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Chapter 1

General introduction

Multiple Sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system that is diagnosed in more than 2 million people worldwide (~10.000 in Belgium). This neurodegenerative autoimmune disease affects the central nervous system (CNS) causing inflammatory lesions in both brain and spinal cord that lead to demyelination and axonal damage/loss in the CNS[1, 2]. This causes a wide spectrum of primary symptoms such as visual/sensory loss, ataxia, limb weakness, but also impaired mobility and cognition. The onset of this disease usually occurs between the age of 20 and 40 years and affects more women (2/3) than man (1/3). Although the exact etiology of MS is still unknown, various environmental (smoking, obesity, low vitamin D, sunlight exposure, toxins) and genetic risk factors appear to be involved[3-5].

Although disease-modifying medication to date reduces the frequency of partial to fully reversible episodes of neurologic disability and limit the accumulation of CNS lesions, MS can still not be cured[2, 5, 6]. Therefore, other treatment strategies that remediate/reduce MS related symptoms and disabilities have become integral parts of overall MS treatment. Such strategies typically involve multidisciplinary various (para)medical approaches including neurology, physiotherapy and occupational therapy that affect a wide range of primary MS related symptoms and secondary complications. Interestingly and similar to other disease populations, secondary consequences include e.g. reduced exercise capacity and muscle strength that are inactivity related and therefore potentially 'treatable' (symptom modifying) by exercise therapy. The effect size of exercise therapy in MS however appears to depend on the intensity of the applied training regimen. Consequently and to further optimize exercise rehabilitation in MS, optimal exercise therapy intensities for MS are currently under investigation.

In keeping with the above described general context, the following paragraphs introduce the pathogenesis of MS, its clinical course, various MS related symptoms, MS treatment and a frequently used animal disease model. Hereafter, we present an overview of various exercise therapy modes that are currently investigated/applied in MS rehabilitation and finally two exercise therapy optimization approaches are introduced.

1. Pathogenesis

Inflammatory processes, accompanied by the formation of inflammatory MS lesions, are believed to be the main pathological characteristics in the early phase of this disease. In the following progressive phase, widespread neurodegeneration is considered to be the main disease mechanism.

Both mechanisms of inflammation and neurodegeneration are described in detail below but it is important to note that until today many important aspects of the MS pathogenesis remain largely unknown[2, 7, 8].

Inflammation

MS is an immune-mediated disease in which inflammatory processes within the immune system result in MS-related nerve-tissue injuries[9]. This inflammation is believed to be caused by autoreactive T cells that become activated in the systemic circulation[5, 6, 9]. Under normal conditions, the CNS is separated from the systemic circulation by the blood brain barrier (BBB) that prevents infiltration of these immune cells into the CNS. However, in MS, degradation of the BBB allows migration of these autoreactive T cells throughout the CNS. Once they have entered the CNS, these autoimmune T cells (Th1 and Th17) are reactivated by antigen presenting cells such as microglia, which then trigger infiltration and migration of macrophages, B cells and cytotoxic CD8+ T cells[2, 10-12]. Although Th1 and Th17 are believed to be the main inducers of this disease, tissue damage is probably mainly

caused by CD8+ T cells that recognize antigens of the major histocompatibility complex (MHC) class I that is expressed during inflammation of oligodendrocytes and neurons, and eventually destruct these neurons[10, 13]. Another disease mechanism involves cytokines that coordinate the entire immune response. In order to maintain homeostasis, pro- and anti-inflammatory cytokines in the CNS and the periphery should be balanced. However in MS, the antigen-presenting cells and reactivated T cells secrete several cytokines in the CNS that may induce an immune response. As such, it is believed that the balance between pro-inflammatory (Th1) and anti-inflammatory (Th2) cytokines in MS is disturbed[14]. Typically, the pro-inflammatory immune response is induced by IL-12, IFN-γ and TNF-α, whilst IL-6 and IL-10 evoke an anti-inflammatory response. Indeed, elevations of IFN-γ, TNF-α, IL-12, IL-6 and IL-10, of which the latter two are of great importance to counteract the elevated pro-inflammatory cytokines, have been reported[14, 15]. However, contradictory literature exists, reporting reductions of several of these pro-inflammatory cytokines in MS, making it difficult to completely understand the pathogenesis of MS. Nevertheless, more recent insights show that IL-17, which produces Th17, is also able to trigger autoimmune inflammation[16]. To date, it is thus clear that both Th1 and Th17 are crucial in the pathogenesis of MS as they and their corresponding cytokines, which exhibit an important role in autoimmunity, are present at the MS inflammation sites[16, 17].

Neurodegeneration

Next to the inflammatory processes that induce nerve-tissue damage, neurodegeneration is considered to be a significant contributor of disability in MS[18, 19]. In this regard, oligodendrocytes apoptosis and axonal pathology, and more specifically axonal swelling and transection that cause neurodegeneration, have been described in MS lesions[20, 21]. Furthermore, MS is not only characterized by white matter demyelination but also by extensive axonal loss and gray matter disturbances that all contribute to

the various primary MS symptoms[8, 21]. Interestingly, brain plasticity is able to compensate this observed neuronal loss, causing only minor clinical disabilities, at least in the early stage of the disease. Nevertheless, in the following progressive phase, neural plasticity is no longer able to restore these disabilities finally resulting in an irreversible neurological decline[22].

