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DOCTORAL DISSERTATION

Exercise therapy in multiple sclerosis: the impact of exercise intensity on glucose disposal and muscle contractile properties

Doctoral dissertation submitted to obtain the degree of doctor of Biomedical Science, to be defended by:

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List of Abbreviations

ANOVA:	Analysis of Variance
ANS:	Autonomic Nervous System
ATP:	Adenosine Triphosphate
AUC:	Area Under the Curve
BBB:	Blood Brain Barrier
BDNF:	Brain Derived Neurotrophic Factor
BMI:	Body Mass Index
CFA:	Freund's Complete Adjuvant
CNS:	Central Nervous System
CON:	Control group
CP:	Chronic progressive
CSA:	Cross Sectional Area
CVD:	Cardiovascular Disease
DMT:	Disease Modifying Therapy
EAE:	Experimental Autoimmune Encephalomyelitis
ECG:	Electrocardiogram
EDL:	m. Extensor Digitorum Longus
EDSS:	Expanded Disability Status Scale
ELISA:	Enzyme-Linked Immunosorbent Assay
EX:	Exercising group
FDA:	Food and Drug Administration
GLUT4:	Glucose Transporter 4
HC:	Healthy Control
$H_{CT}R$:	High intensity Continuous Training and Resistance training
HDL:	High Density Lipoprotein
$H_{IT}R$:	High intensity Interval Training and Resistance training
HR:	Heart Rate
IGT:	Impaired Glucose Tolerance
IR:	Insulin Resistance
LBM:	Lean Body Mass
LDL:	Low Density Lipoprotein
LTPA:	Leisure Time Physical Activity
MBP:	Myelin Basic Protein
MS:	Multiple Sclerosis
MSD:	Meso Scale Discovery
MRI:	Magnetic Resonance Imaging
MRT:	Mean Response Time
mRNA:	Messenger Ribonucleic Acid
NS:	Not Significant
OGTT:	Oral Glucose Tolerance Test

PASIPD:	Physical Activity Scale for Individuals with Physical Disabilities
PBS:	Phosphate Buffered Saline
pMS:	Persons with Multiple Sclerosis
PP:	Primary Progressive MS
PR:	Progressive Relapsing MS
RPE:	Ratings of Perceived Exertion
RPM:	Rotations Per Minute
RER:	Respiratory Exchange Ratio
RR:	Relapsing Remitting MS
SED:	Sedentary subgroup
SE:	Standard Error
SOL:	m. Soleus
SP:	Secondary Progressive MS
tAUC:	Total Area Under the Curve
TG:	Triglyceride
TR^L:	Low intensity Treadmill group
TR^M:	Moderate intensity Treadmill group
TR^H:	High intensity treadmill group
TA:	m. Tibialis Anterior
TNFα:	Tumor Necrosis Factor alfa
VE:	Expiratory Volume
VO_2:	Oxygen Consumption, Oxygen Uptake
W:	Watt
WHO:	World Health Organisation

Chapter I:

General Introduction

Multiple Sclerosis

Multiple Sclerosis (MS) is a neurodegenerative disease that predominantly affects young and middle-aged adults and almost twice as many women as men [1] (WHO - Neurological disorders). It is an inflammatory disorder of the central nervous system (CNS) with subsequent destruction of myelin, oligodendrocytes and axons in the brain, brain stem and spinal cord [2, 3]. With an estimated total of 2.5 million MS patients worldwide, it's the most common disease of the CNS in young adults [1] that is precipitated by environmental factors in a genetically predisposed host [4].

Susceptibility to MS is multifactorial. It has already been suggested by migration studies that geography is important and that several environmental factors, such as viral infections (Epstein-Barr virus), sunlight exposure, vitamin D and smoking are involved in the development of MS [5]. Furthermore, in monozygotic and dizygotic twins the genetic risk to develop MS is 25-30% in the first case and 3-5% in the latter, underlining genetic predisposition to MS. The risk to develop MS in a first-degree relative of a MS patient is 2-4% [6].

The heterogeneous and complex symptoms of MS often lead to a more sedentary lifestyle [7]. This may result in disuse-related loss of muscle strength and exercise capacity, which in turn can affect the quality of life [3, 8] and may cause inactivity-induced secondary health complications.

Despite the progressive character of the disease, 80% of all MS patients live with the disease for more than 35 years [9], whereas the life expectancy is reduced by 5 to 10 years [10]. Recently developed pharmacological therapies are able to slow disease progression, but MS is still not curable. Consequently, MS rehabilitation is an important factor of overall MS treatment. In this respect it is important to note that new scientific findings have changed MS rehabilitation dramatically during the last decade. Moreover observational [11, 12] as well as interventional studies [13, 14], clearly document that MS rehabilitation is able to improve muscle strength, exercise tolerance, functional capacity and health-related quality of life. Unfortunately, the impact of MS rehabilitation on secondary health complications and muscle contractile characteristics is not yet clear.

1. Pathogenesis

Inflammation of the CNS is believed to be the primary cause of damage in MS, inducing demyelination in the white matter of the brain and spinal cord, followed by neurodegeneration during the progressive phase of the disease [15, 16].

The inflammatory process in MS is characterized by degradation of the blood brain barrier (BBB), allowing the migration of autoreactive T-cells. In the CNS, these T-cells are reactivated by antigen presenting cells, such as microglia, triggering an immunological cascade and resulting in the recruitment of B-cells, cytotoxic $CD8^+$ T-cells and macrophages in the CNS [2, 17-19]. The latter will induce disturbances of the local expression of pro- and anti-inflammatory cytokines and chemokines and will cause demyelination, accompanied by a varying degree of axonal injuries and axonal loss [15, 16]. This neurodegeneration is suggested to be a major cause of MS related disabilities [20].

It has become clear that MS is not only characterized by white matter demyelination, but that it is also featured by extensive axonal loss and gray matter pathology [21], all contributing to diverse MS symptoms. During the early stage of the disease course, brain plasticity is able to compensate for the neuronal loss, resulting in only small clinical disabilities. However, during the progressive stage of the disease, neural plasticity is no longer able to palliate these disabilities, resulting in neurological decline, which is irreversible [21].

2. An MS animal model: Experimental Autoimmune Encephalomyelitis

The Experimental Autoimmune Encephalomyelitis (EAE) animal model mimics an inflammatory demyelinating disease of the CNS, often used to investigate demyelination of the CNS in general, and MS in particular [22]. Briefly, after injection of a myelin antigen, myelin reactive T-cells are activated, crossing the BBB and causing inflammation after reactivation in the CNS [23-25]. This model can be induced in mice [26], rats [27], hamsters [28], marmosets [29], rabbits [30], goats [31], sheep [31] and dogs [32] and can, depending on the genetic background of the animal strain and the myelin protein, follow an acute monophasic or a more chronic relapsing-remitting course. Clinically, exacerbations are characterized by hindquarter paralysis, starting at the tip of the tail and progressing to the trunk, and are scored on a scale ranging from 0 (no signs) to 5 (death) [33].

3. Clinical disease course and progression of MS

MS expresses itself in 5 different clinical courses (Figure 1.1). **(1)** Relapsing-remitting MS (RRMS) is the most common MS type, affecting more women than men. This MS type is characterized by relapses followed by (often almost complete) remission and lack of further disease progression until the next relapse [34]. **(2)** The second most common MS type is secondary progressive MS (SPMS), which occurs in 40-65% of RRMS patients and is characterized by an initial relapsing-remitting disease course followed by progression with or without occasional relapses, minor remissions and plateaus [34]. **(3)** Primary progressive MS (PPMS) is the third MS type, characterized by a gradual increase of symptoms or clinical signs over time [2, 3]. It has been suggested that RRMS is predominantly characterized by inflammation and the formation of white matter lesions, whereas in PPMS new inflammatory demyelinating lesions are rare and it is characterized by diffuse atrophy of gray and white matter [15]. Patients with PPMS are, in general, older at onset and a higher proportion are men, compared to RRMS patients [35]. Primary and secondary progressive MS subtypes are often grouped as chronic progressive (CP) MS. **(4)** Progressive-relapsing MS (PRMS) is the fourth and most rare MS type, characterized by acute relapses and continuous progression of the level of disability in between [34]. **(5)** Finally, a significant subgroup of MS patients experience little disease progression and minimal or no MS-induced disability. This MS type is called benign MS and is characterized by full functionality in all neurologic systems 15 years after disease onset [34, 36].

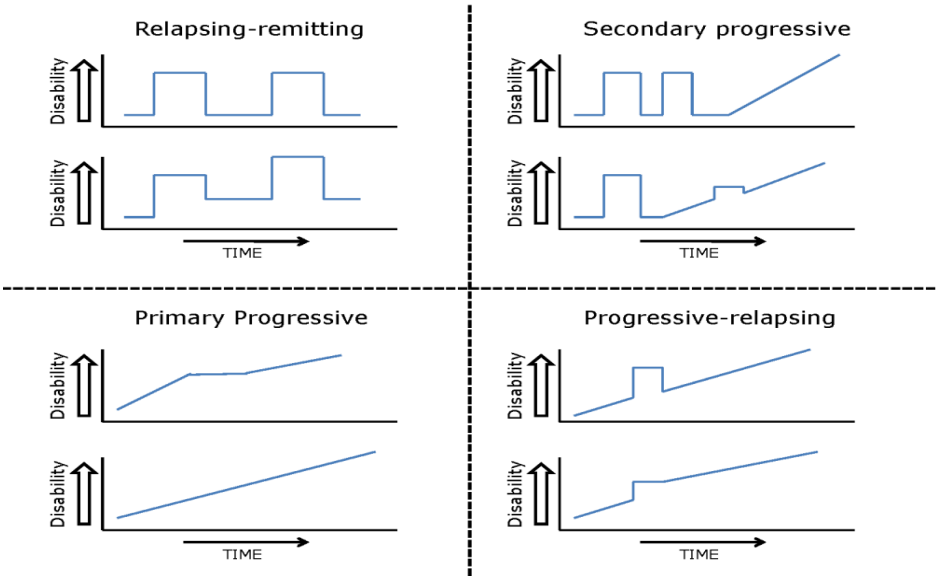

Figure 1.1: *Clinical disease courses of MS.*

During the last years, a number of instruments have been developed to describe the clinical severity and the functional deficit in MS. These measurements are often used as an endpoint in clinical trials to assess the effectiveness of therapeutic interventions. The most common and widely accepted scale is the Expanded Disability Status Scale (EDSS) of Kurtzke [37], a clinician-administered assessment scale evaluating the functional systems of the CNS, describing disease progression of MS patients. It consists of an ordinal rating system, ranging from 0 (normal neurological status) to 10 (dead due to MS), in 0.5 increasing intervals (after reaching EDSS 1). The lower scale values measure impairments based on a neurological examination, whereas the upper range of the scale measures disabilities of persons with MS.

4. MS Symptoms

Due to demyelination and axonal loss of the CNS and the variable distribution of the damage in the myelin sheath of the nerves the disease is characterized by numerous and heterogeneous symptoms and a number of 'hallmark symptoms' [2, 3, 38]. RRMS is characterized by unilateral optic neuritis, diplopia, sensory disturbances, clumsiness, ataxia, bladder and bowel problems and Lhermittes's sign (neck flexion that evokes trunk and limb paraesthesia). Progressive MS on the other hand often induces slow evolving upper-motor neuron symptoms such as quadriparesis, cognitive decline (memory loss, impaired attention), visual loss (optic neuritis), brainstem syndromes, cerebral, sexual, bowel and bladder dysfunction [2, 3, 38].

Persons with MS are often confronted with decreased aerobic capacity [39, 40] and reduced muscle strength [41-51], which, in combination with the earlier mentioned symptoms, often lead to a reduced functional capacity [52, 53]. Furthermore, general fatigue and muscular weakness are also prevalent symptoms in MS. As a consequence of the latter, people with MS are often more inactive than matched healthy controls [7], resulting in an inactivity related physiological profile that is characterized by an even greater weakness, fatigue and associated health risks than provided by the disease *per se* [54, 55], often referred to as 'secondary MS symptoms'.

In healthy people, a limited level of physical activity is associated with an increased risk of several chronic pathologies [56, 57] and risk factors associated with the metabolic syndrome, a term clustering the combined presence of several cardiovascular risk factors such as hypertension, elevated glucose concentrations and dyslipidaemia [58]. As such impaired glucose tolerance (IGT) and insulin resistance (IR), which confers increased risk of developing type II diabetes, and in turn increases the cardiovascular disease (CVD) risk markedly, frequently occur [59]. However, it has not been clearly established

whether MS patients in general are at greater risk of developing CVD and the metabolic syndrome than matched controls.

The symptoms, investigated in this project, are described more in detail below.

4.1 Impaired glucose tolerance

IGT and IR both indicate impaired whole body glucose regulation and refer to a metabolic state intermediate between normal glucose homeostasis and type II diabetes [60-62]. IR reflects limited insulin-mediated glucose uptake in peripheral tissues while the β-cell secretory capacity of the pancreas remains unaffected, resulting in hyperinsulinemia to maintain the glucose homeostasis [60]. Additional limitations of the glucose homeostasis are characterized by decreased insulin sensitivity, mirrored by a decreased number of insulin receptors and glucose intolerance, as a result of the inability of normal disposal of glucose depending on insulin secretion, peripheral glucose uptake, hepatic glucose uptake, as well as on inhibition of hepatic glucose output [63]. Risk factors to develop IGT and IR include, amongst others, a low cardiorespiratory fitness, low muscle strength, high body mass index, large amount of visceral fat, smoking and a low physical activity level [62].

So far, literature regarding the prevalence of IGT in MS is conflicting, with some studies reporting an increased [64-66], similar [10] or decreased [67, 68] prevalence. Moreover, Hussein and co-workers retrospectively investigated the medical records of MS patients, of which 7% were diagnosed with diabetes type II, stating that this was higher than the general population at that time [66]. Warren and Warren concluded that there was a relation between MS and any type of diabetes, since more MS patients, compared to controls, were diabetic or had blood values indicating diabetes [65]. Kang and co-workers reported that 9% of people with MS were diagnosed with type II diabetes mellitus, which was stated to be comparable to the general population [69]. On the other hand, some studies reported a lower prevalence of diabetes in MS patients [67, 68]. Fleming *et al.* retrospectively investigated comorbidities in elderly MS patients and compared that to a matched control group. It was reported that some comorbidities, including diabetes type II, were more prevalent in the control group. Moreover, uncomplicated diabetes type II was reported in 3% of the included MS population, and in 6% of the control group [67]. Comparable conclusions were drawn by Allen and co-workers, reporting a difference between prevalence of any type of diabetes in 11% of the MS patients and 19% of the matched healthy controls [68].

Several studies only reported fasting glucose concentrations [70-74], which is insufficient to indicate IGT according to the European Society of Cardiology, who are recommending a 2h oral glucose tolerance test (OGTT) as the preferable screening tool for IGT. Slawta and co-workers (2003) concluded, that the incidence of elevated glucose concentrations was similar to the (elsewhere reported) general population [70], showing elevated fasting glucose concentrations in 8% of the female MS patients. However, the latter was not confirmed in another study from the same group, reporting normal fasting glucose concentrations in inactive female MS patients [71]. Also, White *et al.* and Mähler *et al.* reported normal fasting glucose concentrations in small samples of MS patients [72, 73]. Furthermore, the latter stated that glucose tolerance was not impaired in persons with MS [73]. Recent research of Sternberg and co-workers showed lower plasma glucose concentrations in MS patients, compared to controls. It was further reported that positive correlations between glucose concentrations and EDSS and between glucose concentration and the rate of clinical relapses existed [74], warranting further research.

4.2 Muscular symptoms

A number of studies have examined skeletal muscle characteristics of MS patients, with some studies [49, 50, 75], but not all [45, 47, 76], reporting loss of muscle mass and decreased [43, 45-47, 49, 77] or comparable [50] maximal muscle strength. The mechanisms underlying the often observed strength deficit include increased axonal conduction times [78], reduced motor unit recruitment and firing rates [45, 79, 80].

At the cellular level, the impact of MS on muscle fiber characteristics, such as cross sectional area (CSA) and muscle fiber proportion remains conflicting. Some authors reported reduced muscle fiber size in MS patients compared to healthy controls [49, 50], whereas others did not report any alterations [45, 47] or indicated alterations, but did not include a direct comparison to healthy controls [81], clearly suggesting a need for well-powered studies to clarify the muscular influence of MS. Furthermore, corresponding to immobilized healthy controls [82-84], a shift from type I to type IIa and IIax fibers was reported in a small group of MS patients by Kent-Braun *et al.*[50], but this was not confirmed by others [45, 47, 49]. The most important characteristics of skeletal muscle fiber type I, IIa and IIx are summarized in Table 1.1 [85, 86].

Table 1.1: *Muscle fiber characteristics of type I, IIa and IIx fibers.*

Properties	Type I	Type IIa	Type IIx
Motor unit type	Slow oxidative	Fast oxidative/glycolytic	Fast glycolytic
Colour	Red	Red	White
Twitch speed	Slow	Fast	Fast
Resistance to fatigue	High	Intermediate high	Low
ATPase activity	Low	High	High
Oxidative enzyme capacity	High	Intermediate high	Low
Myoglobin	High	High	Low
Mitochondrial density	High	High	Low
Capillary density	High	Intermediate	Low
Glycolytic capacity	Low	Intermediate high	High
Glycogen content	Low	High	High
Insulin sensitivity	High	Low	Low
Exercise	Endurance	Endurance/resistance	Resistance

Skeletal muscles are important glucoregulatory sites. Therefore, intramyocellular mechanisms, such as changes in the insulin signalling and/or sensitivity cascade and disturbed muscle energy metabolism may be involved in IGT in MS. The exact mechanisms, however, are unclear. Because a large part of the glucose disposal is directed towards the skeletal musculature, the level of physical exercise and muscular activity can alter insulin sensitivity and glucose tolerance in skeletal muscle [87, 88], mirrored by changes in, amongst others, muscle glucose transporter (GLUT4) expression and glycogen storage capacity [59, 89]. However, this was never investigated before in MS.

5. Treatment of MS

At present, the pharmacological treatment of MS can slow down disease progression but is so far still unable to cure the disease. Treatment of MS patients can be subdivided in disease specific and symptomatic therapies. As such, overall MS treatment constitutes a multi-disciplinary approach directed towards maintaining a MS patients' functional status. In general they comprise pharmacological, rehabilitation and exercise therapy.

5.1 Pharmacological therapy

The pharmacological treatment of MS includes treatment of acute relapses, and disease-modifying treatments. **(1)** Acute relapses are treated using glucocorticosteroids, shortening the duration of the relapse and diminishing the severity of the relapse-related symptoms [90]. In particular, corticosteroids inhibit lymphocyte proliferation and the synthesis of pro-inflammatory cytokines [91]. Nevertheless, the long-term effects on disease activity are unclear [90]. **(2)** There are currently 10 Food and Drug Administration (FDA)-approved disease-modifying medications available for relapsing forms of MS, whereas no agents are FDA-approved for the progressive forms of MS. Four

preparations of interferon beta (IFN-β; Avonex®, Rebif®, Betaseron® and Extavia®) and Glatiramer acetate (GA, Copaxone®), often referred to as 'first line therapies', are frequently applied immunomodulatory therapies, reducing the relapse rate by ~30%, by amelioration of the relapse severity, which in turn leads to a delay of the progression of disability in RRMS patients [90]. Patients who do not respond to GA or IFN-β are treated with, so called 'second line therapy', natalizumab (Tysabri®), a recombinant humanized antibody, or the recently approved oral medications fingolimod (Gilenya®), teriflunomide (Aubagio®) and dimethyl fumurate (Tecfidera®) [92], reducing the relapse rate in RRMS. Furthermore, patients who deteriorate on the application of established therapies (often patients with secondary progressive MS [93]) are sometimes treated with immunosuppressive agents such as mitoxantrone (Novantrone®).

Unfortunately, the current pharmacological treatments are only able to slow down the inflammatory disease process and are only effective in subgroups of MS patients. Furthermore, some of the side effects can be substantial.

So far, it remains unknown whether the reported impairments of MS patients are consequences of the disease per se [94], are caused by inactivity [7, 95] or are affected by a combination of both. Impairments as a result of the disease per se are probably not reversible, whereas inabilities developed as a consequence of MS-induced inactivity could be improved by means of physical exercise. Therefore, a symptomatic treatment, including rehabilitation and/or exercise therapy to minimize the impact of MS on daily life activities, is warranted.

5.2 Rehabilitation and exercise therapy

Rehabilitation programs aim to improve the quality of life, societal participation and the independence of MS patients. Physiotherapy, occupational therapy, psychology and physical exercise are frequently used disciplines in the multidisciplinary setting, of which only the latter will be discussed into detail below.

For many years, MS patients were advised not to participate in physical exercise due to fatigue and/or symptom instability [96], which is frequently caused by the exercise-induced increase in body temperature [96]. However, it has been demonstrated that the worsening of these sensory symptoms, experienced by more than 40% of all MS patients after exercise, is temporal and will disappear within 30 min after termination of the exercise in 85% of all MS patients [96]. Furthermore and as mentioned earlier, physical inactivity directly contributes to the cascade of events that lead to the expression of the 'exercise deficient phenotype', resulting in abdominal fat accumulation,

higher levels of triglyceride, lower levels of high-density-lipoprotein and reduced insulin sensitivity [59].

During the last decades, however, exercise therapy has become an accepted part of overall MS treatment, since scientific research has clearly shown its beneficial effects [41, 97-100]. Several exercise intervention studies already reported significant improvements in almost every aspect of the physiological profile of MS patients, suggesting that a substantial part of the impairments are inactivity related, rather than a result of non-reversible tissue injury. The research and development of rehabilitation programs for MS patients are, therefore, a topic of potential therapeutic interest. Furthermore, given the ability of exercise to reduce the risk of developing IGT and IR [101-105] and to improve insulin sensitivity in other populations [106-108], exercise may be a very important non-pharmacological intervention targeting the CVD risk in MS patients.

Basic physical exercise modalities can be subdivided in endurance training, resistance training and combined training.

5.2.1 Endurance training in MS

Endurance or cardiorespiratory training in MS has been researched extensively, investigating the effect on a wide range of functional, physiological and psychological parameters [13, 39, 109-111]. In particular, an improved endurance capacity/maximal oxygen consumption (VO_2max), lower blood lactate levels and higher aerobic thresholds [41], as well as altered body composition [13], functional capacity and gait velocity [112-115] following low-to-moderate intensity training have been reported. Furthermore, only a few studies investigated the effect of endurance training on muscle strength and reported no or only small changes [115-117].

Interestingly, several authors suggested that MS patients could benefit even more from higher aerobic training intensities, but it is unclear whether this could be tolerated [41, 100, 118, 119]. In healthy subjects, however, high intensity exercise poses the potential to further enhance training outcomes [120-132].

Based on the existing literature it is recommended to exercise MS patients at an initial intensity of 50-70% of VO_2max, corresponding to 60-80% of maximal heart rate, for 10 to 60 min 2-3 times/week [41, 133]. Training volume should be increased to obtain training progression during the first 2 to 6 months [41]. Next, to ensure an optimal training stimulus, a higher training intensity, exceeding the anaerobic threshold, could be advised. However, as stated earlier, it is not known whether MS patients can tolerate high intensity endurance training.

Given the fact that the influence of high intensity endurance exercise on functional capacity, disease symptoms and muscle contractile properties has never been investigated before in MS, the use of the EAE model seems as an appropriate initial approach [134]. However, it is still unclear if results obtained in EAE studies can be directly extrapolated to MS patients, since EAE only mimics the inflammatory process of MS.

So far, research investigating the impact of EAE and/or physical exercise during EAE on muscle contractile properties and EAE progression is scarce, conflicting but also promising [135-138]. De Haan and co-workers explored the impact of EAE on muscle contractile properties. Compared to healthy controls, EAE rats were not only less physically active, they also showed significant loss of body and Gastrocnemius muscle mass (-21 and 33%, respectively), reduced muscle fiber CSA of all fiber types (-40 to 50%), as well as lower maximal muscle force and power (-58 and 73%, respectively) [135]. Furthermore, Le Page and co-workers demonstrated that treadmill running after immunization, delayed the onset and duration of hindquarter paralysis that is associated with chronic EAE. However, maximal clinical scores were not affected and these investigators did not examine changes in muscle CSA [137]. More recently, in mice with chronic EAE less severe neurological deficits and a less pronounced spinal loss in the striated neurons after voluntary, not structured, wheel running have been reported [138].

5.2.2 Resistance training in MS

Several studies have demonstrated the benefits of resistance training [14, 118, 139-149] in MS. However, the methodological quality (lack of control group, low statistical power, home-based intervention, …) of several studies was rather low, resulting in inconclusive findings and a difficulty to formulate general guidelines [14]. Furthermore, the applied training programs were relatively short, remaining the effects of long-term resistance training unclear.

The impact of resistance training on muscle contractile properties in MS has only been investigated using MRI techniques [141, 142] and by Dalgas and co-workers, who used an immunohistochemical staining protocol [81]. The latter reported increased m. Vastus Lateralis mean fiber CSA combined with improved muscle strength following 12 weeks of progressive resistance training. Despite the importance of understanding the effects of exercise on muscle fiber characteristics to optimize exercise and rehabilitations programs in MS, the impact of other training modalities and intensities on muscle fiber CSA and fiber type proportion in MS, has not been investigated yet.

Interestingly, many MS patients develop asymmetric leg strength [150]. In stroke patients, progressive unilateral resistance training has already been applied to optimize the training stimulus [151, 152], based on the larger

neuromuscular adaptations in weaker versus stronger muscles after resistance training in healthy subjects [153]. To our knowledge, only one study applied an unilateral training method [139], whereas all other existing studies applied a bilateral approach [118, 142, 144].

5.2.3 Combined exercise

Theoretically, most benefit is suggested to be gained from exercise combing both cardiovascular and resistance training, since this would positively affect impairments of both the cardiovascular system and muscle strength/activation [41]. However, only a limited number of studies [154, 155] evaluated the impact of a combined exercise intervention in MS. Consequently, the impact a combined exercise intervention remains poorly understood in MS patients.

5.2.4 Physical exercise and impaired glucose tolerance in MS

Given the ability of exercise to reduce the risk of developing IGT and IR [105], and to improve insulin sensitivity in other populations [107], it can be hypothesized that a disturbed glycemic control in MS patients could be improved. The work of Slawta *et al.* [71] and White and co-workers [143] support this hypothesis by demonstrating that exercise may be an important tool in MS-rehabilitation, not only at the level of physical capacity, but also to decrease potential secondary health problems. However, because the above mentioned studies have some methodological shortcomings (fasting blood glucose vs. OGTT [71, 143]), the impact of physical exercise on glucose and insulin profiles in MS patients remains elusive.

To understand the impact of exercise on glycemic control, the exact amount and intensity of physical exercise to achieve health-related benefits should be taken into account. Some investigators reported that low-to-moderate exercise intensity improved glucose tolerance in other populations, whereas others suggested that this intensity was insufficient to improve insulin sensitivity [156]. Sandvei *et al.* reported a decrease in glucose tolerance after 8 weeks of sprint interval training, whereas the glucose response remained unchanged after 8 weeks of continuous running. However, fasting glucose concentrations were significantly reduced in both groups [157]. Similarly, Tjonna and co-workers demonstrated a significant improvement in fasting blood glucose and insulin sensitivity after 16 weeks of aerobic interval training (90% of maximal heart rate) in metabolic syndrome patients, whereas no improvements were found after moderate continuous exercise (70% of maximal heart rate)[158], suggesting that high intensity exercise is probably also more adequate in MS, warranting further research.

Chapter II:

Experimental work and results

Objectives and general outline

The studies performed during this PhD are represented in Figure 2.1 and involved both animal and human research. The obtained results are reported in 8 first-author original research papers, that have been published or are under review for publication in peer-reviewed international journals, focused on clinical and neurological rehabilitation in MS. These 8 papers fit in the objectives of this PhD research, investigating IGT and skeletal muscle characteristics in MS.

Despite many epidemiological studies examining comorbidity in people with MS, there are conflicting opinions on whether the incidence of CVD and its precursors is higher in MS patients compared to the general population. Therefore **study 1** aims to systematically review the existing literature about CVD risk and risk factors constituting the metabolic syndrome in MS. All other studies will be based on the results of this literature search, highlighting the needs for future research on CVD risk in general and IGT in particular in this PhD thesis.

As mentioned earlier, the complex MS symptoms often lead to a more inactive lifestyle than matched controls [7]. In healthy people, a limited level of physical activity is associated with an increased risk of IGT and IR, facilitating the risk of developing type II diabetes, and in turn increases the CVD risk markedly [59]. Based on these findings **study 2** evaluates the hypothesis that the prevalence of IGT is elevated in MS.

Furthermore, given the ability of mild-to-moderate intensity exercise to reduce the risk of developing IGT and IR [105] and to improve insulin sensitivity in other populations [107], **study 3** investigates whether this is also the case in MS patients.

Based on the suggestion that high intensity exercise is functionally probably more adequate in MS, the application of high intensity exercise in MS warrants further research. However, given the fact that high intensity exercise has never been investigated before in MS, the application of the EAE model seems appropriate (study 4 and 5). In particular, **study 4** aims to investigate the impact of low, moderate and high intensity exercise on muscle contractile properties and disease progression of healthy and acute EAE rats, hypothesizing that exercise improves muscle contractile properties and delays the onset of hindquarter paralysis, in an intensity-dependent manner.

Next, **study 5** aims to investigate the impact of high intensity aerobic exercise on disease course and muscle morphology in EAE and healthy animals, immediately before (experiment 1) and after (experiment 2)

hindquarter paralysis. We hypothesize that high intensity aerobic exercise affects the disease course and muscle contractile properties, keeping in mind that paralysis induced inactivity may temper these effects.

Based on studies 4 and 5 a high intensity training program is applied in **study 6** in MS patients, investigating the effect of a high intensity interval and continuous cardiovascular training, both in combination with resistance training, on glucose tolerance and skeletal muscle GLUT4 content. We hypothesize that high intensity exercise improves glucose tolerance and increases muscle GLUT4 content in persons with MS.

Given the muscular symptoms in MS, a number of studies already examined skeletal muscle characteristics of MS patients, with some studies [49, 50, 75], but not all [45, 47, 76], reporting loss of muscle mass and decreased [43, 45-47, 49, 77] or comparable [50] maximal muscle strength. Furthermore, the impact of MS on muscle fiber characteristics, such as CSA and muscle fiber proportion remains conflicting [45, 47, 49, 50]. To clarify the heterogeneous results of the existing literature in small groups of MS patients, **study 7** aims to investigate the effect of MS on muscle fiber CSA and proportion, muscle strength and muscle mass in MS patients and healthy referent subjects. It is hypothesized that MS would negatively affect skeletal muscle characteristics, by decreasing muscle strength and muscle mass and reducing muscle fiber cross sectional area.

So far, the impact of exercise on muscle contractile properties in MS has only been investigated by Dalgas and co-workers [81]. Despite the importance of understanding the effects of exercise on muscle fiber characteristics to optimize exercise and rehabilitations programs in MS, the impact of other training modalities and intensities on muscle fiber CSA and fiber type proportion in MS, has not been investigated yet. Therefore, **study 8** aims to investigate the impact of high intensity interval or continuous cardiovascular exercise, both in combination with resistance training, on muscle contractile characteristics, such as muscle fiber CSA and proportion, muscle strength and muscle mass in MS patients.

Based on the existing literature it is clear that exercise therapy is able to improve several MS symptoms. Interestingly, training mode, exercise intensity, duration, frequency and progression are important factors to induce improvements. However, these parameters could vary, based on the target or goal, the health status and the functional capacity of the MS patients [159]. Therefore, **study 3, 6 and 8** investigates and compares the influence of different exercise intensities and modalities on muscle strength, endurance capacity and body composition of MS patients.

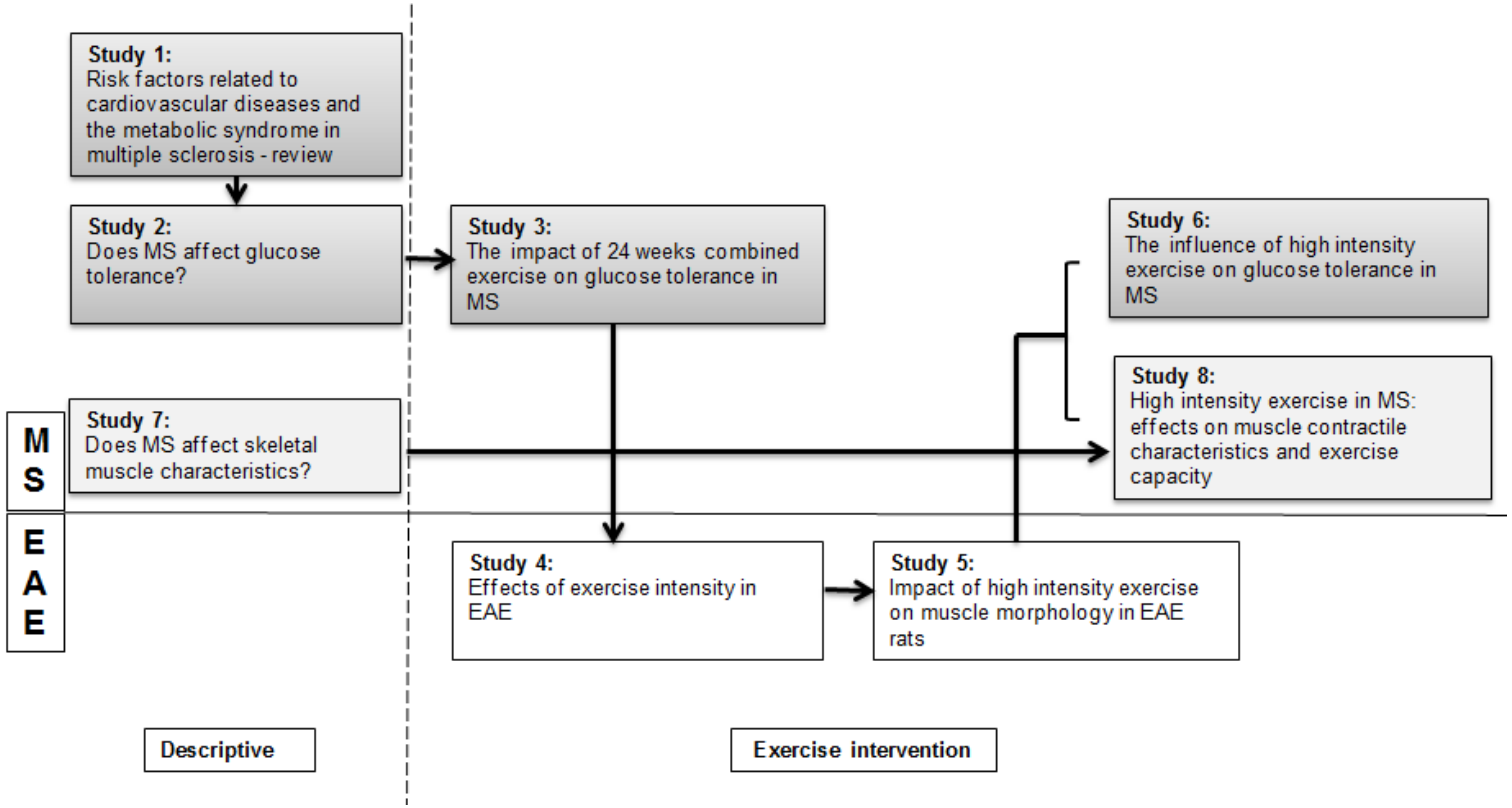

Figure 2.1: *Schematic overview of the performed studies.*

STUDY 1

Risk factors related to cardiovascular diseases and the metabolic syndrome in multiple sclerosis – a systematic review

Based on: Wens I., Dalgas U., Stenager E., Eijnde BO.

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Abstract

Despite many epidemiological studies examining comorbidity in people with multiple sclerosis (pMS), there are conflicting opinions on whether pMS are at more or less risk of cardiovascular disease (CVD) and the metabolic syndrome compared with the general population. As pMS can now expect longer survival, this as an important question both at an individual and public health level. This study aimed to systematically review the literature linking MS to CVD risks and to the risk factors constituting the metabolic syndrome. This systematic review is based on a comprehensive literature search of six databases (Swemed+, Pubmed, Embase, Cochrane, PEDro and CINAHL). In total 34 studies were identified. Despite the high number of identified papers, only limited and inconsistent data exist on the risk factors of the metabolic syndrome and MS. Overall, the data suggest an increased CVD risk in pMS. From the existing studies it is not clear whether the increased risk of CVD is related to an increased risk of obesity or changes in body composition, hypertension, dyslipidaemia or type II diabetes in pMS, indicating the need for future research in the field, if we are to advise pMS adequately in avoiding preventable comorbidity.

