, cryosections were stored at -20°C. Immunofluorescence analysis was used to visualize different fiber types via Myosin Heavy Chain (MyHC) expression**.** Antibodies BA-F8, SC-71, 6H1 were purchased from the Developmental Studies Hybridoma Bank (DSHB). Laminin and secondary antibodies were purchased from ThermoFisher**.**

Cryosections were dried for ten minutes and circled with a hydrophobic DAKO pen. Subsequently, samples were washed in phosphate buffered saline (PBS) with 0.05% triton (PBST) for five minutes. Next, sections were blocked using 10% goat serum (GS) in PBST for 60 minutes. Primary mouse-anti-mouse (BA-F8, IgG2B; SC-71, IgG1; 6H1, IgM. 1/100) and rabbit-anti-mouse (laminin, IgG. 1/200) antibodies were diluted in PBST with 10% GS and applied for 120 minutes. Samples were washed three times for five minutes in PBST. Hereafter, secondary antibodies, in PBST with 10% GS, were added for 60 minutes. Secondary antibodies were Alexa Fluor (AF) 350, AF488 and AF555 (1/250) and AF 647 (1/250). These antibodies were complementary with the primary antibodies (Table 1). Sections were washed three times for five minutes in PBST. After washing, cryosections were dried, mounted and stored in 4°C. Complete protocol was performed at room temperature.

Table 1. List of antibodies and cocktail configurations used for MyHC staining of human skeletal muscle

Primary antibodies	Host species	MyHC reactivity	Secondary antibodies
BA-FB (1/100)	Mouse	I	AF 350 IgG2B (1/250)
SC-71 (1/100)	Mouse	IIa	AF 488 IgG1 (1/250)
6H1 (1/100)	Mouse	IIx	AF 555 IgM (1/250)
Laminin (1/200)	Rabbit	Cell border	AF 647 IgG (1/250)

BA-F8 was added for the detection of type I oxidative fibers. For the detection of the type IIa or intermediate glycolytic fibers, SC-71 was added. 6H1 was added for the detection of type IIx or glycolytic fibers. Laminin was added to every sample to stain cell borders.

Samples were visualized using a Leica fluorescence microscope. Fluorescence signals were recorded using DAPI (MyHC-I), FITC (MyHC-IIa), TRITC (MyHC-IIx), and CY5 (laminin) filters with a Leica DM4000 B LED microscope and a Leica EL6000 external light source (Leica Microsystems, Wetzlar, Germany). Samples were imaged at 10x magnification for quantitative analysis.

3.4.2 Muscle fiber oxidative capacity (SDH staining)

Immediately after cutting, cryosections were air dried for 15 minutes. Thereafter, they were embedded for 20 minutes in a solution of sodium phosphate buffer (SPB, 37mM, pH 7.6), sodium succinate (74mM) and titanium (IV) butoxide (TNBT, 0.4mM). Here, succinate is oxidized to fumarate by the mitochondrial succinate dehydrogenase (SDH) enzyme. Muscle fibers stained blue due to the reaction, with more intense (dark blue) staining indicating higher levels of fumarate and therefore SDH activity. The reaction was stopped by submerging the coupes in an HCl (0.01M) solution. Stained muscle sections were stored at 4°C and images were taken within ten days after staining with a 660nm filter (absorbance, grey value images).

3.4.3 Muscle capillarity (CD31 endothelial staining)

After preparation, coupes were stored at -20°C. Sections were dried (30 min) and washed (PBS, 5 min). Hereafter, primary mouse-anti-CD31 (DAKO, M0823, clone JC70A, IgG1; 1/100) and rabbit-anti-laminin (Sigma L9393; 1/200) antibodies were added overnight at 4°C, diluted in 1% BSA in PBS. Coupes were washed three times in PBS for five minutes. Secondary goat-anti-mouse (AF 488 IgG, 1/250) and goat-anti-rabbit (AF 555 IgG, 1/250) antibodies were diluted in 1% BSA in PBS and added for 60 minutes at room temperature. Afterwards, coupes were washed three times for five minutes in PBS. DAPI was added for ten minutes to stain cell nuclei. Hereafter, coupes were washed again in PBS (3x5min) and mounted.

Samples were visualized using a Leica fluorescence microscope. Fluorescence signals were recorded using a Leica DM4000 B LED microscope and a Leica EL6000 external light source (Leica Microsystems, Wetzlar, Germany). Samples were imaged at 10x magnification for quantitative analysis.

Muscle fiber composition, fiber size, SDH activity and capillarity were analysed using the *ImageJ Fiji software*. Both researchers worked in a standardized manner, following written instructions. Data-extraction and analysation was performed by both researchers. Blinding was not possible for data-extraction and analysis.

3.4.4 Part 2. High Intensity Concurrent Training

Six MS patients were withheld to participate in an exercise intervention, appertaining to the second research question. Patients followed an endurance training combined with force training, namely a High Intensity Concurrent Training (HICT). The endurance training was performed on a cycle ergometer, under supervision, over a period of 12 weeks and took place five times per two weeks. Furthermore, strength training of the lower limbs was performed (leg press, leg extension and leg curls). The intensity and volume increased throughout the training program from 1 x 10 repetitions to 2 x 20 repetitions, with a specific maximal load for each individual.

4. Outcomes

4.1 Primary outcome measures

Outcomes for fiber type composition analysis were subtracted from MyHC stained muscle sections. At least 150 fibers were analysed per muscle sample series per patient. MyHC fiber types (I, I+IIa, IIa and IIa+x) were manually outlined, yielding muscle fiber types, proportions, CSA (μm^2) and minimal Feret diameter (µm). Hybrid fiber type I+IIa was analysed, but not interpreted in the results because of the negligible amount present.

The muscle fiber oxidative activity of the different fiber types (I, IIa, IIa+x) was manually subtracted by matching the same SDH stained section with the analysed MyHC stained section. Researchers made sure that at least 20 type I and IIa fibers and 15 IIa+x fibers were analysed per sample. The integrated density (IntDen) of the grey value was used as a measure of oxidative capacity. After subtraction, outcomes were divided by 12.000 to correct for tissue thickness (10µm) and incubation time (1200s). The muscle fiber oxidative capacity is expressed as ΔA660/µm tissue/s.

Capillary density was measured by dividing the number of capillaries with the mm^2. An area of at least 100 fibers was investigated. Next, the capillary to fiber ratio was measured by dividing the number of capillaries by the number of muscle fibers in this area.

4.2 Secondary outcomes

A maximal exercise test was performed at baseline and after the HICT training, corresponding to the first and second research question respectively. During the first minute of the maximal test, participants started at 20 Watt (W) and 30 Watt for females and males, respectively. Thereafter the workload increased by 10W (female) and 20W (male) per minute until exhaustion. The maximal oxygen uptake (VO_{2max}, mL/kg/min) and maximal cycling resistance (W_{max}, Watt) obtained during the exercise test were used as clinical outcomes.

5. Statistical analysis

All data were analysed using SAS JMP version PRO 14. All data are presented as means ± SD. The threshold for statistical significance is set at $p<0.05$. Before the statistical analysis, data was checked for normality (Shapiro-Wilk test), homoscedasticity (Brown-Forsythe test) and independence.

5.1 Part 1

To compare baseline characteristics and outcome measures, normally distributed data were subjected to an unpaired student's t-test to analyse the mean differences between MS patients and HC. If data was not normally distributed, the Wilcoxon Rank Sum test was used. Secondly, a mixed model (group x fiber type) analysis was performed with fiber CSA, minimal Feret diameter, fiber distribution and oxidative capacity. MS patients and HC were chosen as groups. Fiber types were selected as random effects. Lastly, correlations between primary outcomes, clinical outcomes and patient characteristics in both groups were analysed using the Spearman and Pearson correlation for non-parametric and parametric data, respectively.

5.2 Part 2

Pre-post differences in the MS group were analysed using a Wilcoxon Rank Sum test ($n<10$).

To familiarize with the image analysis and to minimize measurement errors, both researchers analysed the full data set (MyHC, SDH and CD31 staining). Afterwards, the analysis of the CD31 stained samples of both data sets was compared with each other using an unpaired student's t-test. Subsequently, a representative group ($n=5$) of the analysed data of MyHC and SDH stained samples was compared using a Wilcoxon Rank Sum test.
Since no significant difference was found between the analysed data set (CD31) and representative group (MyHC and SDH) of the two researchers ($p>0.05$), it can be concluded that the data was comparable to each other and only one data set was used for statistical analysis of MyHC, SDH and CD31 data.