2. Clinical disease course

The clinical course of MS can be divided into five subtypes (Figure 1)[1, 23, 24]. **(1)** Relapsing-remitting MS (RMMS) is the most common form of MS (~85%) and is defined by a relapsing disease course where periods between relapses (with full or partial recovery) are characterized by a lack in disease progression. **(2)** Primary progressive (PPMS) is the second most common form of MS (~10%) and involves a more gradual increase of clinical signs or symptoms throughout time. **(3)** Secondary progressive (SPMS) is the third form of MS and is the result of the transition from relapsing-remitting to the progressive form of MS (80%). The initial relapsing-remitting disease course is followed by gradual progression, with or without episodic relapses, plateaus and minor remissions. **(4)** The fourth type is progressive relapsing (PRMS, <5%) which includes a progressive disease course with an onset of acute relapses, with or without full recovery. Here, periods between relapses show continuous progression of disability. **(5)** A fifth, though rare, type is the benign MS (<1%), where all neurologic systems remain fully functional up to fifteen years after onset of the disease.

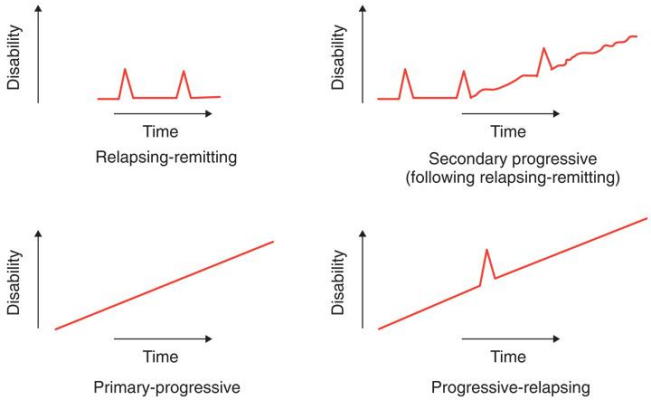

Figure 1. Clinical disease courses of MS

3. An animal MS model: Experimental Autoimmune Encephalomyelitis

Very often new therapeutic approaches are first explored/applied using animal disease models. In MS, Experimental Autoimmune Encephalomyelitis (EAE)[25] is a frequently used animal model with either an acute experimental disease model (inducing only one disease exacerbation and full recovery, Figure 2) or a chronic model (with a more chronic symptomatic disease course and partial recovery, Figure 3). These models induce key pathological features of MS such as inflammation, demyelination and axonal loss. Therefore, EAE is a useful alternative to investigate demyelinating diseases of the CNS, such as MS[26]. In contrast to MS however, EAE requires external immunization via myelin antigen injection to gradually develop CNS inflammation and axonal damage. Briefly, after induction of EAE, an inflammatory period without clinical symptoms occurs (day 0-10), followed by gradual, acute and monophasic hindquarter paralysis (day 11-14). Paralysis starts at the tip of the tail, progresses to the trunk, and is scored from 0 (no clinical signs) to 5 (death)[27]. In the acute EAE model, hindquarter paralysis is than followed by almost full recovery (day 15-17). The chronic EAE model however, is characterized by

a chronic disease course were animals experience one exacerbation and then remain symptomatic for the following experimental period, although at lower levels compared to the peak score (~day 18)[25, 26, 28].

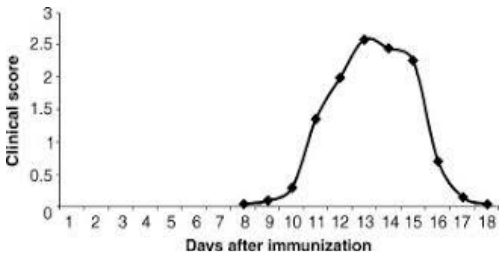

Figure 2. Acute EAE model by Muhallab et al. 2002

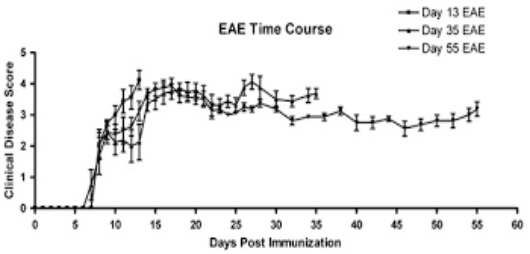

Figure 3. Chronic EAE model by Ziehn et al. 2010. Reprinted with permission of the authors.