Introduction

Multiple Sclerosis (MS) is characterized by heterogeneous symptoms, often leading to a more inactive lifestyle than matched healthy controls (HC) [7], resulting in more weakness, fatigue and associated health risks [11, 54]. In HC, physical inactivity is associated with an increased risk of the metabolic syndrome [160-166], a term describing the combined presence of cardiovascular risk factors hypertension, dyslipidaemia, elevated glucose concentrations and obesity [58]. Many metabolic syndrome patients also suffer from insulin resistance, which confers an increased risk of developing type II diabetes, which in turn increases the cardiovascular disease (CVD) risk markedly [59].

However, it remains unclear whether or not people with MS (pMS) are more likely to have CVD or the risk factors comprising the metabolic syndrome, when compared to HC. Consequently, this study aimed to systematically review the literature evaluating 1) the CVD risk and 2) the risk factors constituting the metabolic syndrome in pMS. In particular, abdominal obesity, high-density lipoprotein (HDL) cholesterol, hypertension and glucose intolerance, were included [59]. Other parameters such as the pro-inflammatory, prothrombotic state [59] and CVD induced by medication, were beyond the scope of this review.

Methods

This systematic review was based on a comprehensive literature search of 6 databases (Swemed+, Pubmed, Embase, Cochrane, PEDro and CINAHL). The databases were searched using subject headings corresponding to the risk factors contributing to the development of CVD and the metabolic syndrome (see Table 2.1.1).

The original search was performed in March 2012, and updated in February 2013. The study inclusion is visualised in Figure 2.1.1. To be included studies had to describe one or more of the mentioned risk parameters related to the metabolic syndrome or CVD in pMS. Consequently, studies evaluating the influence of parameters of the metabolic syndrome on the risk of developing MS were not included in this review. The included studies had to be peer reviewed and had to be written in English. Comments, case reports, reviews and book chapters were excluded as were studies regarded irrelevant to the topic. Finally, study quality was evaluated by an estimation of the level of evidence, based on the Oxford Centre for Evidence-based Medicine rating system [167]. Table 2.1.2 gives an overview of the included articles.

Table 2.1.1: *Overview of the applied search terms and retrieved manuscripts.*

Database	Articles retrieved	Search terms (MeSH etc.)
SveMed+	15	"Multiple Sclerosis" AND "Cardiovascular diseases"
PubMed	718	"Multiple Sclerosis" AND ("Glucose Metabolism disorders" OR "Body Constitution" OR "Dyslipidemias" OR "Hypertension")
Embasse	1449	"Multiple Sclerosis" AND ("Hyperglycemia" OR "Insulin Resistance" OR "Metabolic Syndrome X" OR "Obesity" OR "Dyslipidemia" OR "Hypertension" OR "Non Insulin Dependent Diabetes mellitus" OR "Impaired Glucose Tolerance")
Cochrane	14	"Multiple Sclerosis" AND ("Glucose Metabolism Disorders" OR "Body Constitution")
PEDro	144	"Multiple Sclerosis" AND ("Glucose Metabolism Disorders" OR "Hypertension" OR "Body Constitution" OR "Hyperlipidemia")
CINAHL	237	"Multiple Sclerosis" AND ("Glucose Metabolism Disorders" OR "Hypertension" OR "Body Constitution" OR "Hyperlipidemia")

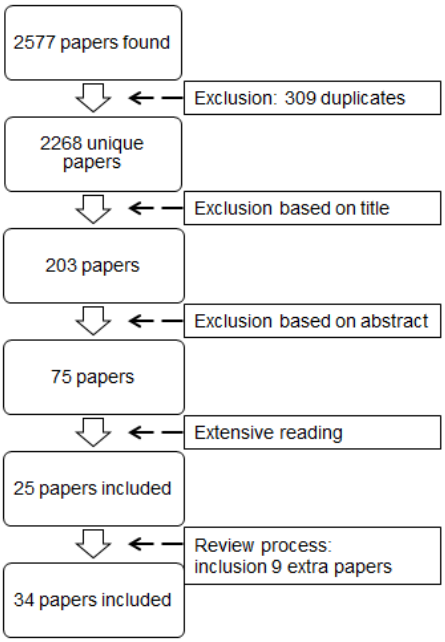

Figure 2.1.1: *Flowchart illustrating the inclusion of literature.*

Table 2.1.2: *Overview of included studies*

Study	Sample size	Study design / setting	Level of Evidence	EDSS	CVD	Obesity/ body composi-tion	Diabetes / Glucose	Hyper-tension	Dyslipi-daemia	Main relevant findings
Allen *et al.*, **2008**	9949 pMS 19 898 CON	Case control (Hospital based)	3b	NR	X	X	X	X	X	pMS, compared to CON, were less likely to experience CVD risk factors, like hypertension, hypercholesterolemia, diabetes and obesity.
Anema *et al.*, **1991**	34 pMS 63 CON	Cohort study (Controlled)	2b	3.8 ± 2.1				X		Blood pressure, in response to standing, was abnormal in 13% of pMS, whereas normal values were obtained from CON.
Bronnum-Hansen *et al.*, **2004**	9881 pMS	Cohort study (controlled)	1b	NR	X					MS was associated with an ~threefold increase of death risks. The mortality rate due to CVD was higher than in the (elsewhere reported) general population.
Buchanan *et al.*, **2006**	1518 pMS	Cross-sectional (Survey based, non-controlled)	2c	NR				X	X	High blood pressure, diabetes and high cholesterol were the most common reported diseases in the included pMS.
Çomoglu *et al.*, **2004**	22 pMS 16 CON	Case series (Controlled)	4	0.5 – 5.5 (range)		X			X	There was no difference between BMI / lean body mass of pMS and CON. Body fat percentage of male pMS was lower, compared to male CON. Total cholesterol levels were slightly higher in MS than in CON, but this was not statistically significant.
Dallmeijer *et al.*, **2009**	146 pMS	RCT	1b	2.5 (median)	X					40% of the MS group reported any type of comorbidity, like CVD (5%) and diabetes (4%), which were associated with aging and EDSS.
Fleming and Blake, 1994	5384 pMS 7168 CON	Case control (Hospital based)	3b	NR	X		X	X		Some conditions, like hypertension, heart failure and diabetes, were less prevalent in pMS, compared to CON.
Formica *et al.*, **1997**	71 female pMS 71 female CON	Cohort study (Controlled)	2b	6.8 ± 0.2		X				BMI of pMS was lower, compared to BMI of CON. Fat mass and fat-free mass were comparable between pMS and CON, except for fat-free mass of nonambulatory pMS.

Study	Sample size	Study design / setting	Level of Evidence	EDSS	CVD	Obesity/ body composi-tion	Diabetes / Glucose	Hyper-tension	Dyslipi-daemia	Main relevant findings
Giubilei *et al.*, **2002**	18 pMS	Cross-sectional (non-controlled)	2c	1.5 (median)					X	A positive correlation was found between number of enhancing lesions and plasma levels of total and LDL cholesterol.
Hussein *et al.*, **2006**	1206 pMS	Letter (Retrospective, controlled)	5	NR			X			The prevalence of type II diabetes was 6.75%, which was higher than in the (elsewhere reported) general population at that time, perhaps due to the use of glucocorticoids.
Kang *et al.*, **2010**	898 pMS 4490 CON	Case control (Retrospective, population based)	3b	NR	X		X	X	X	pMS had a higher risk to develop vascular disorders, hypertension, hyperlipidemia and type II diabetes, compared to CON.
Khurana *et al.*, **2009**	4703 MS veterans	Cross-sectional (observational, questionnaire based)	2c	NR		X				Veterans with MS had a higher prevalence of overweight (~35 %) and a lower prevalence of obesity (~23%), compared to (elsewhere reported) veterans in general. Overweight and obesity were associated with, amongst others, age, smoking, being male, married and employed.
Koch-Hendriksen *et al.*, **1998**	6068 pMS	Cohort study (Register case based, controlled)	1b	NR	X					pMS have an increased risk of dying from heart- or vascular diseases, perhaps ascribed to a lower level of physical activity.
Lalmohamed *et al.*, **2012**	1270 pMS 7648 CON	Cohort study (population based)	2b	NR	X	X				MS patients had a 3.5-fold increased mortality rate for all-cause mortality and the mortality rate ratio was 2.4-fold increased for deaths related to CVD, compared to CON. Increased mortality rates were related with smoking and respiratory diseases.
Lambert *et al.*, **2002**	17 female pMS 12 female CON	Case series (controlled)	4	4.0 (median)		X				No differences in body composition were found between pMS and CON.
LaVela *et al.*, **2012**	1142 MS veterans 31 500 CON veteran 68 357 CON general	Case control (Retrospective, survey based)	3b	NR	X			X	X	Veterans with MS had a higher prevalence of hypertension, hypercholesterolemia, diabetes and coronary heart disease, compared to CON veterans and general CON. Explanatory variables were age, race, smoking and education.

Study	Sample size	Study design / setting	Level of Evidence	EDSS	CVD	Obesity/ body composi-tion	Diabetes / Glucose	Hyper-tension	Dyslipi-daemia	Main relevant findings
Mähler *et al.*, **2012**	16 pMS 16 CON	Case series (controlled)	4	2.0 (median)		X	X			Fasting and postprandial glucose conc were not different between pMS and CON. Glucose tolerance was not impaired in pMS, compared to CON.
Marrie *et al.*, **2008**	8983 pMS	Cross-sectional (Questionnaire based, non-controlled)	2c	NR				X	X	The prevalence of comorbidities was relatively common, ~77% reported at least one comorbidity, like hypercholesterolemia and hypertension. Comorbidity risk was associated with being male, age, race and socioeconomic status.
Marrie *et al.*, **2011**	8983 pMS	Cross-sectional (Questionnaire based, non-controlled)	2c	NR	X			X	X	Vascular comorbidities, like hypercholesterolemia, hypertension, diabetes or heart disease, were reported in ~53% of all participants and were associated with visual disability progress in MS.
Mojtahedi *et al.*, **2008**	29 female pMS	Case series (non-controlled)	4	2.9 ± 1.2		X				BMI and body fat percentage showed that participants were overweight.
Nuyen *et al.*, **2006**	241 pMS	Case control	3b	NR			X	X		~10% of included pMS had hypertension. Furthermore, an inverse relation between MS and diabetes mellitus was reported.
Pepin *et al.*, **1996**	104 pMS 25 CON	Cohort study (controlled)	2b	NR				X		Systolic, diastolic and mean arterial pressure were lower in pMS, compared to CON.
Petajan *et al.*, **1996**	54 pMS	RCT	1b	3.4 ± 0.3		X			X	Exercise was able to decrease skinfolds, triglyceride conc and very-low-density-lipoproteins in included pMS.
Salemi *et al.*, **2010**	40 pMS 80 CON	Case series (controlled)	4	3.0 (median)					X	pMS, compared to CON, showed an increased conc of cholesterol and HDL-cholesterol.
Sanya *et al.*, **2005**	13 pMS 18 CON	Case series (controlled)	4	1 - 4.5 (range)				X		pMS showed abnormale heart rate and blood pressure responses to baroreflex stimulation. Furthermore, similar resting blood pressure values in pMS and CON were reported.
Sioka *et al.*, **2011**	68 pMS 114 CON	Case series (controlled)	4	2.3 ± 2.2		X				Fat percentage and amount of fat free mass of whole body, arms and trunk was not different between pMS and CON.

Study	Sample size	Study design / setting	Level of Evidence	EDSS	CVD	Obesity/ body composi-tion	Diabetes / Glucose	Hyper-tension	Dyslipi-daemia	Main relevant findings
Slawta *et al.*, **2002**	123 female pMS	Case series (non-controlled)	4	NR		X	X		X	LTPA was associated with lower waist circumference, lower TG- and lower glucose concentrations.
Slawta *et al.*, **2003**	123 female pMS	Cross-sectional (Survey based, non-controlled)	2c	NR		X	X		X	The prevalence of obesity and unfavourable glucose conc or dyslipidemia levels was comparable to those of the (elsewhere reported) general population of women without MS.
Snook *et al.*, **2006**	34 pMS	Case series	4	NR		X				This study suggest that inactivity plays an important role in body composition in pMS.
Sterman *et al.*, **1985**	22 pMS 20 CON	Case series (controlled)	4					X		Abnormalities of neural cardiovascular regulation was frequent, but heterogeneous, in pMS, due to scattered plaques.
Sternberg *et al.*, **2013**	206 pMS 142 CON	Case control (Retrospective chart review)	3b	4.1 ± 2.2			X	X	X	pMS had higher total cholesterol and high density lipoprotein conc, but lower glucose conc and lower systolic blood pressure, compared to CON.
Warren *et al.*, **2009**	335 pMS	Cross-sectional (Retrospective, survey based, non-controlled)	2c	NR				X		17% of pMS reported hypertension as one of the 8 most prevalent comorbidities, which was associated with health-related quality of life.
Warren S and Warren KG, 1981	100 pMS 100 CON	Case control (interview based)	3b	NR			X			A relation was found between MS and diabetes mellitus. In particular, more pMS, compared to CON, were diabetic or reported at least one blood relative with diabetes.
White *et al.*, **2006**	12 female pMS	Case series (non-controlled)	4	4.0 ± 1.4		X	X	X	X	Exercise was able to decrease TG conc, whereas body weight, fatness, glucose levels, blood pressure and cholesterol levels remained unchanged.

Abbreviations used: pMS: persons with multiple sclerosis, CON: healthy controls, EDSS: expanded disability status scale, CVD: cardiovascular disease, NR: not reported, RCT: randomized controlled trial, conc: concentration, BMI: body mass index, HDL: high density lipoprotein, LDL: low density lipoprotein, LTPA: leisure time physical activity, TG: triglyceride.

Results

1. Study quality

A total of 34 studies fulfilled the inclusion criteria. The average level of evidence ranged from 1b to 5 and 75% of all included papers ranged between 2c and 5. Only 50% of the identified studies were controlled studies and the majority of the studies reported one or more of the parameters related to the metabolic syndrome or CVD in pMS as a secondary outcome measure only. In summary, the overall quality of the existing literature was low.

2. Cardiovascular disease risk

Bronnum-Hansen and co-workers reported that 15% pMS died from CVD, which was higher than in a matched general population [168]. The same tendency was also reported by Koch-Henriksen *et al.* [9] and Kang *et al.* [69], showing that pMS were more likely to develop vascular disorders. Furthermore, MS veterans showed a higher coronary heart disease prevalence (11%), compared to the general population (3%) [64]. Similarly, recent research reported a 3.5-fold increased mortality rate in pMS, compared to HC, which was mainly caused by increased deaths due to CVD (2.4-fold) [169]. In contrast, both Fleming and Blake and Allen *et al.* concluded that CVD were less common in pMS, when compared to HC [67, 68]. Furthermore, Marrie *et al.* (2011) reported that 7% of the included pMS had heart diseases and 2.4% suffered from vascular diseases, corresponding to existing data for the general population [170]. Finally, a prospective study of Dallmeijer and co-workers found that only 5% of pMS were affected by CVD [171].
In summary, literature is limited and the existing data is inconsistent. However, the most well-designed studies, characterized by an higher level of evidence, suggest a slightly increased risk of dying from CVD and prevalence of CVD in pMS.

3. Obesity/body composition.

Body composition has been investigated in several MS studies [13, 68, 70-73, 75, 169, 172-177]. Most of these compared data to matched HC [68, 73, 75, 169, 172, 175, 176] or to normative data of HC [70, 174]. Others did not include any reference group [13, 71, 72, 173, 177]. Several techniques were applied, including dual energy X-ray absorptiometry [75, 173, 175, 177], skinfold measurements [13, 70-72, 176], whole body air displacement plethysmography [73, 172] or cohort/database data [68, 174]. The three most common reported variables were body mass index (BMI), body fat amount and amount of lean body mass (LBM).

Body mass index: In a study of Slawta *et al.* (2003) mean BMI of female pMS exceeded levels recommended by the WHO. Almost 50% of these participants were overweight, and half of these overweight pMS were obese [70], comparable to general population values reported in the NHANES III study [178]. The same comparisons and conclusions were made by others [169, 177]. Other studies have reported a higher prevalence of obesity in female pMS [71, 72, 172, 173] and in veterans with MS [174]. In contrast, a number of studies were not able to detect any group difference between BMI values [13, 73, 175, 176], and, indeed, some studies reported either a lower prevalence of obesity [68] or lower BMI values in male [175] and female [75] pMS, compared to HC.
In summary the existing data on BMI are inconsistent with some studies reporting slightly elevated BMI values, while others report normal or slightly lowered BMI values in pMS.

Body fat: Sioka *et al.* reported that total body (and sub-regions) fat percentage was similar between male and female pMS and HC, except for the slightly higher values of the lower extremities in female pMS [175]. A number of other studies reported similar findings [73, 75, 172], also after a sub analysis between ambulatory and non-ambulatory female pMS [75]. Çomoglu *et al.* a found significantly lower body fat percentage in male pMS compared to HC while no differences were seen in women. However, women, but not men had a higher ratio of central to peripheral body fat distribution when compared to gender-matched HC [176]. Studies not including a matched control group [13, 70-72, 173, 177] reported body fat percentages of 26-41% in female pMS, which corresponded to the values in pMS reported by the controlled studies (33-48%) [75, 172, 175].
In summary, only a few studies have compared body composition in pMS to matched HC, with existing data showing either similar or lower fat percentage in pMS.

Lean body mass: Most studies showed no difference between LBM of all body compartments in both mixed [73, 175, 176] and female [75, 172] groups of pMS compared to matched HC, except in one study showing lower values in the lower limbs of female pMS [175]. Furthermore, Formica *et al.* reported a significant loss of fat free mass in non-ambulatory female pMS, compared to ambulatory female pMS and female HC [75].
In summary most studies have shown no difference in whole-body LBM between MS and HC, with some studies indicating possible regional differences and an influence of disability level.

4. Glucose intolerance

Conflicting results on the prevalence of type II diabetes exist, with studies showing similar [69] or higher prevalence in pMS [64-66]. Based on medical records Hussein *et al.* found a type II diabetes prevalence of 7% in pMS, which was higher than the reported incidence in the general population [66], while Warren and Warren concluded that more pMS than HC had either diagnosed diabetes (any type) or had blood values indicating diabetes [65]. A few studies based on medical records reported a lower prevalence of type II diabetes (MS:3% vs. HC:6%) [67] or any type of diabetes (MS:10% vs. HC:19%) [68] in pMS.
Several studies only reported fasting or postprandial glucose concentrations [70-74], rather than a diagnosis of diabetes or impaired glucose tolerance, with studies showing that the incidence of elevated glucose concentrations (female MS: 8%) was similar to the (elsewhere reported) general population [70] or that normal fasting glucose concentrations were observed in minor groups of pMS [71-73]. A recent study showed lower plasma glucose concentrations in pMS compared to HC, and found a positive correlation between glucose concentrations and EDSS as well as rate of clinical relapses. In particular, an EDSS increase with one unit, resulted in a 1.5 ± 0.6 fold increase of the plasma glucose concentration [74].
In summary, most studies have reported that the prevalence of type II diabetes in pMS is similar, or slightly higher than in the general population.

5. Hypertension

The NARCOMS [20] study demonstrated that 30% of the included pMS reported hypertension, corresponding to the accepted figures for the general population [170, 179] and to other published results [69]. The Canadian community health survey showed that hypertension affected 17% pMS [180], while a survey of Buchanan *et al.* stated that 47-62% of pMS had comorbidities, of which hypertension was 'very common' [181]. Diverging results are reported in other studies [67, 68, 74, 180, 182], with studies showing that hypertension affected only 10% of the included patients [182], that HC had higher prevalence of hypertension [67, 68] or systolic blood pressure [64] than pMS, or that hypertension in male MS veterans (47%) is elevated compared to the general population [64].
Only a few studies reported data on actual blood pressure values in pMS [72, 183-185]. In particular, studies have reported similar resting blood pressure values in pMS and HC (pMS:123/78mmHg vs. HC:127/75mmHg) [74], (pMS:140/80mmHg vs. HC:140/74mmHg) [183], (pMS:139/80mmHg vs. HC:143/77mmHg) [184], (pMS:119/72mmHg vs. HC:122/71mmHg) [185]. Also, it has been reported that blood pressure in response to standing was abnormal in 13-50% of the included pMS [183, 186], and that systolic and diastolic blood pressure showed lower values after handgrip exercise in pMS [184].

In summary, the existing data on hypertension in MS is limited and inconclusive with studies reporting either similar, increased or decreased prevalence of hypertension when compared to HC.

6. Dyslipidaemia

The NARCOMS registry study reported that 37% of the pMS suffered from hypercholesterolemia which was higher than the elsewhere reported data from the general population. However, the difference was eliminated after including only those pMS who reported being treated for hypercholesterolemia [170, 179]. A number of studies have shown elevated cholesterol concentrations in pMS, when compared to a control group [64, 69, 74, 181, 187, 188], and this has also been confirmed in male veterans with MS compared to the general population [64]. Contrasting results exist showing that hypercholesterolemia is less frequent in pMS compared to HC (pMS:3% vs. HC:5.1%) [68]. A neutral effect is suggested by other studies showing that the frequency of higher total cholesterol [13, 71, 176], higher TG [70, 72, 176] and lower HDL [70, 72, 176] and higher LDL [176] concentrations in women with MS was comparable to norm data.
In summary, data on dyslipidaemia in MS are inconsistent, but the most well-designed studies showed normal or slightly elevated total cholesterol and LDL and reduced HDL values.

Discussion

The present review identified 34 papers linking MS to the risk factors constituting the metabolic syndrome and/or to the risk of developing CVD. Despite the high number of identified papers, only limited data exist on CVD risk and MS, generally suggesting an increased CVD risk in pMS. However, from the existing studies it is not clear whether an increased risk of CVD is attributable to an increased risk of obesity, changes in body composition, hypertension, dyslipidaemia or type II diabetes in pMS.

General study quality and methodological issues

Overall the study quality was low, as evaluated by an estimation of the level of evidence by the Oxford Centre for Evidence-based Medicine rating system (Table 2.1.2). Many studies lacked a matched control group and several studies included only small and/or non-representative populations. Furthermore, most studies were not specifically designed to evaluate the different risk-factors of interest in the present study, making these secondary outcomes. Also, the included studies did not always report in- and exclusion criteria, and those who did often differed substantially and included data from different cultures further complicating comparison and interpretation of the studies.
Several studies reported comorbidities based on medical records, death-certificates and self-reports. However, the validity of these data sources varies depending on the research question of the corresponding research setting [189]. In particular, the presented data were not always a result of a scientific research process, characterized by strict criteria and requirements, but were often secondary outcome measures of a study designed to investigate something else, making it difficult to draw solid conclusions. Furthermore, the impact of physical disabilities/EDSS and cognitive impairments, needs to be assessed before a valid interpretation of applied questionnaires can be performed. Nevertheless, research already stated that pMS are able to accurately report disease related factors [190, 191], and it has been shown that agreement between patient-reported comorbidities and those reported in medical records are high for diabetes, hypertension and hyperlipidaemia [192].
Since comorbidities may increase as the disease progresses, the selection of pMS should be performed carefully to allow generalization. Based on this reasoning we recommend inclusion of patients covering a wide range of disability levels in future studies. Next, it should be kept in mind that biometrical data like sex, age and race can modify the association between MS outcomes and comorbidities. Finally, long-term follow-up studies are required, investigating the metabolic syndrome and the CVD risk factors co-existing with MS, and further exploring the consequences of medication use, an inactive lifestyle and lifestyle interventions in pMS.

Possible explanatory factors

The observed risk of CVD in MS may, in part be explained by readily identifiable factors such as medication, lifestyle interventions and physical inactivity. However, comprehensive analyses of these topics were beyond the scope of this review and will only be discussed briefly.
Some of the drugs applied in MS treatments may impact components of the metabolic syndrome. Corticosteroids [193], are known to lead to elevated fasting glucose- and insulin concentrations and to insulin resistance in HC [194]. The role of repeated corticosteroids in explaining the apparent higher prevalence of type II diabetes in MS is an area warranting further research.
Lifestyle factors, such as excessive weight gain, dietary factors, smoking and sleep deprivation are considered as important modifiable risk factors in the development of the metabolic syndrome [195-199], but their potential role in the setting of MS requires further evaluation.
Physical inactivity, previously encouraged in pMS to limit fatigue [96], could also impact factors of the metabolic syndrome. Physical inactivity directly contributes to the cascade of events that lead to the expression of the 'exercise deficient phenotype', resulting in abdominal fat accumulation, higher levels of TG, lower levels of HDL and reduced insulin sensitivity [59]. Consequently, exercise may be a very important non-pharmacological intervention targeting the CVD risk in pMS.

Conclusion

Studies linking MS to the risk factors constituting the metabolic syndrome and CVD risk were generally of low methodological quality. The limited existing data suggested that CVD risk is increased in pMS, but it is not clear whether an increased risk of CVD is related to an increased risk of obesity or changes in body composition, hypertension, dyslipidaemia or type II diabetes in pMS. As current therapies and treatment strategies seek to minimise the impact of MS itself, it becomes more important for physicians to be aware of non-MS factors which may negatively affect a patient's well-being. We identify areas of uncertainty regarding the risk profile of pMS for one of the major causes of morbidity in the western world, highlighting the need for further research to permit evidence based information to be given to patients.

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STUDY 2

Does multiple sclerosis affect glucose tolerance?

Based on: Wens I., Dalgas U., Deckx N., Cools N., Eijnde BO.
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Abstract

Background: Based on current literature, it is not clear if multiple sclerosis (MS) patients are at increased risk to develop impaired glucose tolerance (IGT).

Methods: Eighty-one MS patients and 45 healthy controls (HC) performed an oral glucose tolerance test. IGT was defined as a fasting glucose concentration of 6.1-6.9mmol/l and 2h post load glucose of 7.8-11.1mmol/l.

Results: The prevalence of impaired fasting glucose concentrations (17% vs. 2%) and IGT (11% vs. 0%) was higher in MS patients than HC. Accordingly, the areas under the glucose and insulin curves were higher in MS patients.

Conclusion: The current study demonstrates an elevated IGT-prevalence in MS.

Introduction

Multiple Sclerosis (MS) is often characterized by a more sedentary lifestyle compared to healthy controls (HC) [7].
Although physical inactivity in HC is associated with an increased risk of impaired glucose tolerance (IGT) [59], contributing to the development of type II diabetes, existing MS-literature is conflicting [200], with studies reporting an increased [64-66], similar [69] or decreased [67, 68] prevalence of IGT and type II diabetes in MS.

In addition, several studies reported only fasting glucose concentrations [70-72, 74], which is insufficient to indicate IGT since, according to the European Society of Cardiology, a 2h oral glucose tolerance test (OGTT) is the preferable screening tool. Slawta and co-workers reported elevated fasting glucose concentrations in 8% of their female patients, which was similar to percentages reported for the general population [70]. However, they were not able to confirm these results in a second study, demonstrating normal fasting glucose concentrations [71]. Likewise, White *et al.* and Mähler *et al.* reported normal fasting glucose concentrations in small cohorts of MS patients [72, 73]. In addition, the latter stated that, after an OGTT, glucose tolerance was not impaired in 16 MS patients [73]. In contrast, Sternberg and co-workers reported lower glucose concentrations in MS patients, compared to HC and found positive correlations between glucose concentrations and EDSS as well as the rate of clinical relapses [74].

In an attempt to clarify the heterogeneous results of the literature, the present cross-sectional controlled study aimed to investigate the prevalence of IGT in a large cohort of MS patients, using an OGTT.

Methods

Eighty-one MS patients, diagnosed according to McDonald criteria (EDSS 0-6.0) and >18 years, and 45 HC participated in this study, providing a ~2:1 match for gender, age and body mass index (BMI). Exclusion criteria were diabetes mellitus type II and glucose lowering therapies, other disorders (cardiovascular, pulmonary, renal diseases and cancer), pregnancy, participation in other studies or an MS-exacerbation 6 months prior to the study. Participants gave written informed consent in accordance with the Declaration of Helsinki and the protocol was approved by the ethical committee (clinicaltrials.gov NCT01845896 and NCT01718392).

An OGTT (1g glucose/kg bodyweight), including determination of blood glucose (Analox-GM7 Micro-stat) and serum insulin (Mercodia Insulin ELISA) levels, was performed to investigate glucose profiles. Whole-blood glucose concentrations were converted to plasma concentrations using a multiplier of 1.11 [201]. IGT was defined by the WHO as a fasting plasma glucose concentration of 6.1-6.9mmol/l and 2h post load plasma glucose of 7.8-11.1mmol/l. Glucose and insulin responses were expressed as the total areas under the curve (tAUC), calculated according to the trapezoidal rule. Glucose tolerance was evaluated using the homeostasis model assessment of insulin resistance (HOMA-IR=fasting plasma glucose(mmol/l) x fasting serum insulin(mU/l)/22,5).

All data were analysed using SAS-9.2-software (SAS Institute Inc, Cary, USA). First normality was checked using the Shapiro-Wilk test for all variables. Glucose and insulin profiles, based on repeated measures equidistant in time, were analysed by a mixed model repeated measures ANOVA, to evaluate a possible disease-effect over time. tAUC and HOMA-IR between groups were analysed by an unpaired student's t-test. Categorical data were compared using Chi-squared and Fisher's exact tests. Finally, a Pearson correlation analysis was performed. All data are presented as mean ± SE and $p<0.05$ represents the threshold for statistical significance.

Results

Subject and disease characteristics are presented in Table 2.2.1.

Table 2.2.1: *Subject and disease characteristics.*

	HC	MS	p-value
No. (M/F)	45 (14/31)	81 (31/50)	/
Age (y)	46 ± 1.8	48 ± 1.1	NS
Height (m)	1,69 ± 0.01	1,69 ± 0.01	NS
Weight (kg)	70,0 ± 1.8	73,0 ± 1.5	NS
BMI (kg/m^2)	24,4 ± 0.5	25,3 ± 0.5	NS
Smoker	5%	11%	NS
Physical activity (MET*h/week)	18.9 ± 2.7	18.9 ± 1.7	NS
EDSS	/	3 ± 0.14	/
Type MS (RR/CP)	/	65% / 35%	/
MS treatment (general)	/	85%	/
Immunomodulatory MS treatment	/	77%	/
Immunosuppressive MS treatment	/	8%	/
Hypertension treatment	2%	10%	NS
Cholesterol-lowering treatment	2%	7%	NS
Beta-blocker	4%	3%	NS
Antidepressant	2%	18%	<0.05
Muscle-relaxing drug	0%	19%	<0.05
Analgesics	2%	22%	<0.05
Somnifacient	0%	6%	NS
Anticonvulsant	0%	13%	<0.05

Values are means ± SE.
Abbreviations used: MS, multiple sclerosis; HC, healthy controls; M, male; F, female; BMI, body mass index; RR, relapsing remitting; CP, chronic progressive; EDSS, expanded disability status scale; MET, metabolic equivalents; Immunosuppressive = alemtuzumab, corticosteroids, mitoxantrone; Immunomodulatory = interferon ɣ, glatiramer acetate, fingolimod, natalizumab; NS, not significant.

Compared to HC, plasma glucose concentrations of MS patients were significantly higher at multiple time points during the OGTT (disease*time, $p<0.05$). In particular, 17% of the MS patients showed elevated fasting plasma glucose concentrations, compared to 2% of the HC ($p<0.05$). In addition, 5% of MS patients showed plasma glucose concentrations ≥11.1mmol/l after 2h, while none of the HC displayed diabetic blood values ($p=0.1$). Furthermore, 11% of the MS patients and none of the HC showed IGT ($p<0.05$). Accordingly, glucose tAUC was higher in MS ($p<0.05$). Similarly, serum insulin concentrations (disease*time, $p<0.05$) and insulin tAUC ($p<0.05$, Figure 2.2.1) were higher in MS. Compared to HC, the HOMA-IR tended to be higher in MS ($p=0.07$).

Glucose and insulin tAUC of male and female MS patients were significantly higher, compared to their respective HC ($p<0.05$). Within HC and within MS, glucose and insulin tAUC did not differ between sexes, albeit insulin tAUC of male MS patients was significantly higher compared to insulin tAUC of female MS patients.

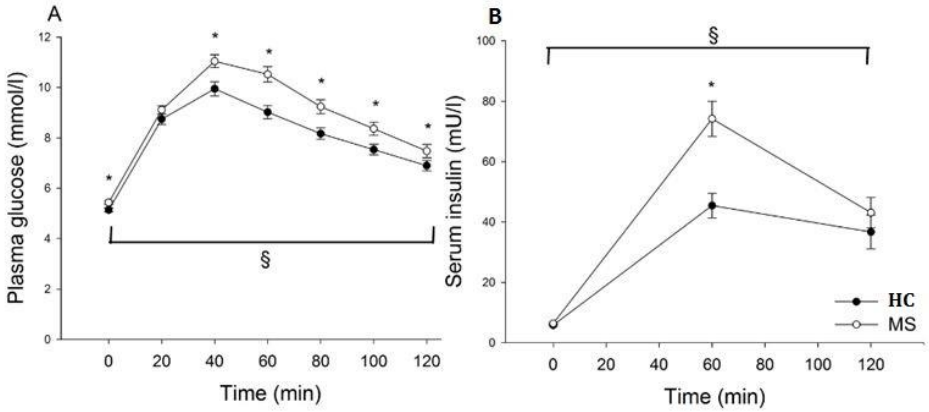

Figure 2.2.1: *Plasma glucose (A) and serum insulin (B) concentration profiles of MS patients and HC after a 2h OGTT (1g glucose/kg body weight).*
Data are given as mean ± SE.
** p<0.05 compared to corresponding HC values, § p<0.05, overall interaction effect.*

Interestingly, glucose tAUC was higher in chronic-progressive MS patients, compared to relapsing-remitting patients ($p<0.05$), whereas insulin tAUC was comparable between both groups ($p>0.05$). Furthermore, MS patients with EDSS>3 had higher glucose and insulin tAUC, than patients with EDSS≤3 ($p<0.05$). Accordingly, EDSS and glucose tAUC ($r=0.32$, $p<0.0001$) and EDSS and insulin tAUC ($r=0.27$, $p<0.0001$) were positively correlated. In MS, insulin tAUC correlated with BMI ($r=0.24$, $p<0.005$) and was significantly affected by gender ($r=-0.26$, $p<0.0001$), whereas glucose tAUC only correlated with age ($r=0.45$, $p<0.0001$). In HC, glucose tAUC correlated with BMI ($r=0.24$, $p<0.005$) and age ($r=0.4$, $p<0.0001$), whereas insulin tAUC only correlated with BMI ($r=0.35$, $p<0.005$).

Discussion

The present cross-sectional study compared the prevalence of IGT in 81 MS patients and 45 HC and observed a higher prevalence in the MS group.

To our knowledge, only one controlled trial showed that glucose tolerance was not impaired in 16 MS patients, contrasting to the present work [73]. Age, gender, obesity, disease type of MS, physical disability (EDSS), medication and lifestyle factors, such as smoking or physical inactivity may be confounding factors in the development of IGT, affecting study outcomes.

Despite the different conclusion of the present study and the study of Mähler and co-workers [73], there were no differences in age, gender and BMI [73]. However, differences in samples size (16 vs. 81), MS-subtypes (relapsing-remitting vs. all types), EDSS (median 2, range 1-4.5 vs. median 3, range 0-6) and medication (glatiramer acetate vs. several treatments) were detected [73]. Interestingly, our findings support a possible association between the glucose profile and MS-subtype, indicated by a less impaired glucose profile in relapsing-remitting patients, compared to chronic-progressive patients. Furthermore, the impact of physical disabilities/EDSS on the glucose profile needs to be considered, given the correlation between glucose concentrations and EDSS [74]. In the present study, this was mirrored by higher glucose and insulin profiles in MS patients with EDSS>3, compared to patients with EDSS≤3.

The use of some disease modifying therapies (DMT) [66, 202], as well as non-DMT [203], could be associated with elevated blood glucose and/or insulin levels, which probably becomes more pronounced as the disease progresses. Noteworthy, none of our participants were treated with glucocorticoids, while MS patients used significantly more antidepressant, muscle relaxing drugs, analgesics and anticonvulsants, than HC.

Since physical inactivity directly contributes to the cascade of events that leads to the 'exercise-deficient phenotype', resulting in reduced insulin sensitivity and glucose intolerance [59], the overall daily activity level might also affect the glucose and insulin profiles in MS. However, in the present study, the self-reported level of physical activity did not differ between MS patients and HC, when measured by the PASIPD questionnaire. Additionally, other lifestyle factors, including smoking, were comparable between MS patients and HC. Finally, since the skeletal muscle is the most important site of glucose disposal, intramyocellular mechanisms, such as decreased insulin signalling/sensitivity and disturbed muscle energy metabolism, independent of inactivity *per se*, may also contribute to IGT in MS and warrants further research.