6. Results

MyHC, SDH and CD31 staining

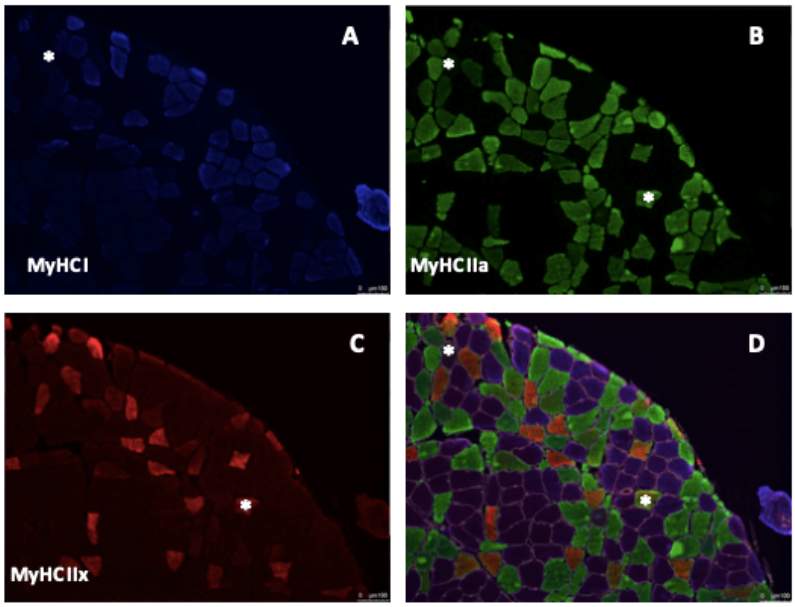

Figure 1. *Representative images of a human vastus lateralis muscle showing MyHC expression. In panel D, all fibers are shown through an overlay. Hybrid fibers, such as type I+IIa (blue and green) and IIa+x (green and red) are shown by a *. Scale bar represents 1*

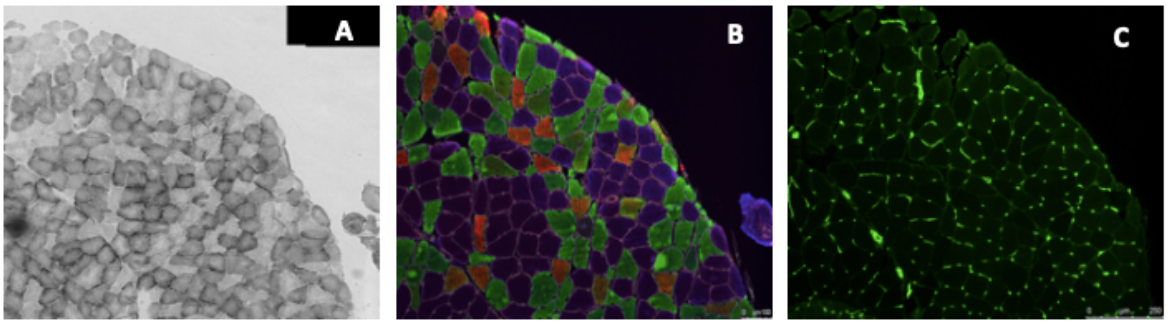

Figure 2*. Representative images of a human vastus lateralis muscle showing SDH (A) and CD31 (C) expression. In panel A, the section is showing SDH activity staining. Panel B was used to identify and compare activity staining per fiber type. In panel C, the section is showing capillaries through the light green colour. Scale bar represents 250 µm.*

6.1 Part 1. Muscle characteristics of MS patients and HC

Table 2. **Subject characteristics**

	MyHC		SDH		CD31	
	MS (n=16)	**HC (n=15)**	**MS (n=12)**	**HC (n=15)**	**MS (n=16)**	**HC (n=15)**
Gender (m/f)	6/10	6/9	6/6	6/9	6/11	6/9
Age (y)	54 ± 7	49 ± 11	55 ± 8	49 ± 11	54 ± 7	49 ± 11
EDSS	3.41 ± 1.36	/	3.67 ± 1.40	/	3.35 ± 1.33	/
Weight (kg)	68 ± 11	67 ± 12	67 ± 12	67 ± 12	67 ± 11	67 ± 12
Length (m)	1.69 ± 0.08	1.69 ± 0.09	1.71 ± 0.08	1.69 ± 0.09	1.68 ± 0.08	1.69 ± 0.09
BMI (kg/m^2)	23.89 ± 3.73	23.61 ± 2.32	23.08 ± 2.08	23.61 ± 2.32	23.88 ± 3.61	23.60 ± 2.32
PASIPD	14.43 ± 11.04	19.40 ± 11.89	13.43 ± 8.70	19.40 ± 11.89	15.40 ± 11.43	19.40 ± 11.89
Smoke (yes/no)	3/13	2/9	2/9	2/9	3/14	2/9
RRMS	11	/	7	/	11	/
SPmS	4	/	4	/	5	/
PPmS	1	/	1	/	1	/

Abbreviations: m (male), f (female), BMI (body mass index), EDSS (expanded disability status scale), RRMS (relapse remitting MS), SPMS (secondary progressive MS), PPMS (primary progressive MS)
Note: data are expressed as means ± SD and represent characteristics of MS patients and healthy controls.

Subject characteristics displayed in table 2 did not differ significantly between groups ($p>0.05$), except for VO_{2max} and W_{max} ($p<0.05$) (table 3). One person of the HC group was excluded due to missing baseline data. Sixteen MyHC, twelve SDH and fifteen CD31 samples of MS patients were analysed.

The majority of patients suffered from relapse remitting MS (n=11), five from secondary progressive MS and one patient was diagnosed with primary progressive MS. The mean EDSS score of all patients 3.46, with a score range of 2 to 6. Furthermore, fifteen HC samples were used for all analyses. Mean PASIPD score was 14.42 for MS patients and 19.4 for HC.

Table 3. **Quantitative analysis of the vastus lateralis muscle and clinical outcomes of MS patients and healthy controls**

	Type I		Type IIa		Type IIa+x	
	MS	**HC**	**MS**	**HC**	**MS**	**HC**
CSA (μm^2)	5105 ± 1844	4839 ± 1072	4650 ± 2045	4077 ± 1393	3330 ± 1676	3117 ± 1803
Feret diameter (µm)	105.7 ± 23.8	102.9 ± 11.5	102.9 ± 23.8	95.9 ± 16.8	88.5 ± 23.2	83.7 ± 21.7
Fiber distribution (%)	39.6 ± 12.0	41.8 ± 11.6	35.1 ± 13.3	36.9 ± 9.6	27.5 ± 10.6	21.0 ± 8.2
SDH (ΔA660/µm/s)	0.087 ± 0.028	0.091 ± 0.028	0.054 ± 0.024	0.059 ± 0.02	0.037 ± 0.017	0.033 ± 0.017
	MS		**HC**			
Capillary density (mm^{-2})	241 ± 78*		311 ± 62*			
Capillary/fiber ratio	1.442 ± 0.442		1.499 ± 0.395			
VO_{2max} (mL/min/kg)	21.77 ± 7.87*		36.28 ± 6.35*			
W_{max} (Watt)	107.67 ± 46.94*		188.00 ± 57.19*			

Abbreviation: CSA (cross-sectional area)
Note*:* data are expressed as means ± standard deviation. * is used to indicate a significant difference ($p<0.05$).

A negligible amount of hybrid fiber I+IIa was found in 48% of all muscle samples and was not included in the analysis. An average of 323.6 ± 117.3 fibers was analysed (data not shown). Two data points of the analysis of capillary density were excluded as statistically significant outliers.

When comparing means of both groups (table 3), no significant results were found for fiber CSA, minimal Feret diameter, fiber distribution, oxidative capacity and c/p ratio. A trend ($p=0.079$) was observed of a higher type IIa+x fiber distribution in MS patients. Moreover, the capillary density was significantly higher in HC vs MS patients ($p=0.0134$).

Subsequently, a mixed model analysis was performed. No interaction effects were found between fiber CSA, min. Feret diameter or fiber distribution and groups. Moreover, no group effect was observed, indicating that these outcomes were similar for MS patients and HC (Figure 3). Fiber types, however, differed significantly for these outcomes. For fiber CSA, type I fibers differed from type IIa+x fibers ($p<0.0001$) and type IIa fibers ($p=0.0361$). A significant difference was also found between type IIa fibers and type IIa+x ($p<0.0001$). When observing the min. Feret diameter of type I and IIa fibers, same results were found. The Feret diameter of type I and type IIa fibers were higher than type IIa+x ($p<0.0001$). The fiber distribution was also significantly different between fiber types. Type I and type IIa were higher than type IIa+x, with $p<0.001$ and $p=0.002$, respectively.

Lastly, a significant difference between the oxidative capacity (SDH intensity) of all fiber types was observed. The oxidative capacity of type I fibers was significantly higher than IIa and IIa+x ($p<0.0001$). Furthermore, oxidative capacity of type IIa fibers was higher than IIa+x ($p<0.0001$).