4. MS diagnosis and disease severity

Diagnosis of MS is based on the internationally accepted McDonald criteria[29] and is preferably performed by an experienced neurologist. Diagnosis involves MRI, spinal fluid and evoked potentials measurements to quantify white matter disease activity over time. In order to describe disease progression of MS patients, several instruments/scales have been developed. Here, the most common and widely accepted scale is the Expanded Disability Status Scale (EDSS) first described by Kurtzke[30]. This assessment scale evaluates the functional systems of the CNS and describes clinical severity and functional deficits of MS patients. It consists of an ordinal scale ranging from 0 (normal neurological status) to 10 (dead due to MS). The EDSS is often used as an outcome measure in clinical trials to assess effectiveness of therapeutic interventions. Throughout this PhD project, MS patients with EDSS<6 were included. This scores indicates that patients are ambulant and still mobile without the need for an assistive device.

5. MS symptoms

The scattered demyelination nerve lesions that are caused by the inflammatory processes throughout the CNS, induce a wide range of clinical symptoms that typically depend on the lesion site. These symptoms are often defined as primary MS symptoms[2]. In the relapsing-remitting form, these symptoms include sensory disturbances, unilateral optic neuritis, ataxia, clumsiness, diplopia, bladder and bowel problems[1, 2]. Progressive MS on the other hand is characterized by slow evolving upper-motor neuron symptoms such as cognitive decline (impaired attention, memory loss) visual loss (optic neuritis), quadriparesis, brainstem syndromes, sexual, cerebral, bowel and bladder dysfunctions[1, 2, 8]. Furthermore, disturbed autonomic control is often present in MS[31-34], causing gastrointestinal problems, inappropriate sweating and cardiovascular dysfunction. It is believed that autonomic dysfunction in MS is caused by the demyelinating plaques, which damage the vasomotor centres in brainstem, but furthermore interfere with descending fibres of the autonomic nervous system in the spinal cord[31-34].

The above described primary MS symptoms very often also lead to various secondary functional consequences such as decreased exercise capacity[35-38] and reduced muscle strength[39-43] further decreasing functional capacity in MS[44, 45]. In combination with increased general fatigue this results in higher levels of inactivity compared to healthy controls[46] and thus an inactivity related physiological profile that causes even greater muscle weakness, increased fatigue and associated health risks independent of the disease process *per se.*

In healthy persons, such an inactive lifestyle is associated with an increased risk to develop cardiovascular diseases (CVD)[47, 48]. Risk factors associated with such an increased risk for CVD include hypertension, disturbed whole body glucose regulation, altered lipid profile and changes in body composition. Interestingly, it was previously shown that several of these CVD risk factors are elevated in MS compared to healthy controls[49, 50] and

that MS patients indeed have an increased risk for the development of CVD[51, 52]. However, it still remains unclear which specific CVD risk factors contribute to this increased risk for the development of CVD in MS[49]. Given the increased CVD related mortality in MS patients[51] it is very relevant to investigate CVD risk factors in this population.

6. MS treatment

Although disease-modifying medication clearly reduces the frequency of reversible (fully or partial) episodes of neurologic disability and limit the accumulation of white matter lesions, MS is currently incurable. Therefore, MS treatment to date typically consists of a multidisciplinary approach that involves neurologists, health workers, specialists in physical medicine and rehabilitation[1, 2] and is directed towards maintenance of the functional status of MS patients. As such, treatment strategies in MS consist of both pharmacological treatment (disease and symptom modifying) and rehabilitation/exercise therapy (symptom modifying).

6.1 Pharmacological treatment

Pharmacologic treatment of MS is divided into the management of acute episodic relapses and disease-modifying treatment. To treat acute relapses, glucocorticosteroids are used to minimize the relapse duration and relapse-related symptoms (symptomatic management). Nevertheless, long-term effects of glucocorticosteroids on disease activity are not clear, and in some cases not effective[53]. To date, the Food and Drug Administration (FDA) has approved 15 disease-modifying medications for MS. Five preparations of interferon-β; two of glatiramer acetate; the monoclonal antibodies natalizumab, alemtuzumab, daclizumab and ocrelizumab; and the oral agents fingolimod, dimethyl fumarate and teriflunomide. All these medications reduce the likelihood of developing new white-matter lesions, clinical relapses, and progressive accumulation of disability[2]. It is however

important to note that ocrelizumab, besides its positive effects in RRMS, is the only effective drug in the progressive form of MS. All other disease-modifying treatments are exclusively administered in the relapsing-remitting forms. Furthermore, several recently approved drugs (targeting histaminic, cholinergic and adrenergic pathways, and nuclear hormone receptor) are being tested for myelin protection and remyelination[2]. Although pharmacological treatment reduces the frequency of fully or partial reversible episodes of neurologic disability and limit the accumulation of white matter lesions, MS can still not be cured. Therefore, alternative treatment strategies that remediate or reduce MS related symptoms and functional disability, such as exercise therapy, are mandatory.

6.2 Rehabilitation and exercise therapy in MS

MS rehabilitation aims to improve physical independence, quality of life and social participation of individuals with this disease. To achieve this, a multidisciplinary approach involving occupational therapy, psychology and exercise therapy is used. Given the scope of the present PhD project however we will only discuss the latter. Exercise therapy aims to restore and improve secondary consequences and functional capacity in individuals with MS and hereby improve their quality of life. It is important to note that exercise therapy is especially of great importance in the (symptomatic) treatment of progressive MS, as it is known that only one sort of pharmacologic intervention (ocrelizumab) exists for this specific form. In general, exercise therapy contains training of balance, gait, transfers, coordination and mobilization together with endurance and/or strength training[54-56].