In conclusion, under the conditions of the present study and compared to HC, we demonstrated a higher prevalence of IGT in MS, emphasising the need for adjusted guidelines for future patient management and follow-up.

Acknowledgements

We thank all MS patients, as well as healthy controls, for participating in this study. Our gratitude goes to Anne Bogaers for logistical assistance and blood sample collection and to Niel Hens for statistical advice. Biological material obtained for this study is stored in the University Biobank Limburg.

STUDY 3

The impact of 24 weeks combined exercise on glucose tolerance in multiple sclerosis patients

Based on: Wens I., Hansen D., Verboven K., Deckx N., Kosten L., Stevens A., Cools N., Eijnde BO.

Am J Phys Med Rehab, under review

Abstract

Purpose: Recently, we reported an increased impaired glucose tolerance prevalence in multiple sclerosis (MS) patients, compared to healthy controls, indicating metabolic defects that may increase comorbidity. Furthermore, MS often leads to a more inactive lifestyle, increasing the likelihood to develop fat accumulation, muscle wasting and weakness, and exercise intolerance. In other populations, these health complications can, partly, be reversed by physical exercise, which is often used as the primary treatment strategy. The impact of a long-term combined exercise program on these parameters in MS, however, remains unclear.

Methods: MS patients were randomized to an exercise intervention group (n=29) or a sedentary control group (n=15). Glucose tolerance and serum brain-derived neurotrophic factor (BDNF) levels, as well as muscle strength (isometric knee extensor and flexor strength), exercise tolerance and body composition to validate the applied exercise program, were determined in both groups, at baseline and after 6, 12 and 24 weeks of combined endurance and resistance training.

Results: Although 6 months of combined exercise improved muscle strength (+10-50%), exercise tolerance (mean response time, heart rate, blood lactate concentration and ratings of perceived exertion), body composition (lean tissue mass +2 ± 0.6%) and serum BDNF concentration (+14 ± 5%) within the intervention group as compared to baseline, no effect on blood glucose and serum insulin profiles were detected. In the control group, no changes were detected.

Conclusion: Although our secondary outcome measures were improved following a long-term combined endurance and resistance exercise program in MS patients, glycaemic control was not affected.

Introduction

The heterogeneous and complex symptoms of multiple sclerosis (MS) often lead to a more sedentary lifestyle [7]. This may result in disuse-related loss of muscle strength and exercise capacity, which in turn can affect the quality of life [3, 8].

In healthy people, physical inactivity can contribute to the development of secondary health problems, such as cardiovascular diseases, obesity and diabetes type II, preceded by impaired glucose tolerance (IGT).[101] Recently, we reported an increased IGT prevalence in MS patients, compared to matched healthy controls, indicating metabolic defects that may increase comorbidity [200, 204].

In other populations, these secondary health complications can, partly, be reversed by physical exercise, which is often used as the primary treatment strategy [101-105]. Given this ability of exercise to reduce the risk of developing IGT and insulin resistance [105] and to improve insulin sensitivity in other populations [107], it can be hypothesized that a disturbed glycaemic control in MS patients could also be improved after exercise. The impact of physical exercise on IGT in MS, however, remains scarce and conflicting since previously reported data were based on fasting blood glucose levels [71, 143] instead of, according to the European Society of Cardiology, the more adequate 2h oral glucose tolerance test (OGTT). The latter was never investigated before in MS and warrants further research.

Evidence suggests that exercise therapy may impact the pathogenesis of MS [205]. However, the underlying mechanisms of physical activity on disease pathogenesis remain unclear. In this respect, the neurothrophin brain-derived neurotrophic factor (BDNF) is suggested as an important mediator in this process due to its activity-dependent regulation, neuroprotective potential and its important role in neurogenesis and synaptic plasticity [206-208]. Research already reported no changes in exercise-induced serum BDNF levels, following an 8-week moderate aerobic training in MS patients [111, 209]. Furthermore, Rojas *et al.* [210] showed that 10 min of moderate aerobic cycling was insufficient to increase the serum BDNF concentration above the pre-exercise level, whereas a single bout of maximal incremental exercise did result in a significant increase in serum BDNF. In accordance with these results Ferris *et al.* [211] indicated that the fold increase of serum BDNF levels during exercise is dependent on the intensity of the exercise. These findings have increased the interest to further investigate the beneficial disease-modifying effects of long-term exercise in MS and the underlying neurological basis of these benefits.

Increasing evidence favours exercise therapy as a method of overall symptom management [212]. Observational [11, 12] as well as interventional studies [13, 14] reported benefits of 4 to 20-week exercise programs, including improvements in muscle strength, exercise tolerance, functional capacity and health-related quality of life. From a theoretical point of view, most benefit is suggested to be gained from combined exercise because this would positively affect impairments of both the cardiovascular system and muscle strength/activation [41]. However, only a limited number of studies [154, 155] evaluated the impact of a long-term (>20 weeks) combined exercise intervention on the above-mentioned parameters in MS. Consequently, the impact of long-term exercise intervention remains poorly understood in MS patients.

In keeping with the above line of reasoning and to further unravel the full potential of exercise training in MS, the present study investigates the impact of a 24-week combined exercise intervention on glycemic control and serum BDNF release in MS. To validate the applied exercise program muscle strength, exercise tolerance and body composition were measured. We hypothesize that a long-term combined exercise program improves the described parameters.

Methods

1. Subjects

Forty-five MS patients, diagnosed according to McDonald criteria (EDSS range 0-6) and aged >18 years, were included following written informed consent (Figure 2.3.1). Subjects were excluded if they had physician-diagnosed diabetes mellitus type II, other chronic diseases (cardiovascular, pulmonary and/or renal), were pregnant, participated in another study, had contra-indications to perform physical exercise or had an acute MS exacerbation 6 months prior to the start of the study. The study was approved by the ethical committee, performed in accordance with the Declaration of Helsinki and was registered at ClinicalTrials.gov (NCT01718392).

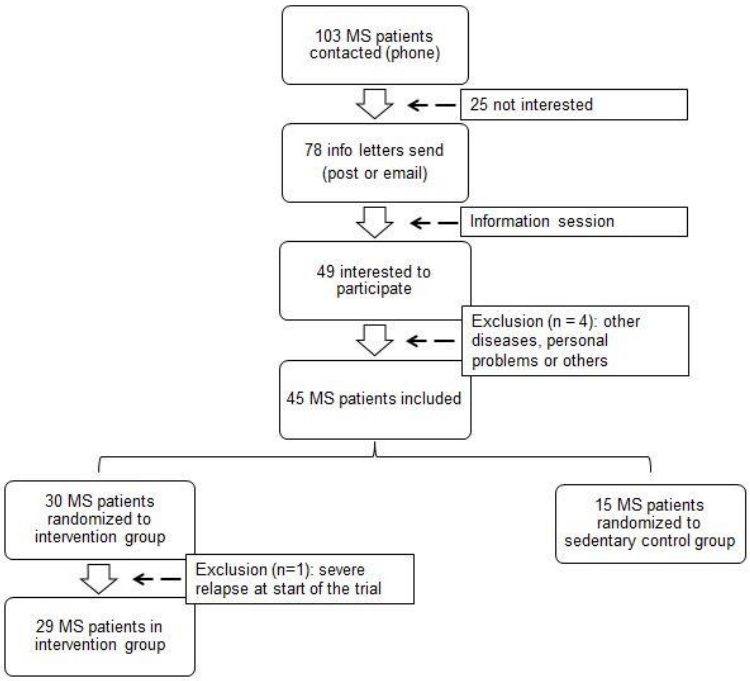

Figure 2.3.1: *Flowchart of MS patients' inclusion.*

2. Study design

At baseline, glucose tolerance, serum BDNF levels, muscle strength, exercise tolerance and body composition and were assessed. Hereafter, MS patients were randomized in a 2:1 ratio to an exercise intervention group (EX, n=29) or a sedentary control group (SED, n=15). Due to the nature of the trial, neither patients nor researchers involved in the project could be blinded to group allocation. EX were enrolled in a combined exercise program during 24 weeks. SED did not participate in any training program and were asked to continue their habitual physical activity level (usual care). Tests were repeated after 6, 12 and 24 weeks in both groups.

3. Exercise training program

Patients in the intervention group participated in a supervised 24-week combined training program at a frequency of 5 sessions per 2 weeks. Each session started with a cardiovascular part, consisting of cycling and treadmill walking or running (Technogym®). Session duration and intensity increased as the program proceeded, starting from 1x6 min/session to 3x10 min/session. The second part consisted of resistance training (leg press, leg curl, leg extension, vertical traction, arm curl and chest press, Technogym®). Resistance training of the lower limb was performed unilaterally, due to bilateral strength differences between legs of MS patients. To improve muscle fitness, sets of repetitions gradually increased during intervention, from 1x10repetitions to 4x15repetitions, with repetition maximum.

All exercises were performed at a mild-to-moderate workload corresponding to 12-14 ratings of perceived exertion on 20-point Borg scale (RPE) and adjusted to individual heart rate and disability level. Continuous encouragement and supervision by the instructors during the training period led to a systematic increase of the training load over the 24-week training period. The sessions were finished by stretching, and RPE was recorded.

4. Primary outcome measure

<u>Oral glucose tolerance test</u>

Glycaemic control of MS patients was investigated using an OGTT. At 8 a.m., following a 10h overnight fasting period, all participants received a 1g glucose/kg body weight glucose bolus. Before and after glucose administration, capillary blood samples were collected from a hyperaemic earlobe at 20 min intervals during a 2h period, to measure whole-blood glucose concentrations immediately (Analox GM7 Micro-stat, Analox instruments Ltd, London, UK). Whole-blood glucose concentrations were

converted to plasma concentrations using a multiplier of 1.11 [201]. IGT was defined by the WHO as a fasting plasma glucose concentration of 6.1-6.9mmol/l and 2h post load plasma glucose of 7.8-11.1mmol/l [213]. To determine serum insulin levels, 4cc of venous blood was collected in serum separation tubes (SST, BD Vacutainer®, Becton Dickinson, Erembodegem, Belgium) at 1h intervals. After 30 min, allowing blood coagulation, samples were centrifuged during 10 min at 3500rpm. The obtained serum was frozen and stored at -80°C for batch analysis of serum insulin levels (Mercodia Insulin ELISA, Uppsala, Sweden). Glucose and insulin responses were expressed as the total area under the curve (tAUC), calculated according to the trapezoidal rule.

5. Secondary outcome measure

BDNF measurements

Serum BDNF concentrations were determined by electrochemoluminiscence enzyme linked immunosorbent assay (ELISA) using Meso Scale Discovery (MSD) plates spotted with anti-BDNF antibodies (Meso Scale Discovery, USA). Briefly, after blocking of the plate, patients' sera and calibrators were dispensed into the plate and incubated for 2h at room temperature. Plates were subsequently washed and a SULFOTAG-labelled secondary anti-BDNF antibody was dispensed into the plate. Following a 2h incubation step, 2X read buffer T was added and plates were measured on a SECTOR® Imager.

6. Validation of exercise program

Isometric muscle strength

After 5 min of warming-up and habitation, the maximal voluntary isometric and dynamic muscle strength of the knee extensors and flexors (45° and 90° knee angle) were measured by means of an isokinetic dynamometer (System 3, Biodex, ENRAF-NONIUS, New York, USA), as reported previously [139]. Two maximal isometric extensions (4s) and flexions (4s), followed by a 30s rest interval, were performed. The highest isometric extension and flexion peak torques (Nm) were selected as the maximal isometric strength. Baseline results were used to classify the legs of each patient as weakest or strongest leg. This subdivision was maintained in further analysis, replacing a conventional left-right classification.

Submaximal exercise test on a cycle ergometer

Patients performed a submaximal cardiopulmonary exercise test on an electronically braked cycle ergometer (eBike Basic, General Electric GmbH, Bitz, Germany). After a 3 min period to obtain resting data, subjects were

instructed to cycle at a rate of 70 rotations per minute (rpm), against a resistance corresponding to 25% of predicted maximal cycling power output (W_{max}), for 6 min [214]. Next, subjects remained seated on the bike for 6 min of rest, where after a subsequent 6 min exercise bout, at the same intensity as the first, was performed. Predicted W_{max} was based on gender, age, body weight and height, and calculated by previously published equations [215].
Oxygen uptake (VO_2, ml/min) and expiratory volume (VE, l/min) were assessed breath-by-breath (Jaeger Oxycon, Erich Jaeger GmbH, Germany). Results were averaged every 10s. Heart rate was continuously monitored by a 12-lead ECG device. Predicted maximal heart rate was calculated by 220 minus age. Exercise-onset VO_2 kinetics were calculated and expressed as mean response time (MRT), as previously described by us [216].

Following each exercise bout, capillary blood samples were obtained from the fingertip to analyse blood lactate concentrations (mmol/l) using a portable lactate analyser (Accutrend Plus®, Roche Diagnostics Limited, Sussex, UK) and RPE was recorded.

Body composition

A Dual Energy X-ray Absorptiometry scan (Hologic Series Delphi-A Fan Beam X-ray Bone Densitometer, Vilvoorde, Belgium) was performed. Fat and lean tissue mass were obtained for whole body, legs, trunk, gynoid and android region. Waist-to-hip fat mass ratio (android fat (g)/gynoid fat (g) ratio) and fat mass of the trunk/fat mass of the limbs ratio were calculated.

7. Statistical analysis

All data were analysed using SAS 9.2 software (SAS Institute Inc, Cary, USA). First, normality was verified using the Shapiro-Wilk test for all variables. Baseline differences between groups were analysed using an unpaired student's t-test. To evaluate possible changes over time, a mixed model repeated measures ANOVA was used. Relations between parameters were analysed by Pearson correlations. All data are presented as mean ± SE and considered statistically significant when $p<0.05$.

Results

1. Subject characteristics

Baseline subject characteristics: No baseline differences in subject and disease characteristics (Table 2.3.1) or in primary and secondary measures were found between groups.

Intervention adherence: Adherence to the intervention program was good with participants attending approximately 90% (range 80-100%) of the 60 supervised sessions. No severe symptom exacerbations and/or adverse events were reported.

Table 2.3.1: *Baseline subject and disease characteristics*

	EX	**SED**	**p-value**
Age (y)	48 ± 2	49 ± 2	NS
Height (m)	1.7 ± 0.02	1.7 ± 0.02	NS
Weight (kg)	71.5 ± 2.9	72.5 ± 3.9	NS
BMI (kg/m^2)	24.6 ± 0.9	24.3 ± 1.3	NS
Gender (m/f)	12/17	7/8	NS
Type MS (RR/CP)	17/12	11/4	NS
EDSS	3.25 ± 0.2	3.36 ± 0.4	NS

Values are means ± SE.
Abbreviations used: EX, exercise group; SED, sedentary group; BMI, body mass index; RR, relapsing remitting; CP, chronic progressive; EDSS, expanded disability status scale.

2. Primary outcome measure

Oral glucose tolerance test

At baseline, 13.6% of all included patients showed IGT, distributed equally between EX and SED. No differences were observed in glucose and insulin tAUC between EX and SED after 6, 12 and 24 weeks of combined exercise or usual care, respectively (Figure 2.3.2). Interestingly, sub-analysis in the IGT-group only showed also no changes after 24 weeks of mild-to-moderate intensity exercise.

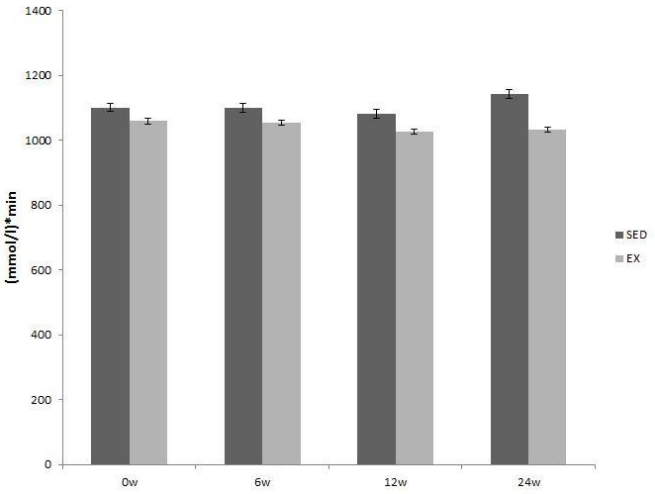

Figure 2.3.2: *Overview of OGTT based total area under the glucose curve of exercised (EX) and sedentary (SED) MS patients. No changes were observed between baseline values and after 6, 12 and 24 weeks (p>0.05).*

3. Secondary outcome measure

Serum BDNF levels

For serum BDNF levels, a tendency for an overall interaction effect (time*intervention, p=0.06) was found. Within EX, serum BDNF concentrations increased with 8 ± 5% and 14 ± 5% after 12 and 24 weeks, respectively (p<0.05), compared to baseline (EX baseline: 11176 ± 792 pg/ml, 12w: 12610 ± 1552 pg/ml, 24w: 13725 ± 1520 pg/ml). In SED, serum BDNF concentrations remained stable.

4. Validation of exercise program

Isometric muscle strength

Within EX, muscle strength improved over time (Figure 2.3.3). In particular, after 24 weeks of exercise and compared to baseline, quadriceps and hamstrings strength (at 45° and 90°) of the weakest legs of EX improved with approximately 23% (baseline 45°: 108 ± 8Nm, 24w 45°: 121 ± 8Nm, p<0.05) and 50% (baseline 45°: 54 ± 5Nm, 24w 45°: 71 ± 5Nm, p<0.05), respectively. Furthermore, approximately a 10% (baseline 45°: 126 ± 8Nm, 24w 45°: 136 ± 8Nm, p<0.05) and 29% (baseline 45°: 65 ± 5Nm, 24w 45°: 79 ± 5Nm, p<0.05) increase in, respectively, the extension and flexion strength (at 45° and 90°) of the strongest legs of EX was observed. An overall interaction effect (time*intervention) was seen in knee 45° extension and flexion of the strongest leg (p<0.05), whereas a tendency to an

interaction effect was seen in knee 45° muscle strength of the weakest legs (p≤0.1). Muscle strength of SED remained stable during 24 weeks as compared to baseline.

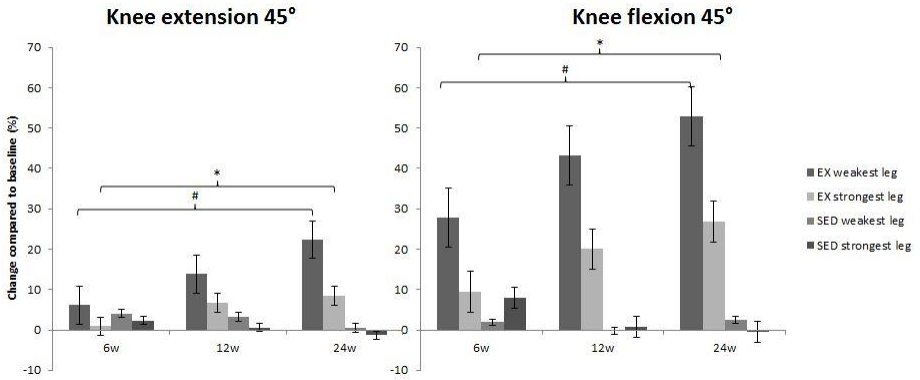

Figure 2.3.3: *Percentage change of knee 45° muscle strength of exercised (EX) and sedentary (SED) MS patients after 6, 12 en 24 weeks of combined exercise or usual care, compared to baseline. In EX, muscle strength improved the most in the weakest leg(#), whereas the strongest leg improved less pronounced (*). In SED muscle strength remained stable. Comparable results were seen for knee 90° muscle strength (data not shown).*

Exercise tolerance

Within EX several parameters describing aerobic capacity improved over time as compared to baseline (Table 2.3.2). In particular, MRT of the first exercise bout improved by 11s ($p<0.05$), whereas MRT of the second exercise bout tended to improve ($p\leq0.1$). Furthermore, heart rate, blood lactate concentration and RPE decreased in EX, after both exercise bouts, as compared to baseline ($p<0.05$). The above-mentioned parameters remained stable in SED.

Body composition

Body weight did not change during 24 weeks in EX and SED. Within EX, lean tissue mass increased with 2 ± 0.6% (baseline: 41.8 ± 1.7 kg, 24w: 42.4 ± 1.8 kg, $p<0.05$), whereas body fat percentage tended to decrease with 3 ± 1% ($p\leq0.1$), as compared to baseline. Furthermore, other adipose and lean tissue mass indices remained stable in both groups.

5. Correlations

No significant correlations were found between changes in primary and secondary outcome measures.

Table 2.3.2: *Overview of parameters describing physical fitness at rest, after the first exercise bout, during recuperation and after the second exercise bout. These variables were measured at baseline, after 6, 12 and 24 weeks, to evaluate the influence of a 24w combined exercise program or usual care in EX and SED, respectively.*

		0w		***6w***		***12w***		***24w***	
		EX	***SED***	***EX***	***SED***	***EX***	***SED***	***EX***	***SED***
Rest	*HR*	*82 ± 2*	*77 ± 4*	*78 ± 2*	*75 ± 4*	*78 ± 2*	*80 ± 4*	*80 ± 2*	*84 ± 5*
	Lact	*2.6 ± 0.1*	*2.6 ± 0.2*	*2.7 ± 0.1*	*3.0 ± 0.2*	*2.4 ± 0.1*	*2.7 ± 0.2*	*2.2 ± 0.1 **	*2.3 ± 0.2*
After Bout 1	*MRT1*	*55.3 ± 2*	*52.5 ± 2*	*50.3 ± 3*	*53.1 ± 4*	*46.9 ± 2*	*52.1 ± 2*	*43.8 ± 2 **	*48.3 ± 3*
	HR	*111 ± 4*	*107 ± 5*	*106 ± 2*	*108 ± 4*	*105 ± 2*	*114 ± 4*	*105 ± 2 * ɣ*	*114 ± 5*
	Lact	*3.0 ± 0.1*	*3.1 ± 0.2*	*3.1 ± 0.2*	*3.5 ± 0.3*	*2.7 ± 0.1*	*3.6 ± 0.2*	*2.6 ± 0.1 * ɣ*	*3.3 ± 0.3*
	Borg	*11.5 ± 0.3*	*11.0 ± 0.5*	*10.4 ± 0.4*	*10.8 ± 0.3*	*10.1 ± 0.3*	*11.4 ± 0.6*	*9.5 ± 0.3 * #*	*10.6 ± 0.4*
Recup.	*HR*	*87 ± 3*	*81 ± 5*	*84 ± 3*	*85 ± 4*	*84 ± 2*	*86 ± 4*	*84 ± 2*	*89 ± 5*
After Bout 2	*MRT2*	*90.3 ± 4*	*89.5 ± 6*	*88.4 ± 4*	*89.2 ± 6*	*81.8 ± 3*	*85 ± 2*	*80.2 ± 2*	*83.8 ± 3*
	HR	*114 ± 4*	*110 ± 5*	*110 ± 3*	*113 ± 4*	*108 ± 2*	*118 ± 4*	*109 ± 3 **	*117 ± 5*
	Lact	*2.8 ± 0.2*	*3.1 ± 0.2*	*3.1 ± 0.2*	*3.5 ± 0.2*	*2.5 ± 0.1*	*3.3 ± 0.2*	*2.5 ± 0.1 * ɣ*	*3.1 ± 0.2*
	Borg	*11.9 ± 0.4*	*11.7 ± 04*	*10.7 ± 0.4*	*11.5 ± 0.4*	*10.4 ± 0.4*	*12.3 ± 0.6*	*9.9 ± 0.4 * #*	*11.0 ± 0.4*

Data are presented as mean ± SE.
** p<0.05, within group effect over 24w*
ɣ p<0.05, # p<0.1, difference between EX and SED at 24w
Abbreviations used: HR, heart rate (beats per minute); Lact, lactate (mmol/l); MRT, mean response time (seconds); Recup, recuperation.

Discussion

In the present randomized controlled trial, we investigated the impact of 24-weeks combined exercise on glucose tolerance and serum BDNF release in MS patients. Furthermore, to validate the effect of 24 weeks of mild-to-moderate intensity combined exercise, muscle strength, exercise tolerance and body composition were assessed. Long-term combined exercise did not affect glucose and insulin profiles but significantly improved BDNF levels within EX as compared to baseline. In addition, the applied exercise intervention improved muscle strength, exercise tolerance and body composition substantially whereas it remained stable in SED.

The influence of exercise on IGT in MS:

To date, knowledge regarding the influence of physical exercise on inactivity-induced medical complications in MS remains elusive. Given the previously observed elevated prevalence of IGT in MS [204] and the ability of physical activity to reduce the risk of developing IGT and to improve insulin action in other patient populations [107], it was hypothesized that whole body glycaemic control could also be improved in MS patients upon exercise. However, following the applied program, no decrease in glucose and insulin profiles was detected. In contrast, Slawta *et al.* [71] showed that low-to-moderate intensity leisure time physical activity was associated with lower fasting glucose levels and White *et al.* [143] reported a trend towards decreased fasting glucose concentrations after 8 weeks of lower extremity progressive resistance training.

Although the variety amongst the applied exercise programs between studies complicates comparison, other variables may also account for the inconsistent findings regarding the effect of exercise on glycaemic control between different studies. First, given the correlation between glucose concentrations and EDSS [74] physical disability status may affect study outcome. Next, patient characteristics, such as age, gender and BMI can alter the outcomes of this kind of research. Finally, baseline activity levels may also contribute to the contrasting results in the different studies. It has been noted that the above mentioned factors differ between the present study and the work of White *et al.* [143] and Slawta and co-workers [71].

Exercise intensity probably also affects training outcome. Some investigators reported that low-to-moderate exercise intensity improved glucose tolerance in other populations, whereas others suggested that this intensity was insufficient to improve insulin sensitivity [156]. Sandvei *et al.* reported a decrease in glucose AUC after 8 weeks of sprint interval training, whereas the glucose response remained unchanged after 8 weeks of continuous running. However, fasting glucose concentrations were significantly reduced

in both groups [157]. Similarly, Tjonna and co-workers demonstrated a significant improvement in fasting blood glucose and insulin sensitivity after 16 weeks of aerobic interval training (90% of maximal heart rate) in metabolic syndrome patients, whereas no improvements were found after moderate continuous exercise (70% of maximal heart rate)[158]. Clearly, future studies are needed to point out whether a high intensity interval exercise is more effective to improve insulin sensitivity than continuous moderate training, as applied in this study.

The influence of exercise on BDNF in MS:

To investigate possible underlying biological mechanisms of exercise on the disease pathogenesis of MS, we assessed serum BDNF. Several studies reported that physical exercise is able to increase the BDNF production [209, 217] and that the fold increase of the BDNF concentration is exercise intensity-dependent [211]. Accordingly, we demonstrated increased levels of serum BDNF in the exercised group, suggesting possible involvement of BDNF in the mechanism of action of exercise therapy in MS and possibly influencing the pathogenesis of MS [205].

Combined exercise in MS:

Several studies have demonstrated the benefits of resistance training [14] or endurance training [13] in MS. However, the influence of a combined exercise program remains unexplored. Here, we demonstrated improvement of physical parameters in EX, whereas these factors remained stable in SED. This is in agreement with Carter and co-workers who reported improved muscle strength and a reduced level of effort of walking after 12 weeks of combined training. Likewise, Motl *et al.* reported significant improvements on walking ability after 8 weeks of supervised combined exercise [218]. In contrast, Surakka *et al.* [155] and Romberg and co-workers [154] observed no or modest improvements in knee extensor and knee flexor muscle strength and no changes in aerobic capacity upon a 26-week home-based combined training program. However, exercise diaries reported that adherence to resistance training was only 59%, whereas the adherence to endurance training was 185% [155]. We have set forth that by implementing a supervised exercise program, a higher degree of standardization could be obtained as evidenced by a training adherence of approximately 90% in the present study. Furthermore, we applied a mild-to-moderate intensity program based on an individual approach taking into account bilateral differences between limbs of MS patients. Consequently, it was possible to gain more muscle strength in the weakest leg, whereas improvements of the strongest leg were less pronounced.

The effect of a combined exercise program on body composition in MS was never investigated previously. We reported an increased lean tissue mass and a tendency to a decreased fat percentage in EX. Similarly, Petajan *et al.* reported a significant decrease in skinfold and a tendency to reduced body fat percentage after 15 weeks of aerobic exercise [13]. Furthermore, leisure-time physical activity was associated with lower waist circumference in women with MS [71], whereas Snook and co-workers reported a negative correlation between physical activity and body fatness in MS patients [177]. In contrast, White *et al.* reported no changes in body weight and fatness after 8 weeks of lower-extremity progressive resistance training in women with MS [143].

Overall, and in agreement with previous studies [154, 155], we conclude that the applied combined exercise program was well tolerated in MS.

Limitations:

The present study had some limitations, resulting in recommendations for future research. Since we were, to our knowledge, the first who investigated the influence of physical exercise on glucose tolerance in MS, we were not able to perform a pre-trial power analysis, due to the absence of a defined effect size. Furthermore, only 14% of the included MS patient showed IGT at baseline, possibly explaining the absence of some of the expected results on the glucose and insulin profile after 24 weeks of exercise. A sub analysis in the IGT-group only, however, showed also no changes after 24 weeks of mild-to-moderate intensity exercise. Since some of the reported improvements were only statistical significant within EX, whereas the overall interaction effect (time*intervention) was absent, future studies should include a larger cohort of a more homogeneous subpopulation of MS patients. Finally, given the nature of the design, social interactions (such as peer pressure, physical activity outside the program, the awareness of being involved in an exercise intervention study, changing the food pattern,…) between MS patients could possibly affect study results, by improving intervention outcomes.

Conclusion

In conclusion, long-term exercise was not able to affect glycaemic control in MS albeit that muscle strength, exercise tolerance and body composition were improved within the exercise group. Moreover, an exercise-induced increase of BDNF levels was detected, suggesting a positive effect of exercise on disease pathogenesis. No changes were observed in the control group.

Acknowledgements

The authors would like to thank all MS patients for participating in this study. Our gratitude goes to Anne Bogaers for logistical assistance and blood sample collection, to Prof. Dr. Niel Hens (Interuniversity Institute for Biostatistics and Statistical Bioinformatics, Hasselt University, Belgium and Centre for Health Economics Research & Modelling Infectious Diseases, Vaccine and Infectious Disease Institute, University of Antwerp, Belgium) for statistical advice, to Prof. Dr. Ulrik Dalgas of Aarhus University Denmark, for providing valuable feedback and comments during the preparation of the manuscript and Devid Muys, without whose help and support this study would not have been possible.

STUDY 4

Effects of exercise intensity in experimental autoimmune encephalomyelitis

Based on: Wens I., Broekmans T., Hendriks JJA., Savelberg HH., Hesseling MK., Eijnde BO.
BMC Physiology, under review

Abstract

Background. Muscle contractile properties and disease progression following experimental autoimmune encephalomyelitis (EAE) and physical exercise have not been investigated.

Objective. To investigate the effect of exercise intensities on muscle contractile properties and hindquarter paralysis during EAE in Lewis rats.

Methods. A control and EAE group were divided in sedentary, light, moderate and high intensity running subgroups. During EAE course, hind limb paralysis, body weight and food intake were registered. Following EAE recovery isokinetic foot extensor strength was measured during 115 maximal contractions and fiber characteristics of m. Tibialis Anterior (TA) and m. Extensor Digitorum Longus (EDL) were analysed.

Results. EAE reduced CSA of type IIb+x fibers of TA and EDL, while type I and IIa fibers CSA were not affected by EAE. Exercise did not change CSA of type I, IIa and IIb+x fibers of EDL nor TA, except for TA type IIa fibers CSA, which increased in EAE moderate and EAE high intensity groups. Muscle work peak was absent in all EAE animals during isokinetic muscle contractions. Intense exercise delayed onset of hindquarter paralysis in EAE, while disease peak and remission were not improved by exercise.

Conclusion. This study suggests that EAE reduces CSA of type IIb+x fibers of TA and EDL. This possibly explains the absence of peak muscle work during the first of a series of isokinetic muscle contractions. Furthermore, exercise was not able to reduce muscle fiber atrophy, whereas high intensity exercise delayed onset of hindquarter paralysis.

Introduction

Experimental autoimmune encephalomyelitis (EAE) is an inflammatory demyelinating disease model of the central nervous system (CNS) often used to investigate demyelination in the CNS in general, and Multiple Sclerosis (MS) in particular [22]. So far, research investigating the impact of EAE and/or physical exercise during EAE on muscle contractile properties and EAE progression is scarce, conflicting but also promising [135-138]. De Haan and co-workers explored the impact of EAE on muscle contractile properties. Compared to healthy controls, EAE rats were not only less physically active, they also showed significant loss of body weight and Gastrocnemius muscle mass (-21 and 33% respectively), reduced muscle fiber cross sectional area (CSA) of all fiber types (-40 to 50%), as well as lower maximal muscle force and power (-58 and 73% respectively) [135]. Furthermore, Le Page and co-workers demonstrated that treadmill running after immunization, delayed the onset and duration of hindquarter paralysis, associated with chronic EAE. However, maximal clinical scores were not affected and these investigators did not examine changes in muscle CSA [137]. More recently, in mice with chronic EAE less severe neurological deficits and a less pronounced spinal loss in the striated neurons after voluntary, not structured, wheel running have been reported [138].

In MS, muscle contractile property research is conflicting [45, 47, 49, 50] and only the impact of moderate progressive strength training on it has been investigated [81]. Interestingly, several authors [41, 100, 118, 119] suggested that MS patients could further benefit from higher intensity exercise, but it is unclear whether this could be tolerated. To further investigate the above described issues the use of an animal MS model seems appropriate [134].

In accordance with the above line of reasoning, the current study aimed to investigate the impact of low, moderate and high intensity exercise on muscle contractile properties and disease progression of healthy and acute EAE rats. It is hypothesized that exercise improves muscle contractile properties and delays the onset of hindquarter paralysis.

Methods

1. Animals

In total 64 female Lewis rats (age 6-7 weeks, body weight 120-170 g, Harlan CPB, Zeist, The Netherlands) were maintained on a constant light:dark cycle (12:12), a temperature of 22°C and a relative humidity of 22-24%, in the animal facilities of Hasselt University. Rats were fed ad libitum with normal rat pellets (Carfil RN-01-K12, Harlan). The animal Ethics Committee of Hasselt University approved the study protocol in accordance with the national and European legislation. Furthermore, the National Research Council's guide for the care and use of laboratory animals was followed.

2. Study design

Following acclimatization and adaptation rats were enrolled in a treadmill running training program (TR, n=64, low to high intensity training) and subdivided in a sedentary (TR^{SED}), light (TR^{L}), moderate (TR^{M}) and high intensity (TR^{H}) running group (Figure 2.4.1). During habituation animals were familiarized to treadmill running (day -14 to -1) at progressively increased training durations and intensities. Animals were encouraged to walk/run by means of low intensity electrical shocks. Shocks, if required, lasted <1s and usually occurred a few times per training session. After habituation TR^{L} rats were able to walk 1h at 5m/min (0° inclination). TR^{M} and TR^{H} rats ran 1h at respectively 11m/min (15° inclination) and 18m/min (25° inclination). Although not measured, running intensity for TR^{H} rats was probably just below the anaerobic threshold [219, 220]. TR^{SED} animals were subjected to similar daily manipulation. From the habituation period onwards, daily food intake and body weight were registered. Following habituation (day 0), all sedentary and training groups were divided in a healthy control (CON) and EAE group (EAE induction). Hereafter TR^{L}, TR^{M} and TR^{H} rats were subjected to daily (1h/day) physical exercise, until progressive hindquarter paralysis (~day 11) prevented this. However, if an animal developed hindquarter paralysis before day 11, preventing daily exercise, the exercise program was immediately terminated, in accordance with the designated endpoint of exercise, where after the animal was excluded and humanely euthanized. After (partial) recovery (day 17) animals were anaesthetized using an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body weight). Following determination of repetitive isokinetic foot extensor performance of the left hind limb, m. Extensor Digitorum Longus (EDL) and m. Tibialis Anterior (TA) of the right hind limb were dissected and freed of connective tissue and visible blood. Hereafter, the mid-part of each muscle was mounted in embedding tissue (Tissue-Tek® OCT™ Compound, Miles Laboratories, Inc., Elkhart, Indiana,

USA), frozen in isopentane (Sigma-Aldrich, St. Louis, MO, USA), cooled in liquid N_2, and stored at -80°C until further analysis were performed. Finally, rats were sacrificed by an intracardial injection of pentobarbital sodium.