A. B.

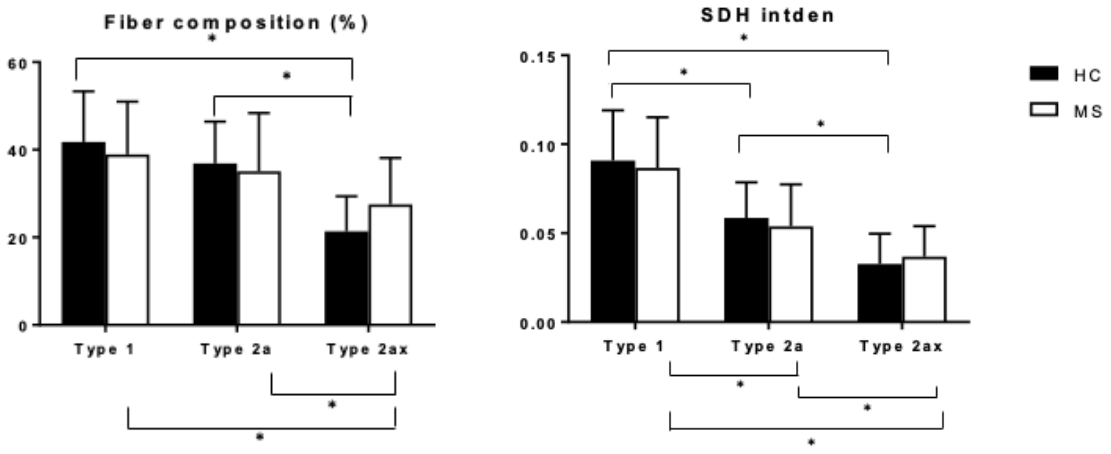

Figure 3. *A. fiber type composition and B. Fiber type oxidative capacity of MS patients and HC. No significant differences were found between both groups ($p<0.05$). The composition and oxidative capacity between fiber types differed significantly ($p<0.05$). * indicates a significant difference of $p<0.05$.*

Within the MS group, no significant correlations were observed between the EDSS score and muscle characteristics, such as fiber type proportions or fiber oxidative capacity (Figure 4.A). Furthermore, VO_{2max} or W_{max} were not significantly correlated with any muscle property. The BMI was positively correlated with multiple muscle characteristics. This parameter correlated with the CSA (r=0.55, p=0.044) and Feret diameter (r=0.55, p=0.019) of type IIa fibers (Appendix Figure 6), as well with the oxidative capacity of type I fibers (r=0.59, p=0.045). These findings were also present in healthy controls.

When combining MS and HC groups, further positive correlations were observed. BMI correlated with the capillary to fiber ratio (r=0.39, p=0.032) (Figure 4.B) and oxidative capacity of type IIa fibers (r=0.46, p=0.018) and type IIa+x fibers (r=0.50, p=0.01).

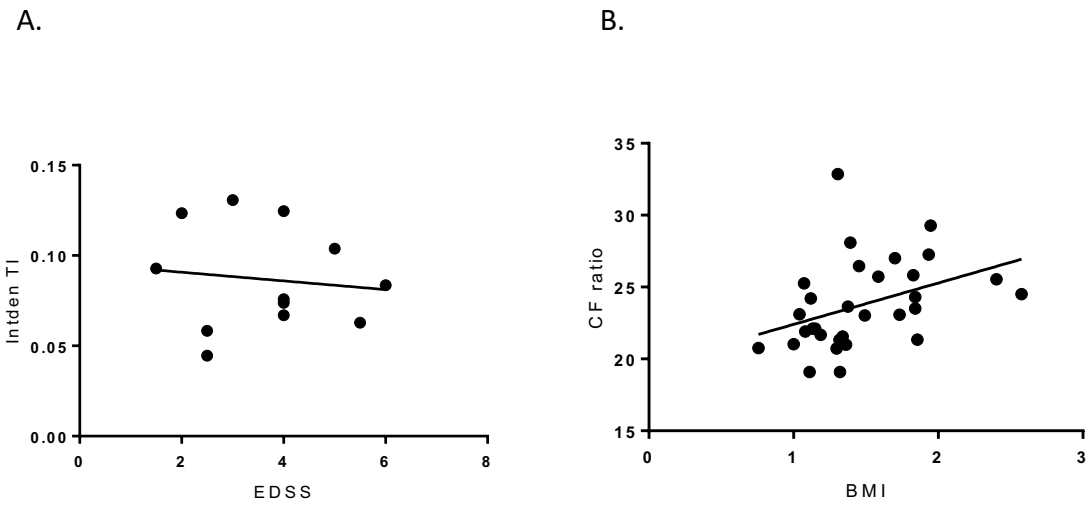

Figure 4. *A. No significant correlation is observed between the EDSS score and oxidative capacity of type I fibers in MS patients (p=0.89, r=-0.05). B. When combining both groups (MS + HC) a significant positive correlation (p<0.05) is found with the c/f ratio (p=0.032, r=0.39).*

6.2. Part 1. Conclusion

A significant difference between capillary density was found between muscle characteristics of MS patients and HC. Moreover, the VO_{2max} and W_{max} was significantly higher in HC. Although significant effects of the different fiber types were observed on the oxidative capacity, CSA and Feret diameter of fibers, no interaction effects or group effects were present. Furthermore, no significant correlations were present between the EDSS score of patients and muscle properties. However, BMI correlated positively in both the MS group and combined groups with multiple muscle properties. Correlations were found with type IIa fiber properties, SDH activity of type I fibers. Subsequently, BMI correlated significantly with the c/f ratio when combining both groups. No significant correlations were found between the clinical outcomes and muscle properties.

6.3. Part 2. Effects of the training program

Table 4. **Subject characteristics**

	MyHC	**SDH**	**CD31**
	MS pre-post (n=6)	**MS pre-post (n=4)**	**MS pre-post (n=5)**
Gender (m/f)	2/4	2/2	2/3
Age (y)	54 ± 5	53 ± 7	53 ± 6
EDSS	3.17 ± 1.37	3.25 ± 1.56	3.40 ± 1.39
Weight (kg)	60 ± 11	64 ± 12	62 ± 11
Length (m)	1.67 ± 0.09	1.70 ± 0.09	1.68 ± 0.09
BMI (kg/m^2)	21.53 ± 1.93	21.92 ± 2.36	21.68 ± 2.11
Smoke (yes/no)	0/6	0/4	0/5
RRMS	3	2	2
SPMS	3	2	3
PPMS	0	0	0

Abbreviations*:* m (male), f (female), BMI (body mass index), EDSS (expanded disability status scale), RRMS (relapse remitting MS), SPMS (secondary progressive MS), PPMS (primary progressive MS)
Note*:* data are expressed as means ± standard deviation and represent characteristics of MS patients. * is used to indicate a significant difference ($p<0.05$).

To investigate the effect of a 12-week HICT program, six MyHC, four SDH and five CD31 samples were compared pre and post intervention. The mean EDSS score was 3.27, ranging from 1.5 to 4. Subject characteristics (table 4) did not differ significantly between groups ($p>0.05$).

Table 5. **Quantitative analysis of the vastus lateralis muscle of MS patients after a HICT intervention**

	Type I		Type IIa		Type IIa+x	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
CSA (μm^2)	4192 ± 2179	4250 ± 1816	3351 ± 1495	3336 ± 1421	2423 ± 1190	2270 ± 774
Feret diameter (µm)	92.78 ± 27.45	95.05 ± 22.44	87.50 ± 19.78	85.31 ± 18.06	74.80 ± 18.75	71.08 ± 13.94
Fiber distribution(%)	40.8 ± 12.0	51.0 ± 12.2	37.8 ± 16.4	32.3 ± 12.9	21.3 ± 11.8	16.7 ± 10.0
SDH (ΔA660/µm/s)	0.077 ± 0.027	0.073 ± 0.029	0.049 ± 0.015	0.042 ± 0.015	0.034 ± 0.012	0.029 ± 0.013
	Pre			**Post**		
Capillary density (mm^{-2})	332 ± 153			256 ± 77		
Capillary/fiber ratio	1.495 ± 0.632			1.134 ± 0.369		
VO_{2max} (mL/min/kg)	25.17 ± 8.36*			32.13 ± 10.70*		
W_{max} (Watt)	120.83 ± 52.95*			135.83 ± 52.39*		

Abbreviation: CSA (cross-sectional area), VO_{2max} (maximal oxygen uptake), W_{max} (maximal cycling resistance)
Note: data are expressed as means ± standard deviation. * is used to indicate a significant difference ($p<0.05$).