During the last two decades, exercise therapy has become an integral part of the clinical care and rehabilitation of MS patients. However, until ~2005 it was believed that the increase in body temperature caused by exercise in MS induced symptom instability and increased fatigue. Therefore, persons with MS were advised not to participate in physical exercise and even to limit physical activity to a minimum[39, 57]. However, since then it is clear that sensory symptoms following exercise therapy, that are experienced by more than 40% of all MS patients, are temporal and normalize within 30min after cessation of the activity in 85% of all patients[57]. Moreover, during the last decade the benefits of exercise therapy have clearly been demonstrated in MS[58-60], including improvements in walking capacity, symptoms of fatigue, depression, balance, cognitive function and parameters of functional capacity (e.g. exercise tolerance and muscle strength)[37, 61, 62], all contributing to an improved quality of life. It is important to note that to date exercise therapy in MS is considered save and well-tolerated. Furthermore and as indicated above the decreased activity level in MS, leading to inactivity related secondary symptoms are major rehabilitation topics that can be successfully addressed by exercise therapy interventions. Such exercise therapy interventions typically involve resistance training, that mainly stimulates muscular strength, or endurance training, that mainly stimulates the cardiorespiratory system[63]. Very recently, higher intensity exercise training in MS has also gained much attention[38, 64, 65].

6.2.1 Resistance training in MS

Resistance training induces physiological, morphological, biochemical and neural changes of the musculoskeletal system. More specifically, resistance training has beneficial effects on the daily activity level, systolic and diastolic blood pressure, isokinetic and isometric muscle strength, muscle fibre characteristics, bone mineral density, fatigue levels and risk for depression[39]. Although the benefits of resistance training in MS have been demonstrated in various studies[39], the low methodological quality (lack of

control groups, home-based intervention, different protocols) makes it difficult to formulate general guidelines for resistance training in MS, as stated before by Kjolhede et al[66]. Nevertheless, various resistance/strength training interventions (8-12w) have shown significant increases in muscle strength (+5-36%) and improved fatigue (8%), functional capacity (22%) and quality of life (8%) in MS[37, 66-70]. Therefore, strength training in the rehabilitation of MS patients appears to be a useful exercise therapy tool and is recommended.

6.2.2 Endurance training in MS

Endurance or cardiorespiratory exercise training has been researched extensively in MS reporting benefits on various functional, physiological and psychological parameters[35, 37, 71-73]. Such endurance training that typically uses low-to-moderate exercise intensities, improves the efficiency of the cardiorespiratory system by increasing the heart size, stroke volume, cardiac output and VO_2max in MS leading to an increased exercise capacity/tolerance which is a major determinant of functionality and quality of life. Furthermore, it induces lower blood lactate levels, higher anaerobic thresholds[39] and improves body composition[71]. Currently, recommendations regarding endurance training in MS include a training frequency of 2-3 sessions per week at an intensity of 50-70% $VO2_{max}$, corresponding to 60-80% of the maximal heart rate, with an initial duration of 10-40min, depending on the disability level of the MS patient. Progression during the first 2-6 months should be provided by increasing training volume (longer training duration or adding extra sessions)[39].

6.2.3 Concurrent training in MS

Combining both endurance and resistance exercise in one training session, often referred to as concurrent training, in other populations (e.g. elderly) has been shown to significantly improve body composition, muscle strength, overall health and cardiovascular fitness compared to endurance training alone[74-78]. Concurrent training in MS however has not been extensively investigated[79-81]. A recent standardized exercise intervention including 24 weeks of endurance and strength training however was able to improve quadriceps and hamstrings muscle strength (+23%), exercise capacity (+21%) and lean tissue mass (-2%) in MS patients (24w, 3x/w, 1x10 to 4x15 repetitions at maximal attainable load)[37]. Therefore, combining both endurance and resistance training in the rehabilitation of MS patients appears to positively affect the cardiovascular system and functional parameters as muscle strength and exercise capacity[38, 39].

6.2.4. High intense exercise therapy

Interestingly, it was already suggested that MS patients might also benefit from higher exercise intensities (e.g. interval training using intensities of up to 90% $VO2_{max}$)[39]. Indeed in 2015, Wens et al.[38] were the first to successfully implement high intensity exercise in MS patients. Not only did the high intense (90-100% maximal heart rate) training appear to be safe and well tolerated in MS, but when comparing the results of both studies, it also seemed to induce superior effects on functional parameters such as muscle strength (up to +45%) and exercise capacity (+17%) compared to moderate intensity training interventions[37]. This warrants further investigation and optimization of high intense exercise therapy in the rehabilitation of MS patients.