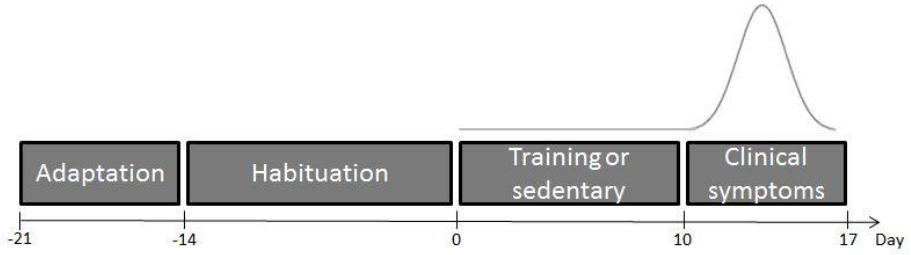

Figure 2.4.1: *Study design. Following adaptation rats were familiarized to treadmill running during habituation. After EAE induction (day 0) TR^L, TR^M and TR^H rats were subjected to daily physical exercise, until progressive hindquarter paralysis prevented this (day 10). After (partial) recovery (day 17) isokinetic foot extensor performance was measured, where after muscle samples of EDL and TA were collected. Finally, rats were sacrificed.*

3. EAE induction

EAE was induced in EAE subgroups by a single percutaneous injection in both footpads (100µl/foot) under isoflurane anaesthesia and consisted, per animal, of 24µl purified myelin basic protein (MBP, 25mg/ml) in combination with 25µl 7RA heat-killed mycobacterium tuberculosis (20mg/ml, Difco), 120µl complete Freunds adjuvant (CFA, Difco) and 31µl phosphate-buffered saline (PBS) [33].

4. Primary outcome measures

Fiber CSA and distribution

To quantify type I and IIa muscle fiber CSA and distribution, serial transverse sections (8µm) from the obtained muscle samples were cut at -20°C and stained by means of triple-staining. Air-dried (30 min) cryosections were washed (5 min) with 0.5% Triton-100, added to PBS and then washed (5 min) with PBS. Next, sections were incubated 60 min at room temperature with a mix of 2 mouse monoclonal antibodies against myosin heavy chain I (1:25; A4.840 supernatant, Developmental Studies Hybridoma Bank, Iowa, USA) and IIA (1:25; N2.261 supernatant, Developmental Studies Hybridoma Bank, Iowa, USA) and 1 rabbit polyclonal laminin antibody (1:100; L-9393, Sigma, Zwijndrecht, Belgium). Then, slides were washed 3 times (5 min) with PBS, followed by an incubation period of 45 min at room temperature with a mixture of secondary antibodies (1:500, Goat anti-Mouse IgM AlexaFluor 555; 1:200 Goat anti-Mouse IgG_1 AlexaFluor 488

and 1:130 Goat anti-RabbitIgG AlexaFluor 350; Molecular probes, Invitrogen, Breda, The Netherlands) diluted in PBS. Hereafter, sections were washed 3 times (5 min) with PBS and mounted in Fluorescent Mounting Medium (Dako, North America, California, USA). Muscle fibers were examined and recorded using a Nikon Eclipse 90i fluorescence microscope (Nikon, Boerhavedorp, Germany). The fluorescence signals were recorded using a TRITC and FITC filter for type I and IIa muscle fibers, respectively, and DAPI filter for cell membrane. The regions who were not fluorescent, including type IIb and IIx fibers, were grouped together and called type IIb+x fibers. Digital images (x20 magnification, exposure time for TRITC and FITC 400ms, DAPI 800ms) were analysed using NIS Elements® BR 3.0 software (LIM, Prague, Czech Republic).

Hindquarter paralysis

After EAE induction all EAE rats were examined daily at 8.30 a.m. for the development of clinical symptoms. Typically, hindquarter paralysis developed 12 to 14 days after induction, where after rats partly recovered (day 17). Symptoms were blinded scored on a scale ranging from 0 to 5: 0, no signs; 0.5: partial loss of tail tonus (defined as the disease onset); 1.0: complete loss of tail tonus; 2.0: hind limb paresis; 3.0: hind limb paralysis; 4.0: moribund; 5.0: death due to EAE [33]. The clinical endpoints were based on the clinical scores. If an animal was not able to eat or drink independently, the animal was excluded and euthanized. Overall hindquarter paralysis was expressed as the average of the summated daily scores per group.

5. Secondary outcome measures

Isokinetic foot extensor strength

After general anaesthesia at day 17, left hind limb foot extensor muscle strength was assessed during fatiguing isokinetic muscle contractions, as described elsewhere [221, 222]. Briefly, percutaneous needle electrodes were placed on the common peroneal nerve fusing 115 consecutive concentric isokinetic foot extensions (50°/s, 1 mA, 250ms, 3s rest intervals) after standardized fixation of knee and ankle on a custom build Ashton-Miller like rat dynamometer [223]. Work fatigue was expressed as a percentage, compared to the highest work, which was performed during the first 30 consecutive contractions and set at 100%.

Body weight & food intake

Daily body weight and food intake was registered using an automatic/ digital balance (Sartorius®, Goettingen, Germany) at 8 a.m.

6. Statistical analysis

All data were analysed using SAS software (SAS Institute Inc, Cary, USA). First *normality* was checked using the Shapiro-Wilk test for all variables. *Body weight and food intake* were analysed by a (Group [CON; EAE] x Activity [TR^{SED}; TR^{L}; TR^{M}; TR^{H}] x study phase [habituation phase; induction phase; exercise phase; paralysis phase]) mixed model ANOVA. *Muscle fatigue of isokinetic foot extensor* data was analysed using a (Group [CON; EAE] x Activity [TR^{SED}; TR^{L}; TR^{M}; TR^{H}] x Contraction [number of dynamic muscle contractions]) mixed model ANOVA. *Muscle fiber type area and distribution* were analysed using a 2x4 (Group [CON; EAE] x Activity [TR^{SED}; TR^{L}; TR^{M}; TR^{H}]) mixed model ANOVA. *Hindquarter paralysis* was analysed using a (EAE Group [EAE-TR^{SED}; EAE-TR^{L}; EAE-TR^{M}; EAE-TR^{H}] x Time [Days 0-17]) mixed model ANOVA, with body weight as confounding factor. To analyse disease onset, which was defined as a hindquarter paralysis score equal to 0.5, a time to event analysis was used for all EAE groups. When appropriate, post hoc pre-planned contrast tests were applied. All data are presented as mean ± SE, the threshold for statistical significance was set at $p<0.05$.

Results

1. Primary outcome measures

Muscle fiber cross sectional area and distribution

Type I and IIa muscle fiber CSA of both muscles in all groups were not affected by EAE. Furthermore, type IIb+x fiber CSA of EDL and TA were significantly reduced, respectively ~22% and ~40%, due to EAE ($p<0.05$). Fiber type distribution in EDL and TA did not differ between CON and EAE (Table 2.4.1).

In CON and EAE exercise did not affect CSA of type IIb+x fibers of EDL nor TA, compared to corresponding TR^{SED}. Comparable results were detected, in all groups, for type I and IIa fibers, except for TA type IIa fiber CSA of EAE-TR^{M} and EAE-TR^{H} which increased, respectively, 16% and 23% ($p<0.05$), compared to EAE-TR^{SED}. Furthermore, exercise did not affect fiber distribution in EDL nor TA (Table 2.4.1).

Hindquarter paralysis

Compared to the corresponding SED groups, disease onset, peak and remission did not differ between intensity groups, except for EAE-TR^{H} disease onset, which was significantly delayed (EAE-TR^{H}: day 11.6 ± 0.3, EAE-TR^{SED}: day 11.0 ± 0.1, $p<0.05$, Figure 2.4.2).

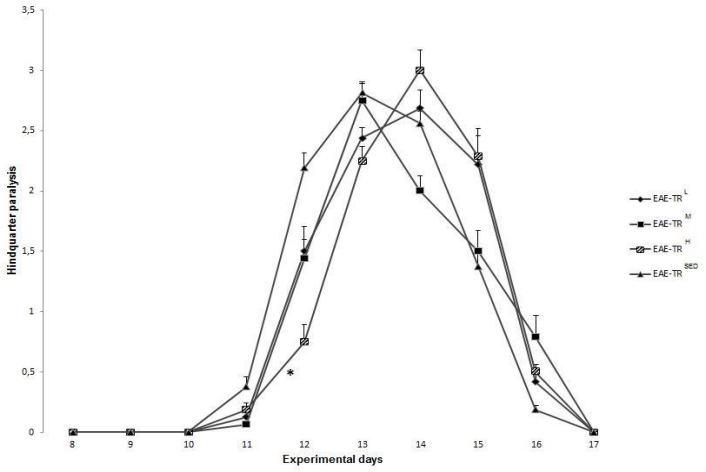

Figure 2.4.2: *Effect of experimental autoimmune encephalomyelitis and different exercise intensities on hindquarter paralysis.*
Values are means ± SE and express the level of hindquarter paralysis, on a scale from 0 to 5, in sedentary and exercised EAE animals.
**p<0.05 onset of hindquarter paralysis (score 0.5) of EAE-TR^{SED} compared to EAE-TR^{H}.*

Table 2.4.1: *Effect of experimental autoimmune encephalomyelitis and treadmill exercises on cross sectional area and muscle fiber composition of Extensor Digitorum Longus (EDL) and Tibialis Anterior (TA)*

	Type I			Type IIa			Type IIb		
EDL									
	n	**Area (µm²)**	**p**	**Distr (%)**	**p**	**Area (µm²)**	**p**	**Distr (%)**	**p**	**Area (µm²)**	**p**	**Distr (%)**	**p**
CON-TR^{SED}	**8**	511 ± 46.6		5.7 ± 0.9		699 ± 55.7		26.1 ± 2.4		996 ± 59.2		68.1 ± 2.4	
CON-TR^{L}	**8**	516 ± 42.7		5.0 ± 0.8		693 ± 44.3		26.9 ± 2.4		1137 ± 63.4		68.2 ± 2.8	
CON-TR^{M}	**7**	514 ± 46.9	0.64	6.4 ± 0.8	0.18	654 ± 39.7	0.65	20.0 ± 3.1	0.70	988 ± 57.5	0.04	73.7 ± 3.3	0.94
CON-TR^{H}	**8**	559 ± 63.0		6.0 ± 0.8		658 ± 60.5		26.3 ± 2.9		1003 ± 62.7		67.8 ± 3.0	
EAE-TR^{SED}	**8**	552 ± 24.8		5.9 ± 0.9		680 ± 55.6		24.9 ± 2.0		772 ± 51.2*		69.2 ± 1.8	
EAE-TR^{L}	**5**	562 ± 95.0		7.2 ± 1.2		685 ± 24.4		23.6 ± 3.6		800 ± 109.8*		69.2 ± 3.9	
EAE-TR^{M}	**7**	624 ± 43.2		4.6 ± 0.5		735 ± 60.9		23.4 ± 3.6		832 ± 74.2		72.0 ± 3.6	
EAE-TR^{H}	**6**	539 ± 35.0		6.3 ± 1.4		619 ± 35.8		25.5 ± 3.2		767 ± 72.1*		69.2 ± 2.7	

Table 2.4.1 (continued):

		Type I			Type IIa			Type IIb		
		TA								
	n	Area (µm²)	p	Distr (%)	p	Area (µm²)	p	Distr (%)	p	Area (µm²)	p	Distr (%)	p
CON-TR^{SED}	**7**	682 ± 62.6		1.3 ± 0.3		797 ± 40.1		16.8 ± 2.9		1384 ± 84.2		81.9 ± 2.9	
CON-TR^{L}	**7**	724 ± 29.8		1.3 ± 0.2		723 ± 36.1		15.5 ± 1.4		1198 ± 37.2		83.2 ± 1.4	
CON-TR^{M}	**8**	875 ± 40.8	0.45	3.2 ± 1.2	0.15	774 ± 39.3	0.08	15.3 ± 1.7	0.88	1267 ± 35.4	0.03	81.5 ± 1.5	0.87
CON-TR^{H}	**8**	746 ± 43.5		2.0 ± 0.3		774 ± 22.1		19.5 ± 2.4		1244 ± 64.8		78.5 ± 2.5	
EAE-TR^{SED}	**8**	807 ± 56.8		2.6 ± 1.0		751 ± 41.2		18.2 ± 2.9		762 ± 47.4*		79.2 ± 3.1	
EAE-TR^{L}	**6**	740 ± 89.4		1.7 ± 0.5		778 ± 37.3		18.7 ± 2.8		786 ± 73.2*		79.6 ± 2.6	
EAE-TR^{M}	**7**	804 ± 95.2		1.4 ± 0.3		872 ± 42.3†		18.9 ± 2.4		786 ± 78.9*		79.8 ± 2.4	
EAE-TR^{H}	**6**	769 ± 54.5		1.6 ± 0.2		922 ± 46.3*†		19.5 ± 2.7		818 ± 72.4*		78.9 ± 2.7	

Values are means ± SE and express muscle fiber cross sectional area (µm²) and fiber type composition (Distr %) in Extensor Digitorum Longus (EDL) and Tibialis Anterior (TA) of sedentary (TR^{SED}), low- (TR^{L}), moderate- (TR^{M}) and High- (TR^{H}) training intensity healthy (CON) and experimental autoimmune encephalomyelitis (EAE) rats. p-values represent group (CON, EAE) x activity (TR^{SED}, TR^{L}, TR^{M}, TR^{H}) effects.
** p<0.05 compared to corresponding CON value. † p<0.05 compared to EAE-TR^{SED}*

2. Secondary outcome measures

Isokinetic muscle strength

Group x contraction analysis, indicated that the muscle work curves of EAE and CON rats significantly differed (Figure 2.4.3). More particular, for the treadmill groups, muscle work of CON peaked and then declined (-36 ± 1%) during the first 30 contractions while in EAE muscle work remained stable (-5 ± 0.2%).

Body weight and food intake

EAE decreased body weight and food intake by, respectively, ~10% and ~57%, immediately after EAE induction ($p<0.05$). Hereafter, body weight and food intake gradually recovered until the onset of hindquarter paralysis at day 11. Then, body weight and food intake of EAE groups decreased ($p<0.05$), on average, by ~19% and ~69% respectively (data not shown). Furthermore, exercise was able to reduce body weight loss and food intake drop during the paralysis phase. Here, an exercise effect ($p<0.05$) between EAE-TR^{H} and EAE-TR^{SED} on body weight and food intake was detected (data not shown).

3. Drop out

In total, five EAE animals died (EAE-TR^{L} n=2; EAE-TR^{M} n=1; EAE-TR^{H} n=2) during the study course with no significant differences in survival rate between groups.

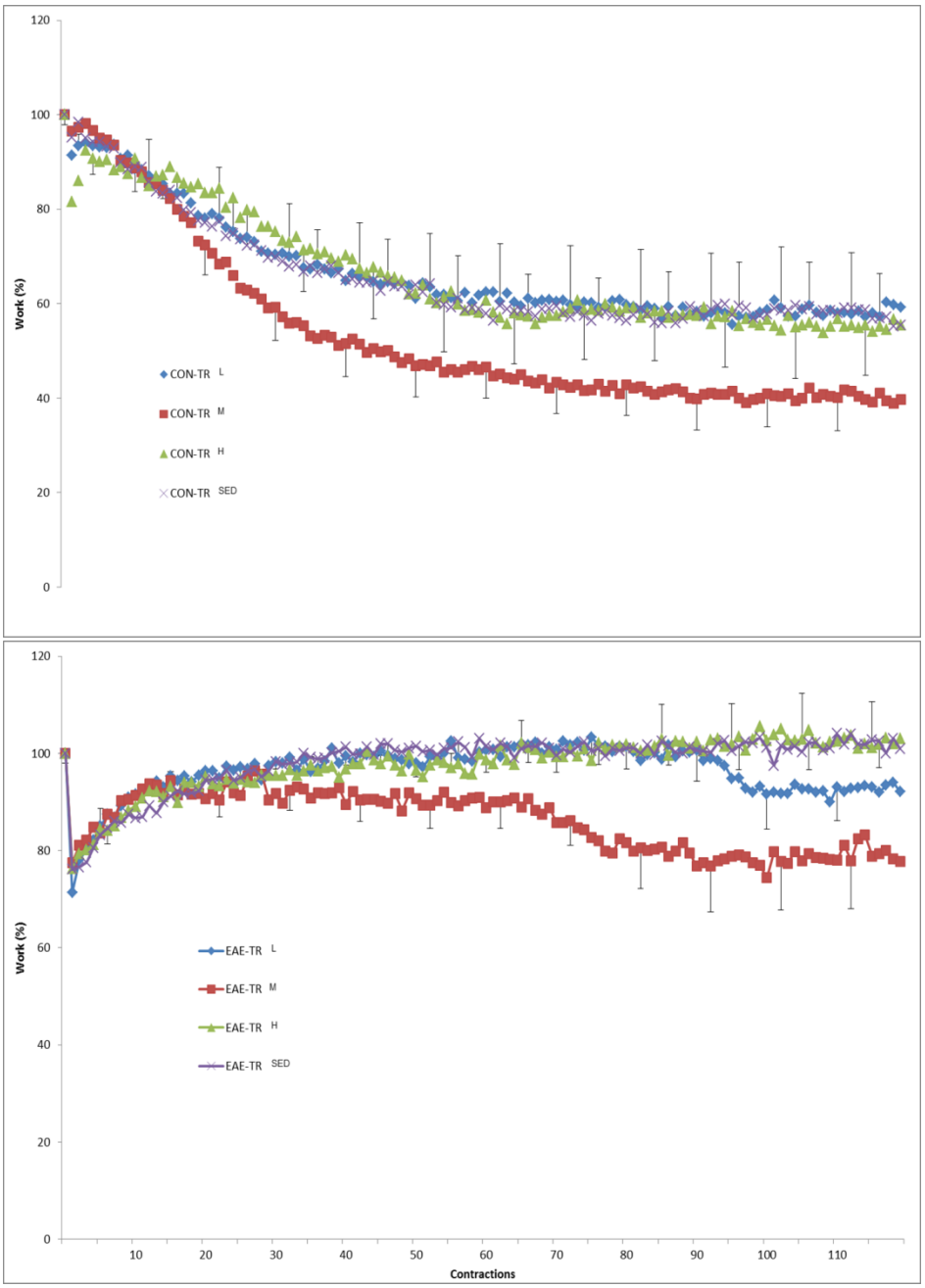

Figure 2.4.3: *Effect of different treadmill exercise intensities on isokinetic muscle work in healthy and EAE rats.*
Values are means ± SE and express muscle work (%) during 115 consecutive maximal muscle contractions (1mA, 150Hz, 250ms) in healthy (CON) rats and EAE rats, subdivided in different exercise intensity groups (TR^{SED}: sedentary; TR^{L}: low; TR^{M}: moderate; TR^{H}: high intense running exercise).

Discussion

The current study was the first to investigate the impact of low, moderate and high intensity endurance training on muscle contractile characteristics and hindquarter paralysis during/following acute EAE. EAE reduced CSA of type IIb+x muscle fibers in TA and EDL, which probably explains the absence of peak force during a series of 115 isokinetic muscle contractions in EAE [224, 225]. Under the conditions of the present study, treadmill exercise was not able to reduce type IIb+x muscle fiber atrophy. Finally, and with the exception of high intensity running exercise that was able to delay paralysis onset, peak and remission of hindquarter paralysis did not change after low and moderate intensity exercise.

Under the conditions of the present study the CSA of EDL and TA type IIb+x fibers in EAE animals was decreased. Muscle fiber distribution was not affected by EAE. De Haan and co-workers previously investigated the impact of EAE on the medial Gastrocnemius, a muscle comprising ±80% type I fibers, and also demonstrated no muscle fiber shifts but reduced CSA of all fiber types [135]. In rats it is documented that running exercise can reverse muscle fiber atrophy [226-228]. In the present study high intensity running exercise tended to reduce muscle fiber atrophy only in EAE-TR^H rats, suggesting an exercise effect. However, other exercise intensities were not able to reduce EAE-induced fiber atrophy in any fiber type of the other groups. Interestingly, in the healthy control rats exercise did also not alter muscle fiber characteristics. This suggests that one week of inactivity during the paralysis period of EAE rats, which was also applied in the control rats, may have tempered training effects. Therefore, muscle sampling immediately after the training period and before the onset of hindquarter paralysis might give better insight into the effect of exercise on muscle contractile properties during EAE. Furthermore, it is also interesting to investigate the effect of resistance training on muscle fiber characteristics, since EAE is able to reduce the CSA of type IIb muscle fibers, that are more susceptible to strength training [229-231].

As depicted in Figure 2.4.3, and as previously reported by Wakatsuki *et al.* [232] in healthy rats, muscle work in CON subgroups peaked during the first 20-30 contractions and then progressively declined. This was also reported by De Haan *et al.* in control and EAE rodents during a series of repeated maximal isometric contractions of m. Gastrocnemius [135]. In the EAE subgroups of the present study muscle work did not peak. This might be explained by the fact that different muscle groups were analysed. More specific, TA and EDL with predominately glycolytic muscle fibers [233] were used in the current study, versus medial Gastrocnemius with a proximal region, containing all muscle fibers, and a distal region, containing only type IIx and IIb fibers were investigated in the study of De Haan and co-workers

[135]. Because fast glycolytic type IIb muscle fibers largely contribute to peak force production [224, 225] the decreased CSA of type IIb+x fibers probably explains the absence of peak muscle work in EAE during the first 30 repetitive isokinetic contractions in the present study.

Interestingly, high intensity treadmill running was able to delay the onset of EAE-induced hindquarter paralysis in EAE-TR^H. This was paralleled by reduced body weight loss during the paralysis period. Low and moderate intensity endurance training, however, did not change EAE disease course. Other authors also reported delayed onset of clinical EAE signs following voluntary wheel running in chronic EAE mice [138] and treadmill running in rats [136, 137]. It is difficult to compare different studies due to application of different exercise intensities (lower versus higher intensities) or due to the use of voluntary exercise versus quantified training load and intensity. The training intensities used in the present study were probably higher compared to the work of Le Page and Rossi. In fact, in the present study EAE-TR^H and CON-TR^H exercise intensity is probably near the anaerobic threshold [219, 220]. Therefore, the present findings and the above-described work of others suggest that exercise intensity determines the impact of training on the course of EAE and that the optimal endurance training intensity in EAE is probably near the anaerobic threshold. As such and given the fact that the effects of exercise therapy on a variety of functional parameters in MS is at present under investigation, it is worthwhile to investigate this hypothesis in MS patients.

The present study had some limitations, resulting in a few recommendations for future research. The absence of an explicit exercise effect, could, possibly, be explained by the sedentary week after a relatively short training period. Therefore, it is suggested to collect muscle samples immediately after the training period and before the onset of hindquarter paralysis, to further investigate the influence of exercise on muscle contractile properties in EAE. Furthermore, this research and the work of others suggested that the optimal exercise intensity is near the anaerobic threshold. However, during the present study lactate concentrations and/or VO_2 kinetics were not measured. Therefore, it is recommended to quantify exercise intensity in future studies. Finally, since forced treadmill running can induce stress, which could influence the EAE symptoms, it is recommended to measure stress hormone levels in future research.

In conclusion, the present study showed that intense treadmill running delayed the onset of hindquarter paralysis in EAE. Low and moderate running exercise had no effect. Furthermore, EAE reduced rat CSA type IIb+x fibers of TA and EDL. This probably explains the absence of peak muscle work during the first 30 contractions of a series of isokinetic muscle performance in EAE.

STUDY 5

Impact of high intensity exercise on muscle morphology in EAE rats

Based on: Wens I., Dalgas U., Verboven K., Kosten L., Stevens A., Hens N., Eijnde BO.
Phys Research, under review

Abstract

Introduction: The impact of high-intensity exercise on disease progression and muscle contractile properties in experimental autoimmune encephalomyelitis (EAE) remains unclear.

Methods: Control (CON) and EAE rats were divided into sedentary and exercise groups. Before onset (experiment 1, n=40) and after hindquarter paralysis (experiment 2, n=40), isokinetic foot extensor strength, cross sectional area (CSA) of Tibialis Anterior (TA), Extensor Digitorum Longus (EDL) and Soleus (SOL) and brain-derived neurotrophic factor (BDNF) levels were assessed.

Results: EAE reduced muscle fiber CSA of TA, EDL and SOL. In general, exercise was not able to affect CSA, whereas it delayed hindquarter paralysis peak. CON muscle work peaked and declined, while it remained stable in EAE. BDNF-responses were not affected by EAE or exercise.

Conclusion: EAE affected CSA-properties of TA, EDL and SOL, which could, partly, explain the absence of peak work during isokinetic muscle performance in EAE-animals. However, exercise was not able to prevent muscle fiber atrophy.

Introduction

Muscle weakness and muscle fatigue are two frequently occurring symptoms in Multiple Sclerosis (MS). Consequently, many MS patients show a reduced daily life physical activity level, often leading to inactivity related loss of muscle strength and muscle mass and reduced quality of life [8, 97]. Similar to other pathologies, it is clear that muscle weakness in MS can, at least in part, be reversed by physical exercise [7, 41, 82, 139, 234, 235]. Several studies already investigated muscle properties in MS patients [45-47, 49, 51, 77]. The effects of MS *per se* on muscle fiber characteristics such as cross sectional area (CSA) and fiber proportion, however, remain unclear [45, 47, 49, 50]. The impact of physical exercise on muscle fiber characteristics in MS has only been investigated by Dalgas and co-workers, reporting increased m. Vastus Lateralis mean fiber CSA (8 ± 15%) after 12 weeks of progressive resistance training [81]. The impact of other training modalities such as aerobic training on muscle morphology has not been investigated yet. Furthermore, several authors suggested that MS patients could benefit more from higher aerobic training intensities [41, 100, 118, 119]. However, before evaluating the effects of high intensity aerobic training on muscle morphology and function in MS patients, it seems relevant to investigate this first in a MS animal model, experimental autoimmune encephalomyelitis (EAE).

Literature investigating the impact of EAE and physical exercise on muscle morphology is sparse and partly conflicting, but results are promising [135-138, 236-238]. De Haan and co-workers demonstrated a significant EAE-induced loss of Gastrocnemius muscle mass (33%), reduced muscle fiber CSA of all fiber types (40-50%) and reduced maximal muscle force and power (58 and 73%, respectively) [135]. We previously reported comparable results for muscle strength and type IIb fiber CSA of m. Tibialis Anterior (TA) and m. Extensor Digitorum Longus (EDL)[238]. In accordance to Le Page and co-workers [137] we also demonstrated that daily high intensity (1h/day) treadmill exercise, during the inflammatory period (days 1 to 10 after EAE induction), delayed EAE associated hindquarter paralysis onset, while low and moderate exercise intensities had no effect on the disease course [236]. Furthermore, inactivity, due to EAE-induced paralysis, prevented exercise effects on muscle fiber characteristics [238].

These findings warrant further exploration of the beneficial disease modifying effects of exercise and the underlying neurological basis of these benefits, since the underlying mechanisms of the therapeutic effects of exercise in MS patients are not clear yet. In this respect, it is important to mention that exercise can affect the production of brain-derived neurotrophic factor (BDNF) [209, 217], a neurotrophin that plays an important role in neurogenesis [208] and synaptic plasticity [205, 206, 217, 239, 240]. In fact, BDNF is an important

key-regulation mediator in this process due to its activity-dependent regulation [206] and neuroprotective potential [207]. Interestingly, Rojas Vega *et al.* [210] showed that 10 min of moderate aerobic cycling was not sufficient to increase the serum BDNF concentration above the pre-exercise level, whereas a single bout of maximal incremental exercise resulted in a significant increase in serum BDNF. In accordance with these results, Ferris *et al.* [211] indicated that the magnitude of the increase in serum BDNF concentration during exercise is dependent on exercise intensity. In MS, Gold and co-workers showed that 30 min of moderate aerobic exercise significantly induced BDNF production in MS patients to the same extent as in healthy controls [241]. However, Schulz *et al.* [111] and Castellano and White [209] investigated the effect of 8-weeks of aerobic bicycle training of moderate intensity in MS patients and found no changes in basal nor exercise-induced serum BDNF levels. Moreover, an investigation of the impact of high intensity aerobic exercise on BDNF levels in MS patients is warranted. Since tolerance to high intensity aerobic exercise may be limited in persons with MS, initial studies applying the EAE model seem appropriate.

Consequently, the current study aimes to investigate the impact of high intensity aerobic exercise on disease course, muscle morphology and BDNF release in EAE animals, immediately before (experiment 1) and after (experiment 2) hindquarter paralysis. We hypothesized that high intensity aerobic exercise affects disease course, muscle contractile properties and BDNF release, keeping in mind that paralysis-induced inactivity may temper these effects.

Methods

1. Animals

Eighty female Lewis rats (age 6-7 weeks, body weight 100-120 g, Harlan CPB, Zeist, The Netherlands) were individually housed, in the animal facilities at Hasselt University, on a constant light:dark cycle (12h:12h), a temperature of 22°C and a relative humidity of 22-24%. Rats were fed ad libitum with normal rat pellets (Carfil RN-01-K12, Harlan). The animal Ethics Committee of Hasselt University approved the study protocol in accordance with the national and European legislation. The National Research Council's guide for the care and use of laboratory animals was followed.

2. Study design

Following acclimatization and adaptation (day -21 to -15), animals were randomized into two experiments (n=40 per experiment, Figure 2.5.1). On day -14 rats were, in each experiment, divided into two subgroups: a sedentary group (SED, n=20) and an exercise group (EX, n=20). Due to the nature of the trial, the researchers involved in the daily progression of the project could not be blinded to group allocation. As performed previously [236-238], EX animals were familiarized to treadmill running during the habituation period (day -14 to -1) by progressively increasing running duration and intensity, until a running duration of 1h and a running speed of 18m/min (25° inclination) was reached. During this habituation period EX animals were encouraged to run by means of short electrical shocks. These shocks lasted <1s and usually occurred a few times during the training sessions. In order to induce comparable levels of stress, SED animals were seated on the stationary treadmill (1h) on a daily basis. From the habituation period onwards, daily food intake and body weight were registered. At day 0, SED and EX groups were subdivided into a healthy control group (CON^{SED}, CON^{EX}) and an EAE group (EAE^{SED}, EAE^{EX}), followed by EAE induction. Hereafter, EX rats exercised daily for 1h/day, during 10 consecutive days, until progressive hindquarter paralysis prevented this. At day 0 and 9 arterial blood samples were collected from the tail and serum samples were stored at -80°C.

Experiment 1: At day 10, before onset of clinical symptoms, treadmill training was terminated. To avoid acute exercise-induced training effects, tests were performed 48h after the last exercise bout. First, an additional arterial blood sample from the tail was collected, and all animals were then anaesthetized using an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body weight). Following determination of repetitive isokinetic foot extensor performance of the left hind limb (see details later), m. Extensor Digitorum Longus (EDL), m. Tibialis Anterior (TA) and m. Soleus

(SOL) of the right hind limb were dissected and freed of connective tissue and visible blood. The mid-part of each muscle was mounted in embedding tissue (Tissue-Tek®, Miles Laboratories), frozen in 2-methylbutane (Sigma-Aldrich, St. Louis, MO, USA), cooled in liquid N_2, and stored at -80°C until further analysis were performed. Finally, animals were sacrificed by an intracardial injection of pentobarbital sodium.

Experiment 2: After termination of treadmill exercise, all rats lived sedentary (day 11 to 17), enduring hindquarter paralyses (EAE group, day 11 – 17) and (partial) recovery (day 17). At day 13 and 17 additional arterial blood samples were collected from the tail and serum samples were stored at -80°C. Finally, isokinetic foot extensor performance and muscle sampling were performed, whereupon animals were sacrificed.

During the complete study animal well-being was monitored. If an animal developed hindquarter paralysis earlier than expected, restricting the animal to run, the exercise program was immediately terminated, in accordance with the designated endpoint of exercise, where after the animal was excluded and humanly euthanized.

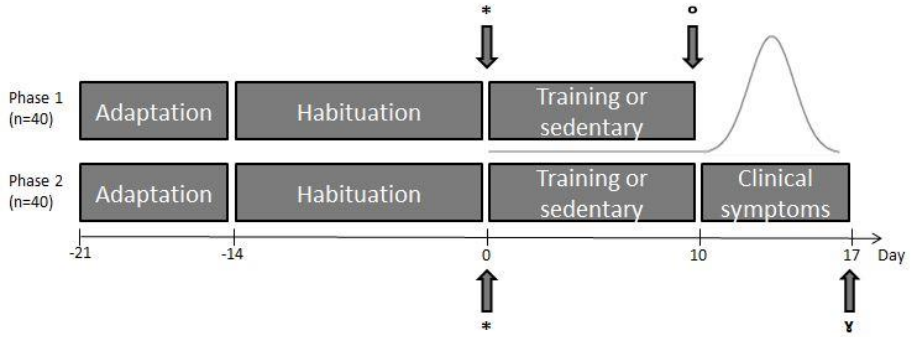

Figure 2.5.1: *Study design of the protocol. Following adaptation rats were familiarized to treadmill running during habituation. From the habituation period onwards, daily food intake and body weight were registered. After EAE induction (*****), CON^{EX} and EAE^{EX} rats were subjected to daily physical exercise, during 10 days. During phase 1, isokinetic foot extensor performance was measured and muscle samples of EDL, TA and SOL were collected before onset of clinical symptoms (°). During phase 2, isokinetic foot extensor performance and muscle sampling were performed after (partial) hindquarter paralysis recovery (¥). Finally, all rats were sacrificed.*

3. EAE induction

EAE was induced in EAE subgroups by a single percutaneous injection in both footpads (100µl/foot) under isoflurane anaesthesia [33] and consisted, per animal, of 24µl purified myelin basic protein (MBP, 25mg/ml) in combination with 25µl 7RA heat-killed mycobacterium tuberculosis (20mg/ml, Difco), 120µl complete Freunds adjuvant (CFA, Difco) and 31µl phosphate-buffered saline (PBS).

4. Primary outcome measures

Fiber CSA and proportion

To quantify muscle fiber CSA and proportion of TA and EDL, serial transverse sections (8µm) from the obtained muscle samples were blinded cut at -20°C and stained by means of triple-staining. Briefly, sections were incubated during 45 min with a mix in PBS of 2 mouse monoclonal antibodies against myosin heavy chain I and IIA (1:25; A4.840 supernatant and 1:25; N2.261 supernatant, Developmental Studies Hybridoma Bank, Iowa, USA) and 1 rabbit polyclonal laminin antibody (1:100; L-9393, Sigma, Zwijndrecht, Belgium). Next, the second incubation comprised a mixture of secondary antibodies (1:500, Goat anti-Mouse IgM AlexaFluor 555; 1:200 Goat anti-Mouse IgG_1 AlexaFluor 488 and 1:130 Goat anti-Rabbit IgG AlexaFluor 350; Molecular probes, Invitrogen, Breda, The Netherlands) , diluted in PBS. The fluorescence signals were recorded using a TRITC and FITC filter for type I and IIa muscle fibers, respectively, and a DAPI filter for cell membrane, using a Nikon E800 fluorescence microscope (Nikon, Badhoevedorp, The Netherlands). The regions that were not fluorescent, representing type IIx and IIb fibers, were grouped together and called type IIx+b fibers. Digital images (x20 magnification, exposure time for TRITC and FITC 400ms, DAPI 800ms) were analysed using NIS Elements software (Nikon, Badhoevedorp, The Netherlands).

Due to cross-reaction the above described protocol was not able to stain muscle fibers of SOL, therefore, a second technique was used to quantify fiber CSA and proportion of SOL. Serial transverse sections (10µm) from the obtained muscle samples were blinded cut at -20°C and stained by means of ATPase histochemistry, after preincubation at pH 4.4, 4.65 and 10.3, essentially following the procedure of Brooke and Kaiser [86]. The serial sections were visualized and analysed using a Leica DM2000 microscope (Leica, Stockholm, Sweden) and a Leica Hi-resolution Color DFC camera (Leica, Stockholm, Sweden) combined with image-analysis software (Leica Qwin ver. 3, Leica, Stockholm, Sweden). This software was able to automatically draw a fiber mask at the stained sections. Afterwards, this mask was fitted manually to the cell borders of selected fibers. Only fibers

cut perpendicularly to their longitudinal axis were used for the determination of fiber size.

Hindquarter paralysis

After EAE induction all EAE rats were examined daily at 8.30 a.m. for the development of clinical symptoms. Typically, hindquarter paralysis developed 12 to 14 days after induction, followed by partly recovery (day 17). Symptoms were blind-scored on a scale ranging from 0 to 5: 0, no signs; 0.5: partial loss of tail tonus (defined as the disease onset); 1.0: complete loss of tail tonus; 2.0: hind limb paresis; 3.0: hind limb paralysis; 4.0: moribund; 5.0: death due to EAE [33]. Disease peak was defined as the highest clinical score of each animal. The clinical endpoints were based on the clinical scores. If an animal exceeded a score of 4, suffering complete paralysis of hind limbs and midriff the animal was excluded and humanely euthanized. If an animal was not able to eat or drink independently, the animal was excluded and euthanized as well. Overall hindquarter paralysis was expressed as the average of the daily scores per group.