The results of a twelve week intervention program are shown in table 5. A negligible amount of hybrid fiber I+IIa was found in 54.5% of the samples and was not included in the analysis. An average of 388.27 ± 235.87 fibers was analysed per patient (data not shown).

While no significant differences were found comparing muscle characteristics, a trend for an increase in type I fibers was present (p=0.1417 two-tailed, p=0.07 one-tailed) (Figure 5). Furthermore, a significant increase of the $VO2_{max}$ (p=0.0313) and W_{max} (p=0.0313) was observed after twelve weeks.

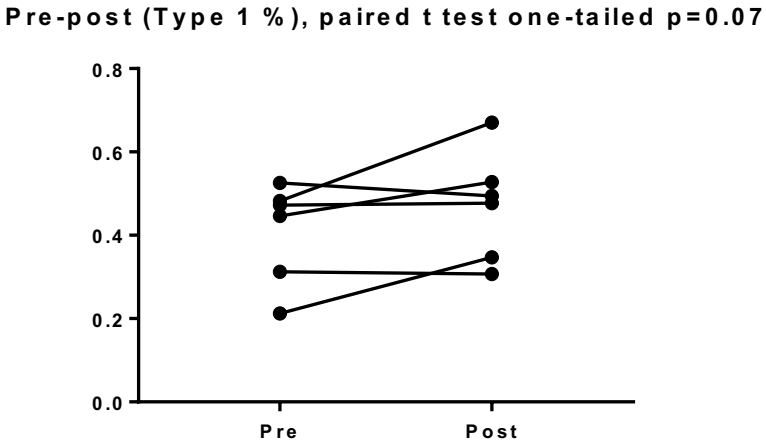

***Figure 5**. An increasing trend of type I fiber distribution was observed after a twelve week HICT intervention.*

6.4. Part 2. Conclusion

After a twelve week intervention, a significant improvement of $VO2_{max}$ and W_{max} was observed. No significant findings were presented in the muscle characteristics used in this research.

7. Discussion

This study shows a significant difference between capillary density, $VO2_{max}$ and W_{max} of MS patients and HC. But no difference in fiber CSA, min. Feret diameter, fiber distribution, oxidative activity and capillary/fiber ratio was observed. Furthermore, significant improvements in VO_{2max} and W_{max} were observed after a twelve week exercise intervention. However, no improvements in muscle characteristics were found.

7.1. Part 1. Comparing healthy controls and MS patients

7.1.1. Muscle fiber type composition

Wens et al. (2014) observed a significantly smaller mean fiber CSA as well as a smaller CSA of the separate fiber types. This applied to all fiber types (type I, IIa), except type IIx. These results were not perceived in this research, which confirms the results of Chad et al. (2005) who concluded corresponding fiber CSA between mildly impaired MS patients and sedentary controls. Moreover, no significant differences in fiber type distribution were found, which Chad et al. (2005) also reported. The present study as well as the study of Chad et al. (2005) compared the effects of MS to healthy, but sedentary individuals. This may explain why no differences were found. Fiber hierarchy (type I > IIa > IIa+x) stayed the same in the MS samples, as others reported in similarly disabled MS patients (de Haan et al. 2000; Kent-Braun et al. 1997; Wens et al. 2014).

When looking at the previously executed literature study focussing on the EAE animal MS model, a shift to a higher type IIa+x fiber distribution in MS patients' skeletal muscles was expected. An increase of intermediate fibers (type IIx+b) was found in the soleus (SOL) and extensor digitorum longus (EDL) muscles of EAE rats (Wens et al. 2015b; Luque et al. 2015). The SOL muscle mainly consists of oxidative fibers (type I) and EDL muscles consist mostly out of glycolytic fibers (type II) (Novak et al. 2010; Talbot & Maves, 2016). This study used biopsies of the human vastus lateralis muscle, which also contains for the most part oxidative fibers. However, there must be caution in transferring or comparing results from animal studies in comparison with humans. Effects of EAE and MS on muscles can differ.

De Haan et al. (2000) did observe a shift from oxidative to glycolytic fibers (type II) evoked by chronic disuse in MS patients. Lastly, shifts in fiber type I to II could be present caused by oxidative stress (Moylan & Reid, 2007). These findings were not present in this research, only a trend ($p=0.0786$) of a larger type IIa+x distribution in MS patients was observed.

A post hoc analysis revealed that the sample size of this study should have consisted of at least 35 MS patients to achieve a significant effect of fiber type IIa+x distribution (power=0.8, $p<0.05$). This accords with the sample size of Wens et al. (2014), where 34 MS patients participated.

7.1.2. Muscle fiber oxidative capacity

No differences in fiber oxidative capacity were observed in the present study. Kent-Braun et al. (1997) found a decreased SDH activity in tibialis anterior muscles of MS patients, which was mostly present in type I fibers. The reduction of oxidative activity accorded with SDH activity levels of subjects suffering from spinal cord injuries. Furthermore, Tobore (2019) concluded a presence of a higher production of reactive oxygen species (ROS) in skeletal muscle cells, due to inflammation and oxidative stress. ROS influences the mitochondrial health and reduces oxidative capacity. An important buffer of oxidative stress is carnosine, which was significantly lower in MS patients compared to HC (Keytsman et al. 2017). Perhaps the presence of lower carnosine concentrations indicates higher oxidative stress levels accompanied by ROS, which then influences the mitochondria and can result in a lower oxidative capacity. In addition, oxidative stress can influence fiber type composition as mentioned above (Moylan & Reid, 2007).

Furthermore, Hansen et al. (2015b) noted a significant difference of basal levels of AMP-activated protein kinase (AMPK) between HC and MS patients. AMPK is activated by oxidative stress, hypoxemia or ischemia and stimulates the mitochondrial biogenesis. When activated by ATP depletion, AMPK switches to catabolic pathways to gain ATP (Hardy et al. 2002). AMPK levels were significantly higher in MS patients ($p<0.05$), which may explain why no differences in oxidative capacity were observed in the present study.

The influence of MS on muscle biochemistry was not investigated in the present study, hence no clear statements could be made upon the underlying mechanisms that could possibly influence the oxidative activity.

7.1.3. Muscle fiber capillarity

This study compared the muscle characteristics of mildly affected MS patients (n=16) and HC (n=15), where only a significant difference between capillary density was to be found. Moro et al. (2019) discovered a positive correlation between capillary to fiber ratio and activity levels (r=0.57, p=0.019) in healthy older individuals, measured in steps/day. MS patients have lower overall physical activity levels than healthy persons (Ellis & Motl. 2013). However, this study found no differences between the PASIPD scores of both groups. The mean scores were 14.42 and 19.40 for the MS and HC group respectively. Both groups had low physical activity levels, which can explain why no significant difference was found. Furthermore, the present study also investigated VO_{2max} as the outcome of one's aerobic exercise capacity. This outcome plays an important role in one's physical activity and was significantly lower in the MS group. This could possibly explain why the ratio and therefore capillary density was significantly lower compared to the HC.

7.1.4. Secondary outcomes

The present study used VO_{2max} as an outcome for aerobic exercise capacity, which is considered the golden standard**.** There may be a possibility that MS patients were not able to perform a maximal effort during the maximal exercise test. Langeskov-Christensen et al. (2014) has proven that a valid VO_{2max} test can be performed with mildly to moderate affected MS patients. The mean EDSS score of the participating subjects of this study was 3.46, which makes findings of the study of Langeskov-Christensen et al. (2014) applicable. Strong correlations were found between measurements of VO_{2max} and the validity criteria. However, a difference of more than 10% should be perceived as a real difference between groups. This research concluded a difference in this outcome of 66.7% between HC and MS patients. Furthermore, an improvement of 27.7% was found after the exercise intervention. Hence, making the term VO_{2max} appropriate.

Mitchell et al. (2018) found a positive correlation of the VO2 peak and capillary to fiber ratio, as well with the fiber proportion and CSA of type I fibers ($p<0.05$). However, these correlations were not significant when taken body weight into account. In addition, these findings were presented in healthy, athletic males which makes comparisons difficult.

No correlations were found between fiber CSA and the EDSS score, which was in accordance with the study of Wens et al. (2014). However, the present study found a significant positive correlation between BMI and the CSA of type IIa fibers ($r=0.55$, $p<0.05$). Lower physical activity levels are commonly present in MS patients (Keegan, & Noseworthy. 2002), which can lead to an increase in BMI. Therefore, the consequences of the disease possibly play an important role, influencing muscle characteristics.

7.2. Part 2. Exercise interventions, clinical outcomes and muscle properties

A significant improvement of clinical outcomes was observed after a HICT intervention on MS patients. Similar results were found in the study of Wens et al. (2015a), who conducted a High Intensity Interval Training (HIIT) and a High Intensity Continuous Cardiovascular Training (HICCT). The study concluded a significant increase of W_{max} and VO_{2max} in the HIIT group, where the same training modalities were applied as in the present study. The HIIT group improved 15.4%, while the HICT in the present study improved 27.7%.