Exercise training outcome and efficiency is highly correlated to exercise modalities such as intensity and duration. Briefly, exercise intensity and duration are determinants of specific physiological alterations during exercise, and are inversely related: the higher the intensity, the shorter the duration[63]. As, in the general population, one of the most cited barriers for engagement in regular physical activity remains 'lack of time'[82-84], development of more time-efficient exercise strategies is warranted. As such, currently high intensity interval training (HIIT) is gaining more and more attention, not only in healthy subjects but furthermore in various disease populations. HIIT is described as (very) short sessions of physical activity characterized by brief, intermittent bursts of vigorous activity, interspersed by periods of rest/recovery of low-intensity exercise[85]. HIIT protocols are highly variable as the intensity (90-100% of the maximal heart rate, all-out), the number (1 to 5) and duration (6s-4min) of the intervals, as well as the duration of recovery periods (30s-4min) can constantly be adjusted. Interestingly, despite a significant reduced time commitment and low total exercise volume, HIIT has been shown to induce similar physiological remodelling compared to classic moderate intensity endurance training[86]. However, in order to integrate and optimize such high intense exercise therapy in the rehabilitation of MS, it is important to understand the exact physiological mechanisms that occur during this type of vigorous exercise.

During exercise activities skeletal muscles adequately respond to the demands of the central nervous system by providing contractions of the appropriate force and sequence in order to produce e.g. limb/body movements required for that specific exercise task. To do so, the skeletal muscle uses several pathways to provide sufficient energy under the form of adenosine triphosphate (ATP). The pathways of ATP provision during exercise are categorised as aerobic, requiring oxygen (O_2) and anaerobic, not requiring O_2. These mechanisms are often referred to as oxidative and substrate phosphorylation respectively. In both pathways, the muscle converts adenosine diphosphate (ADP) and inorganic phosphate (Pi) into

ATP. This ATP is then used to create shortening/contraction of the muscle fibres, resulting in the requested body movement. Although aerobic energy production is the most prevalent provision of ATP in the plurality of exercise modes, ATP provision at the onset of exercise and during high intense exercise modalities depends on anaerobic pathways. Anaerobic metabolism is defined as the ability of the muscular metabolic pathways to produce ATP, without the use of O_2. Sources of anaerobic ATP production are the degradation of phosphocreatine (PCr hydrolysis) and the processes of anaerobic glycogenolysis and glycolysis that typically result in the formation of lactate and H+ (byproducts of anaerobic glycolysis)[87, 88], which are important contributors to (subjective) fatigue during exercise. Interestingly, higher resting lactate concentrations have already been reported in MS compared to HC[89]. Whether MS patients also present higher lactate accumulation during (high intense) exercise however, is not known.

During high-intensity intermittent exercise, the anaerobic systems provide a significant portion of ATP, as the required power output during this type of exercise is very high, and the time for aerobic energy contribution is rather short[90, 91]. During 3 maximal sprints of 30s each, interspersed with 4min recovery, on a cycle ergometer, in the initial 6s of the first bout, PCr hydrolysis and anaerobic glycolysis provide equal amounts of ATP, whilst the aerobic metabolism contributes very little. Between 6s and 15s of the sprint, the contribution of PCr decreases, the provision of the anaerobic glycolysis remains stable (becoming the main contributor of ATP) and aerobic energy provision increases. In the final 15s, the aerobic metabolism becomes the dominant source of energy supply, whilst the contribution of anaerobic pathways strongly reduces. After such sprints, muscle PCr and glycogen content is reduced and both lactate and H^+ increases. Following a 4min recovery period, a second 30s sprint and a second 4min rest period, PCr stores recover and glycogen remains stable, whilst lactate and H^+ concentrations remain elevated. As such, prior to the third sprint, PCr is resynthesized and able to provide ATP again, whilst the byproducts of the anaerobic glycolysis of the previous two sprints are still present. As a

consequence, during the third sprint, the first 6s of energy are provided by PCr alone, with no contribution of anaerobic glycolysis. Furthermore, the aerobic metabolism significantly contributes more energy in the third bout compared to the first. These findings emphasize the inability of the anaerobic system to recover from extended bouts of high intense exercise, which is probably due to the extreme acidosis and fatigue in the muscle caused by the glycolytic byproducts (lactate and H^+). Following the above line of reasoning, we can thus conclude that during repeated bouts of high intense exercise, anaerobic (PCr and glycolysis) ATP provision is essential during the first seconds of exercise, followed by predominantly aerobic energy supply in the subsequent bouts of exercise[87].