5. Secondary outcome measures

Isokinetic foot extensor performance

After general anaesthesia, left hind limb foot extensor muscle strength was assessed during fatiguing isokinetic muscle contractions, as described elsewhere [221, 222]. Briefly, percutaneous needle electrodes were placed on the common peroneal nerve fusing 130 consecutive concentric isokinetic foot extensions (50°/s, 1 mA, 250ms, 3s rest intervals) after standardized fixation of knee and ankle on a custom build Ashton-Miller like rat dynamometer [223]. Muscle performance of all animals is reported as muscle work (mJ).

BDNF measurement

Serum BDNF concentrations were blinded determined by electrochemoluminiscence ELISA using Meso Scale Discovery (MSD) plates spotted with BDNF-specific capture antibodies according to manufacturer's instructions (Meso Scale Discovery, USA). Briefly, after blocking of the plate, rats' sera and calibrators were dispensed into the MSD plate and incubated for 2h at room temperature. The plates were subsequently washed to remove unbound material and a BDNF-specific MSD SULFOTAG-labelled detection antibody was dispensed into the MSD plate. Following a 2h incubation step 2X read buffer T was added and plates were measured on the SECTOR® Imager.

Body weight & food intake

Daily body weight and food intake was registered using a digital balance (Sartorius®, Germany) at 8 a.m.

6. Statistical analysis

All data were analysed using either SAS software (SAS Institute Inc, Cary, USA) or R 2.15.2 software [242]. Additive and linear mixed models and unpaired student t-tests were used to analyse data. For the unpaired student t-tests, normality was checked using the Shapiro-Wilk test. For the generalized linear mixed model, diagnostics were based on the studentized residuals.

In particular, *body weight and food intake* were analysed by a (Group [CON; EAE] x Activity [SED; EX] x Time) mixed model ANOVA. To analyse the *course of hindquarter paralysis*, a (EAE Group [SED; EX] x Time [Days 0-17]) mixed model ANOVA was used. To analyse disease onset, which was defined as a hindquarter paralysis score equal to 0.5, overall symptom intensity was compared between groups by an unpaired student's t-test. *Muscle fiber type area and proportion* were analysed using a 2x2 (Group [CON; EAE] x Activity [SED; EX]) ANOVA. *BDNF profiles* were analysed by a (Group [CON; EAE] x Activity [SED; EX] x Time) mixed model ANOVA. *Muscle fatigue of isokinetic foot extensor data* was analysed using a (Group [CON; EAE] x Activity [SED; EX] x Contraction [number of dynamic muscle contractions]) mixed model ANOVA. Furthermore, (generalized) additive mixed models were fitted using thin-plate regression splines to model the contraction effect and interactions with both the disease status and whether or not an intervention took place. Testing effects, starting with the interaction effect, was performed using approximate F-tests. Correlations were analysed by means of Pearson's correlation analysis. All data are presented as mean ± SE and the threshold for statistical significance was set at $p<0.05$.

Results

1. Primary outcome measures

Muscle fiber cross sectional area and proportion

Muscle fiber CSA of the different muscles is represented in Figure 2.5.2 and 2.5.3. Compared to CON^{SED}, EAE reduced CSA of type I, IIa and IIx+b fibers of all muscles in both experiments. In experiment 1, exercise did not affect CSA of the different fiber types of CON^{EX}, compared to CON^{SED}, except for type IIa muscle fiber CSA of SOL. Furthermore, in EAE^{EX}, compared to EAE^{SED}, exercise was able to increase fiber type IIa and IIx+b CSA in SOL and tended to increase mean CSA and muscle fiber type IIx+b CSA of TA ($p<0.1$). In experiment 2, exercise did not affect CSA of the different fiber types of CON^{EX}, compared to CON^{SED}, except for type IIa and IIx+b muscle fiber CSA of SOL. Furthermore, in EAE^{EX}, compared to EAE^{SED}, exercise increased mean CSA and muscle fiber type IIx+b CSA of TA, whereas other muscle fiber types remained unaffected by exercise.

Muscle fiber proportions of the different muscles are represented in Figure 2.5.4. Compared to CON^{SED}, EAE decreased the proportion of EDL,TA and SOL type I fibers and increased the proportion of type IIx+b of SOL in experiment 2, whereas during experiment 1 muscle fiber distribution remained unaffected by EAE. Furthermore, exercise did not affect the proportion of the different fiber types in experiment 1, except for type I distribution of SOL in CON^{EX}, which increased, compared to CON^{SED}. In experiment 2 exercise did not affect the proportion of the different fiber types in CON^{EX}. In EAE^{EX} on the other hand, compared to EAE^{SED}, exercise increased the proportion of EDL and TA type I and IIa fibers, whereas the proportion of type IIx+b fibers decreased after treadmill running in EDL, TA and SOL.

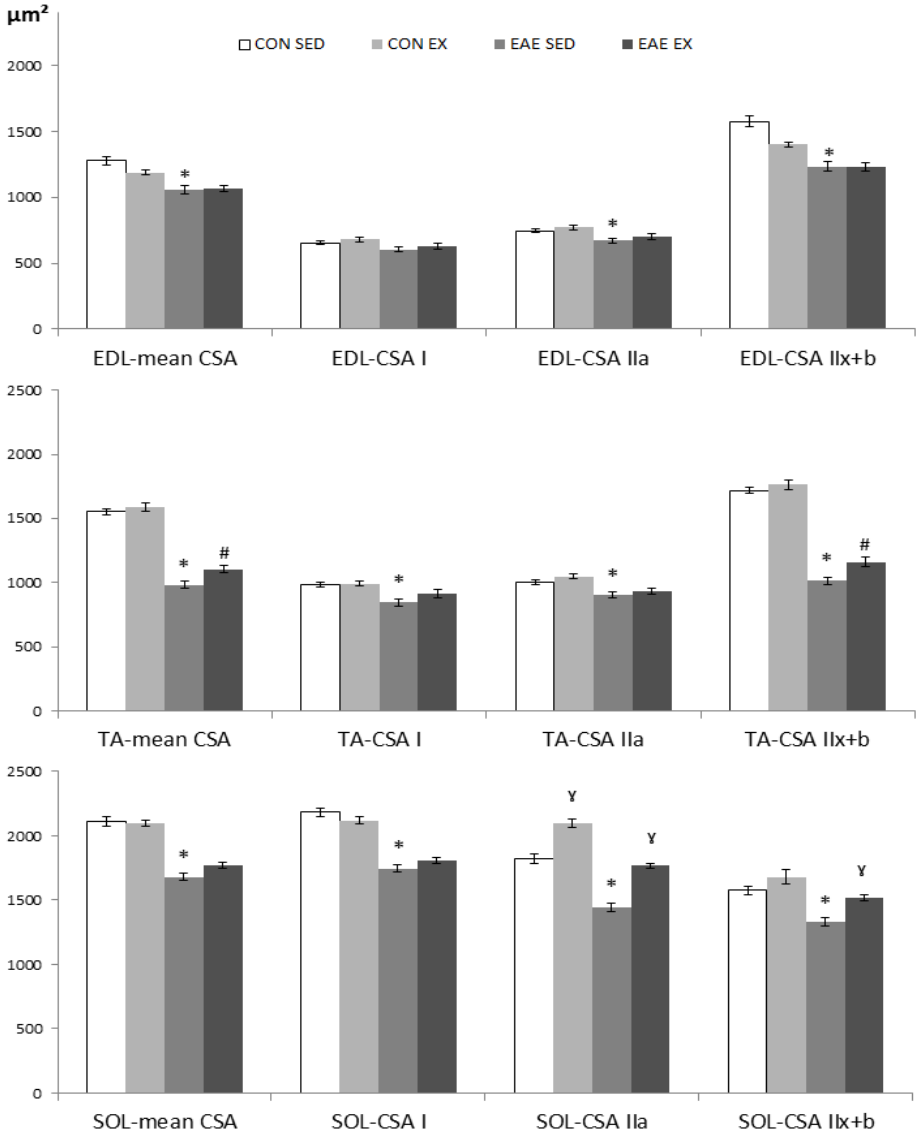

Figure 2.5.2: *Effect of EAE and treadmill exercises on cross sectional area (CSA) of Extensor Digitorum Longus (EDL), Tibialis Anterior (TA) and Soleus (SOL), during experiment 1, comprising 10 days of exercise.*
Data are presented as mean ± SE.
** = disease effect, comparison between CON^{SED} and EAE^{SED}*
ɣ = intervention effect, compared to corresponding SED group
 # = p ≥ 0.05, but < 0.1, intervention effect, compared to corresponding SED group

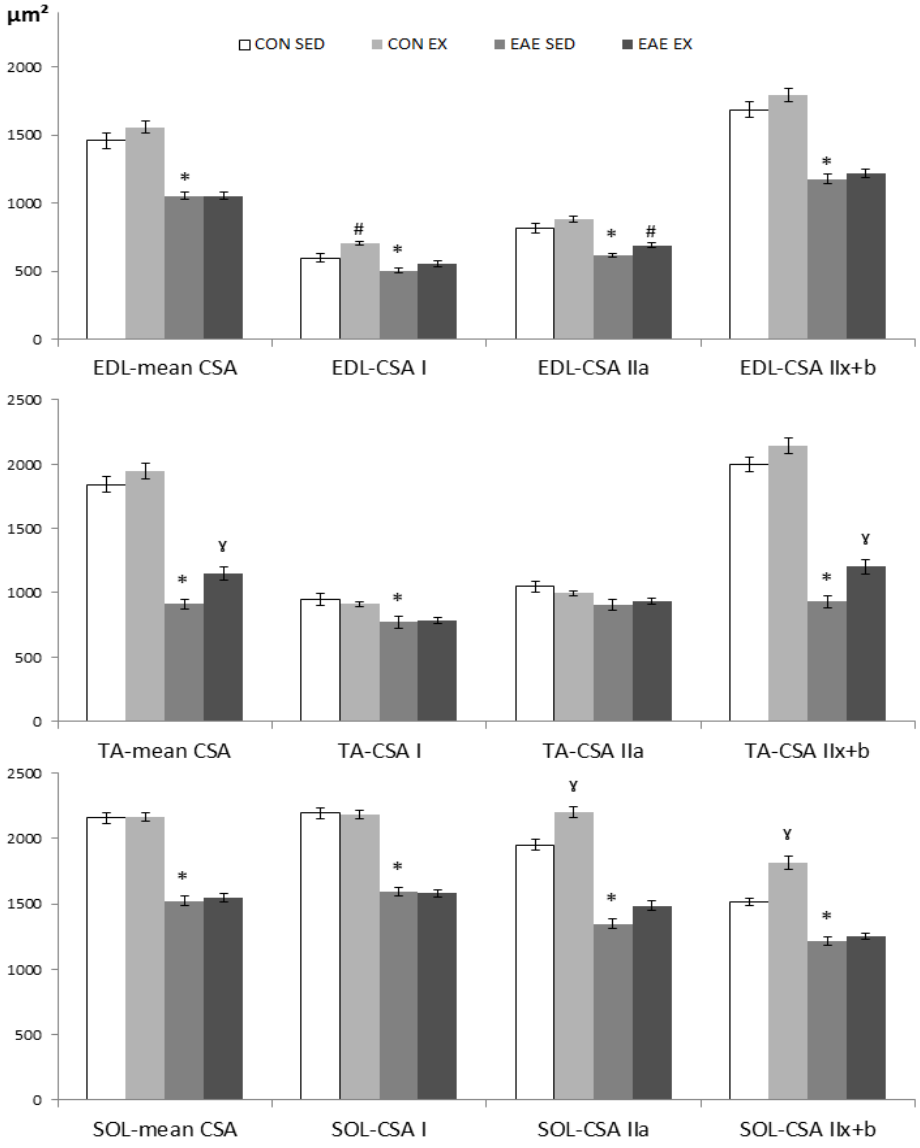

Figure 2.5.3: *Effect of EAE and treadmill exercises on cross sectional area (CSA) of Extensor Digitorum Longus (EDL), Tibialis Anterior (TA) and Soleus (SOL), during experiment 2, comprising of 10 days of exercise and 7 sedentary days.*
Data are presented as mean ± SE.
** = disease effect, comparison between CON^{SED} and EAE^{SED}*
ɣ = intervention effect, compared to corresponding SED group
 # = p ≥ 0.05, but < 0.1, intervention effect, compared to corresponding SED group

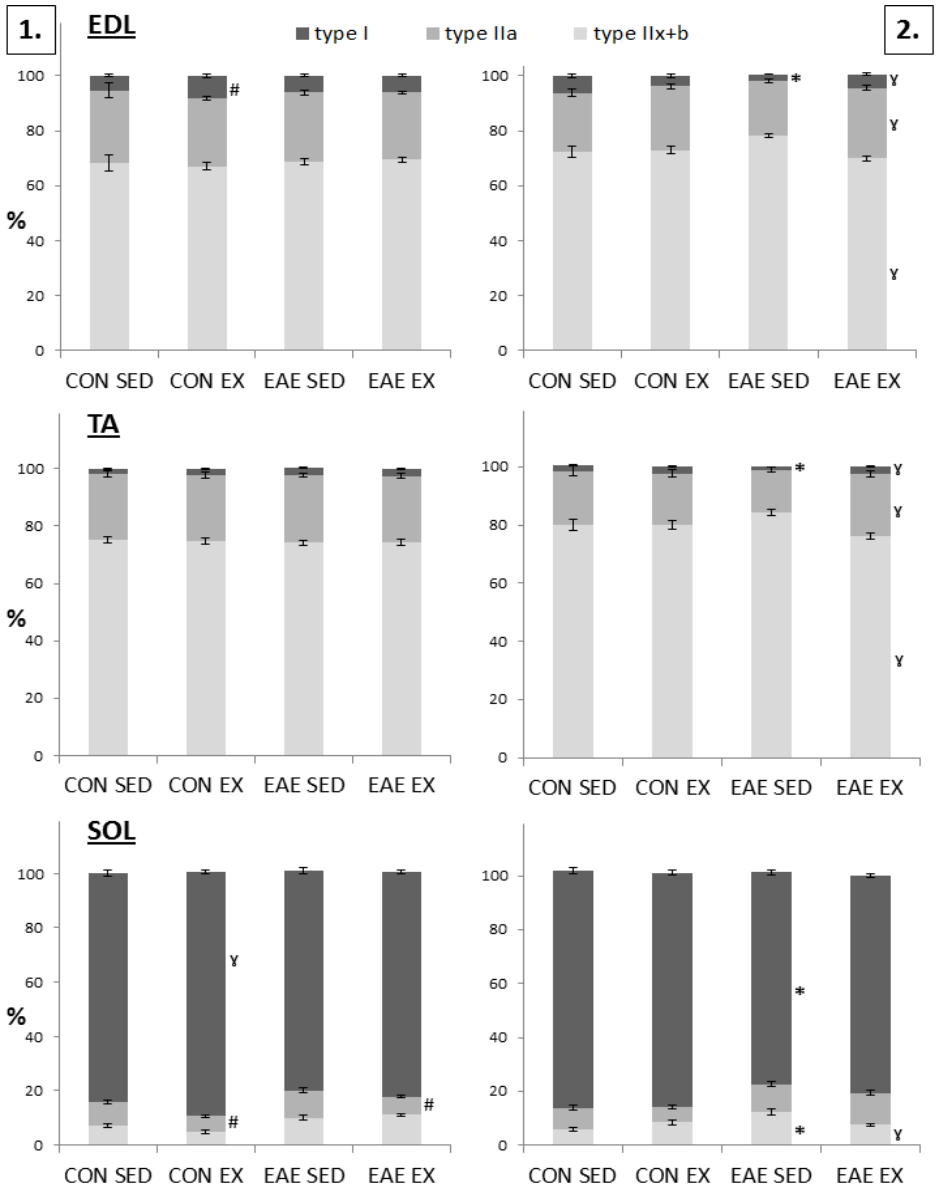

Figure 2.5.4: *Effect of EAE and treadmill exercises on muscle fiber proportion of Extensor Digitorum Longus (EDL), Tibialis Anterior (TA) and Soleus (SOL), during experiment 1 and 2. Data are presented as mean ± SE.*
** = disease effect, comparison between CON^{SED} and EAE^{SED}*
ɣ = intervention effect, compared to corresponding SED group
 # = p ≥ 0.05, but < 0.1, intervention effect, compared to corresponding SED group

Hindquarter paralysis (experiment 2)

Overall, the hindquarter paralysis curve of EAE^{SED} and EAE^{EX} tended to differ over time (intervention x time, p=0.07). Moreover, exercise delayed the occurrence of the disease peak of EAE^{EX}, compared to EAE^{SED}, by 1.0 ± 0.3 days (p<0.05), whereas the severity of the disease peak (2.4 ± 0.3) was comparable between both groups. Furthermore, compared to EAE^{SED}, disease onset tended to be delayed by 0.7 ± 0.4 day in EAE^{EX} (p=0.1, Table 2.5.1). Finally, no animals reached the human endpoint (score > 4 or inability to eat or drink independently).

Table 2.5.1: *Overview of disease onset and peak of EAE^{SED} and EAE^{EX} during experiment 2. Data are reported as mean ± SE.*

	EAE^{SED}	**EAE^{EX}**	**difference**	**p-value**
Disease onset				
Day	11.2 ± 0.3	11.9 ± 0.4	0.7 ± 0.4	0.1
Score	0.5	0.5	/	NS
Disease peak				
Day	13 ± 0.3	14 ± 0.3	1.0 ± 0.3	0.01
Score	2.38 ± 0.2	2.38 ± 0.3	/	NS

2. Secondary outcome measures

Isokinetic muscle strength

In both experiments muscle work of CON peaked during the first 30 contractions, then declined and remained stable throughout the remaining contractions. In EAE, muscle work did not peak but was stable from onset (disease x contraction, p<0.05, Figure 2.5.5).

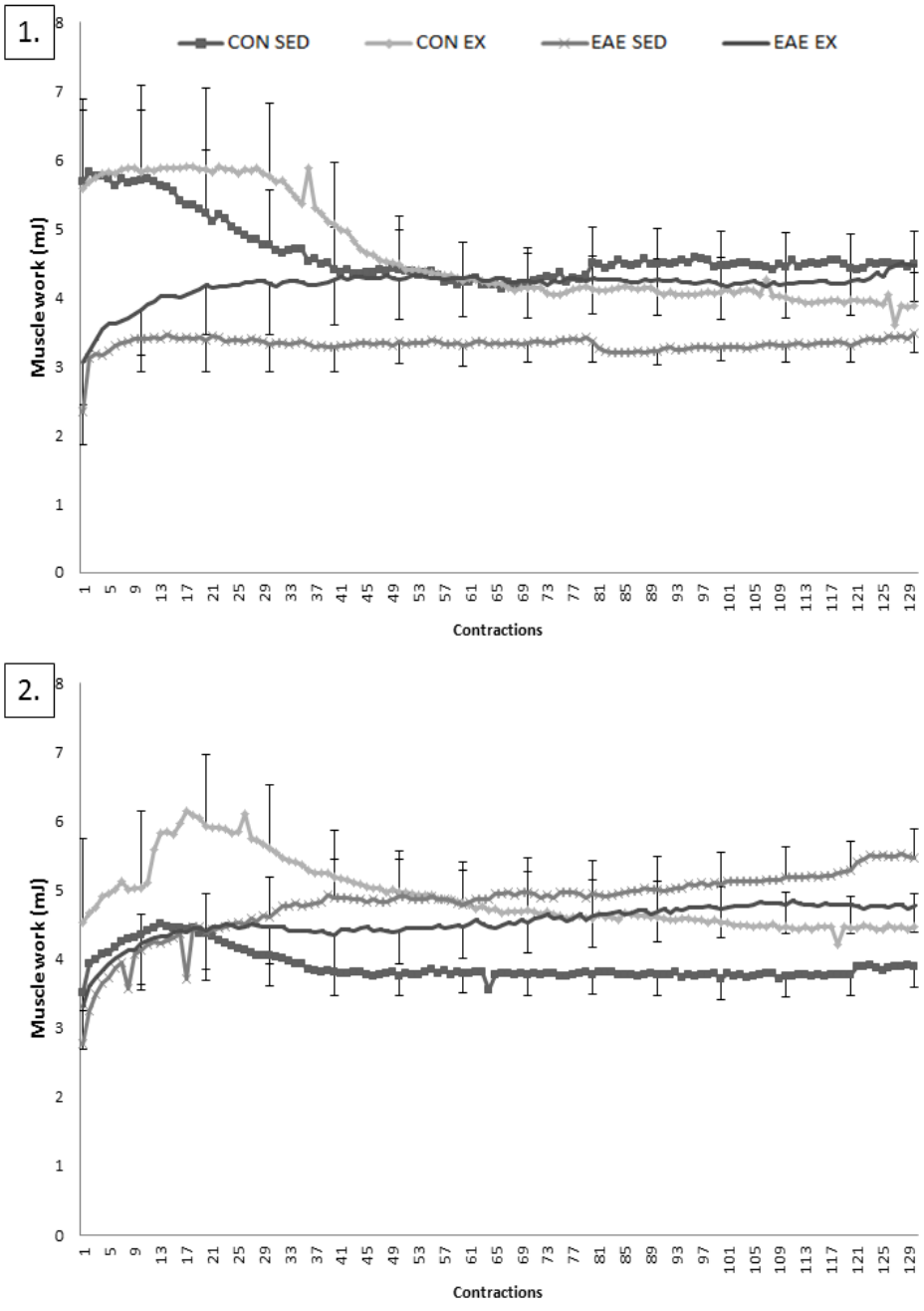

Figure 2.5.5: *Isokinetic muscle work in healthy and EAE rats, during experiment 1 and 2.*
Values are mean ± SE and express muscle work during 130 consecutive maximal muscle contractions (1mA, 150Hz, 250ms) in CON and EAE rats.

BDNF response

During both experiments, BDNF concentrations of all groups increased over time ($p<0.05$). In particular, BDNF concentrations of all animals remained stable during the first 9 days, where after it increased during both experiments (Table 2.5.2).

Table 2.5.2: *Overview of BNDF concentrations (pg/ml), in CON and EAE animals, during experiment 1 and 2.*

BDNF conc (pg/ml)	CON^{SED}	CON^{EX}	EAE^{SED}	EAE^{EX}
Experiment 1				
Day 0	1906 ± 88	1740 ± 66	1918 ± 86	1753 ± 45
Day 9	1843 ± 78	1683 ± 89	1593 ± 67	1619 ± 86
Day 11	2573 ± 111*	2213 ± 76*	2219 ± 89*	2031 ± 79*
Experiment 2				
Day 0	1468 ± 220	1717 ± 202	1785 ± 227	1597 ± 227
Day 9	1880 ± 126	1775 ± 112	1790 ± 70	1748 ± 104
Day 13	2468 ± 265	2379 ± 194	2428 ± 165	2394 ± 239
Day 17	2508 ± 235*	2696 ± 277*	2484 ± 147*	2603 ± 225*

*Data are presented as mean ± SE. * = within group effect*

Body weight and food intake

Body weight and food intake decreased immediately after EAE induction in both experiments, on average ~10% and ~65%, respectively, ($p<0.05$). Hereafter, body weight and food intake recovered gradually until the onset of hindquarter paralysis on day 11. During hindquarter paralysis (experiment 2), body weight and food intake of EAE groups decreased, on average, ~15% and ~60%, respectively ($p<0.05$). Here, exercise was able to temper reduced food intake ($p<0.05$).

3. Correlations

In EAE groups, mean CSA of TA and SOL were negatively correlated with the disease peak (TA: r=-0.60 and SOL: r=-0.63, $p<0.05$), as well as TA fiber IIx+b CSA, SOL fiber I and IIa CSA (r=-0.58, -0.64, -0.51, respectively, $p<0.05$). Furthermore, TA mean CSA and TA fiber type IIx+b CSA were positively correlated with the day of the disease onset (r=0.52 and 0.49, respectively, $p<0.05$). CSA of EDL and BDNF profiles did not correlate with disease onset or disease peak in EAE.

4. Drop out

In total, 1 EAE animal died, unrelated to EAE, during experiment 1 of the study. Overall, there were no significant differences in survival rates between groups.

Discussion

This study investigated the impact of high intensity treadmill exercise on muscle morphology, disease course and BDNF release in EAE animals, immediately before the onset (experiment 1) and after (experiment 2) hindquarter paralysis. During both experiments, EAE reduced mean fiber CSA, as well as type I, IIa and IIx+b fiber CSA in TA, EDL and SOL. Under the conditions of the present study exercise was, in general, not able to prevent muscle fiber atrophy in both experiments, with the exception of some minor changes in CON^{EX} and EAE^{EX}. Muscle work of CON peaked during the first 30 contractions and then progressively declined during the remaining contractions, while muscle work of EAE remained stable. High intensity exercise delayed hindquarter paralysis peak. Finally, the present study indicated that the BDNF response was not affected by EAE or exercise.

EAE and hindquarter paralysis

The applied high intensity aerobic exercise was able to delay hindquarter paralysis peak and tended to delay disease onset. This was mirrored by a reduced food intake drop during paralysis. These findings confirm our previously reported results in EAE animals [236]. Also, other authors reported delayed onset of clinical EAE symptoms [136-138]. However, it remains difficult to compare studies due to the application of different exercise protocols in terms of exercise intensity, volume and type (voluntary exercise versus quantified training load and intensity). Our applied training intensity was estimated near the anaerobic threshold [219, 220], which is probably higher, compared to the work of Le Page [136, 137] and Rossi [138]. However, lactate concentrations and VO_2 kinetics were not measured. As such, our present and former findings and the above-described work of others suggest that exercise intensity is the key determinant of the impact of exercise training on the course of EAE. Moreover, it would be interesting to investigate this hypothesis in MS patients.

Muscle morphology and exercise in EAE

The present study showed that CSA of all muscle fiber types in EDL, TA and SOL decreased due to EAE. We [236-238] and De Haan and co-workers, who investigated the impact of EAE on the medial Gastrocnemius [135], previously reported similar results.

It is clear that treadmill running is able to reverse muscle fiber atrophy in other rat populations [226-228]. In the present study, however, exercise was, in general, not able to affect CSA nor fiber proportion significantly, with some minor exceptions in, mainly, SOL. Interestingly, it was expected that an exercise effect in experiment 1 would be seen by excluding the tempering

effect of the sedentary week of experiment 2. However, the expected exercise effect was absent in all CON and EAE groups, suggesting that the applied exercise duration was too short to induce the hypothesized muscle fiber alterations. Furthermore, this may imply that aerobic exercise is not optimal to induce adaptations in muscle fiber CSA and proportion in EAE rats. Therefore it is suggested to investigate the influence of resistance training on muscle fiber characteristics, since EAE is able to reduce type IIx+b fiber CSA, which (in humans) are more susceptible to resistance training [229-231].

Muscle work and exercise in EAE

Muscle work of CON animals peaked, during the first 30 contractions and then progressively declined, whereas muscle work of EAE remained stable. These findings confirm our previously reported results in EAE and CON animals [237]. However, these results contradicts data reported by De Haan and co-workers [135], who reported that muscle work of both CON and EAE peaked and then declined during a series of repeated isometric maximal contractions of m. Gastrocnemius. The difference can, possibly, be explained by the fact that different muscle groups were analysed. Moreover, TA and EDL having predominately glycolytic muscle fibers [233] in the present study versus medial Gastrocnemius having a proximal region, containing all muscle fibers, and a distal region, containing only type IIx and IIb fibers [135] in the work performed by De Haan. Because fast glycolytic type IIb muscle fibers largely contribute to peak force production [224, 225] decreased CSA of type IIx+b fibers probably explains the absence of peak muscle work in EAE during the first 30 repetitive isokinetic contractions. As mentioned earlier, it was expected that an exercise-induced effect in experiment 1 would be present by excluding the tempering effect of the sedentary week. Moreover, in experiment 1, muscle work results suggested an exercise-induced effect in both CON and EAE groups. In experiment 2, on the other hand, muscle work curves only suggested an exercise effect in CON. However, statistical significance was not reached in both experiments due to large variations between muscle work of different animals. Therefore, future research should include larger sample sizes to increase power.

BDNF and exercise in EAE

Since BDNF is a neuroprotective mediator during remyelination after a relapse [243, 244] and because it is suggested that BDNF could play an important role in the therapeutic effect of exercise in MS [207, 210, 211, 241], an elevation of the BDNF levels in the exercising rats was expected. However, BDNF profiles did not differ between groups, suggesting that the improved clinical parameters are ameliorated by exercise per se and not by elevated neuroplasticity. These findings are in accordance with recent research,

investigating the effect of a 10 day forced treadmill exercise on neurotrophic factors in EAE and healthy animals [245]. The lack of difference between sedentary and exercised animals can possibly be explained by the short duration of the exercise program in our study and in the study of Patel *et al.* In MS it is already proven that 8 weeks of exercise is not able to change BDNF levels [111, 209]. These findings make it interesting to investigate the effect of a long term exercise program in EAE and MS. Furthermore, contradictory to our hypothesis, BDNF profiles of all groups increased. This could, possibly, be explained by the exposure of stress and elevated levels of stress hormones, since all animals experienced the same level of stress during the study. Research already demonstrated that short periods (15-60 min) of stress could induce an increased BDNF mRNA expression, leading to elevated BDNF protein concentrations and suggesting some degree of neuronal plasticity to deal with new stimuli [246, 247]. However, stress hormone concentrations were not measured during the present study. Therefore, it is recommended to further investigate the influence of stress hormones in the future. Finally, since there are no differences between CON and EAE, nor between SED and EX BDNF profiles, our results suggest that high intensity aerobic exercise does not worsen the disease, indicating that high intensity aerobic exercise is tolerable in EAE.

Limitations and future directions

The present study had some limitations, resulting in a few recommendations for future research. The lack of an explicit exercise effect, could, possibly, be explained by the short duration of the applied exercise program. Furthermore, aerobic exercise was perhaps not the optimal exercise modality to induce improvements of the investigated parameters in EAE rats. Therefore, it is suggested to investigate the long term effect of physical exercise in chronic EAE and the influence of resistance training on muscle contractile properties and BDNF release in EAE rats.

Furthermore, since the optimal exercise intensity is not known yet, it is recommended to quantify exercise intensity in future studies, measuring lactate concentrations and/or VO_2 kinetics. Next, since forced treadmill running can induce stress, which could influence the EAE symptoms, it is recommended to measure stress hormone levels in future research.

Conclusion

In conclusion, the present study demonstrates that EAE reduces muscle fiber CSA of TA, EDL and SOL, which could in part, explain the absence of peak muscle work during the first 30 contractions of isokinetic muscle performance in EAE animals. Furthermore, exercise increases muscle fiber type IIa and IIx+b CSA in SOL and is able to delay the onset and peak of EAE-induced hindquarter paralysis. Finally, there was no difference between BDNF profiles of CON and EAE, suggesting that high intensity exercise does not worsen the disease.

STUDY 6

The influence of high intensity exercise on glucose tolerance in multiple sclerosis

Based on: Wens I., Dalgas U., Verboven K., Hansen D., Deckx N., Cools N., Eijnde BO.
Metabolism, under review

Abstract

Introduction: The prevalence of impaired glucose tolerance (IGT) is elevated in persons with multiple sclerosis (MS) compared to matched healthy controls. In other populations IGT can, at least partly, be reversed by intense physical exercise, but the influence of intense physical exercise on IGT in MS remains unknown.

Aim: To investigate the effect of high intensity aerobic interval or continuous cardiovascular training, both in combination with resistance training, on glucose tolerance and skeletal muscle GLUT4 content.

Methods: Thirty-four MS patients were randomized into a sedentary control group (SED, n=11) and 2 exercise groups that performed 12 weeks of high intensity interval ($H_{IT}R$, n=12) or high intensity continuous aerobic training ($H_{CT}R$, n=11), both in combination with resistance training. Before and after 12 weeks of training, glucose tolerance and skeletal muscle GLUT4 content were determined by an oral glucose tolerance test (OGTT) and harvesting of a biopsy from m. Vastus Lateralis, respectively.

Results: All outcome measures remained stable in SED. Within $H_{IT}R$ (-7 ± 2%) and $H_{CT}R$ (-11 ± 3.5%) the total area under the glucose curve (tAUC) significantly decreased. Insulin tAUC decreased (-12 ± 14%) within $H_{CT}R$ and muscle GLUT4 content increased (+7 ± 2%) in $H_{IT}R$.

Conclusion: Twelve weeks of high intensity aerobic exercise in combination with resistance training was well tolerated and induced changes in glucose and insulin concentrations, as well as in muscle GLUT4 content.

Introduction

The heterogeneous symptoms of multiple sclerosis (MS) often lead to a more sedentary lifestyle [7]. In healthy people, physical inactivity can contribute to the development of secondary health problems, such as cardiovascular diseases, obesity and diabetes type II, preceded by impaired glucose tolerance (IGT) [101]. Recently, we reported an elevated prevalence of IGT in MS, when compared to matched healthy subjects [204]. In other populations, these secondary health complications can, partly, be reversed by physical exercise, which is often used as the primary treatment strategy [101-105]. The impact of physical exercise on IGT in MS, however, remains scarce and conflicting.

One previous study from our group showed unchanged glucose and insulin profiles in MS patients after 24 weeks of moderate intensity combined (aerobic and resistance) exercise [248] (Wens *et al.*, under review). However, in other populations, some studies reported that low-to-moderate exercise intensity improved glucose tolerance [156, 249], whereas other findings suggested that this level of intensity was not sufficient to improve insulin sensitivity [250, 251]. Interestingly, Sandvei *et al.* [157] reported improved glucose profiles after 8 weeks of sprint interval training in young healthy subjects, whereas glucose responses remained unchanged after 8 weeks of continuous running (70-80% of maximal heart rate). Fasting glucose concentrations were, however, significantly reduced in both groups. Similar findings were reported by Tjonna and co-workers [252] who showed a significant improvement in fasting blood glucose and insulin sensitivity after 16 weeks of aerobic interval training (90% of maximal heart rate) in metabolic syndrome patients, whereas no improvements were found after moderate continuous exercise (70% of maximal heart rate) [252]. These findings suggest that high intensity interval exercise might be more effective to improve glucose tolerance than continuous moderate intensity training.

Skeletal muscle membrane glucose transport is almost exclusively mediated by GLUT4 glucose transporter translocation following insulin stimulation and/or muscle contractions [253]. Failure of this GLUT4 translocation can induce the development of IGT, insulin resistance and type II diabetes [254]. In other populations, physical exercise has been shown to increase GLUT4 content [88, 255, 256], which could be associated with changes in insulin stimulated glucose uptake [257, 258]. However, skeletal muscle GLUT4 content as well as the effects of exercise on GLUT 4, have currently not been investigated in persons with MS.

Accordingly, the present study aimed to investigate, the effect of a high intensity interval and continuous cardiovascular training, both in combination with resistance training, on glucose tolerance and skeletal muscle GLUT4 content. We hypothesized that high intensity exercise improves glucose tolerance and increases muscle GLUT4 content in persons with MS.

Methods

1. Subjects

Thirty-four MS patients participated in this study. Inclusion criteria on admission were: male and female, older than 18 years and physician-diagnosed MS (EDSS score between 0-6). Subjects were excluded if they had physician-diagnosed diabetes mellitus type II, had other disorders, were pregnant, participated in another study, had contra-indications to perform physical exercise or had an acute MS exacerbation 6 months prior to the start of the study. The study was approved by the ethical committee, performed in accordance with the Declaration of Helsinki and was registered at ClinicalTrials.gov (NCT01845896). Written informed consent to participate in the study was obtained from all participants.

2. Study design

At the start of this randomized controlled trial, all MS patients were randomized into a sedentary control group (SED, n=11) and 2 exercise groups that performed 12 weeks of a high intensity interval training followed by resistance training ($H_{IT}R$, n=12) or intense continuous endurance training followed by resistance training ($H_{CT}R$, n=11). Before and after 12 weeks of exercise, the glucose tolerance (oral glucose tolerance test, OGTT) and muscle GLUT4 content (m. Vastus Lateralis biopsy) were determined. Muscle strength (isokinetic dynamometer, System 3, Biodex, ENRAF-NONIUS, New York, USA), exercise capacity (cycle ergometry, eBike Basic, General Electric GmbH, Bitz, Germany) and body composition (Dual Energy X-ray Absorptiometry scan, Hologic Series Delphi-A Fan Beam X-ray Bone Densitometer, Vilvoorde, Belgium) were also assessed. A detailed description of these methods and results are reported elsewhere (Wens *et al.*, under review). Neither the patients nor the researchers (assessors) involved in the project were blinded to group allocation. SED subjects remained sedentary during the study course and were instructed to continue their current level of physical activity during the period of the study.