To date, exercise therapy has become more important in the rehabilitation of MS patients. Various studies have proven the significance of exercise on multiple health related parameters in MS (Campbell et al. 2018; Keytsman et al. 2019; Kjolhede et al. 2012; Mostert et al. 2002). However, different exercise modalities are used with little to no consensus on the effects on the skeletal muscles. If the effects of MS on these health-related parameters - including muscle health - are mapped, exercise therapy can be improved. Studies found significant results on other muscle properties after exercise interventions. The mean CSA of both intervention groups increased significantly after twelve weeks (Wens et al. 2015a).

Muscle fiber type I increased significantly in the HICCT group, whereas fiber type IIa increased in the HIIT group. Furthermore, studies did find effects of exercise on muscle health in healthy individuals, in contrary of the present study (Gavin et al. 2004; Gavin et al. 2007)**.** Moro et al. (2019) showed that angiogenesis would occur in healthy individuals with a lower muscle capillarization, which was the case in MS patients, when exercising. This process improves the vascular diffusion capacity after 12 weeks of resistance exercise training. Consequently, the oxidative capacity increased. These results were not observed in this research, keeping in mind a different exercise intervention was used, what could be an influencing factor on the outcomes.

Perhaps other determinants, not investigated in this research, could explain the improvements in VO_{2max} and W_{max}. While no significant changes were observed in the muscle properties used in the present study, other systems of the body could be positively influenced by exercise. More specifically other aspects of the periphery system and the respiratory or circulatory system. Whereas the study of Dalgas et al. (2010) performed solely a strength training, it concluded significant improvements of knee flexor and extensor muscle strength ($p<0.05$) in fast velocities (180°/s). Wens et al. (2015a), who performed two types of interval training, found similar results. Keytsman et al. (2019) used the same training modalities and found a significant improvement of the isometric and isokinetic muscle force of quadriceps muscles after the intervention. Additionally, the maximal expiratory volume (VE) also increased significantly ($p<0.05$). Mostert et al. (2002) found significant changes in the Forced Vital Capacity (FVC) and peak expiratory flow rate (PEFR) after four weeks of aerobic exercise training ($p<0.05$). Hansen et al. (2015a) found that long time aerobic exercise decreased blood lactate content significantly ($p<0.05$). Perhaps patients would perform better on the maximal exercise test. These outcome measures were not used in the present study, which should be considered for future research.

A post hoc power analysis showed that the sample size should consist of at least 12 persons to detect a significant effect between type I fiber distribution after this exercise intervention (power=0.8, $p<0.05$). This indicates that the used sample size in this study was not appropriate.

7.3. Future research and limitations

This research encountered a small sample size (n=6) when examining the pre-post samples of six patients. Consequently, findings of this study should be confirmed in a larger sample. Another recommendation could be to further investigate the effects of MS on the biochemistry of cells.

This study used one sample per subject to analyse mean CSA and fiber proportion. However, these parameters can have a large variability along the depth and length of the muscle. According to Lexell et al. (1989), at least three biopsies should be extracted to reduce the sampling error. From every biopsy, 150 fibers should be analysed from different depths (superficial, middle and deeper regions). Keeping this in mind, the ethical responsibility of taking multiple samples of subjects can be questioned. This study aimed to analyse 150 fibers of each sample, which did not succeed with four subjects' data**.** However, these data were statistically compared and were not seen as outliers**.** The fact that the researchers were not blinded during the data extraction and analysis should be taken into consideration. Furthermore, another possible bias is the fact that participants were recruited by local advertisement which may decreased generalization.

This study does have multiple strengths, both researchers performed 100% of the data extraction and analysis**.** Data sets of both researchers were statistically checked by performing an unpaired t-test (n=5) for homogeneity. Furthermore, the baseline characteristics corresponded well between MS patients and HC. The duration of the exercise intervention was long enough to examine possible effects. Moreover, supervision during exercise could diminish lack of motivation. Lastly, a comparison was made with a control group. However, no comparison with a sedentary control group was used when looking at effects of an exercise intervention. This should be present in future research.

8. Conclusion

This research investigated the muscle characteristics of mildly affected MS patients and compared it to healthy, but sedentary controls (HC). Although baseline characteristics were matched, the physical fitness of MS patients ($VO2_{max}$ and W_{max}) was significantly lower than HC. Regarding muscle characteristics, a significant difference was found in the capillary density (CD31 stained samples) of MS patients. However, no differences were observed when comparing the MyHC and SDH stained muscle samples. Only a trend ($p=0.079$) was found of a higher type IIa+x fiber distribution in MS patients.

Furthermore, this research found no correlations between the disease and fiber CSA, min. Feret diameter, fiber distribution, oxidative capacity or capillarity. However, correlations with BMI have been found. This may be explained by the negative impact of MS on physical activity levels, which can lead to a higher BMI.

A twelve week HICT intervention significantly improved the $VO2_{max}$ and W_{max} of MS patients. However, these improvements could not be seen in muscle characteristics. Only a trend for an increase in type I fibers was present ($p=0.1417$ two-tailed, $p=0.07$ one-tailed). Perhaps when looking at other systems of the body, explanations of these improvements can be found.

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10. Appendix

Table 6. Overview of MyHC staining protocol at room temperature

Protocol	Time
Muscle cryosections of 10 µm stored at -80°C	
Air drying	10 min
Washing in PBST	5 min
Block with 10% goat serum in PBST	60 min
Primary antibody cocktail application	120 min
PBST wash	3 x 5 min
Secondary antibody cocktail application	60 min
PBST wash	3 x 5 min
Air drying, mounting and storage at 4°C	

Table 7. Overview of SDH staining protocol at room temperature

Protocol	Time
Muscle cryosections of 10 µm stored at -80°C	
Air drying	15 min
Embedment in SPB + sodium succinate + TNBT	20 min
Submerging in HCl solution + Air drying, mounting and storage at 4°C	

Table 8. Overview of CD31 endothelial staining protocol at room temperature

Protocol	Time
Muscle cryosections of 10 µm stored at -20°C	
Air drying	30 min
Washing in PBST	5 min
Primary antibody cocktail application at 4°C	Overnight
Washing in PBST	3 x 5 min
Secondary antibody cocktail application	60 min
Washing in PBST	3 x 5 min
Application of DAPI	10 min
Washing in PBST	3 x 5 min
Air drying, mounting and storage at 4°C	

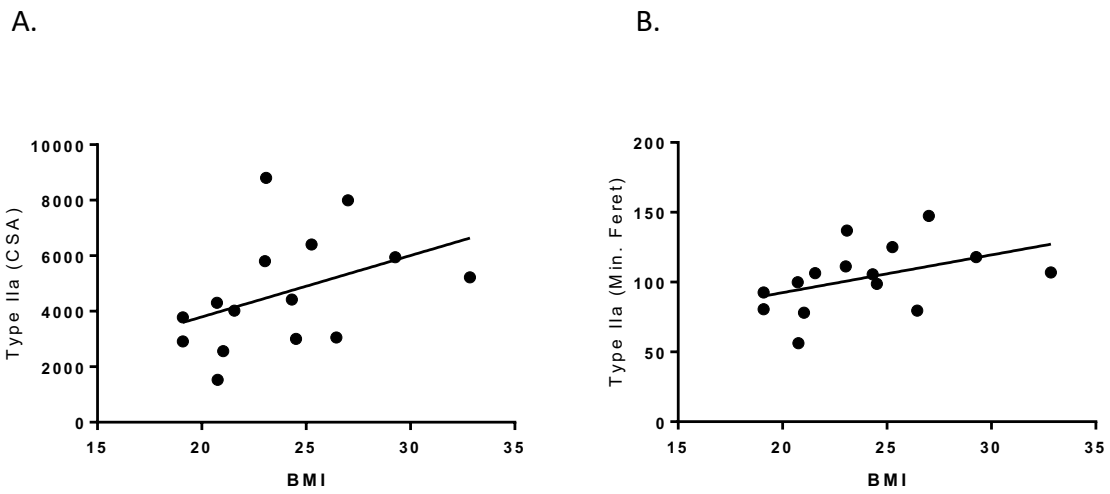

Figure 6*. A significant correlation is observed between BMI and (A) fiber CSA of type II fibers (r=0.55, p=0.044) and (B) min. Feret diameter (r=0.55, p=0.019) of type IIa fibers.*

Addenda

www.uhasselt.be
Campus Hasselt | Martelarenlaan 42 | BE-3500 Hasselt
Campus Diepenbeek | Agoralaan gebouw D | BE-3590 Diepenbeek
T + 32(0)11 26 81 11 | E-mail: info@uhasselt.be