Interestingly, it was shown that improvements in VO2 peak appeared to be correlated with the time spent at a high level of oxygen uptake[92]. Indeed, various studies have reported higher improvements (up to double the increase) of VO2 peak/max following HIIT interventions compared to classic moderate intensity continuous training[93-96]. It was even shown that with every 10% increase in exercise intensity, an increase of 1ml/kg/min in VO2 peak/max is achieved[96, 97]. Besides superior effects on functional measures[85, 94, 98] (exercise capacity and muscle strength) and quality of life[92, 96] in healthy subjects, obese and stroke/cardiac patients, HIIT has shown to positively affect various health related parameters such as body composition[93, 99, 100] (weight, subcutaneous and abdominal fat, waist circumference), blood glucose and insulin sensitivity[94, 95, 101], cardiac function[92, 102] (intrinsic heart pump activity, ventricular function, endothelial function), systolic and diastolic blood pressure[93, 100] and parameters of the blood lipid profile[96] (HDL, triglycerides). Furthermore, the higher VO2 peak/max following HIIT contributes to a higher cardiorespiratory fitness (CRF), which is associated with improved survival rates and decreased incidence of cardiovascular diseases or comorbidities including hypertension, diabetes and heart failure[93-96, 103]. As such, HIIT is able to both prevent and positively affect cardiovascular risk factors and secondary cardiovascular diseases in other populations.

6.2.5. High intense exercise therapy in MS

In MS, HIIT has gained more and more attention as it substantially enhances exercise capacity (+22%) and muscle strength (+44%)[38]. Furthermore, it has been shown to improve cognitive performance (+8%)[64] in this population, and moreover is related to improved functionality and quality of life[92, 96] in other diseases. As such, these few studies show that HIIT has the potential to become an integral part of MS rehabilitation. Nevertheless, more research regarding the potential of HIIT in MS is warranted because next to superior improvements in exercise capacity and functional parameters, it is currently not known whether HIIT is also able to improve health related parameters such as cardiovascular risk factors, in MS.

Despite these recent positive findings and the fact that HIIT appears to be save and well-tolerated in MS[38, 64, 65], the effective implementation of such high intense exercise interventions in actual rehabilitation programs is difficult. Probably, rehabilitation regimens consisting of high intensity exercise alone are too demanding (e.g. 1-5 maximal exercise bouts ranging from 6s to 4min interspersed by recovery periods of 30s–4min, 90-100% maximum heart rate) and thus require self-guidance and external motivational factors to adhere to longer term HIIT interventions especially in a patient population. As such, this may result in suboptimal HIIT performance and reduced therapy adherence, leading to decreased clinical exercise outcomes. Indeed, higher exercise intensities appear to negatively influence training adherence in patients, leading to decreased exercise output[104] and thus reduced clinical outcome. Therefore, any strategy that improves high intense exercise performance and feasibility/adherence in MS is worthwhile investigating.

6.3. Optimizing high intense exercise in MS

From the above it is clear that high intense exercise therapy appears to be a very promising new exercise mode that has the potential to become an integral part of MS rehabilitation. However, in order to facilitate integration of HIIT in current MS rehabilitation, further optimization of high intense exercise interventions, to improve both HIIT performance and feasibility in MS, is mandatory. In the exercise/sports community, high intense exercise performance is frequently optimized by ergogenic supplements and training periodization.

6.3.1. Optimizing high intense exercise performance in MS: muscle carnosine

In the high performance exercise/sports community ergogenic supplementation, and more specifically β-alanine supplementation which induces muscle carnosine loading, is a popular strategy that is used to improve HIIT performance. Carnosine, a dipeptide composed of β-alanine and L-histidine, is found in high concentrations in mammalian skeletal muscle[105]. Variants of carnosine are anserine and ophidine, the methylated analogs with the same bioactivity, which are exclusively found in animals. Other carnosine analogs are homocarnosine (β-alanine replaced by GABA), acetylcarnosine (β-alanine is acetylated) and carcinine (L-histidine is replaced by histamine). Together with anserine or ophidine/balenine, carnosine forms the histidine-containing dipeptides (HCD)[105]. The synthesis of carnosine, through β-alanine and L-histinde, is mediated by the enzyme carnosine synthase, which is situated in the skeletal and heart muscle, and certain brain regions. Methylation of carnosine, required to form the methylated analogs anserine and ophidine/balenine, occurs by the enzyme carnosine N-methyltransferase (CMT) or by enzymatic condensation of β-alanine with Npimethylhistine through carnosine synthase. Degradation of carnosine, and its related compounds, takes place by their own hydrolytic enzymes, called carnosinases. Two types of carnosinases can be

differentiated: CN1 and CN2. CN1, the serum carnosinase, is highly active in adults, leading to undetectable concentrations of circulating carnosine. Importantly, CN1 is not present in animals. CN2, which is called the tissue carnosinase, has a much lower catalytic rate compared to CN1, and it is still unknown whether CN2 leads to hydrolysis of carnosine in tissues holding large carnosine content. Skeletal muscles hold 99% of carnosine of the human body, but it also found in the olfactory bulb (brain). Furthermore, carnosine is measurable in other brain regions and tissues, but 10- to 1000 times lower compared to the skeletal muscle. Skeletal muscle carnosine concentrations can be measured through a muscle biopsy or MR spectroscopy. The physiological role of carnosine is related to contractile function in general and more specific to Ca^{++} handling, buffering of exercise–induced acidosis (pH buffering)[106], affection of mitochondrial respiration[107] and protection against (exercise-induced) oxidative stress[107, 108], by reducing reactive oxygen species (ROS) and free radicals. Furthermore, carnosine prevents the formation of advanced lipoxidation end-products (ALE's) and advanced glycoxidation end-products (AGE's) which are involved in the aging process and oxidative induced chronic diseases (diabetes, atherosclerosis, Alzheimer's).