3. Exercise training program

After the baseline measurements, the subjects were enrolled in a well-controlled supervised training program. Subjects were instructed to participate in 5 sessions per 2 weeks. To ensure adequate recovery, training sessions were interspersed by at least 48h of rest. All exercises were performed at a high workload corresponding to a perceived exertion rating of 14-16 on the 20-point Borg scale (RPE), and the exercise programs were adjusted to fit the individual disability level. All sessions were ended by stretching of the extremities, and RPE level was recorded.

$H_{IT}R$ program: Each session started with a 5 min warm up on a cycle ergometer. Hereafter, high intensity cycle interval training was performed. During the first 6 weeks exercise duration gradually increased from 5x1 min of maximal exercise interspersed by 1 min rest intervals to 5x2 min and 1 min rest intervals. Exercise intensity was defined as the workload, corresponding to 100% of the maximal heart rate (measured during a maximal endurance test on the ergometer). During the second 6 week training period, duration remained stable at 5x2 min and the workload increased to reach a level corresponding to 100-120% of the maximal heart rate, which was determined by the watt loading. The second part consisted of moderate-to-high intensity resistance training (leg press, leg curl, leg extension, vertical traction, arm curl and chest press, Technogym®). In order to exercise at similar relative workload, resistance training of the lower limbs was performed unilaterally, due to the frequent bilateral strength differences seen between the legs of MS patients [259]. Training intensity and volume were adjusted from 1x10 repetitions to 2x20 repetitions at maximal attainable load.

$H_{CT}R$ program: Each session started with a cardiovascular part, consisting of cycling and treadmill walking/running (Technogym®). Session duration and exercise intensity increased as the intervention progressed, starting from 1x6 min/session to 2x10 min/session, at a workload corresponding to 80-90% of the maximal heart rate. If necessary, adjustments were made according to individual capabilities. The second part of the training session comprised the same resistance training program as described in the $H_{IT}R$ program.

4. Primary outcome measure

Oral glucose tolerance test

Glucose tolerance of MS patients was investigated using an oral glucose tolerance test (OGTT), including determination of blood glucose and serum insulin levels. After a 10h overnight fasting period, all participants received a 1g glucose/kg bodyweight glucose bolus. Capillary blood samples, to immediately measure whole blood glucose concentrations (Analox GM7 Micro-stat, Analox instruments Ltd, London, UK), were collected from a hyperaemic earlobe, before and after glucose administration, and at 20 min intervals during the following 2h period. To determine serum insulin levels, 4 ml of venous blood was collected in serum separation tubes (SST, BD Vacutainer®, Becton-Dickinson, Erembodegem, Belgium) at 1h intervals. After 30 min, allowing blood clotting, samples were centrifuged during 10 min on 3500rpm. Thereafter, the obtained serum was frozen and stored at -80°C until batch analysis of serum insulin levels, according to manufacturer's instructions, was performed (Mercodia Insulin ELISA, Uppsala, Sweden).

Whole-blood glucose concentrations were converted to plasma concentrations using a multiplier of 1.11 as determined previously [201]. Impaired glucose tolerance was defined by the WHO as a fasting plasma glucose concentration of 6.1-6.9mmol/l and 2h post load plasma glucose of 7.8-11.1mmol/l [213]. Glucose and insulin responses were expressed as the total area under the glucose and insulin curves (tAUC), calculated according to the trapezoidal rule.

5. Secondary outcome measure

Muscle GLUT content

To investigate GLUT4 content, muscle biopsies from the middle part of the m. Vastus Lateralis (Bergström needle technique) of the weakest leg (based on isometric muscle strength measurements) were taken by an experienced medical doctor. Following 12 weeks of exercise or usual care, the muscle biopsy was taken 2-3cm proximal to the biopsy taken at baseline. Muscle samples were immediately frozen in isopentane cooled with liquid nitrogen and stored at -80°C, until further analysis using the BlueGene Human Glucose Transporter 4 Elisa kit.

6. Statistical analysis

All data were analysed using SAS 9.2 software (SAS Institute Inc, Cary, USA). First normality was checked using the Shapiro-Wilk test for all variables. Differences between groups (SED, $H_{CT}R$ and $H_{IT}R$) were analysed by a one-way ANOVA, whereas within group differences (post minus pre) were analysed with a paired student's t-test. Relative changes due to the intervention were calculated as the mean of the individual changes and expressed as a percentage. Finally, correlations between changes of the primary and changes of the secondary outcome measures on grouped data from all groups were calculated as Pearson's correlation coefficients. All data are presented as mean ± SE and $p<0.05$ represents the threshold for statistical significance.

Results

1. Baseline subject characteristics and adherence to the intervention

At baseline, no differences in general subject and disease characteristics (Table 2.6.1) as well as outcome measures were found between groups. Approximately 90% of the 30 supervised training sessions were attended in both exercise groups and no severe symptom exacerbations and/or adverse events were seen. No patient drop out was noted.

Furthermore, at baseline, 18% of all MS patients showed elevated fasting glucose concentrations, 9% showed IGT and 6% showed 2h post-load diabetic glucose concentrations.

Table 2.6.1: *Baseline subject and disease characteristics*

	SED (n=11)	**$H_{CT}R$ (n=11)**	**$H_{IT}R$ (n=12)**	**p-value**
Age (y)	47 ± 3	47 ± 3	43 ± 3	NS
Height (m)	1.67 ± 0.02	1.69 ± 0.02	1.70 ± 0.02	NS
Weight (kg)	75.8 ± 3.6	70.2 ± 3.7	76.9 ± 4.1	NS
BMI (kg/m^2)	27.0 ± 1.4	24.4 ± 1.2	26.1 ± 1.14	NS
Gender (m/f)	2/9	5/6	5/7	NS
MS type (RR/CP)	8/3	8/3	10/2	NS
EDSS	2.5 ± 0.3	2.7 ± 0.3	2.3 ± 0.3	NS

Data are presented as mean ± SE.
Abbreviations used: SED, sedentary (usual care); $H_{CT}R$, high intensity continuous exercise + resistance training; $H_{IT}R$, high intensity interval training + resistance training, BMI, body mass index; RR, relapsing remitting; CP, chronic progressive; EDSS, expanded disability status scale

2. Primary outcome measure

Oral glucose tolerance test

After 12 weeks of high intensity exercise, fasting glucose concentrations of $H_{IT}R$ and $H_{CT}R$ significantly decreased, 7 ± 7% and 9 ± 6% respectively ($p<0.05$), while it remained stable in SED. In addition, tAUC tended to differ between the three groups (overall interaction effect, $p=0.07$). In particular, within $H_{IT}R$ and $H_{CT}R$ glucose tAUC significantly decreased, 7 ± 2% and 11 ± 3.5%, respectively ($p<0.05$), whereas it remained stable in SED (Figure 2.6.1).

Furthermore, in none of the groups we observed a change in fasting insulin concentrations following 12 weeks of high intensity exercise or usual care. Interestingly, 1h post-load insulin concentrations significantly decreased in $H_{CT}R$ (71 ± 14mU/l to 47 ± 7mU/l, $p<0.05$), whereas changes in $H_{IT}R$ (80 ± 10mU/l to 63 ± 13mU/l) did not reach statistical significance. These results are mirrored by insulin tAUC (Figure 2.6.2).

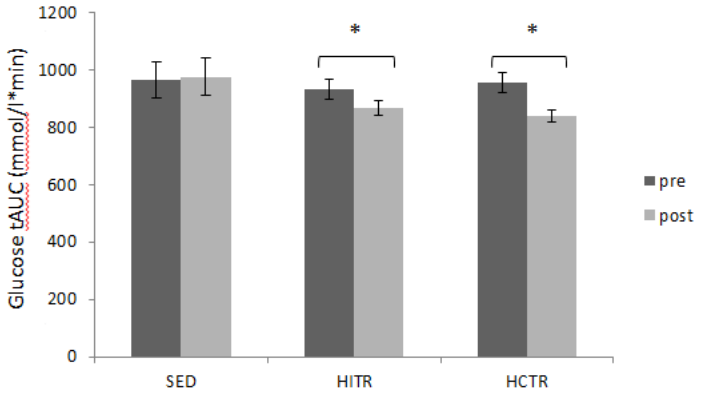

Figure 2.6.1: *Glucose total area under the curve.*
Plasma glucose total area under the curve before (PRE) and after (POST) 12 weeks of sedentary living (usual care, SED), high intensity continuous training + resistance training ($H_{CT}R$) and high intensity interval training + resistance training ($H_{IT}R$).
Data are reported as mean ± SE.
** $p<0.05$, compared with pre-intervention value, within group*

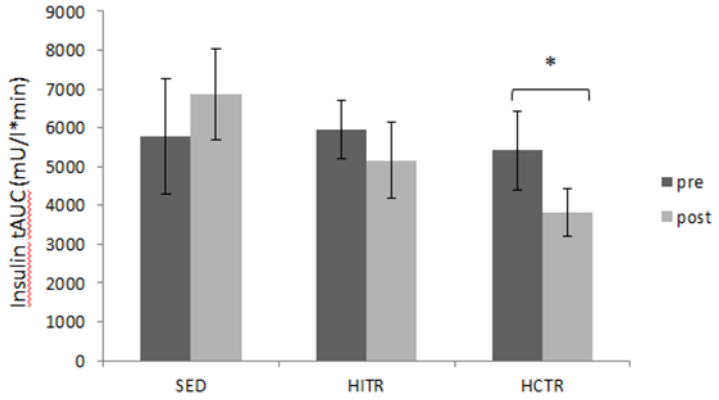

Figure 2.6.2: *Insulin total area under the curve.*
Serum insulin total area under the curve before (PRE) and after (POST) 12 weeks of sedentary living (usual care, SED), high intensity continuous training + resistance training ($H_{CT}R$) and high intensity interval training + resistance training ($H_{IT}R$).
Data are reported as mean ± SE.
** $p<0.05$, compared with pre-intervention value, within group*

3. Secondary outcome measure

Muscle GLUT4 content

In SED GLUT4 content remained stable throughout the study course (Figure 2.6.3). After 12 weeks of high intensity exercise, muscle GLUT4 content significantly increased in $H_{IT}R$ (7 ± 2%, $p<0.05$), whereas increases in $H_{CT}R$ did not reach statistical significance ($p=0.2$).

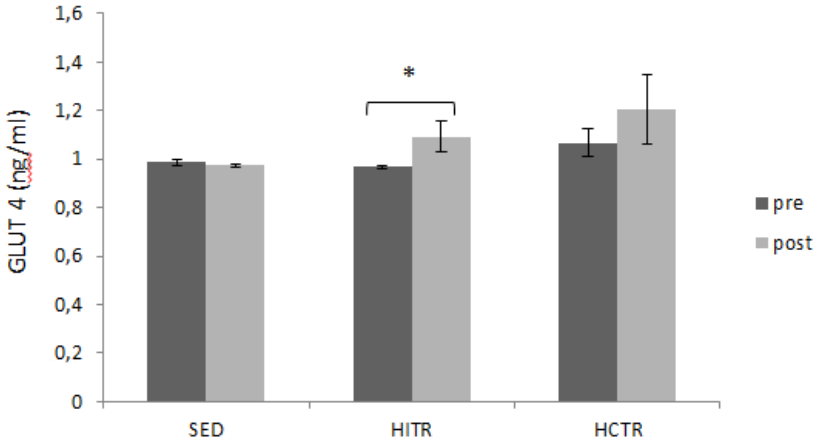

Figure 2.6.3: *Muscle GLUT4 content.*
Muscle biopsy GLUT4 content after 12 weeks of sedentary living (usual care, SED), high intensity continuous training + resistance training ($H_{CT}R$) and high intensity interval training + resistance training ($H_{IT}R$).
Data are reported as mean ± SE.
** p<0.05, compared with pre-intervention value, within group*

4. Correlations

Overall, no significant correlations were found between the change of the primary and secondary outcome measures on pooled data.

Discussion

The present randomized controlled trial investigated the influence of high intensity exercise on glucose tolerance and GLUT4 content in MS patients. Whereas all outcome measures remained stable in SED, glucose tAUC significantly decreased within $H_{IT}R$ and $H_{CT}R$, and insulin tAUC only decreased within $H_{CT}R$. Furthermore, muscle GLUT4 content only increased in $H_{IT}R$, whereas increases in $H_{CT}R$ did not reach statistical significance.

Safety and tolerability

Despite the fact that high intensity training has been shown to substantially increase glucose tolerance in other populations [157, 250-252], its impact on glucose disposal in MS has never been investigated before. The latter could be explained by safety concerns regarding the symptom instability of MS patients often seen during/after high intensity exercise, which is frequently caused by the exercise-induced increase in body temperature [96]. Interestingly, we previously reported positive effects of high intensity training on exercise capacity and muscle strength in MS (Wens et al, under review) with no dropout or adverse events during the study course, demonstrating that mild-to-moderately impaired MS patients tolerate intense exercise programs well. However, the long-term effects of high intensity exercise needs to be further elucidated in future research.

Exercise and IGT

Given the previously observed elevated prevalence of IGT in MS [204] and the inability of mild-to-moderate physical activity to improve the glucose- and insulin profiles (Wens *et al.*, under review), it was hypothesized that high intensity exercise could improve whole body glucose disposal in MS. Under the conditions of the present study both $H_{IT}R$ and $H_{CT}R$ improved glucose tAUC, whereas insulin tAUC was only decreased following $H_{CT}R$. Furthermore, muscle GLUT4 content increased in $H_{IT}R$ only. In general, these results are comparable with data reported in literature from other populations demonstrating that exercise results in enhanced insulin- and contraction-stimulated glucose transport capacity, mirrored by an exercise-induced increase in skeletal muscle GLUT4 content [88, 256, 260-262]. This indicates that high intensity exercise is probably more effective to improve glucose tolerance than moderate intensity training [156-157], as applied in our former research[248] (Wens et al, under review) and the work of others [71, 143] and suggests that the effect of exercise on health related parameters such as glucose tolerance in MS is intensity related. The insulin tAUC and GLUT4 changes who did not reach statistical significance in the present study are difficult to explain. Possibly they reflect the large inter-subject variability in the responses to exercise, as also suggested by others [263].

Continuous vs. interval training

As reported elsewhere (Wens et al, under review) improvements in muscle strength, endurance capacity and body composition were higher following $H_{IT}R$ compared to $H_{CT}R$. Interestingly, decreased glucose profiles did not differ between $H_{IT}R$ and $H_{CT}R$.

Limitations

Because this study is the first to investigate the effect of high intensity exercise on IGT in MS, we were not able to perform a pre-trial power analysis, due to the absence of a defined effect size. Interestingly, even though we did not include only persons with IGT, we were able to detect some general improvements on glucose tolerance. However, since some of the changes did not reach statistical significance and due to the potential presence of a large inter-subject variability in the response to exercise, future studies, taking into account inclusion of a larger cohort of MS patients, as well as more stringent inclusion criteria, are warranted.

Conclusion

The present study indicates that 12 weeks of high intensity cardiovascular exercise in combination with resistance training is able to induce changes in blood glucose and insulin profiles as well as in muscle GLUT4 content.

Acknowledgements

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STUDY 7

Does multiple sclerosis affect skeletal muscle characteristics?

Based on: Wens I., Dalgas U., Vandenabeele F., Grevendonk L., Eijnde BO.
Plos One, under review

Abstract

Background: The impact of multiple sclerosis (MS) on skeletal muscle characteristics, such as muscle fiber cross sectional area (CSA), fiber type proportion, muscle strength and whole muscle mass, remains conflicting.

Methods: In this cross sectional study, body composition, muscle strength of the quadriceps and self-reported physical activity levels were assessed in 34 MS (EDSS: 2.5 ± 0.19) patients and 18 matched healthy controls (HC). Hereafter a muscle biopsy (m. Vastus Lateralis) was taken.

Results: Compared to HC, mean muscle fiber CSA of all fibers, as well as CSA of type I, II and IIa fibers were smaller and muscle strength of the quadriceps was lower in MS patients. Whole body composition and physical activity levels were comparable between groups. However, compared to HC, the biopsied leg tended to have a higher fat percentage ($p=0.1$) and a lower lean body mass ($p=0.06$) in MS patients.

Conclusion: MS seems to negatively influence skeletal muscle fiber CSA, muscle strength and muscle mass of the lower limbs of mildly affected MS patients. This emphasises the need for rehabilitations programs focusing on muscle preservation of the lower limb.

Introduction

Multiple sclerosis (MS) is characterized by complex and heterogeneous symptoms, often leading to reduced quality of life [264] and impaired functional capacity [53]. The latter is related to reduced muscle strength of predominately the lower limbs [265, 266]. The mechanisms underlying the observed strength deficits are of muscular [47, 49, 50] as well as neural origin [45, 46].

At the whole muscle level a number of studies have examined skeletal muscle characteristics of MS patients, with some studies [49, 50, 75], but not all [45, 47, 76], reporting loss of muscle mass and decreased [43, 45-47, 49, 77] or comparable [50] maximal muscle strength. Neurologically, reduced motor unit recruitment and firing rates are reported [45]. At present it remains unknown whether the reported observations are consequences of the disease *per se*, are caused by inactivity or are affected by a combination of both.

At the cellular level the impact of MS on muscle fiber cross sectional area (CSA) and muscle fiber proportion remains conflicting. On the one hand two small studies (n=9 [50] and n=6 [49]) have reported reduced muscle fiber size in MS patients compared to healthy controls (HC) [49, 50], while one study indicated alterations, but did not include a direct comparison to HC [81]. On the other hand two small studies (n=7 [47] and n=16 [45]) did not report any alterations [45, 47], clearly suggesting a need for well-powered studies to clarify the muscular influence of MS. Furthermore, corresponding to immobilized HC [82-84], a shift from type I to type IIa and IIax fibers was reported in a small group of MS patients by Kent-Braun *et al.* [50], but this was not confirmed by others [45, 47, 49].

To clarify the heterogeneous results of the existing literature in small groups of MS patients, the present cross sectional study aimed to investigate the effect of MS on muscle fiber CSA and proportion, muscle strength, body composition and self-reported activity levels in a larger group of MS patients and compared with HC. It was hypothesized that MS would negatively affect skeletal muscle characteristics.

Methods

1. Subjects

Thirty-four MS patients diagnosed according to the McDonald criteria (EDSS range 0-6) and 18 matched healthy controls (HC), aged >18 years, were included following written informed consent, providing a ~2:1 match for gender, age and body mass index (BMI). Subjects were excluded if they had other disorders (cancer, cardiovascular, pulmonary and/or renal diseases), were pregnant, participated in another study and, in case of MS patients, have had an acute MS exacerbation 6 months prior to the start of the study. The study was approved by the ethical committee and was registered at ClinicalTrials.gov (NCT01845896). All tests were performed in accordance with the Declaration of Helsinki.

2. Primary outcome measure

Skeletal muscle fiber cross sectional area and fiber type proportion

Muscle biopsies were obtained from MS patients and HC from the middle part of the m. Vastus Lateralis (Bergström needle technique), by an experienced medical doctor. Because we aimed to evaluate the impact of MS on muscle fiber characteristics, the muscle biopsies of the MS patients were obtained from the weakest leg, as assessed by a preceding isometric muscle strength test performed on an isokinetic dynamometer (System 3, Biodex, ENRAF-NONIUS, New York, USA). The biopsied leg of HC was randomized. The collected tissue was freed from connective tissue and immediately embedded in Tissue-Tek, frozen in isopentane cooled with liquid nitrogen and stored at -80°C, until further analysis was performed.

Serial transverse sections (9µm) from the obtained muscle samples were cut at -20°C and stained by means of ATPase histochemistry, after preincubation at pH 4.4, 4.6 and 10.3, essentially following the procedure of Brooke and Kaiser [86]. The serial sections were visualized and analysed using a Leica DM2000 microscope (Leica, Stockholm, Sweden) and a Leica Hi-resolution Color DFC camera (Leica, Stockholm, Sweden) combined with image-analysis software (Leica Qwin ver. 3, Leica, Stockholm, Sweden). This software was able to automatically draw a fiber mask at the stained sections. Afterwards, this mask was fitted manually to the cell borders of the selected fibers. Only fibers cut perpendicularly to their longitudinal axis were used for the determination of fiber size. On average 170 ± 10 fibers were calculated and included in the CSA and fiber type analyses.

Calculation of the fiber CSA was performed for the major fiber types (I, II, IIa and IIx) and for the mean fiber CSA, since the number of fibers

expressing the minor fiber types (IIax and IIc) was too small for statistical comparison and CSA calculation.

3. Secondary outcome measures

Approximately 1 to 2 weeks before the muscle biopsy was performed, body composition, isometric muscle strength of the quadriceps and self-reported physical activity level were assessed from all subjects.

Body composition

A Dual Energy X-ray Absorptiometry scan (GE Hologic Series Delphi-A, Vilvoorde, Belgium) was performed. Fat and lean tissue mass were obtained for the whole body as well as for different regions covering the legs, the trunk, the gynoid and the android region. Waist-to-hip fat mass ratio (android fat (g)/gynoid fat (g) ratio) and fat mass of the trunk/fat mass of the limbs ratio were calculated.

Isometric muscle strength of the quadriceps

Following 5 min of warming-up on a cycle ergometer and after habitation, the maximal voluntary isometric muscle strength of the knee extensors (45° and 90° knee angle) were measured, as reported elsewhere [139], using an isokinetic dynamometer (System 3, Biodex, ENRAF-NONIUS, New York, USA) in all MS patients and 50% of HC. Briefly, two maximal isometric extensions (4s), separated by a 30s rest interval, were performed. The highest isometric extension peak torque (Nm) was selected as the maximal isometric strength. Because we aimed to evaluate the impact of MS on muscle strength, the muscle strength of the weakest, biopsied, leg of MS patients was reported, whereas the tested leg of HC was randomized. Muscle strength of the quadriceps was reported as the mean of the knee extensions at 45° and 90°.

Physical activity level

Self-reported physical activity level was measured using the Physical Activity Scale for Individuals with Physical Disabilities (PASIPD) [267].

4. Statistical analysis

All data were analysed using SAS 9.2 software (SAS Institute Inc, Cary, USA). First normality was checked using the Shapiro-Wilk test for all variables. Differences between MS patients and HC were analysed by unpaired t-tests. Correlations were analysed by means of Pearson's correlation analysis. All data are presented as mean ± SE and $p<0.05$ represents the threshold for statistical significance.

Results

1. *Subject characteristics*

No differences in general subject characteristics were found between MS patients and HC (Table 2.7.1).

Table 2.7.1: *Subject and disease characteristics and overview of secondary outcome measures in MS patients and healthy controls.*
Data are reported as mean ± SE.

	Healthy controls	MS patients	p-value
Number (f/m)	18 (13 / 5)	34 (22 / 12)	NS
Age (y)	47.5 ± 1.9	45.7 ± 1.7	NS
EDSS	/	2.5 ± 0.19	/
Type MS (RR / CP)	/	26 / 8	/
Height (m)	1.71 ± 0.02	1.70 ± 0.01	NS
Total body:			
Total body weight (kg)	73.8 ± 3.2	74.4 ± 2.2	NS
BMI (kg/m^2)	25.0 ± 1.0	25.8 ± 0.7	NS
Total fat mass (kg)	26.1 ± 2.5	26.4 ± 1.4	NS
Total fat percentage (%)	34.5 ± 2.2	36.1 ± 1.4	NS
Total lean tissue (kg)	47.9 ± 1.9	46.2 ± 1.5	NS
Leg muscle biopsy:			
Leg mass (kg)	12.9 ± 0.6	12.3 ± 0.4	NS
Leg fat mass (kg)	4.7 ± 0.5	4.9 ± 0.3	NS
Leg fat percentage (%)	35.6 ± 2.6	38.9 ± 1.7	0.1
Leg lean tissue (kg)	8.2 ± 0.4	7.4 ± 0.9	0.06
Isometric muscle strength (Nm)	138 ± 8	108 ± 8	0.04
Physical activity (MET*h/week)	23.7 ± 3.6	18.4 ± 2.6	NS

Abbreviations used: MS, multiple sclerosis; m, male; f, female; BMI, body mass index; RR, relapsing remitting; CP, chronic progressive; EDSS, expanded disability status scale; MET, metabolic equivalents; NS, not significant.

2. *Primary outcome measure*

Skeletal muscle fiber cross sectional area and fiber type proportion

Compared to HC, mean muscle fiber CSA, as well as CSA of type I, II, and IIa fibers were significantly smaller in MS patients ($p<0.05$), whereas muscle fiber CSA of type IIx was comparable between both groups. Furthermore, type II fibers experienced a larger atrophy (-20%), compared to type I fibers (-15%) in MS ($p<0.05$). Compared to women, men had a higher CSA for almost all fiber types in both HC and MS. Compared to HC, fiber type I proportion tended to be lower in MS ($p=0.1$), whereas type IIa proportion tended to be higher ($p=0.1$, Table 2.7.2).

Table 2.7.2: *Muscle fiber distribution and cross sectional area (CSA) of MS patients and matched healthy controls.*

	Healthy controls			MS		
	All	Women	Men	All	Women	Men
Fiber type distribution (%)						
Type I	46.1 ± 2.8	44.7 ± 2.1	49.7 ± 9.2	41.6 ± 2.3[b]	45.7 ± 2.6	35.2 ± 3.3[c]
Type IIa	32.6 ± 2.7	29.0 ± 2.2	41.9 ± 6.5[e]	36.4 ± 2.1[b]	34.3 ± 2.6	39.7 ± 3.3
Type IIx	23.2 ± 2.9	27.7 ± 2.9	10.1 ± 3.9[e]	21.8 ± 1.9	20.1 ± 2.6	24.4 ± 2.5
Fiber CSA (μm^2)						
Mean	4621 ± 302	4422 ± 321	5660 ± 474[e]	3827 ± 200[a]	3512 ± 199	4326 ± 354[c]
Type I	4880 ± 313	4682 ± 383	5398 ± 514	4109 ± 223[a]	3912 ± 226	4422 ± 425[d]
Type II	4353 ± 332	3875 ± 315	5595 ± 616[e]	3502 ± 219[a]	3065 ± 193	4192 ± 395[c]
Type IIa	4985 ± 342	4448 ± 313	6380 ± 592[e]	3862 ± 234[a]	3340 ± 193	4689 ± 415[c]
Type IIx	3566 ± 277	3360 ± 327	4181 ± 459[f]	3165 ± 226	2765 ± 221	3766 ± 391[c]

Data are reported as mean ± SE.
[a] $p<0.05$, [b] $p\leq0.1$, compared to healthy controls
[c] $p<0.05$, [d] $p\leq0.1$, compared to female MS patients
[e] $p<0.05$, [f] $p\leq0.1$, compared to female healthy controls

3. Secondary outcome measure

Body composition

Total body composition did not differ between MS patients and HC. In particular, there were no differences between total body weight, adipose and lean tissue mass of MS and HC (Table 2.7.1). However, compared to HC, the lower limb, of which the muscle tissue was collected, tended to have a higher fat percentage ($p=0.1$) and a lower lean body mass ($p=0.06$) in the MS patients.

Isometric muscle strength of the quadriceps

Compared to HC, MS patients showed reduced isometric muscle strength of the quadriceps of the biopsied leg (-22%, $p<0.05$, Table 2.7.1).

Physical activity level

MS patients and HC reported comparable levels of self-reported daily physical activity (Table 2.7.1).

4. Correlations

Mean CSA as well as type I, II, IIa and IIx CSA were highly correlated with muscle strength of the quadriceps (r values between 0.70 and 0.81, $p<0.05$). All muscle fiber types CSA and mean CSA of MS patients and HC correlated positively with total body mass (r values between 0.38 and 0.76, $p<0.05$), total lean body mass (r values between 0.37 and 0.74, $p<0.05$), as well as with the biopsied leg lean tissue (r between 0.42 and 0.75, $p<0.05$). Interestingly, only in MS patients, fiber type II and IIa CSA were negatively correlated with the fat percentage of the biopsied leg ($r=-0.4$ and -0.45, respectively, $p<0.05$).

Furthermore, mean CSA and fiber type II and IIa CSA were negatively correlated to gender in MS and HC, whereas type IIx fiber CSA was only negatively correlated with gender in MS (r values between -0.36 and -0.61, $p<0.05$). Muscle fiber type IIa and IIx CSA were also negatively correlated with age in MS and HC (r values between -0.40 and -0.51, $p<0.05$).

No correlations were detected between muscle fiber CSA and total fat mass, total fat percentage and physical activity level, in both groups. Finally, in case of MS patients, there was no correlation between muscle fiber CSA and EDSS or type of MS.

Discussion

This study compared skeletal muscle characteristics of 34 MS patients and 18 matched HC and indicated quantitative as well as qualitative changes in the skeletal muscle characteristics of mildly affected MS patients. In particular, mean muscle fiber CSA, as well as CSA of type I, II and IIa fibers were significantly smaller in MS patients, independent of MS type, disease severity and physical activity level. Furthermore, MS patients showed reduced leg extensor muscle strength and tended to have a higher leg fat percentage and a lower leg lean tissue mass, compared to HC.

Muscle fiber CSA: In accordance with the present work, Kent-Braun *et al.* and Garner *et al.* reported reduced muscle fiber size in 9 and 7 MS patients, respectively [49, 50]. Furthermore, in male MS patients we found a muscle fiber CSA hierarchy of type I and IIa > IIx, which differed from the hierarchy of CSA type IIa > I and IIx, seen in our healthy men. In female MS patients a muscle fiber CSA hierarchy of type I > IIa > IIx was found, which also differed from the patterns of CSA type I and IIa > IIx, seen in our healthy women. These differences suggest a selective type II(a) atrophy in MS patients, as also indicated by others [49, 50, 81]. Selective type II atrophy is considered to be an effect of ageing [268] or inactivity [269, 270]. Interestingly, in the present study average age and physical activity levels were similar between MS patients and HC, suggesting that the reported observations could be consequences of the disease *per se*.

Contrary to the present study and the work of Kent-Braun *et al.* [50] and Garner *et al.* [49], other studies did not report alterations in muscle fiber CSA in MS patients and HC [45, 47]. These differences could be explained by the small sample sizes and/or the use of other techniques (anterior leg compartment, visualized by magnetic resonance imaging [45]).

Muscle fiber proportion: The present study also showed a tendency towards a higher proportion of type IIa fibers, at the expense of type I fibers, as previously reported in MS patients [50] and in immobilized healthy controls [82-84], suggesting that inactivity also plays a role in MS. Other studies reporting muscle fiber proportions in MS and HC showed inconsistent results [47, 49]. These differences could be related to the use of different muscle tissue (Vastus Lateralis vs. Tabialis Anterior), the different anatomical functions of these muscles and the inclusion of patients with different levels of disability, making it difficult to draw solid conclusions.

Body composition: Body composition, body fat and lean tissue mass in particular, has already been investigated in MS [200]. Similar to our results some studies also reported similar total body fat percentages and total body lean tissue mass in MS patients and HC [73, 75, 172, 175, 176] and slightly higher fat

percentage and lower lean tissue mass in the lower extremities of (female) MS patients [175]. However, the work of others [73, 75, 172, 175, 176] was never correlated to muscle fiber CSA, making it difficult to further compare between studies. In addition, the present study showed that muscle fiber CSA was positively correlated with lean tissue mass and negatively correlated with fat percentage of the biopsied leg, indicating that the preservation of muscle mass of the lower limb of MS patients is very important, already at the early stage of the disease.

Muscle strength: The reduced maximal isometric quadriceps strength of the MS patients was consistent with previous findings [43, 45-47, 49, 77]. Furthermore, muscle fiber CSA was highly correlated with muscle strength of the quadriceps, suggesting that reduced CSA contributes to muscle weakness in MS patients and that changes in skeletal muscle characteristics in MS may affect function.

Physical activity: An association between a reduced physical activity level and mobility disability is commonly accepted in MS [271, 272]. As such, the reported reduced muscle strength and muscle fiber cross sectional areas could be an imported consequence of disuse in MS patients. However, the present study, as well as our former research [204], showed comparable self-reported physical activity levels between mildly affected MS patients and HC. Thus, despite the acknowledgement that self-reported physical activity measures are not perfect measures, the observed reduction in muscle fiber CSA in mildly affected patients is comparable with reductions reported in more disabled MS patients [49, 50]. This may indicate that MS *per se* affects muscle contractile properties.

Limitations: The muscle fiber CSA and proportion are considered to be representative for the whole muscle under investigation. However, it should be kept in mind that the fiber type proportion changes along the length and the depth of the muscle. Therefore, Lexell *et al.* [273, 274] recommended to collect three biopsies from different depths of the muscle and to analyse >150 fibers from each sample to reduce sampling error. Given the ethical concerns we collected only one biopsy and analysed approximately 170 fibers from each sample, being aware of the variation in our study results. Nevertheless, a post hoc power analysis showed that each group should comprise at least 19 subjects, to detect a difference of 20% between mean muscle fiber CSA of HC and MS patients (power=0.8, p=0.05), indicating an appropriate sample size in the present study. Furthermore, given the cross sectional nature of the study, these results do not allow conclusions on causality.

Conclusion

In conclusion, the results of this study suggest that muscle fiber characteristics are altered by MS, irrespective the type or severity of the disease. This emphasises the need for rehabilitation programs focusing on preservation and/or rebuilding of muscle mass of the lower limb, as a means to protect and/or enhance physical function in MS patients.

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STUDY 8

High intensity exercise in multiple sclerosis: effects on muscle contractile characteristics and exercise capacity

Based on: Wens I., Dalgas U., Vandenabeele F., Grevendonk L., Verboven K., Hansen D., Eijnde BO.
Physical Therapy, under review

Abstract

Introduction: Low-to-moderate intensity exercise improves muscle contractile properties and endurance capacity in multiple sclerosis (MS). The impact of high intensity exercise remains unknown.

Methods: Thirty-four MS patients were randomized into a sedentary control group (SED, n=11) and 2 exercise groups that performed 12 weeks of a high intensity interval ($H_{IT}R$, n=12) or high intensity continuous cardiovascular training ($H_{CT}R$, n=11), both in combination with resistance training. M. Vastus Lateralis fiber cross sectional area (CSA) and proportion, knee-flexor/extensor strength, body composition, maximal endurance capacity and self-reported physical activity levels were assessed before and after 12 weeks.

Results: Compared to SED, 12 weeks of high intensity exercise increased mean fiber CSA ($H_{IT}R$:+21 ± 7%, $H_{CT}R$:+23 ± 5%), as well as fiber type I ($H_{IT}R$:+12 ± 9%, $H_{CT}R$:+29 ± 6%), II ($H_{IT}R$:+23 ± 7%, $H_{CT}R$:+21 ± 8%) and IIa ($H_{IT}R$:+23 ± 6%, $H_{CT}R$:+15 ± 5%) CSA. Muscle strength improved in $H_{IT}R$ and $H_{CT}R$ (between +13 ± 7% and +45 ± 20%) and body fat percentage decreased ($H_{IT}R$:-3.9 ± 2.0% and $H_{CT}R$:-2.5 ± 1.2%). Furthermore, endurance capacity (W_{max} +21 ± 4%, time to exhaustion +24 ± 5%, VO_{2max} +17 ± 5%) and lean tissue mass (+1.4 ± 0.5%) only increased in $H_{IT}R$. Finally self-reported physical activity levels increased 73 ± 19% and 86 ± 27% in $H_{CT}R$ and $H_{IT}R$, respectively.

Conclusion: High intensity cardiovascular exercise combined with resistance training was safe, well tolerated and improved muscle contractile characteristics and endurance capacity in MS.

Introduction

The heterogeneous symptoms of multiple sclerosis (MS) often lead to a more sedentary lifestyle [7]. This may result in disuse-related loss of exercise capacity and muscle strength, which in turn can affect quality of life [3]. Increasing evidence favours exercise therapy as a method for overall symptom management [212]. Observational [12, 55] as well as interventional studies [13, 14, 109, 111] have reported improvements in exercise tolerance, muscle strength, functional capacity and health-related quality of life after low-to-moderate intensity cardiovascular or resistance training. Although combined cardiovascular and resistance training could, from a theoretical point of view, positively affect both the cardiovascular system and muscle strength/activation [41], this type of rehabilitation/exercise therapy has not been investigated extensively [154, 155, 218, 248] (Wens *et al.*, under review).

Several authors already suggested that MS patients could benefit more from higher training intensities [41, 100, 119], but so far, no studies on combined exercise have evaluated high intensity training in MS. In healthy controls (HC) and in other populations, high intensity exercise and high intensity interval training (H_{IT}) have previously been investigated, showing profound improvements in endurance performance and muscle strength [275, 276], reduced subcutaneous and abdominal fat [121], improved functional recovery (after stroke) [122] and beneficial effects to the heart [130], emphasising the need to investigate this in MS.