UHASSELT
KNOWLEDGE IN ACTION

INVENTARISATIEFORMULIER WETENSCHAPPELIJKE STAGE DEEL 2

DATUM	INHOUD OVERLEG	HANDTEKENINGEN
19/08/19	introductie analyse coupes	Promotor: Copromotor/Begeleider: Student(e): Corteyn Student(e): Luyts
21/11/19	· overleg + vragen voorvitgang analyse	Promotor: Copromotor/Begeleider: Student(e): Corteyn Student(e): Luyts
07/04/20	· overleg + vragen voorvitgang analyse + statistiek	Promotor: Copromotor/Begeleider: Student(e): Corteyn Student(e): Luyts
22/04/20	· overleg statistiek + resultaten	Promotor: Copromotor/Begeleider: Student(e): Corteyn Student(e): Luyts
01/05/20	· overleg resultaten	Promotor: Copromotor/Begeleider: Student(e): Corteyn Student(e): Luyts
05/05/20	· overleg resultaten	Promotor: Copromotor/Begeleider: Student(e): Corteyn Student(e):
11/05/20	· overleg resultaten	Promotor: Copromotor/Begeleider: Student(e): Corteyn Student(e): Luyts
15/05	Presentatie onderzoeksgroep	Promotor: Copromotor/Begeleider: Student(e): Corteyn Student(e): Luyts
		Promotor: Copromotor/Begeleider: Student(e): Student(e):
		Promotor: Copromotor/Begeleider: Student(e): Student(e):

Verklaring op Eer

Ondergetekende, student aan de Universiteit Hasselt (UHasselt), faculteit Revalidatiewetenschappen aanvaardt de volgende voorwaarden en bepalingen van deze verklaring:

1. Ik ben ingeschreven als student aan de UHasselt in de opleiding Revalidatiewetenschappen en kinesitherapie waarbij ik de kans krijg om in het kader van mijn opleiding mee te werken aan onderzoek van de faculteit Revalidatiewetenschappen aan de UHasselt. Dit onderzoek wordt beleid door Prof. Dr. Op'T Eijnde Bert en kadert binnen het opleidingsonderdeel Wetenschappelijke Stage. Ik zal in het kader van dit onderzoek creaties, schetsen, ontwerpen, prototypes en/of onderzoeksresultaten tot stand brengen in het domein van cardiorespiratoire en neurologische revalidatie. (hierna: "De Onderzoeksresultaten").

2. Bij de creatie van De Onderzoeksresultaten doe ik beroep op de achtergrondkennis, vertrouwelijke informatie[1], universitaire middelen en faciliteiten van UHasselt (hierna: de "Expertise").

3. Ik zal de Expertise, met inbegrip van vertrouwelijke informatie, uitsluitend aanwenden voor het uitvoeren van hogergenoemd onderzoek binnen UHasselt. Ik zal hierbij steeds de toepasselijke regelgeving, in het bijzonder de Algemene Verordening Gegevensbescherming (EU 2016-679), in acht nemen.

4. Ik zal de Expertise (i) voor geen enkele andere doelstelling gebruiken, en (ii) niet zonder voorafgaande schriftelijke toestemming van UHasselt op directe of indirecte wijze publiek maken.

5. Aangezien ik in het kader van mijn onderzoek beroep doe op de Expertise van de UHasselt, draag ik hierbij alle bestaande en toekomstige intellectuele eigendomsrechten op De Onderzoeksresultaten over aan de UHasselt. Deze overdracht omvat alle vormen van intellectuele eigendomsrechten, zoals onder meer – zonder daartoe beperkt te zijn – het auteursrecht, octrooirecht, merkenrecht, modellenrecht en knowhow. De overdracht geschiedt in de meest volledige omvang, voor de gehele wereld en voor de gehele beschermingsduur van de betrokken rechten.

6. In zoverre De Onderzoeksresultaten auteursrechtelijk beschermd zijn, omvat bovenstaande overdracht onder meer de volgende exploitatiewijzen, en dit steeds voor de hele beschermingsduur, voor de gehele wereld en zonder vergoeding:

   - het recht om De Onderzoeksresultaten vast te (laten) leggen door alle technieken en op alle dragers;
   - het recht om De Onderzoeksresultaten geheel of gedeeltelijk te (laten) reproduceren, openbaar te (laten) maken, uit te (laten) geven, te (laten) exploiteren en te (laten) verspreiden in eender welke vorm, in een onbeperkt aantal exemplaren;

[1] Vertrouwelijke informatie betekent alle informatie en data door de UHasselt meegedeeld aan de student voor de uitvoering van deze overeenkomst, inclusief alle persoonsgegevens in de zin van de Algemene Verordening Gegevensbescherming (EU 2016/679), met uitzondering van de informatie die (a) reeds algemeen bekend is; (b) reeds in het bezit was van de student voor de mededeling ervan door de UHasselt; (c) de student verkregen heeft van een derde zonder enige geheimhoudingsplicht; (d) de student onafhankelijk heeft ontwikkeld zonder gebruik te maken van de vertrouwelijke informatie van de UHasselt; (e) wettelijk of als gevolg van een rechterlijke beslissing moet worden bekendgemaakt, op voorwaarde dat de student de UHasselt hiervan schriftelijk en zo snel mogelijk op de hoogte brengt.

- het recht om De Onderzoeksresultaten te (laten) verspreiden en mee te (laten) delen aan het publiek door alle technieken met inbegrip van de kabel, de satelliet, het internet en alle vormen van computernetwerken;
- het recht De Onderzoeksresultaten geheel of gedeeltelijk te (laten) bewerken of te (laten) vertalen en het (laten) reproduceren van die bewerkingen of vertalingen;
- het recht De Onderzoeksresultaten te (laten) bewerken of (laten) wijzigen, onder meer door het reproduceren van bepaalde elementen door alle technieken en/of door het wijzigen van bepaalde parameters (zoals de kleuren en de afmetingen).

De overdracht van rechten voor deze exploitatiewijzen heeft ook betrekking op toekomstige onderzoeksresultaten tot stand gekomen tijdens het onderzoek aan UHasselt, eveneens voor de hele beschermingsduur, voor de gehele wereld en zonder vergoeding.

Ik behoud daarbij steeds het recht op naamvermelding als (mede)auteur van de betreffende Onderzoeksresultaten.

7. Ik zal alle onderzoeksdata, ideeën en uitvoeringen neerschrijven in een "laboratory notebook" en deze gegevens niet vrijgeven, tenzij met uitdrukkelijke toestemming van mijn UHasseltbegeleider Prof. Dr. Op'T Eijnde Bert.

8. Na de eindevaluatie van mijn onderzoek aan de UHasselt zal ik alle verkregen vertrouwelijke informatie, materialen, en kopieën daarvan, die nog in mijn bezit zouden zijn, aan UHasselt terugbezorgen.

Gelezen voor akkoord en goedgekeurd,

Naam: Fonteyn Lena

Adres: Peperstraat 83, 3080 Tervuren

Geboortedatum en -plaats : 02/09/1996, Vilvoorde

Datum: 19/05/2020

Handtekening: Fonteyn

UHASSELT

Verklaring op Eer

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1. Ik ben ingeschreven als student aan de UHasselt in de opleiding Revalidatiewetenschappen en kinesitherapie waarbij ik de kans krijg om in het kader van mijn opleiding mee te werken aan onderzoek van de faculteit Revalidatiewetenschappen aan de UHasselt. Dit onderzoek wordt beleid door Prof. Dr. Op'T Eijnde Bert en kadert binnen het opleidingsonderdeel Wetenschappelijke Stage. Ik zal in het kader van dit onderzoek creaties, schetsen, ontwerpen, prototypes en/of onderzoeksresultaten tot stand brengen in het domein van cardiorespiratoire en neurologische revalidatie. (hierna: "De Onderzoeksresultaten").

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4. Ik zal de Expertise (i) voor geen enkele andere doelstelling gebruiken, en (ii) niet zonder voorafgaande schriftelijke toestemming van UHasselt op directe of indirecte wijze publiek maken.

5. Aangezien ik in het kader van mijn onderzoek beroep doe op de Expertise van de UHasselt, draag ik hierbij alle bestaande en toekomstige intellectuele eigendomsrechten op De Onderzoeksresultaten over aan de UHasselt. Deze overdracht omvat alle vormen van intellectuele eigendomsrechten, zoals onder meer – zonder daartoe beperkt te zijn – het auteursrecht, octrooirecht, merkenrecht, modellenrecht en knowhow. De overdracht geschiedt in de meest volledige omvang, voor de gehele wereld en voor de gehele beschermingsduur van de betrokken rechten.