Because β-alanine has been shown to be the rate-limiting precursor of carnosine synthesis, β-alanine supplementation substantially increases muscle carnosine concentrations[109]. Although various supplementation protocols have been applied, supplementing 3.2-6.4 gr/day for 23 to 46 days seems the most efficient strategy to increase muscle carnosine levels[110-112]. In order to maintain this elevated muscle carnosine concentration, a maintenance dose of 1.2 gr/day seems ideal to keep the carnosine content 30-50% above baseline values for a prolonged period of time[113]. Typically, β-alanine supplementation is beneficial for shorter (high intense) exercise types lasting 1-4min, whereas the effect is less pronounced in longer (lower intensity) duration exercise[109]. In this regard, β-alanine intake is rapidly becoming a popular ergogenic substance in the (clinical) exercise/sports community, as it increases muscle carnosine levels

and hereby augments the fatigue threshold and improves high intense exercise performance[109, 114]. The underlying mechanisms include better excitation-contraction coupling by improved Ca^{++} handling, defence against ROS and buffering of exercise-induced acidosis (lactate buffering)[40, 42, 115]. As such, it seems that impaired muscle carnosine concentrations might reduce optimal high intense exercise performance.

Interestingly, altered tissue carnosine concentrations were shown in neurological diseases such as Amyotrophic Lateral Sclerosis (ALS)[116] and Parkinson's Disease[105, 117]. Moreover, reduced serum carnosinase activity was already found in patients with MS[118] warranting to investigate muscle carnosine and ß-alanine supplementation in MS. However, this was never investigated in this population.

6.3.2 Optimizing high intense exercise feasibility/adherence in MS: training periodization

In the sports/exercise community, sequencing of various training modalities (long- and short duration, low- and high intensity) is used to attain the desired training state, maximize long-term performance and minimize overtraining and injuries[119]. This approach is called training periodization. An important aspect of training periodization is the supercompensation concept that resembles a load-recovery pattern and is divided into 3 phases. In the first phase, a physical load (training session) causes fatigue/exhaustion and an acute reduction in exercise performance. The second phase induces marked fatigue and a pronounced process of recovery. At the end of the second phase, performance/work capacity increases and reaches pre-load levels. Phase 3 is characterized by a continuous increase in work capacity, exceeding the previous levels. This is called the supercompensation phase (Figure 4). Mechanisms responsible for this concept are depletion and restoration of biochemical substances such as glycogen and creatine phosphate[120].

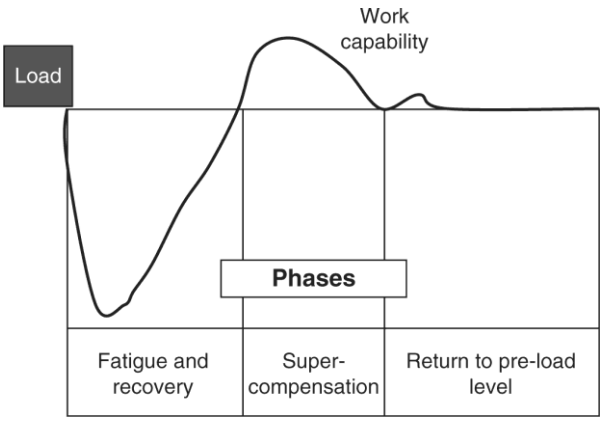

Figure 4. Supercompensation phase by Issurin et al. 2010. Reprinted with permission of the author.

In a periodized training program, variation in training specificity, intensity and volume is organized within shorter cycles or periods within an overall long-term exercise program. Rather than progressive linear training, a prolonged period is divided into periodically alternating blocks of 1-4 weeks with different training goals, modalities and periods of rest/recovery. This approach has been shown to provide superior results compared to classic progressive linear training in athletes[121].

In current MS rehabilitation guidelines, exercise interventions rather exist of uniform workloads with little to no variation in exercise intensity and/or volume over time, or otherwise progressive linear training interventions where intensity and volume progressively increase[39, 62]. Although improving various health-related and functional parameters[61, 122, 123], long-term adherence to such exercise interventions is rather low. Furthermore these exercise modalities have been shown to induce psychological symptoms such as mental fatigue, mood disturbances and reduced motivation, which are directly related to performance and clinical outcomes[124]. As such, alternative and more behaviourally sustainable approaches to exercise prescription in MS are warranted.

In this regard, applying high-performance periodization principles in a rehabilitation setting including varying exercise modalities (high- and moderate intensity), as well as sufficient recovery periods (supercompensation cycle) may promote fitness gains, while preventing physical discomforts such as overtraining, fatigue, performance decrements or injury[124], and thus improve clinical outcome. However, principles of periodization in the rehabilitation of MS were never investigated.