To date the impact of MS on skeletal muscle characteristics, such as muscle fiber cross sectional area (CSA) and proportion remains unclear. Recently, we reported reduced muscle fiber CSA and changed fiber proportions in MS patients, compared to HC (Wens *et al.*, under review). The impact of exercise on muscle contractile properties in MS has only been investigated by Dalgas and co-workers [81]. They reported increased m. Vastus Lateralis mean fiber CSA combined with improved muscle strength following 12 weeks of progressive resistance training. Despite the importance of understanding the effects of exercise on muscle fiber characteristics to optimize exercise and rehabilitations programs in MS, the impact of other training modalities and intensities on muscle fiber CSA and fiber type proportion in MS, has not been investigated yet.

To determine the effects of high intensity exercise in MS, this study aimed to investigate the impact of high intensity interval or continuous cardiovascular exercise, both in combination with resistance training, on muscle contractile characteristics, in terms of muscle fiber CSA/proportion, muscle strength and muscle mass and on endurance capacity in MS. It was hypothesized that the applied intense programs could improve mean muscle fiber CSA and proportion as well as muscle strength and endurance capacity.

Methods

1. Subjects

Thirty-four MS patients diagnosed according to McDonald criteria (EDSS range 0-6), aged >18 years, were included following written informed consent. Subjects were excluded if they had other disorders (cancer, cardiovascular, pulmonary and/or renal), were pregnant, participated in another study, had an acute MS-exacerbation 6 months prior to the start of the study or contra-indications to perform physical exercise. The study was approved by the ethical committee, performed in accordance with the Declaration of Helsinki and was registered at ClinicalTrials.gov (NCT01845896).

2. Study design

All MS patients were randomized into a sedentary control group (SED, n=11) and 2 exercise groups that performed 12 weeks of a high intensity interval + resistance training ($H_{IT}R$, n=12) or high intensity continuous endurance + resistance training ($H_{CT}R$, n=11). M. Vastus Lateralis fiber CSA and proportion, knee flexor and extensor strength, body composition, maximal endurance capacity and self-reported physical activity levels were assessed before and after the intervention. Neither the patients nor the researchers involved in the project were blinded to group allocation. SED remained sedentary during the study course and were instructed to continue their current level of physical activity during the period of the study.

3. Exercise training program

After the baseline measurements, the subjects were enrolled in a well-controlled and supervised training program to increase cardiorespiratory fitness, as well as strength of the major peripheral muscle groups. Subjects participated in 5 sessions per 2 weeks. Training sessions were interspersed by at least one day of rest, to ensure adequate recovery.

$H_{IT}R$ program: Each session started with a 5 min warm-up on a cycle ergometer. Hereafter, high intensity cycle interval training was performed. During the first 6 weeks exercise duration gradually increased from 5x1 min interspersed by 1 min rest intervals to 5x2 min and 1 min rest intervals. Exercise intensity was defined as the workload, corresponding to 100% of the maximal heart rate. During the second 6 weeks, duration remained stable at 5x2 min and the workload increased to reach a level corresponding to 100-120% of the maximal heart rate. The second part consisted of moderate-to-high intensity resistance training (leg press, leg curl, leg extension, vertical traction, arm curl and chest press, Technogym®). In

order to exercise at similar relative workload, resistance training of the lower limb was performed unilaterally, due to the frequent bilateral strength differences seen between the legs of MS patients [259]. Training intensity and volume were adjusted from 1x10 repetitions to 2x20 repetitions at maximal attainable load.

H_{CT}R program: Each session started with a cardiovascular part, consisting of cycling and treadmill walking/running (Technogym®). Session duration and exercise intensity increased as the intervention progressed, starting from 1x6 min/session to 2x10 min/session, at a high workload, corresponding to 80-90% of maximal heart rate and according to individual capabilities. The second part of the training session comprised similar resistance training, as described in the H_{IT}R program.

All exercises were performed at a high workload corresponding to 14-16 ratings of perceived exertion on 20-point Borg scale (RPE) and were adjusted to individual disability level. Continuous encouragement by the instructors led to a systematic increase of the training load over the 12-week training period. All sessions were ended by stretching of the extremities, and RPE-level was recorded.

4. Primary outcome measure

Muscle fiber CSA and proportion

To investigate muscle fiber CSA and proportion, muscle biopsies form the middle part of the m. Vastus Lateralis (Bergström needle technique) of the weakest leg (see isometric muscle strength measurements) were collected by an experienced medical doctor. The second biopsy, following 12 weeks of exercise or usual care, was taken 2-3cm proximal to the biopsy taken at baseline. Muscle samples were immediately mounted with Tissue-Tek, frozen in isopentane cooled with liquid nitrogen and stored at -80°C, until further analysis. The cross-sections of the biopsies, collected at baseline and after 12 weeks, were processed simultaneously.
Serial transverse sections (9µm) from the obtained muscle samples were cut at -20°C and stained by means of ATPase histochemistry, after preincubation at pH 4.4, 4.6 and 10.3, essentially following the procedure of Brooke and Kaiser [86]. The serial sections were visualized and analysed using a Leica DM2000 microscope (Leica, Stockholm, Sweden) and a Leica Hi-resolution Color DFC camera (Leica, Stockholm, Sweden) combined with image-analysis software (Leica Qwin ver. 3, Leica, Stockholm, Sweden). A fiber mask of the stained sections was drawn automatically and afterwards this mask was fitted manually to the cell borders of the selected fibers. Only fibers cut perpendicularly to their longitudinal axis were used for the

determination of fiber size. On average 170 ± 10 fibers were calculated and included in the CSA and fiber type analyses.
Calculation of the fiber CSA was performed for the major fiber types (I, IIa and IIx) and for the mean fiber CSA, since the number of type IIax and IIc fibers was too small for statistical comparison and CSA calculation.

5. Secondary outcome measure

Approximately 1-2 weeks before the muscle biopsy was performed secondary outcome measures were assessed from all subjects.

Isometric muscle strength

After 5 min of warming-up on a cycle ergometer and following habitation, the maximal voluntary isometric muscle strength of the knee extensors and flexors (45° and 90° knee angle) were measured, as described elsewhere [139], using an isokinetic dynamometer (System 3, Biodex, ENRAF-NONIUS, New York, USA). Two maximal isometric extensions (4s) and flexions (4s), followed by a 30s rest interval, were performed. The highest isometric extension and flexion peak torques (Nm) were selected as the maximal isometric strength. Baseline results were used to classify the legs of each patient as weakest or strongest leg. This subdivision was maintained in further analysis, replacing a conventional left-right classification.

Endurance capacity

During the exercise test to volitional fatigue, an electronically braked cycle ergometer (eBike Basic, General Electric GmbH, Bitz, Germany) with pulmonary gas exchange analysis (Jaeger Oxycon, Erich Jaeger GmbH, Germany) was used (cycling frequency: 70 rpm). This test was performed at least 48h separated from the isometric muscle strength test to exclude interference of muscle fatigue. Female and male MS patients started at 20 watt (W) and 30W, respectively, during the first minute. Hereafter, workloads increased, respectively, 10W and 15W per minute. Oxygen uptake (VO_2), expiratory volume (VE), and respiratory exchange ratio (RER) were collected breath-by-breath and averaged every 10s. Using a 12-lead ECG device, heart rate (HR) was monitored every minute. At the end of the test RER values were evaluated to verify the test was maximal. In addition, maximal cycling resistance (W_{max}), maximal heart rate (HR_{max}), test duration and VO_{2max}, defined as the corresponding load, heart rate, amount of minutes and oxygen uptake measured at the level of exhaustion, were reported.

Body composition

A Dual Energy X-ray Absorptiometry scan (Hologic Series Delphi-A Fan Beam X-ray Bone Densitometer, Vilvoorde, Belgium) was performed pre- en post-intervention. Fat and lean tissue mass were obtained for whole body, legs, trunk, gynoid and android region. Waist-to-hip fat mass ratio (android fat (g)/gynoid fat (g) ratio) and fat mass of the trunk/fat mass of the limbs ratio were calculated.

Physical activity level

Before and after the intervention, patients were asked to report their physical activity level by using the Physical Activity Scale for Individuals with Physical Disabilities (PASIPD) [267].

6. Statistical analysis

All data were analysed using SAS 9.2 software (SAS Institute Inc, Cary, USA). First normality was checked using the Shapiro-Wilk test for all variables. Differences between MS groups (SED, $H_{CT}R$ and $H_{IT}R$) were analysed by an one-way ANOVA, whereas within group differences (post minus pre) were analysed with a paired student's t-test. Relative changes due to the intervention were calculated as the mean of the individual changes and expressed as a percentage. Correlations between changes of the primary and changes of the secondary outcome measures on grouped data from all groups were analysed by means of Pearson's correlation analysis. All data are presented as mean ± SE and $p<0.05$ represents the threshold for statistical significance.

Results

1. *Baseline subject characteristics and adherence to the intervention*

At baseline, no differences in general subject and disease characteristics (Table 2.8.1) as well as outcome measures were found between groups. Approximately 90% of the 30 supervised training sessions were attended in both exercise groups and no severe symptoms exacerbations and/or adverse events were reported. Furthermore, no patient drop out was noted.

Table 2.8.1: *Baseline subject and disease characteristics*

	SED (n=11)	**$H_{CT}R$ (n=11)**	**$H_{IT}R$ (n=12)**	**p-value**
Age (y)	47 ± 3	47 ± 3	43 ± 3	NS
Height (m)	1.67 ± 0.02	1.69 ± 0.02	1.7 ± 0.02	NS
Weight (kg)	75.8 ± 3.6	70.2 ± 3.7	75.9 ± 4.1	NS
BMI (kg/m^2)	27.0 ± 1.4	24.4 ± 1.2	26.1 ± 1.14	NS
Gender (m/f)	2/9	5/6	5/7	NS
Type MS (RR/CP)	8/3	8/3	10/2	NS
EDSS	2.5 ± 0.3	2.7 ± 0.3	2.3 ± 0.3	NS

Data is presented as mean ± SE.
Abbreviations used: MS, multiple sclerosis; SED, sedentary group; $H_{CT}R$*,* intense continuous endurance + resistance training*;* $H_{IT}R$*,* high intensity interval training + resistance training*, BMI, body mass index; RR, relapsing remitting; CP, chronic progressive; EDSS, expanded disability status scale.*

2. *Primary outcome measure*

<u>Muscle fiber CSA and proportion</u>

In SED muscle fiber CSA and proportion did not change. Mean CSA, as well as fiber type I, II and IIa CSA significantly increased in $H_{IT}R$ and $H_{CT}R$ following 12 weeks of exercise. Fiber type IIx CSA only increased in $H_{IT}R$ after the intervention, whereas it remained stable in $H_{CT}R$. No changes in fiber type proportion were observed in any exercise group after 12 weeks of exercise. However, within group effects were observed on type I and IIx of $H_{IT}R$ and $H_{CT}R$ and in type IIa of $H_{CT}R$, after comparison of the pre- and post-intervention fiber type proportion values (Table 2.8.2).

Table 2.8.2: *Muscle fiber type proportion and cross sectional area (CSA) at baseline and after 12 weeks of usual care or high intensity aerobic exercise in combination with resistance training.*

	SED		**$H_{CT}R$**		**$H_{IT}R$**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
Fiber type proportion (%)						
Type I	44.2 ± 3.9	47.5 ± 2.9	40.1 ± 4.7	46.9 ± 4.7[a]	41.3 ± 3.0	46.3 ± 2.6[a]
Type IIa	34.2 ± 3.9	34.2 ± 2.3	34.1 ± 2.9	38.9 ± 4.6[b]	40.9 ± 3.8	44.5 ± 2.4
Type IIx	21.2 ± 4.5	17.7 ± 2.0	24.3 ± 2.7	13.5 ± 2.6[a]	18.5 ± 2.8	10.1 ± 2.8[b]
Fiber CSA (μm^2)						
Mean	3738 ± 267	3740 ± 431	3551 ± 351	3905 ± 408[a, c]	4038 ± 321	4892 ±379[a, c]
Type I	4078 ± 384	4050 ± 531	3630 ± 443	4071 ± 470[a, c]	4410 ± 188	4916 ± 399[b, d]
Type II	3487 ± 265	3478 ± 334	3285 ± 321	3622 ± 398[a, d]	3612 ± 429	4551 ± 462[a, d]
Type IIa	3703 ± 306	3729 ± 402	3719 ± 366	4014 ± 522[a, c]	4037 ± 444	5034 ± 447[a, c]
Type IIx	3446 ± 305	3191 ± 318	2771 ± 277	2955 ± 258	3187 ± 438	3920 ± 519[a, d]

Data are reported as mean ± SE.
[a] p<0.05, [b] p ≤ 0.1, compared with pre-intervention value, within group
[c] p<0.05, [d] p ≤ 0.1, pre to post change compared with change from pre to post in SED
Abbreviations used: SED, sedentary (usual care); $H_{CT}R$, high intensity continuous exercise + resistance training; $H_{IT}R$, high intensity interval training + resistance training

3. Secondary outcome measure

Isometric muscle strength

Muscle strength of SED remained stable during 12 weeks of usual care (Figure 2.8.1). Compared to SED, knee flexion and knee extension strength of the weakest leg of $H_{IT}R$ improved by 25 ± 13 to 45 ± 20% ($p<0.05$), whereas only hamstring strength of the strongest leg of $H_{IT}R$ improved by 13 ± 7 to 21 ± 7% ($p<0.05$). Furthermore, $H_{CT}R$ flexion and extension strength improved, from pre- to post trial, in the weakest leg by 19 ± 9 to 33 ± 17% ($p<0.05$), whereas muscle strength of the strongest leg remained stable ($p>0.05$).

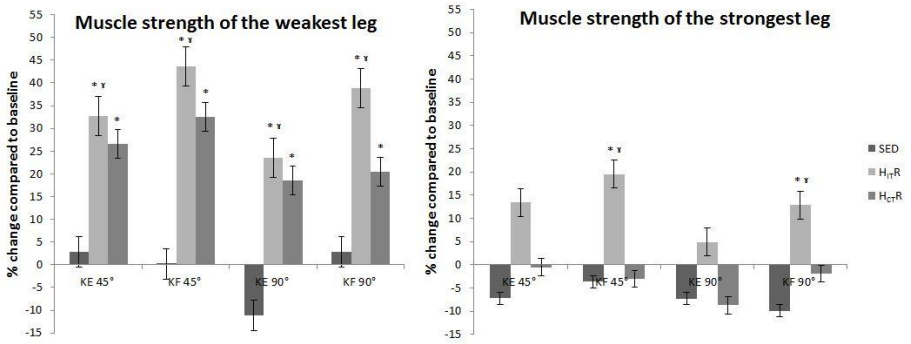

Figure 2.8.1: *Percentage change of knee extension and flexion after 12 weeks of sedentary living (usual care, SED), high intensity continuous training + resistance training (HCTR) and high intensity interval training + resistance training (HITR). Data are reported as mean ± SE.*
** p<0.05, compared with pre-intervention value, within group*
ɣ p<0.05, pre to post change compared with change from pre to post in SED
Abbreviations used: KF, knee flexion; KE, knee extension.

Endurance capacity

After 12 weeks, endurance capacity variables remained stable in SED and $H_{CT}R$. Compared to SED and $H_{CT}R$, W_{max} (+21 ± 4%), test duration (+24 ± 5%) and VO_{2max} (+17 ± 5%) significantly improved ($p<0.05$) in $H_{IT}R$ (Table 2.8.3).

Table 2.8.3: *Exercise capacity, body composition and physical activity level after 12 weeks of usual care or high intensity aerobic exercise in combination with resistance training.*

	SED		**$H_{CT}R$**		**$H_{IT}R$**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
Exercise capacity:						
Maximal cycling resistance (watt)	121 ± 8	115 ± 11	131 ± 18	133 ± 18	158 ± 15	188 ± 15[a,b]
Maximal cycling resistance (watt/kg)	1.6 ± 0.12	1.6 ± 0.15	1.85 ± 0.24	1.9 ± 0.23	2.0 ± 0.17	2.4 ± 0.16[a,b]
Test duration (min)	10.4 ± 0.8	9.9 ± 0.8	9.5 ± 1.0	9.8 ± 0.9	12.1 ± 0.9	14.5 ± 0.9[a,b]
VO_2 max (ml/min)	1647 ± 133	1645 ± 160	1870 ± 238	1969 ± 230	2031 ± 186	2379 ± 197[a,b]
VO_2 max (ml/min/kg)	21.9 ± 1.8	23.6 ± 2.1	26.3 ± 3.1	28.2 ± 3.0	26.6 ± 2.2	30.7 ± 2.1[a,b]
RER max	1.18 ± 0.04	1.17 ± 0.03	1.3 ± 0.19	1.2 ± 0.02	1.2 ± 0.03	1.2 ± 0.02
HR rest (beats/min)	75 ± 4	87 ± 4[a]	76 ± 3	80 ± 4	75 ± 3	84 ± 3[a]
HR max (beats/min)	142 ± 7	153 ± 5[a]	154 ± 6	162 ± 6[a]	160 ± 6	168 ± 5[a]
Body composition:						
Lean tissue mass (kg)	43.2 ± 2.1	43.5 ± 2.1	45.4 ± 2.6	46.2 ± 2.5	48.5 ± 3.1	49.9 ± 3.1[a]
Fat percentage (%)	38.2 ± 2.1	37.3 ± 2.2	33.6 ± 2.8	32.6 ± 2.8[a]	36.2 ± 1.9	34.3 ± 2.0[a]
Physical activity level (MET*h/week)	16 ± 2.6	15.8 ± 3.7	14.7 ± 2.7	23.9 ± 4.4[a,b]	25.8 ± 6.6	37.6 ± 7.2[a,b]

Data are reported as mean ± SE.
a p<0.05, compared with pre-intervention value, within group
b p<0.05 , pre to post change compared with change from pre to post in SED
Abbreviations used: SED, sedentary (usual care); $H_{CT}R$, high intensity continuous exercise + resistance training; $H_{IT}R$, high intensity interval training + resistance training; MET, metabolic equivalents

Body composition

Following 12 weeks of exercise, body weight remained stable in all groups. Within $H_{IT}R$ and $H_{CT}R$, body fat percentage decreased by 3.9 ± 2.0% and 2.5 ± 1.2%, respectively ($p<0.05$). Furthermore, lean tissue mass significantly increased 1.4 ± 0.5% within $H_{IT}R$, whereas it remained stable in $H_{CT}R$ and SED (Table 2.8.3). Finally, other adipose and lean tissue mass indices remained stable in all groups.

Physical activity level

Compared to SED, the physical activity level of $H_{IT}R$ and $H_{CT}R$ significantly increased by 86 ± 27% and 73 ± 19%, respectively ($p<0.05$), following 12 weeks of exercise. In SED the physical activity level remained stable (Table 2.8.3).

4. Correlations

Overall, no significant correlations were found between the change of the primary and secondary outcome measures on pooled data.

Discussion

This study is the first to investigate the impact of high intensity cardiovascular exercise combined with resistance training on muscle contractile characteristics and endurance capacity in MS. Moreover, 12 weeks of the applied high intensity programs were safe, well tolerated and induced beneficial adaptations in MS patients. In particular, muscle fiber CSA, muscle strength of the weaker legs, body fat percentage and self-reported physical activity levels improved following both $H_{IT}R$ and $H_{CT}R$. In addition, further improvements of the endurance capacity, muscle flexion strength of the stronger legs and lean tissue mass were only seen in $H_{IT}R$. These results are clinically relevant, due to the need for exercise programs that are able to counteract reduced endurance capacity, muscle strength and muscle mass of particularly the lower limbs, enhancing physical function in MS patients.

Safety and tolerability
Several studies have already demonstrated the benefits of resistance training [14] or endurance training [13, 109, 111] in MS. The effect of combined training has only been sparsely explored [154, 155, 218, 248] (Wens *et al.*, under review) and the impact of high intensity combined exercise has never been investigated before. The latter could be explained by safety concerns regarding the symptom instability of MS patients often seen during/after high intensity exercise, which is frequently caused by the exercise-induced increase in body temperature [96]. Interestingly, no dropout or adverse events were reported during and after 12 weeks of $H_{IT}R$ and $H_{CT}R$, demonstrating that mild-to-moderately impaired MS patients tolerate intense exercise programs.

Continuous vs. interval training
The present study showed an improvement of the endurance capacity, muscle flexion strength of the stronger legs and lean tissue mass in $H_{IT}R$, and improved muscle strength of the weaker leg, body fat percentage and self-reported physical activity levels in $H_{IT}R$ and $H_{CT}R$, suggesting that exercise efficiency is even higher in $H_{IT}R$. This is in line with what is seen in healthy highly trained endurance athletes where endurance performance was significantly improved after H_{IT}, whereas a submaximal endurance training did not induce an additional increase of the endurance capacity and associated physiological variables [277]. Importantly, and as already suggested by others [41] , the observed training improvements were often larger compared to those reported after mild-to-moderate combined exercise programs [154, 155, 218, 248] (Wens *et al.*, under review). This indicates that higher training intensities are more effective and that training adaptations are intensity related in MS.

Muscular effects

Recently, we reported that MS affects muscle fiber CSA and proportion (Wens *et al.*, under review). To our knowledge, only Dalgas *et al.* investigated the effects of exercise (progressive resistance training) on muscle fiber CSA in MS [81], reporting increased mean muscle fiber CSA (8 ± 15%), predominantly in type II muscle fiber CSA (14 ± 19%) and a tendency towards increased type I CSA [81]. In the present study, mean muscle fiber CSA ($H_{IT}R$: 21 ± 7%, $H_{CT}R$: 23 ± 5%) and lean muscle mass further increased, suggesting an additional value of the high intensity aerobic exercise. This is, partly, in accordance with results reported in sedentary HC, demonstrating a significant increase of the area of type I and IIx fibers after high intensity interval training [278]. In addition, high intensity aerobic exercise induced an increased CSA of both type IIa and IIx fibers and no changes in type I fiber size in elite ice hockey players [279].

Based on an often more inactive lifestyle of MS patients, Dalgas *et al.* expected an inactivity-related higher proportion of type IIx fibers and a possibility to transform type IIx to IIa fibers after progressive resistance training [118, 270]. However, they were not able to report any changes in the proportion of fiber types. In the present study, type IIx proportions decreased after 12 weeks of $H_{CT}R$ and tended to decrease after 12 weeks of $H_{IT}R$, whereas the type IIa proportion tended to increase in $H_{CT}R$. These results are comparable with data reported in healthy elderly populations, reporting a reduction of the type IIx proportion and an increase of the proportion of the type IIa fibers [280, 281]. Interestingly, these studies used higher training frequencies [280] or longer training periods [281], compared to the work of Dalgas *et al.* [81], suggesting that a higher training volume and intensity is required to induce fiber type changes than to induce changes in fiber type CSA.

Limitations

Since this is the first study that investigated the effects of high intensity exercise on muscle fiber CSA and proportion in MS, we were not able to perform a pre-trial power analysis, due to the absence of a defined effect size. Nevertheless, a post-hoc power analysis (R 2.15.2 software) on mean muscle fiber CSA and based on the present results, demonstrated that 5 persons in each group would be sufficient to provide a >80% power to detect a 20% increase of mean muscle fiber CSA after 12 weeks of high intensity exercise ($p=0.05$, $\sigma=7\%$), demonstrating a suitable sample size in the present study. Secondly, given the ethical concerns we collected only one biopsy per test, despite the recommendation of Lexell *et al.* [274] to optimally collect three biopsies from different depths of the muscle and to analyse >150 fibers from each sample to reduce sampling error. Finally, given the nature of the design, social interactions between MS patients could possibly influence intervention outcomes.

Conclusion

The present study showed that 12 weeks of high intensity cardiovascular exercise in combination with resistance training was safe, well tolerated and improved muscle strength and endurance capacity, with interval training seemingly superior to continuous training.

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Chapter III:

General discussion and conclusions

General discussion

During the course of this PhD we demonstrated that MS patients have a higher risk to develop secondary health complications and, in particular, that the prevalence of IGT is higher in MS patients, compared to healthy referent subjects. Furthermore, MS also seems to negatively affect skeletal muscle characteristics, by decreasing muscle fiber CSA, muscle strength and muscle mass of, predominantly, the lower limbs of mildly affected MS patients. Furthermore, we showed that, in this population of moderately affected MS patients, exercise therapy (cardiovascular and resistance training) improves not only endurance capacity, body composition and muscle contractile characteristics but also IGT in an exercise intensity-dependent manner. Finally and because no relapsed and/or adverse events were reported, we are to our knowledge the first to report that high intensity exercise is safe and well tolerated in MS patients.

1. MS and glucose tolerance

Following a systematic literature search **(Study 1)** we concluded that MS patients are at higher risk of developing secondary health complications. During the systematic review we were able to associate MS to several risk factors constituting the metabolic syndrome and CVD risk. The cited studies, however, were generally of low methodological quality and it was not clear whether an increased risk of CVD in MS was attributable to an increased risk of obesity, changes in body composition, hypertension, dyslipidaemia, glucose intolerance or type II diabetes, warranting further investigation. In an attempt to answer some of these questions we have focussed predominantly on glucose tolerance in MS.

1.1. Prevalence of impaired glucose tolerance in MS

The glycaemic control in MS, compared to matched referent subjects, was investigated in **Study 2**, evaluating the hypothesis that the prevalence of IGT is elevated in MS. This cross-sectional study including 81 MS patients and 45 healthy controls demonstrated an elevated prevalence of IGT in MS. To our knowledge, only 8 papers were published on this topic [65-70, 73, 74] so far, reporting glucose and insulin concentrations or information about type II diabetes, as primary or secondary outcome measure. This limited and heterogeneous amount of research studies makes it difficult to compare between studies and draw solid conclusions. Furthermore, most of these studies only reported fasting glucose concentrations, which is insufficient to indicate IGT according to the European Society of Cardiology, whereas we used a 2h OGTT, the preferable screening tool for IGT. Six of these studies [65-69, 74] were based on retrospective analysis of databases and charts. However, none of these sources are regarded as the golden standard, since

the validity of the data sources varies depending on the research question of interest [189]. The results of these studies reported a lower [67, 68, 74], comparable [69] or higher [65, 66] prevalence of type II diabetes or fasting glucose concentrations in MS, compared to healthy controls. One paper was a non-controlled trial, reporting comparable glucose concentrations between MS patients and (elsewhere reported) healthy controls [70]. So far, only one study was a controlled trial that, contrasting to the present work, showed that glucose tolerance was not impaired in MS [73]. This could, in part, be explained by the sample size (n=16) and the included subtype of MS patients (only RRMS) [73]. The present study included 81 MS patients, including all MS types, in an attempt to create a representative sample of the general MS population.

Given the positive correlation between glucose concentrations and EDSS as well as between glucose concentration and the rate of clinical relapses [74], the impact of physical disabilities and EDSS score needs to be assessed before a valid interpretation can be performed. In this respect, we reported higher glucose and insulin profiles in EDSS>3, compared to EDSS≤3, and an association between the glucose profile and MS-subtype. The median EDSS score of the study by Mähler *et al.* was 2.0, ranging from 1.0 to 4.5 [73], while the median EDSS score of study 2 was 2.5, ranging from 0 to 6.0, making it difficult to directly compare between populations. Therefore, the selection of MS patients should be performed carefully in the future, to allow generalization.

Because age can be associated with increased type II diabetes risk, it is important to note that biometrical data such as age, as well as, gender and race can alter study outcomes. Despite the different conclusion of our work and the study of Mähler and co-workers[73], there was no difference in age between the MS patients. Nevertheless, the men-women ratio was different between both studies, where Mähler *et al.* used the same numbers of male and female MS patients [73], study 2 included approximately 40% male and 60% female participants, trying to give a representative image of the general MS population. This difference also complicates direct comparison between the two studies.

Furthermore, factors such as medication, lifestyle interventions and physical inactivity, could, at least in part, explain the observed elevated glucose profile in MS. First, some of the drugs applied in MS treatments may increase blood glucose and insulin concentrations in MS patients. In particular, the use of disease modifying therapies (DMT), which becomes more pronounced as the disease progresses, can be associated with elevated blood glucose and/or insulin levels in MS [66, 202, 282]. Moreover, corticosteroids [193], an important MS drug, can lead to elevated fasting glucose- and insulin concentrations and to IR in healthy controls [194]. Also, Hussein and co-

workers suggested that factors, like the use of corticotropine and glucocorticoids, may in part explain the possible higher prevalence of diabetes type II in MS patients [66]. Furthermore, also the use of non-DMT's, such as antidepressants, can be associated with IGT and type II diabetes [203, 283]. Another difference to the study of Mähler and co-workers was that only MS patients treated with glatiramer acetate were included in their study [73], while the present study included patients using a spectrum of MS-related medication. However, none of the patients were treated with glucocorticoids, since none of the included patients experienced a relapse before or during sample collection.

Modifiable factors, such as excessive weight gain, dietary factors, smoking and sleep deprivation have to be considered in the development of IGT and IR. In particular, weight gain and obesity are important factors in the development of IR [284-286]. Nevertheless, BMI values of the present study were normal in healthy controls and MS patients. The latter was also comparable to the BMI values reported by Mähler and co-workers [73]. Next, smoking is widely accepted as an important risk factor in the development of IR [287-291]. Furthermore, recent research confirmed the association of smoking with type II diabetes [292, 293]. However, in study 2 there were no differences between the amount of smokers in the healthy control group and the MS patients. Finally, insufficient sleep can also affect the glucose metabolism [199, 294, 295]. All these factors need further research in MS.

Finally, physical inactivity, could also induce the development of IGT. Physical inactivity directly contributes to the cascade of events that leads to the expression of the 'exercise-deficient phenotype', resulting in reduced insulin sensitivity as well as in abdominal fat accumulation, higher levels of triglyceride and lower levels of high-density-lipoproteins [59]. Consequently, exercise may be a very important non-pharmacological intervention targeting secondary health risk in MS patients (study 3 and 6).

1.2 Mild-to-moderate intensity exercise and IGT in MS

Study 3 aimed to investigate the impact of a 24-week mild-to-moderate intensity combined exercise intervention on glycaemic control, muscle strength, exercise tolerance, body composition and serum BDNF concentration in MS. Given the ability of physical activity to reduce the risk of developing IGT and to improve insulin action in other patient populations [107], it was hypothesized that whole body glycaemic control could also be improved in MS patients following exercise. However, long-term mild-to-moderate intensity combined exercise did not decrease glucose and insulin profiles in MS patients whereas a significant exercise effect was detected within the secondary outcome measures.

Noteworthy, only 14% of the included MS patients showed IGT at baseline, possibly explaining the absence of some of the expected results on the glucose and insulin profiles after 24 weeks of exercise. Nevertheless, a sub analysis in the IGT-group also showed no changes after 24 weeks of exercise; confirming our results. Furthermore, fasting glucose concentrations of the IGT-group did not decrease after 24 weeks of mild-to-moderate intensity exercise.

In contrast to our work, Slawta *et al.* [71] showed that low-to-moderate intensity leisure time physical activity was associated with lower fasting glucose levels in MS patients and White *et al.* [143] reported a trend towards decreased fasting glucose concentrations after 8 weeks of lower extremity progressive resistance training. The differences between the present work and the work of others could possibly be explained by the fact that the examination of changes in fasting blood glucose levels, as applied by Slawta *et al.* [71] and White *et al.* [143], is not sufficient since a 2h OGTT, as applied in the present work, is the preferable screening tool to detect IGT and exercise-induced changes, according to the European Society of Cardiology. Nevertheless, the exact amount and intensity of physical exercise, to induce health-related benefits, remained unclear and needed further investigation.

1.3 High intensity exercise and IGT in MS

In other populations, some studies reported that low-to-moderate intensity exercise improved glucose tolerance [156, 249], whereas others suggested that this level of intensity was not sufficient to improve insulin sensitivity [250, 251]. Therefore, the influence of high intensity exercise on glucose tolerance was investigated in **Study 6** and we demonstrated that tAUC significantly decreased within the high intensity interval group and the high intensity continuous training group, whereas insulin tAUC only decreased within the high intensity continuous training group.

At baseline, 9% of all included MS patients showed IGT. Interestingly, even though we did not only include persons with IGT, but a general MS population, we were able to detect some general improvements on glucose tolerance, suggesting that exercise-induced changes of IGT are intensity related in MS. Interestingly, a sub analysis in the IGT-group showed that fasting glucose concentrations were decreased and normalised after 12 weeks of high intensity exercise.

Because skeletal muscles are the most important sites of whole body glucose disposal and because disturbed muscle energy metabolism may also contribute to IGT in MS, we examined the impact of exercise on the skeletal muscle membrane glucose transporter (GLUT4). Interestingly, muscle GLUT4 content increased in the high intensity interval group, whereas changes in

the high intensity continuous training group did not reach statistical significance.

In general, these results are comparable with data reported in the literature from other populations demonstrating that exercise results in enhanced insulin- and contraction-stimulated glucose transport capacity, mirrored by an exercise-induced increase in skeletal muscle GLUT4 content [88, 256, 260-262]. This indicates that high intensity exercise is probably more effective to improve glucose tolerance than moderate intensity training [156-157], as applied in our former research [248] and the work of others [71, 143] and suggests that the effect of exercise on health related parameters such as glucose tolerance in MS is intensity related. The insulin tAUC and GLUT4 changes who did not reach statistical significance in the present study are difficult to explain. Possibly, the total volume of the endurance training, being lower in $H_{IT}R$ compared to $H_{CT}R$, could, partly, explain the unchanged insulin levels of $H_{IT}R$ compared to the reduced insulin tAUC of $H_{CT}R$, suggesting that the total exercise duration should be taken into account when designing exercise programs to improve insulin action. Furthermore, the intensity of the endurance training, being higher in the $H_{IT}R$ compared to $H_{CT}R$, could possibly clarify the increased GLUT4 content of $H_{IT}R$ compared to the unchanged GLUT4 levels of $H_{CT}R$, suggesting that exercise intensity should be considered when designing an exercise program with the intent of increasing GLUT4 content. Finally, these results may reflect the large inter-subject variability in the responses to exercise, as also suggested by others [263].

1.4. Limitations and recommendations IGT studies

Because, to our knowledge, we were the first who investigated the effect of physical exercise on glucose tolerance in MS, we were not able to perform a pre-trial power analysis, due to the absence of a defined effect size in study 3 (mild-to-moderate intensity) and 6 (high intensity). In fact, post hoc power analyses showed the need to include more MS patients, underlining the fact to be careful in drawing conclusion and generalise results of the present studies.

Even though we did not only include persons with IGT, we were able to detect an improved glucose tolerance after high intensity exercise, whereas mild-to-moderate intensity seemed not able to induce IGT changes. Still, the use of more stringent inclusion criteria is probably warranted in future research, including, for example, only MS patients with IGT at baseline, since sub-analyses in small groups of MS patients with IGT, as applied in the present work, is not representative for the MS population.

Furthermore, given the nature of the study design, social interactions between MS patients could have affected intervention outcomes. In particular, peer pressure between participants and the awareness of being involved in an exercise intervention study, influencing the food pattern, could improve muscle strength, endurance capacity or body composition. Furthermore, some inactivity-induced MS symptoms may have improved during the exercise intervention program, which could ameliorate quality of life and induce changes in the physical activity level, which in turn could have affected study results. These subtle subjective changes are difficult to investigate. However, the use of some questionnaires, involving quality of life, physical activity and daily routine are recommended in future research.

2. MS and skeletal muscle characteristics

2.1 Skeletal muscle characteristics in MS

To investigate the effect of MS on skeletal muscle characteristics, we assessed muscle strength and body composition and we obtained muscle biopsies from m. Vastus Lateralis biopsies to investigate muscle fiber CSA and proportion in **Study 7.** Mean muscle fiber CSA of all fibers, as well as CSA of type I, II and IIa fibers were smaller and muscle strength of the quadriceps was lower in MS patients, compared to matched healthy controls. Whole body composition was comparable between groups, whereas the biopsied leg tended to have a higher fat percentage and a lower lean body mass in MS patients, compared to healthy controls.

In accordance with our findings, Kent-Braun *et al.* and Garner *et al.* also reported a reduced muscle fiber size [49, 50]. Furthermore, in male MS patients we found a muscle fiber CSA hierarchy of type I and IIa > IIx, which differed from the hierarchy of CSA type IIa > I and IIx, seen in our healthy men. In female MS patients a muscle fiber CSA hierarchy of type I > IIa > IIx was found, which also differed from the patterns of CSA type I and IIa > IIx, seen in our healthy women. These differences suggest a selective type II(a) atrophy in MS patients, as also indicated by others [49, 50, 81]. Selective type II atrophy is often reported as an effect of ageing [268] or inactivity [269, 270]. Interestingly, in the present study average age and physical activity levels were similar between MS patients and HC, suggesting that the reported observations could also be consequences of the disease *per se*.