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---

[1] Vertrouwelijke informatie betekent alle informatie en data door de UHasselt meegedeeld aan de student voor de uitvoering van deze overeenkomst, inclusief alle persoonsgegevens in de zin van de Algemene Verordening Gegevensbescherming (EU 2016/679), met uitzondering van de informatie die (a) reeds algemeen bekend is; (b) reeds in het bezit was van de student voor de mededeling ervan door de UHasselt; (c) de student verkregen heeft van een derde zonder enige geheimhoudingsplicht; (d) de student onafhankelijk heeft ontwikkeld zonder gebruik te maken van de vertrouwelijke informatie van de UHasselt; (e) wettelijk of als gevolg van een rechterlijke beslissing moet worden bekendgemaakt, op voorwaarde dat de student de UHasselt hiervan schriftelijk en zo snel mogelijk op de hoogte brengt.

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8. Na de eindevaluatie van mijn onderzoek aan de UHasselt zal ik alle verkregen vertrouwelijke informatie, materialen, en kopieën daarvan, die nog in mijn bezit zouden zijn, aan UHasselt terugbezorgen.

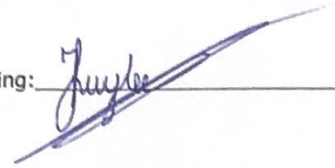
Gelezen voor akkoord en goedgekeurd,

Naam: Ruyters Jonas

Adres: Ketelbutersstraat 16 3990 Peer

Geboortedatum en –plaats : 29-09-1997 Hasselt

Datum:19/05/2020

Handtekening: 

AFSPRAKENNOTA

1. Organisatie

Naam	Universiteit Hasselt/transnationale Universiteit Limburg (Hierna: UHasselt/tUL)
Adres	Martelarenlaan 42 3500 Hasselt
Sociale doelstelling	De UHasselt/tUL is een dynamisch kenniscentrum van onderwijs, onderzoek en dienstverlening.
Werking van de organisatie	**Faculteiten** De UHasselt telt zes faculteiten die het onderwijs en onderzoek aansturen: o faculteit Architectuur en kunst o faculteit Bedrijfseconomische wetenschappen o faculteit Geneeskunde en levenswetenschappen o faculteit Industriële ingenieurswetenschappen o faculteit Rechten o faculteit Wetenschappen Elke faculteit stelt per opleiding een onderwijsmanagementteam (OMT) en een examencommissie samen. **Vakgroepen** Binnen de faculteiten opereren diverse vakgroepen. Zij groeperen alle personeelsleden die onderzoek en onderwijs verrichten binnen eenzelfde discipline. Elke vakgroep bestaat vervolgens uit een of meerdere onderzoeksgroepen. Zij staan in voor de organisatie van het gespecialiseerd onderzoek. Deze klassieke boomstructuur van faculteiten, onderzoeksgroepen en vakgroepen wordt doorkruist door de onderzoeksinstituten. De instituten groeperen onderzoekers uit verschillende onderzoeksgroepen die in bepaalde speerpuntdomeinen onderzoek uitvoeren. Daarbij wordt het volledige onderzoekspectrum afgedekt, van fundamenteel over toegepast onderzoek tot concrete valorisatietoepassingen.
Juridisch statuut	Autonome openbare instelling

Verantwoordelijke van de organisatie, die moet verwittigd worden bij ongevallen.

Naam	Britt Loos
Functie	Jurist en beleidsadviseur
Tel. - GSM	011/268002

2. De vrijwilliger: student-onderzoeker

Naam	Fonteyn Lena
Correspondentieadres	Lena.fonteyn@student.uhasselt.be
Tel. - GSM	0493153434

Naam	Jonas Ruyters
Correspondentieadres	Jonas.ruyters@student.uhasselt.be
Tel. - GSM	0494475145

3. Verzekeringen

Waarborgen	De burgerlijke aansprakelijkheid van de organisatie.
Maatschappij	Ethias
Polisnummer	45009018

Waarborgen	Lichamelijke schade die geleden is door vrijwilligers bij ongevallen tijdens de uitvoering van het vrijwilligerswerk of op weg naar- en van de activiteiten.
Maatschappij	Ethias
Polisnummer	45055074

4. Vergoedingen

De organisatie betaalt geen vergoeding aan de vrijwilliger.

5. Aansprakelijkheid

De organisatie is burgerrechtelijk aansprakelijk voor de schade die de vrijwilliger aan derden veroorzaakt bij het verrichten van vrijwilligerswerk.

Ingeval de vrijwilliger bij het verrichten van het vrijwilligerswerk de organisatie of derden schade berokkent, is hij enkel aansprakelijk voor zijn bedrog en zijn zware schuld.
Voor lichte schuld is hij enkel aansprakelijk als die bij hem eerder gewoonlijk dan toevallig voorkomt.

Opgelet: voor het materiaal dat de vrijwilliger zelf meebrengt, is hij/zij zelf verantwoordelijk.

6. Geheimhoudingsplicht – verwerking persoonsgegevens

De vrijwilliger verleent de UHasselt toestemming om de gegevens die in het kader van zijn/haar inschrijving aan UHasselt werden verzameld, ook te gebruiken voor de uitvoering van deze afsprakennota (de evaluatie van de vrijwilliger alsook het aanmaken van een certificaat). UHasselt zal deze informatie vertrouwelijk behandelen en zal deze vertrouwelijkheid ook bewaken na de beëindiging van het statuut student-onderzoeker. De UHasselt neemt hiertoe alle passende maatregelen en waarborgen om de persoonsgegevens van de vrijwilliger conform de Algemene Verordening Gegevensbescherming (EU 2016/679) te verwerken.

De vrijwilliger verbindt zich ertoe om alle gegevens, documenten, kennis en materiaal, zowel schriftelijk als mondeling ontvangen in de hoedanigheid van student-onderzoeker aan de UHasselt als strikt vertrouwelijk te behandelen, ook indien deze niet als strikt vertrouwelijk werd geïdentificeerd. Indien de vertrouwelijke gegevens van de UHasselt ook persoonsgegevens bevatten dient de stagiair hiertoe steeds de Algemene Verordening Gegevensbescherming (EU 2016/679) na te leven en bij elke verwerking het advies van het intern privacycollege van de UHasselt in te winnen. Hij/zij verbindt zich ertoe om in geen geval deze vertrouwelijke informatie mee te delen aan derden of anderszins openbaar te maken, ook niet na de beëindiging van het statuut student-onderzoeker.

7. Concrete afspraken

Functie van de vrijwilliger
De vrijwilliger zal volgende taak vervullen: assisteren bij het uitvoeren van een experimentele studie, met name bij opstellen dataverwerking van het onderzoek
Deze taak omvat volgende activiteiten: Opstellen, dataverwerking
De vrijwilliger voert zijn taak uit onder verantwoordelijkheid van de faculteit Revalidatiewetenschappen
De vrijwilliger wordt binnen de faculteit begeleid door Drs. Jan Spaas
Zijn vaste werkplek voor het uitvoeren van de taak is REVAL - Rehabilitation Research Center

De vrijwilliger zal deze taak op volgende tijdstippen uitvoeren:

- op de volgende dag(en):
    - maandag
    - dinsdag
    - woensdag
    - donderdag
    - vrijdag
    - zaterdag
    - zondag
- het engagement wordt aangegaan voor de periode van 01/10/2019 tot 01/07/2020 (deze periode kan maximaal 1 kalenderjaar zijn en moet liggen tussen 1 januari en 31 december).

Begeleiding
De organisatie engageert zich ertoe de vrijwilliger tijdens deze proefperiode degelijk te begeleiden en te ondersteunen en hem/haar van alle informatie te voorzien opdat de activiteit naar best vermogen kan worden uitgevoerd.
De vrijwilliger voert de taken en activiteiten uit volgens de voorschriften vastgelegd door de faculteit. Hij/zij neemt voldoende voorzorgsmaatregelen in acht, en kan voor bijkomende informatie over de uit te voeren activiteit steeds terecht bij volgende contactpersoon: Drs. Jan Spaas

De vrijwilliger krijgt waar nodig vooraf een vorming. Het volgen van de vorming indien aangeboden door de organisatie, is verplicht voor de vrijwilliger.

De vrijwilliger heeft kennis genomen van het 'reglement statuut student-onderzoeker' dat als bijlage aan deze afsprakennota wordt toegevoegd en integraal van toepassing is op de vrijwilliger.

Certificaat

Indien de vrijwilliger zijn opdracht succesvol afrondt, ontvangt hij/zij een certificaat van de UHasselt ondertekend door de decaan van de faculteit waaraan de vrijwilliger zijn opdracht voltooide.