Furthermore, the reduced maximal isometric quadriceps strength of the MS patients was consistent with previous findings [43, 45-47, 49, 77]. Interestingly, muscle fiber CSA was highly correlated with muscle strength of the quadriceps, suggesting that reduced CSA contributes to muscle weakness in MS patients and that changes in skeletal muscle characteristics in MS may affect function. Similar to our results, some studies also reported similar

total body fat percentages and total body lean tissue mass in MS patients and healthy controls [73, 75, 172, 175, 176] and slightly higher fat percentage and lower lean tissue mass in the lower extremities of (female) MS patients [175]. However, the work of others [73, 75, 172, 175, 176] was never correlated to muscle fiber CSA, making it difficult to further compare between studies. In addition, the present study showed that muscle fiber CSA was positively correlated with lean tissue mass and negatively correlated with fat percentage of the biopsied leg, indicating that the preservation of muscle mass of the lower limb of MS patients is very important, already at the early stage of the disease and warranting appropriate rehabilitations programs.

2.2 Effects of exercise on skeletal muscle characteristics in MS

a. Muscle strength and muscle mass

Observational [11, 12] as well as interventional studies [13, 14, 39, 41, 109, 111, 118, 141, 142, 144, 146, 148, 296] reported benefits of 4 to 20 weeks exercise interventions, including improvements in muscle strength. From a theoretical point of view, most benefit is suggested to be gained from combined exercise since this would positively affect impairments of both the cardiovascular system and muscle strength/activation [41]. However, only a limited number of studies [154, 155] evaluated the impact of a long-term (>20 weeks) combined exercise intervention on the above-mentioned parameters in MS. Consequently, the impact of long-term exercise intervention was poorly understood in MS patients. Therefore, we investigated the effect of 24 weeks of mild-to-moderate intensity combined exercise on maximal muscle strength and muscle mass of the lower limbs in **Study 4**. In the exercise group muscle strength of the quadriceps (10-23%) and hamstrings (29-54%) improved over time. Furthermore, lean tissue mass increased by 2 ± 0.6%, whereas body fat percentage tended to decrease by 3 ± 1% in the exercise group. All these parameters remained stable in the sedentary control group.

To date, several authors have suggested that MS patients could benefit more from higher training intensities [41, 100, 119], but so far, no studies on combined exercise have evaluated high intensity training in MS. Therefore, we investigated the impact of 12 weeks of high intensity interval or continuous cardiovascular exercise, both in combination with resistance training, on muscle strength and muscle mass in **Study 8**. In particular, muscle strength of the weaker legs (+25 ± 13 to +45 ± 20%) and body fat percentage (-3.9 ± 2.0% and -2.5 ± 1.2%) improved following both high intensity interval and high intensity continuous training, respectively. In addition, further improvements of the muscle flexion strength of the stronger legs (13 ± 7 to 21 ± 7%) and lean tissue mass (+1.4 ± 0.5%) were only seen in the high intensity interval group. Importantly, these exercise-induced improvements were often larger, compared to those reported by others after combined

exercise [154, 155, 218] or resistance training [118, 139] and the improvements after 12 weeks of high intensity exercise (Study 8) were larger than those after 12 weeks of mild-to-moderate intensity exercise (Study 3), suggesting that higher training intensities are more effective and that training adaptations are intensity related in MS.

Noteworthy, MS patients suffer from asymmetric leg strength and classical bilateral strength training is suggested not to provide an optimal training stimulus [150]. To further optimize the applied relative workloads and exercise effects, the applied supervised exercise programs were based on unilateral leg training. As such, muscle strength improvements in the weakest leg were more substantial compared to improvements of the strongest leg. Based on these results it can be suggested that the unilateral exercise approach has the potential to further improve muscle strength of severely affected legs, which is an important clinical finding.

b. Muscle fiber characteristics

To our knowledge, only Dalgas *et al.* investigated the effects of exercise (progressive resistance training) on muscle fiber CSA in MS [81], reporting increased mean muscle fiber CSA (8 ± 15%), predominantly in type II muscle fiber CSA (14 ± 19%) and a tendency towards increased type I CSA [81]. In **Study 8**, mean muscle fiber CSA showed a larger mean increase (22 ± 6%) and an increase in lean muscle mass, suggesting an additional value of high intensity aerobic exercise. This is, partly, in accordance with results reported in sedentary healthy controls, demonstrating a significant increase of the area of type I and IIx fibers after high intensity interval training [278]. In addition, high intensity aerobic exercise induced an increased CSA of both type IIa and IIx fibers and no changes in type I fiber size in elite ice hockey players [279].

Based on an often more inactive lifestyle of MS patients, Dalgas *et al.* expected an inactivity-related higher proportion of type IIx fibers and a possibility to transform type IIx to IIa fibers after progressive resistance training [118, 270]. However, they were not able to report any changes in the proportion of fiber types. In **Study 8**, type IIx proportions decreased after 12 weeks of high intensity continuous training and tended to decrease after 12 weeks of high intensity interval training, whereas the type IIa proportion tended to increase in the high intensity continuous training group. These results are comparable with data reported in healthy elderly populations, reporting a reduction of the type IIx proportion and an increase of the proportion of the type IIa fibers [280, 281]. Interestingly, these studies used higher training frequencies [280] or longer training periods [281], compared to the work of Dalgas *et al.* [81], suggesting that a higher training volume and

intensity is required to induce changes in fiber type proportions than to induce changes in fiber type CSA.

2.3. Limitation on skeletal muscle research

Given our ethical concerns we collected only one biopsy per test in study 6, 7 and 8, despite the recommendation of Lexell *et al.*[274] to optimally collect three biopsies from different depths of the muscle and to analyse >150 fibers from each sample to reduce sampling error. However, this was in line with the methodology applied in most other human biopsy studies.

3. Animal studies

So far, research investigating the impact of EAE and physical exercise during EAE on muscle contractile properties and EAE progression was scarce and conflicting but also promising [135-138]. Furthermore, it is difficult to compare different studies due to application of different exercise intensities (lower versus higher intensities) or due to the use of voluntary exercise versus a quantified training load and intensity. **Study 4** was the first to investigate the impact of several training intensities. In particular low, moderate and high intensity endurance training, on muscle contractile characteristics and hindquarter paralysis during and following acute EAE was investigated. Due to the absence of an explicit exercise effect in study 4, which could, possibly, be explained by the sedentary week after a relatively short training period and given the fact that the results of study 4 and the work of others suggests that the optimal aerobic exercise intensity is near the anaerobic threshold, **Study 5** aimed to investigate the effect of high intensity aerobic exercise on disease course and muscle morphology in EAE animals, immediately before (experiment 1) and after (experiment 2) hindquarter paralysis.

3.1 Muscle morphology and exercise in EAE

In **Study 4** EAE reduced CSA of type IIb+x muscle fibers in TA and EDL, whereas muscle fiber distribution was not affected by EAE. Similarly, during both experiments of study 5, EAE reduced mean fiber CSA, as well as type I, IIa and IIx+b fiber CSA in TA, EDL and SOL. De Haan and co-workers previously investigated the impact of EAE on the medial Gastrocnemius, a muscle comprising ±80% type I fibers, and demonstrated, in accordance with our results, no muscle fiber shifts but reduced CSA of all fiber types [135].

Under the conditions of study 4, treadmill exercise was not able to reduce type IIb+x muscle fiber atrophy, despite the fact that it is documented that running exercise can reverse muscle fiber atrophy in rats [226-228]. Interestingly, high intensity running exercise tended to reduce muscle fiber

atrophy in EAE rats, suggesting an exercise effect. However, other exercise intensities were not able to reduce EAE-induced fiber atrophy in any fiber type of the other groups. In addition, in the healthy control rats exercise did also not alter muscle fiber characteristics, suggesting that one week of inactivity during the paralysis period of EAE rats, which was also applied in the control rats, may have tempered training effects. Therefore, it was suggested to collect muscle samples immediately after the training period and before the onset of hindquarter paralysis, to further investigate the influence of exercise on muscle contractile properties in EAE. This was investigated further into detail in **Study 5**, where an exercise effect was expected in experiment 1 by excluding the tempering effect of the sedentary week of experiment 2 (which was comparable to study 4). Nonetheless, exercise was, in general, not able to prevent muscle fiber atrophy in both experiments of study 5, with the exception of some minor changes in healthy and EAE animals. However, the expected exercise effect was absent in all healthy and EAE groups, suggesting that the applied exercise duration was too short to induce the hypothesized muscle fiber alterations. Consequently, this may imply that aerobic exercise is not optimal to induce adaptations in muscle fiber CSA and proportion in EAE rats. Therefore it is suggested to investigate the influence of resistance training on muscle fiber characteristics, since EAE is able to reduce type IIx+b fiber CSA, which (in humans) are more susceptible to resistance training [229-231].

3.2 Muscle work and exercise in EAE

In **Study 4 and 5**, muscle work in the healthy subgroups peaked during the first 20-30 contractions and then progressively declined, as previously reported by De Haan *et al.* in control and EAE rodents during a series of repeated maximal isometric contractions of m. Gastrocnemius [135]. However, in the EAE subgroups of study 4 and 5 muscle work did not peak. This might be explained by the fact that different muscle groups were analysed. More specific, TA and EDL with predominately glycolytic muscle fibers [233] were used in study 4 and 5, whereas medial Gastrocnemius with a proximal region, containing all muscle fibers, and a distal region, containing only type IIx and IIb fibers were investigated in the study of De Haan and co-workers [135]. Because fast glycolytic type IIb muscle fibers largely contribute to peak force production [224, 225] the earlier reported decreased CSA of type IIb+x fibers probably explains the absence of peak muscle work in EAE during the first 30 repetitive isokinetic contractions in study 4 and 5. As mentioned earlier, it was expected that an exercise-induced effect in experiment 1 of study 5 would be present by excluding the tempering effect of the sedentary week. Moreover, in experiment 1 of study 5, muscle work results suggested an exercise-induced effect in both healthy and EAE groups. In experiment 2 of study 5, on the other hand, muscle work curves only suggested an

exercise effect in the healthy animals. However, statistical significance was not reached in both experiments due to large variations between muscle work of different animals.

3.3 EAE and hindquarter paralysis

Finally, **Study 4 and 5** showed that intense treadmill running delayed the onset of hindquarter paralysis in EAE, whereas low and moderate running exercise had no effect. Other authors also reported delayed onset of clinical EAE signs following voluntary wheel running in chronic EAE mice [138] and treadmill running in rats [136, 137]. As mentioned earlier, it is difficult to compare different studies due to application of different exercise intensities and modalities. The training intensities used in study 4 and 5 were probably higher compared to the work of Le Page [136, 137] and Rossi [138]. In fact, the applied high intensity exercise was probably near the anaerobic threshold [219, 220]. Therefore, the present findings and the above-described work of others suggest that exercise intensity determines the impact of training on the course of EAE and that the optimal endurance training intensity in EAE is probably near the anaerobic threshold.

3.4. Limitation animal studies

Study 4 and 5, as well as the work of others suggested that the optimal exercise intensity is near the anaerobic threshold to induce improvements of the investigated parameters in EAE rats. However, during the present studies lactate concentrations and/or VO_2 kinetics were not measured. As such, true exercise intensity was difficult to quantify.

The lack of an explicit exercise effect, could, possibly, be explained by the short duration of the applied exercise program. Furthermore, aerobic exercise was perhaps not the optimal exercise modality to induce improvements of the investigated parameters in EAE rats. Therefore, it is suggested to investigate the long term effect of physical exercise in chronic EAE and the influence of resistance training on muscle contractile properties.

Furthermore, since forced treadmill running can induce stress, which could influence the EAE symptoms and muscular adaptations, it is recommended to measure stress hormone levels in future research.

4. Recommendations and future perspectives

Based on the results obtained during the course of this PhD project, it has become evident that some aspects of MS rehabilitation are still to be investigated. In the following recommendations for future research are given.

First, based on the results obtained during the present PhD project it is clear that the prevalence of IGT in MS is higher compared to matched referent subjects. Given the above described new findings and given the fact that only some of the cardiometabolic risk factors were already researched retrospectively in MS patients or reported as secondary outcome measures in studies not specifically designed to evaluate these risk factors, it is, at present, difficult to estimate the overall cardiometabolic risk state of MS patients. The cardiometabolic risk state clusters several CVD risk factors, such as dyslipidaemia, hypertension, elevated body fat, glucose intolerance/IR, heart function/autonomic dysfunction and inflammation. To our knowledge, we were the first who investigated IGT in MS as a primary outcome measure. Furthermore, preliminary results (in collaboration with Prof. Jerome Hendriks) showed a smaller HDL and LDL particle size and a disturbed HDL-LDL particle count in MS patients. In accordance with the work of others [73, 75, 172, 175, 176], we reported similar total body fat percentages and total body lean tissue mass in MS patients and healthy controls and a slightly higher fat percentage and lower lean tissue mass in the lower extremities of MS patients [297]. The existing data on hypertension in MS is limited and inconclusive with studies reporting either similar, increased or decreased prevalence of hypertension when compared to healthy controls [64, 67, 68, 74, 180-185]. Due to the multifocal demyelination that also affects the autonomic nervous system (ANS), autonomic dysfunction is not rare in MS, as also reported by our research group [186, 298-303]. Interestingly, elevated blood glucose concentrations, that occurs during IGT, can also cause abnormalities of the ANS, as reported in type II diabetes patients [304], causing damage to, predominantly, the parasympathetic fibers (such as n.vagus), leading to a predominance of the sympathetic nervous system. This results in disturbances in blood pressure and heart rate, worsening of glycaemic control, altered lipolysis and basal metabolic rate, development of diabetic cardiomyopathy, and/or reduced exercise capacity [305, 306]. The relation between autonomic dysfunction, MS and cardiometabolic risk factors, however, was never demonstrated. Another factor contributing to the cardiometabolic risk state is inflammation [307-309], inducing peripheral IR, hypertension, type II diabetes [310-312], disturbed lipid metabolism [313] and playing a key role in the CVD development [314]. Interestingly, in MS, inflammation of the CNS is believed to be the primary cause of demyelination, induced by disturbances of the expression of pro-

and anti-inflammatory cytokines and chemokines [16]. The relation between inflammation, MS and the cardiometabolic risk state is not clear. The combined prevalence of all these risk factors was never investigated before in MS and warrants further research. Due to the progressive nature of the disease, mirrored by an increase of EDSS, inactivity level, age and medication use, it could be hypothesized that the cardiometabolic risk increases as the disease progresses. In this respect, MS patients should be tested regularly, since we need to advise MS patients about preventable comorbidities. Therefore, rehabilitation programs should not only focus on physical impairments, but also on inactivity-induced secondary health complications, which are often reversible or even preventable. In particular, we advise to exercise on prescription, based on a risk screening and individual needs or capacities.

Second, increasing evidence favours exercise therapy as an efficient method for overall symptom management in MS [212], reporting improvements after low-to-moderate intensity cardiovascular or resistance training [12-14, 55, 111]. Given the fact that higher intensity exercise is well tolerated and renders better rehabilitation outcomes, it is warranted to apply supervised and well guided higher intensity exercise.

Third, The present work reported reduced muscle strength and muscle mass and decreased muscle fiber CSA of the lower limbs of MS patients, emphasising the need for rehabilitations programs that also focus on muscle preservation and/or rebuilding of muscle mass. To counteract these impairments, we recommend to use progressive resistance training in MS rehabilitation. In addition, to improve endurance capacity, high intensity interval training was very effective and could also be employed in MS rehabilitation.

Finally, at present it is clear that supervised exercise therapy in controlled research settings improves several MS symptoms. To reduce the costs of supervised exercise programs in a research/clinical setting, individualized home-based exercise may be effective [118]. However, it is unclear whether home-based rehabilitation protocols are also able to improve MS symptoms and/or are able to maintain obtained rehabilitation results. Furthermore, despite the improvements of aerobic fitness, muscle strength, glycaemic control and weight management [315-321] and despite the association between adherence to exercise and the reduced incidence of CVD in other populations, motivation and compliance of the participants are often suboptimal [316]. Nevertheless, based on our own experience and patient contacts it is clear that MS patients need follow up after termination of a supervised study exercise program.

General conclusion

In conclusion, this PhD thesis demonstrated that the prevalence of IGT in MS is higher compared to healthy subjects. Furthermore, this secondary healthy complication, as well as some clinical relevant aspects such as muscle strength, muscle mass and endurance capacity, can be improved by means of combined endurance and resistance exercise, whereas the level of improvements are dependent on the applied exercise intensity.

MS also seems to negatively affect skeletal muscle fiber characteristics, muscle strength and muscle mass of, predominantly, the lower limbs of mildly affected MS patients. This can be improved by high intensity combined exercise, that was demonstrated to be safe and well tolerated in moderately affected MS patients.

These results are clinically relevant because, similar to other populations, adequate exercise therapy is not only able to improve important aspects of health related fitness but is also able to counteract secondary health complications. In combination with other therapeutic strategies this probably further enhances quality of life and physical functioning of MS patients.

Chapter IV:

Related research activities

The work presented in this PhD also resulted in several co-author publications, investigating exercise-onset VO_2 kinetics and heart rate, as well as walking capacity, autonomic control and ventilation-perfusion mismatch during or after exercise.

Hansen D, **Wens I**, Kosten L., Verboven K., Eijnde BO. Slowed exercise-onset VO_2 kinetics during submaximal endurance exercise in subjects with multiple sclerosis.
Neurorehabil Neural Repair. 2013 Jan;27(1):87-95.

Peter Feys, Deborah Severijns, Stefanie Vantenderloo, Kathy Knuts, Dennis Hannes, Domien Gijbels, **Inez Wens**. Changes in spatio-temporal gait parameters according to speed instructions and walking history are dependent on level of ambulatory dysfunction: a study in MS.
Multiple Sclerosis and Related Disorders, July 2013; 2(3):238-246

Hansen D, **Wens I**, Dendale P, Eijnde BO. Exercise-onset heart rate increase is slowed in multiple sclerosis patients: does a disturbed cardiac autonomic control affect exercise tolerance?
NeuroRehabilitation. 2013;33(1):139-46

Dominique Hansen, Peter Feys, **Inez Wens**, Bert O Eijnde. Is walking capacity in subjects with multiple sclerosis primarily related to muscle oxidative capacity or maximal muscle strength? A pilot study
Multiple Sclerosis International, 2014;2014:759030. Epub 2014 Jan 29.

Baert I., Freeman J., Smedal T., Dalgas U., Romberg A., Kalron A., Conyers H., Elorriaga I., Gebara B., Gumse J., Heric J., Jensen E., Jones K., Knuts K., Maertens B., Martic A., Normann B., Op 't Eijnde B., Rasova K., Santoyo Medina C., Truyens V., **Wens I**., Feys P. Responsiveness and clinically meaningful improvement, according disability level, of five walking measures after rehabilitation in multiple sclerosis: a European multi-center study.
Neurorehabilitation and Neural Repair, 2014 Feb 6. [Epub ahead of print]

Hansen D, **Wens I**, Keytsman C., Eijnde BO, Dendale P. Is long-term exercise intervention effective to improve cardiac autonomic control during exercise in subjects with multiple sclerosis? A randomized controlled trial.
European Journal of Physical and Rehabilitation Medicine, 2014 Mar 6. [Epub ahead of print]

Dominique Hansen, **Inez Wens**, Charly Keytsman, Kenneth Verboven, Paul Dendale, Bert O Eijnde. Patients with multiple sclerosis suffer from ventilation-perfusion mismatch during exercise: impact of exercise intervention
Under review

Bert O Eijnde, Charly Keytsman, **Inez Wens**, Dominique Hansen. Is an improved exercise tolerance during whole-body cooling related to improved muscle oxidative capacity in subjects with multiple sclerosis?
Under review

Dominique Hansen, **Inez Wens**, Frank Vandenabeele, Kenneth Verboven, Bert O Eijnde. Altered AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) phosphorylation in skeletal muscle of patients with multiple sclerosis.
In preparation

Chapter V:

Nederlandstalige samenvatting

Multiple Sclerose (MS) is een progressieve auto-immune aandoening van het centrale zenuwstelsel en wordt meestal gediagnostiseerd tussen de leeftijd van 20 en 40 jaar. Wereldwijd worden er meer dan 2,5 miljoen mensen geconfronteerd met deze ziekte. Ondanks intensief wetenschappelijk onderzoek is de onderliggende oorzaak van MS nog steeds niet volledig gekend. De variabele distributie van de schade in de myeline schede van de zenuwen kan leiden tot zeer uiteenlopende symptomen, waaronder spierzwakte en vermoeidheid. Als gevolg van deze laatstgenoemde symptomen zijn mensen met MS vaak minder fysiek actief wat resulteert in een verlies aan functionele spierkracht en inspanningscapaciteit. In andere populaties werd reeds herhaaldelijk aangetoond dat een verminderde hoeveelheid fysieke activiteit in belangrijke mate bijdraagt tot de ontwikkeling van chronische aandoeningen en het ontstaan van risico factoren die deel uitmaken van het metabole syndroom, een term die de gecombineerde aanwezigheid van verschillende cardiovasculaire risico's, zoals verhoogde bloeddruk, glucose intolerantie en hoge cholesterol, groepeert. Zodoende komen verstoorde glucose tolerantie en insuline resistentie frequenter voor, waardoor het risico voor de ontwikkeling van type II suikerziekte en cardiovasculaire aandoeningen sterk toeneemt. Op basis van een systematische literatuur analyse kon in **studie 1** worden geconcludeerd dat MS patiënten inderdaad een verhoogd risico hebben op de ontwikkeling van secundaire gezondheidsproblemen, zoals cardiovasculaire aandoeningen en het metabole syndroom in het algemeen. De methodologie van de geïncludeerde literatuur was echter meestal van een beperkte kwaliteit, waardoor het onduidelijk is waar het verhoogde risico aan toegeschreven kan worden. In een poging enkele van deze vragen te beantwoorden werd dit doctoraatsonderzoek in belangrijke mate gericht op het voorkomen en remediëren van glucose intolerantie in MS. Dit werd onderzocht in een reeks van studies, uitgevoerd bij zowel het MS proefdiermodel als bij mensen met MS. In het onderstaande wordt een beknopt overzicht gegeven van de aard en belangrijkste resultaten van de uitgevoerde studies.

De glucose tolerantie van 81 MS-patiënten werd vergeleken met deze van 45 gezonde controle personen in **studie 2**. In deze studie werd aangetoond dat de prevalentie van glucose intolerantie verhoogd is in personen met MS, in vergelijking met deze van de gezonde controle personen. Verschillende factoren, zoals het type MS, de ernst van de ziekte, het gebruik van medicatie en fysieke inactiviteit, kunnen de ontwikkeling van glucose intolerantie induceren en dienen in de toekomst verder onderzocht te worden.

Ondanks het progressieve karakter van MS leeft meer dan 80% van de patiënten meer dan 35 jaar met de ziekte. Recent ontwikkelde farmacologische therapieën kunnen de voortgang van de ziekte vertragen, maar tot op de dag van vandaag is MS niet geneesbaar. Daarom is revalidatietherapie een belangrijke factor in de behandeling van MS. Hierbij is het belangrijk op te merken dat nieuwe wetenschappelijke bevindingen de MS revalidatie drastisch veranderd hebben gedurende de laatste jaren. Verschillende studies hebben reeds aangetoond dat MS revalidatietherapie in staat is om, onder andere, de spierkracht, het uithoudingsvermogen, de functionele capaciteit en de kwaliteit van leven te verbeteren. Aangezien fysieke activiteit, in andere populaties, reeds wordt aangewend voor de verbetering van insuline resistentie en glucose intolerantie en het verlagen van abdominaal vet en cholesterol, zou fysieke activiteit een belangrijk niet-farmacologische interventie kunnen zijn voor de bestrijding van secundaire gezondheidsproblemen bij personen met MS. In **studie 3** werd daarom de invloed van een 24 weken durend matig intens gecombineerd revalidatieprogramma onderzocht op glucose intolerantie in MS. Daarnaast werd ook de invloed van deze training onderzocht op spierkracht, uithoudingsvermogen en lichaamssamenstelling. Het matig intens gecombineerde revalidatie programma was niet in staat de glucose tolerantie te verbeteren na 24 weken, ondanks de verbetering van de spierkracht en het uithoudingsvermogen en de verandering van de lichaamssamenstelling.

Verder werd in andere populaties reeds gesuggereerd dat matig intense fysieke activiteit vaak niet voldoende is voor de verbetering van glucose intolerantie. Intense fysieke activiteit daarentegen geeft vaak betere resultaten. Aangezien hoge intense fysieke activiteit nooit eerder werd onderzocht in MS, werd het gebruik van een intens programma eerst geëvalueerd in het MS dierenmodel, EAE. **Studie 4 en 5** hebben aangetoond dat hoge intense fysieke activiteit de start van de verlammingssymptomen van de EAE ratten kan vertragen. Bovendien was het gebruik van deze hoge intensiteitstraining veilig en werden er geen neveneffecten gerapporteerd. Gebaseerd op de bevindingen van studie 4 en 5 werd het effect van hoge intensiteitstraining op glucose intolerantie in personen met MS onderzocht in **studie 6**. Deze studie toonde aan dat de glucose tolerantie kon verbeterd worden na een hoog intens gecombineerd programma, wat suggereert dat de verbetering van glucose tolerantie in MS intensiteitsafhankelijk is. Dit programma induceerde ook verbeteringen van de spierkracht, het uithoudingsvermogen en de lichaamssamenstelling. Deze verbeteringen waren vaak groter dan deze gerapporteerd na het matig intense programma, wat suggereert dat de verbeteringen van sommige MS symptomen ook intensiteit gerelateerd zijn. Bovendien toonden wij aan dat het gebruik van een intens trainingsprogramma veilig is voor personen met MS.

Aangezien mensen met MS vaak spier gerelateerde symptomen ervaren werd tijdens dit project het spierweefsel van 34 mensen met MS en 18 gezonde controle personen eveneens onderzocht. Tijdens **studie 7** werden hiervoor spierbiopten genomen en werd er aangetoond dat MS de contractiele kenmerken van skeletspieren negatief beïnvloedt, door het verkleinen van de cross-sectionele doorsnede van de spiervezels en het verminderen van de spierkracht en spiermassa van de onderste ledematen. Ondanks de nood om het effect van fysieke activiteit op skeletspier karakteristieken volledig te begrijpen, werd dit nog niet intensief nagegaan. Daarom werd in **studie 8** de invloed van hoog intens gecombineerde training onderzocht op de spierkarakteristieken van personen met MS. Deze studie toonde aan dat hoge intensiteitstraining de spierkarakteristieken gevoelig kan verbeteren, door het vergroten van de cross-sectionele doorsnede van de spiervezels en het toenemen van de spierkracht en spiermassa.

De resultaten van dit onderzoek zijn klinisch relevant, aangezien revalidatie therapie niet enkel in staat is om belangrijke MS symptomen, maar ook secundaire gezondheidsproblemen, af te remmen en/of te verbeteren. In combinatie met andere therapeutische strategieën kan dit verder de kwaliteit van leven en het fysiek functioneren van personen met MS verbeteren.

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Curriculum Vitae

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2. University degrees:

Master Bio Science engineering (master in de Bio-ingenieurs wetenschappen)
Leuven University, Belgium
Graduated: June 2010

3. Scientific career:

PhD
Start: November 2010
End: June 2014
Title: Exercise therapy in multiple sclerosis: the impact of exercise intensity on glucose disposal and muscle contractile properties
Promotor: Prof. Dr. Bert Op 't Eijnde
Co-promotor: Prof. Dr. Ulrik Dalgas

4. International experiences:

12-14 March 2012:
Systematic literature search
Library Aarhus University, Denmark (Prof. Dr. Ulrik Dalgas)

August – September 2012:
Muscle research (EAE rat samples)
Section of Sport Science, Dep. Public Health, Aarhus University, Denmark (Prof. Dr. Ulrik Dalgas)

12 and 19 march 2013:
How to perform human muscle biopsies
Faculty of Health, Medicine, and Life Sciences, Universiteit Maastricht, The Netherlands (Prof. Dr. Johan Jocken)

August – September 2013:
Muscle research (muscle biopsies MS patients)
Section of Sport Science, Dep. Public Health, Aarhus University, Denmark (Prof. Dr. Ulrik Dalgas)

August 2014 (planned):
New techniques for muscle research (muscle biopsies MS patients), discussion/start postdoc
Section of Sport Science, Dep. Public Health, Aarhus University, Denmark (Prof. Dr. Ulrik Dalgas)

5. Scientific awards:

First price (poster): (300€). 17th annual conference of Rehabilitation in Multiple Sclerosis (RIMS), May 2012, Hamburg, Germany

Nominated top score poster: 29th Congress of the European Commitees for Treatment and Research in Multiple Sclerosis, 2 - 5 October 2013, Copenhagen, Denmark

6. Travel grants and other awards:

International mobility grant 2012 Hasselt University (1000€)

International mobility grant 2013 Hasselt University (500€)

Travel grant + free registration ECTRIMS/RIMS 2013, Copenhagen (620€)

International mobility grant 2014 Hasselt University (500€)

7. Additional skills

Authentic networking: 27/3/2014, Universiteit Hasselt, Diepenbeek

Career Coaching: 17/3 – 31/3 – 29/4 – 20/5/2014

How to Write a Winning Grant Proposal: 2-3/12/2013, VIB, Leuven

Basic statistics: 6-7-8/11/2012 - 13/11/2012 – 20-22/11/2012 – 27/11/2012 – 13/12/2012, KULeuven

Effective scientific communication: 20/9/2012 – 12/10/2012 – 16/10/2012 – 19/10/2012 – 30/10/2012, Universiteit Hasselt, Diepenbeek

Academic English: 14/5/2012 – 21/5/2012 – 4/6/2012 – 18/6/2012 – 25/6/2012, Universiteit Hasselt, Diepenbeek

Organisation symposium "Cytokines and cell trafficking in immunological disorders": 9/2/2012, UHasselt

Good scientific conduct : 22/11/2011, Universiteit Hasselt, Diepenbeek

Lab book taking: 22/11/2011, Universiteit Hasselt, Diepenbeek

Project management: 6/10/2011 - 27/10/2011 - 17/11/2011 - 1/12/2011 - 14/12/2011, Universiteit Hasselt, Diepenbeek

Parametric and non-parametric statistical methods for the life science, part I and II: 7/6/2011, Universiteit Hasselt, Diepenbeek

Biosafety: 25/5/2011, Universiteit Hasselt, Diepenbeek

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Publications

First author papers:

Wens I, Dalgas U, Stenager E, Eijnde BO. Risk factors related to cardiovascular diseases and the metabolic syndrome in multiple sclerosis - a systematic review.
Mult Scler 2013;19(12):1556-1564.

Wens I., Dalgas U., Deckx N., Cools N., Eijnde BO. Does Multiple sclerosis affect glucose tolerance?
Mult Scler, 2013 Dec 17. [Epub ahead of print].

Wens I., Broekmans T., Hendriks JJ., Savelberg HH., Hesselink MK, Eijnde BO. Muscle contractile properties and disease progress in EAE rats: impact of training intensity.
Under review, BMC Physiology

Wens I., Dalgas U., Verboven K., Kosten L., Stevens A., Hens N., Eijnde BO. Impact of high intensity exercise on muscle morphology in EAE rats.
Under review, Physiological Research

Wens I, Hansen D, Verboven K, Deckx N, Kosten L, Stevens A, Cools N, Eijnde BO. The impact of 24 weeks combined exercise on glucose tolerance in MS patients.
Under review, American Journal of Physical Medicine and Rehabilitation

Wens I., Dalgas U., Vandenabeele F., Grevendonk L., Eijnde BO. Does multiple sclerosis affect skeletal muscle characteristics?
Under review, Plos One

Wens I., Dalgas U., Vandenabeele F., Verboven K., Hansen D., Eijnde BO. High intensity exercise in multiple sclerosis: effects on muscle contractile characteristics and exercise capacity.
Under review, Physical Therapy

Wens I., Verboven K., Hansen D., Deckx N, Cools N, Eijnde BO. Impact of high intensity exercise on glucose tolerance in Multiple Sclerosis.
Under review, Metabolism

Co-author papers:

Kint CI, **Wens I**, Verstraeten N, Liebens VR, Fauvart M, Michiels J. Elucidating the structure-function relationship of a bacterial GTPase in DNA replication through random mutagenesis.
Commun Agric Appl Biol Sci 2011.

Kint CI, Verstraeten N, **Wens I**, Liebens VR, Hofkens J, Versées W, Fauvart M, Michiels J. The Escherichia coli GTPase ObgE modulates hydroxyl radical levels in response to DNA replication fork arrest.
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Hansen D, **Wens I**, Kosten L., Verboven K., Eijnde BO. Slowed exercise-onset VO2 kinetics during submaximal endurance exercise in subjects with multiple sclerosis.
Neurorehabil Neural Repair. 2013 Jan;27(1):87-95.

Peter Feys, Deborah Severijns, Stefanie Vantenderloo, Kathy Knuts, Dennis Hannes, Domien Gijbels, **Inez Wens**. Changes in spatio-temporal gait parameters according to speed instructions and walking history are dependent on level of ambulatory dysfunction: a study in MS.
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NeuroRehabilitation. 2013;33(1):139-46

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Multiple Sclerosis International, 2014;2014:759030. Epub 2014 Jan 29.

Baert I., Freeman J., Smedal T., Dalgas U., Romberg A., Kalron A., Conyers H., Elorriaga I., Gebara B., Gumse J., Heric J., Jensen E., Jones K., Knuts K., Maertens B., Martic A., Normann B., Op ‘t Eijnde B., Rasova K., Santoyo Medina C., Truyens V., **Wens I**., Feys P. Responsiveness and clinically meaningful improvement, according disability level, of five walking measures after rehabilitation in multiple sclerosis: a European multi-center study.
Neurorehabilitation and Neural Repair, 2014 Feb 6. [Epub ahead of print]

Hansen D, **Wens I**, Keytsman C., Eijnde BO, Dendale P. Is long-term exercise intervention effective to improve cardiac autonomic control during exercise in subjects with multiple sclerosis? A randomized controlled trial.
European Journal of Physical and Rehabilitation Medicine, 2014 Mar 6. [Epub ahead of print]

Bert O Eijnde, Charly Keytsman, **Inez Wens**, Dominique Hansen. Is an improved exercise tolerance during whole-body cooling related to improved muscle oxidative capacity in subjects with multiple sclerosis?
Under review

An LM Stevens, Dominique Hansen, Lieven Herbots, **Inez Wens**, An Creemers, Paul Dendale, Bert O Eijnde. Exercise training improves glucose tolerance in stable chronic heart failure patients.
Under review

Dominique Hansen, **Inez Wens**, Charly Keytsman, Kenneth Verboven, Paul Dendale, Bert O Eijnde. Patients with multiple sclerosis suffer from ventilation-perfusion mismatch during exercise: impact of exercise intervention
Under review

Dominique Hansen, **Inez Wens**, Frank Vandenabeele, Kenneth Verboven, Bert O Eijnde. Altered AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) phosphorylation in skeletal muscle of patients with multiple sclerosis.
In preparation

Published abstract

First author abstracts:

Wens I., Hansen D., Eijnde BO. The impact of 24 weeks of combined cardiovascular and strength training on glucose tolerance, muscle strength and aerobic capacity in persons with multiple sclerosis.
Mult Scler 2012, 18(5), S35-S36

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Mult Scler 2013, 19(11), 311-312

Co-author abstracts:

Severijns D., Kerkhofs L., **Wens I.**, Feys P. Fatigability of hand grip strength in persons with multiple sclerosis.
Mult Scler 2012, 18(5), 31

Ilse Baert, Jennifer Freeman, Tori Smedal, Ulrik Dalgas, Anders Romberg, Helen Conyers, Iratxe Elorriaga, Benoit Gebara, Johanna Gumse, Adnan Heric, Ellen Jensen, Kari Jones, Alon Kalron, Kathy Knuts, Benoît Maertens, Andrej Martic, Britt Normann, Kamila Rasova, Carmen Santoyo Medina, Veronik Truyens, **Inez Wens**, Peter Feys. Responsiveness and clinically meaningful improvement, according disability level, of five walking measures after rehabilitation in multiple sclerosis: a European multi-center study.
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Co-author presentations: (presented by others)

Nathalie Deckx, **Inez Wens**, Amber Nuyts, Barbara Stein, Zwi Berneman, Viggo Van Tendeloo, Bert Op 't Eijnde, Nathalie Cools. Modulatory effect of physical exercise on immunological mediators of autoimmunity. WOG-MS meeting, Brussels, Belgium, 1/12/2011

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