8. Einde van het vrijwilligerswerk.

Zowel de organisatie als de vrijwilliger kunnen afzien van een verdere samenwerking. Dat kan gebeuren:
- bij onderlinge overeenstemming;
- op vraag van de vrijwilliger zelf;
- op verzoek van de organisatie.

Indien de samenwerking op initiatief van de vrijwilliger of de organisatie wordt beëindigd, gebeurt dit bij voorkeur minstens 2 weken op voorhand. Bij ernstige tekortkomingen kan de samenwerking, door de organisatie, onmiddellijk worden beëindigd.

Datum: 30/09/2019

Naam en Handtekening decaan

Naam en Handtekening vrijwilliger

Fonteyn Lena

Jonas Ruyters

Bijlage 1

Reglement betreffende het statuut van student-onderzoeker[1]

Artikel 1. Definities

Voor de toepassing van dit reglement wordt verstaan onder:
student-onderzoeker: een regelmatig ingeschreven bachelor- of masterstudent van de UHasselt/tUL die als vrijwilliger wordt ingeschakeld in onderzoeksprojecten. De opdrachten uitgevoerd als student-onderzoeker kunnen op geen enkele wijze deel uitmaken van het studietraject van de student. De opdrachten kunnen geen ECTS-credits opleveren en zij kunnen geen deel uitmaken van een evaluatie van de student in ket kader van een opleidingsonderdeel. De onderzoeksopdrachten kunnen wel in het verlengde liggen van een opleidingsonderdeel, de bachelor- of masterproef.

Artikel 2. Toepassingsgebied

Enkel bachelor- en masterstudenten van de UHasselt/tUL die voor minstens 90 studiepunten credits hebben behaald in een academische bacheloropleiding komen in aanmerking voor het statuut van student-onderzoeker.

Artikel 3. Selectie en administratieve opvolging

§1 De faculteiten staan in voor de selectie van de student-onderzoekers en schrijven hiervoor een transparante selectieprocedure uit die vooraf aan de studenten kenbaar wordt gemaakt.
§2 De administratieve opvolging van de dossiers gebeurt door de faculteiten.

Artikel 4. Preventieve maatregelen en verzekeringen

§1 De faculteiten voorzien waar nodig in de noodzakelijke voorafgaande vorming van student-onderzoekers. De student is verplicht deze vorming te volgen vooraleer hij/zij kan starten als student-onderzoeker.
§2 Er moet voor de betrokken opdrachten een risicopostenanalyse opgemaakt worden door de faculteiten, analoog aan de risicopostenanalyse voor een stagiair van de UHasselt/tUL. De faculteiten zien er op toe dat de nodige veiligheidsmaatregelen getroffen worden voor aanvang van de opdracht.
§3 De student-onderzoekers worden door de UHasselt verzekerd tegen:
- Burgerlijke aansprakelijkheid
- Lichamelijke ongevallen

en dit ongeacht de plaats waar zij hun opdrachten in het kader van het statuut uitoefenen.

Artikel 5. Vergoeding van geleverde prestaties

§1 De student-onderzoeker kan maximaal 40 kalenderdagen, gerekend binnen één kalenderjaar, worden ingeschakeld binnen dit statuut. De dagen waarop de student-onderzoeker een vorming moet volgen, worden niet meegerekend als gepresteerde dagen.

§2 De student-onderzoeker ontvangt geen vrijwilligersvergoeding voor zijn prestaties. De student kan wel een vergoeding krijgen van de faculteit voor bewezen onkosten. De faculteit en de student maken hier aangaande schriftelijke afspraken.

Artikel 6. Dienstverplaatsingen

De student-onderzoeker mag dienstverplaatsingen maken. De faculteit en de student maken schriftelijke afspraken over deal dan niet vergoeding voor dienstverplaatsingen. De student wordt tijdens de dienstverplaatsingen en op weg van en naar de stageplaats uitsluitend verzekerd door de UHasselt voor lichamelijke ongevallen.

[1] Zoals goedgekeurd door de Raad van Bestuur van de Universiteit Hasselt op 15 juni 2017.

Artikel 7. Afsprakennota

§1 Er wordt een afsprakennota opgesteld die vooraf wordt ondertekend door de decaan en de student-onderzoeker. Hierin worden de taken van de student-onderzoeker alsook de momenten waarop hij/zij de taken moet uitvoeren zo nauwkeurig mogelijk omschreven.
§2 Aan de afsprakennota wordt een kopie van dit reglement toegevoegd als bijlage.

Artikel 8. Certificaat

Na succesvolle beëindiging van de opdracht van de student-onderzoeker, te beoordelen door de decaan, ontvangt hij een certificaat van de studentenadministratie. De faculteit bezorgt de nodige gegevens aan de studentenadministratie. Het certificaat wordt ondertekend door de decaan van de faculteit waaraan de student-onderzoeker zijn opdracht voltooide.

Artikel 9. Geheimhoudingsplicht

De student-onderzoeker verbindt zich ertoe om alle gegevens, documenten, kennis en materiaal, zowel schriftelijk (inbegrepen elektronisch) als mondeling ontvangen in de hoedanigheid van student-onderzoeker aan de UHasselt, als strikt vertrouwelijk te behandelen, ook indien deze niet als strikt vertrouwelijk werd geïdentificeerd. Hij/zij verbindt zich ertoe om in geen geval deze vertrouwelijke informatie mee te delen aan derden of anderszins openbaar te maken, ook niet na de beëindiging van zijn/haar opdracht binnen dit statuut.

Artikel 10. Intellectuele eigendomsrechten

Indien de student-onderzoeker tijdens de uitvoering van zijn/haar opdrachten creaties tot stand brengt die (kunnen) worden beschermd door intellectuele rechten, deelt hij/zij dit onmiddellijk mee aan de faculteit. Deze intellectuele rechten, met uitzondering van auteursrechten, komen steeds toe aan de UHasselt.

Artikel 11. Geschillenregeling

Indien zich een geschil voordoet tussen de faculteit en de student-onderzoeker met betrekking tot de interpretatie van dit reglement of de uitoefening van de taken, dan kan de ombudspersoon van de opleiding waarbinnen de student-onderzoeker zijn taken uitoefent, bemiddelen. Indien noodzakelijk, beslecht de vicerector Onderwijs het geschil.

Artikel 12. Inwerkingtreding

Dit reglement treedt in werking met ingang van het academiejaar 2017-2018.

COVID-19 Addendum - Masterproef 2

Gelieve dit document in te laten vullen door de promotor en ingevuld toe te voegen aan je masterproef.

Naam promotor(en)
Bert Op 't Eijnde
Jan Spaas

Naam studenten
Lena Fonteyn
Jonas Ruyters

1) Duid aan welk type scenario is gekozen voor deze masterproef:

☐ scenario 1: masterproef bestaat uit een meta-analyse - masterproef liep door zoals voorzien
☒ scenario 2: masterproef bestaat uit een experiment - masterproef liep door zoals voorzien
☐ scenario 3: masterproef bestaat uit een experiment - maar een deel van de voorziene data is verzameld
    ☐ 3A: er is voldoende data, maar met aangepaste statische procedures verder gewerkt
    ☐ 3B: er is onvoldoende data, dus gewerkt met een descriptieve analyse van de aanwezige data
☐ scenario 4: masterproef bestaat uit een experiment - maar er kon geen data verzameld worden
    ☐ 4A: er is gewerkt met reeds beschikbare data
    ☐ 4B: er is gewerkt met fictieve data

2) Geef aan in hoeverre de student(e) onderstaande competenties zelfstandig uitvoerde:

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

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

Datum

Van: **Bert OP 'T EIJNDE** <bert.opteijnde@uhasselt.be>
Date: ma 25 mei 2020 om 11:34
Subject: Re: Correcte versie indiening Masterproef 2
To: Fonteyn Lena <lena.fonteyn@student.uhasselt.be>
Cc: Jan SPAAS <jan.spaas@uhasselt.be>, Jonas Ruyters <jonas.ruyters@student.uhasselt.be>

Dag Lena en Jonas,

Inmiddels hebben Jan en ik jullie werk en diverse documenten kunnen doornemen en geven wij jullie een gunstig advies voor het afleggen van jullie masterproef tijdens de eerste examenperiode 2019-2020.

Met vriendelijke groeten,

Jan Spaas en Bert Op 't Eijnde

Prof. Dr. Bert Op 't Eijnde | EIM - Exercise is Medicine
Exercise physiology, sports medicine & sport/rehabilitation sciences

ADLON Sports Medical Center | BIOMED - Biomedical Research Institute | Faculty of Medicine & Life Sciences I Hasselt University | Agoralaan, Gebouw A | B-3590 Diepenbeek | België/Belgium

+32(0)11269201 (secr.) | bert.opteijnde@uhasselt.be | www.uhasselt.be/glw I www.adlon.be | @bertopteijnde