Chapter 2

Objectives and general outline

Objectives and general outline

This PhD project involves both animal and human research that is presented schematically in Figure 5. These studies are presented as six original research papers that have been published, are under review or submitted for publication in peer-reviewed international journals. The performed studies fit into two research objectives (**A & B**) that represent the main topics of interest of this PhD. First, this PhD project aims to investigate the potential of high intensity interval training (HIIT) as an exercise therapeutical intervention to **improve health-related comorbidities** (e.g. cardiovascular risks) that are induced by MS-related inactivity (**A**). Second, this PhD project aims to further **optimize HIIT as a rehabilitation strategy** in MS (**B**). Here, optimization of overall HIIT performance and functional outcome following HIIT (**B1**) as well as strategies to increase HIIT feasibility/adherence during MS rehabilitation are investigated (**B2**).

To investigate the impact of HIIT on important health-related MS comorbidities such as cardiovascular disease (CVD), that are induced by inactivity related secondary symptoms, it is necessary to investigate what cardiovascular risk factors are disturbed in MS. As described in Chapter 1, other researches previously suggested that MS patients indeed have a higher CVD risk profile compared to healthy controls (HC)[49]. However, it is unclear whether an increased CVD risk is primarily mediated by changes in body composition, blood pressure, blood lipid profile or glycemic control[49] in this population. Therefore, in **Study 1** (**objective A**), we investigate what CVD risk factors primarily contribute to an increased CVD risk in MS and we explore their interrelations.

In other populations high intensity exercise training clearly improves various health-related parameters including cardiovascular risk factors[93, 94, 96, 97, 100, 101]. Based on the results of **Study 1** and because this was never investigated in MS, **Study 2** (**objective A**) explores whether such high intense exercise training regimens have the potential to improve important cardiovascular risk factors in MS patients.

During high intense exercise, accumulation of lactate is an important contributor to subjective fatigue, leading to earlier cessation of the exercise activity, and thus possibly altered exercise therapy outcomes. Recently, it was demonstrated that MS patients have elevated resting serum lactate concentrations compared to HC[89]. Whether lactate concentrations during acute exercise and/or following training (repeated exercise) are also higher in MS is unknown. This is very relevant information because disturbed exercise-induced lactate responses during HIIT may cause higher perceived fatigue perceptions and earlier cessation, leading to inadequate high intensity training stimuli and thus attenuated clinical outcome. Therefore, in **Study 3** (**objective A**), we analyze whether blood lactate accumulation in MS, during submaximal and maximal exercise, is different compared to healthy controls.

The second objective (**B**) of this PhD project involves optimization of overall HIIT performance in MS (**objective B1***)*. This may further enhance exercise capacity and muscle strength and as such improve clinical outcome, functionality and quality of life in this population. Furthermore and given the promising results of HIIT in MS, further optimization of existing HIIT protocols to improve feasibility and long term adherence are necessary to facilitate effective integration of HIIT in current MS rehabilitation strategies (**objective B2***)*. In the exercise/sports community, high intense exercise performance and training optimization are frequently achieved by specific ergogenic supplements (**objective B1**) and training periodization (**objective B2**).

(**Objective B1**) Here, β-alanine intake is rapidly becoming a popular ergogenic substance in the (clinical) exercise/sports community, because it increases muscle carnosine levels and hereby improves exercise performance, especially during high intense intermittent exercise bouts[109, 114]. The underlying mechanisms include improved Ca^{2+} handling and buffering of exercise-induced acidosis[40, 42, 115]. As such, increasing muscle carnosine by β-alanine supplementation, is able to improve HIIT performance. However, before investigating the impact of β-alanine supplementation and elevated muscle carnosine levels on HIIT performance in MS, it is important to understand the impact of MS on muscle carnosine. In **Study 4** (**objective B1***)* we investigate whether muscle carnosine levels of both an animal MS model (Experimental Autoimmune Encephalomyelitis, EAE) and MS patients are different from healthy controls. Because both EAE and MS affect muscle carnosine we explore the effectiveness of carnosine/β-alanine supplementation in EAE animals first (**Study 5, objective B1**).

(**Objective B2**) In high performance sports, training programs are often periodized into sequential phases and cyclical periods to achieve specific performance goals and maximize optimal long-term training/performance stimuli while minimizing overtraining and/or injuries[120, 121]. Current MS rehabilitation programs however very often use monotonous uniform workloads or progressive linear modalities that often induce lack of motivation, mental fatigue, poor adherence, overload injuries and suboptimal training stimuli[39, 124]. Furthermore, despite substantial clinical improvements following HIIT in MS, effective implementation of such high intense exercise interventions in current rehabilitation programs is difficult. Rehabilitation regimens consisting of predominantly HIIT are physically demanding and this appears to affect longer term therapy adherence. Indeed, attenuated clinical effects resulting from reduced compliance following high intensity exercise have already been reported in sedentary adults[104]. Therefore, in **Study 6** (**objective B2***)* we explore whether a periodized exercise intervention, including demanding HIIT sessions, improves therapy adherence and feasibility of HIIT in MS.

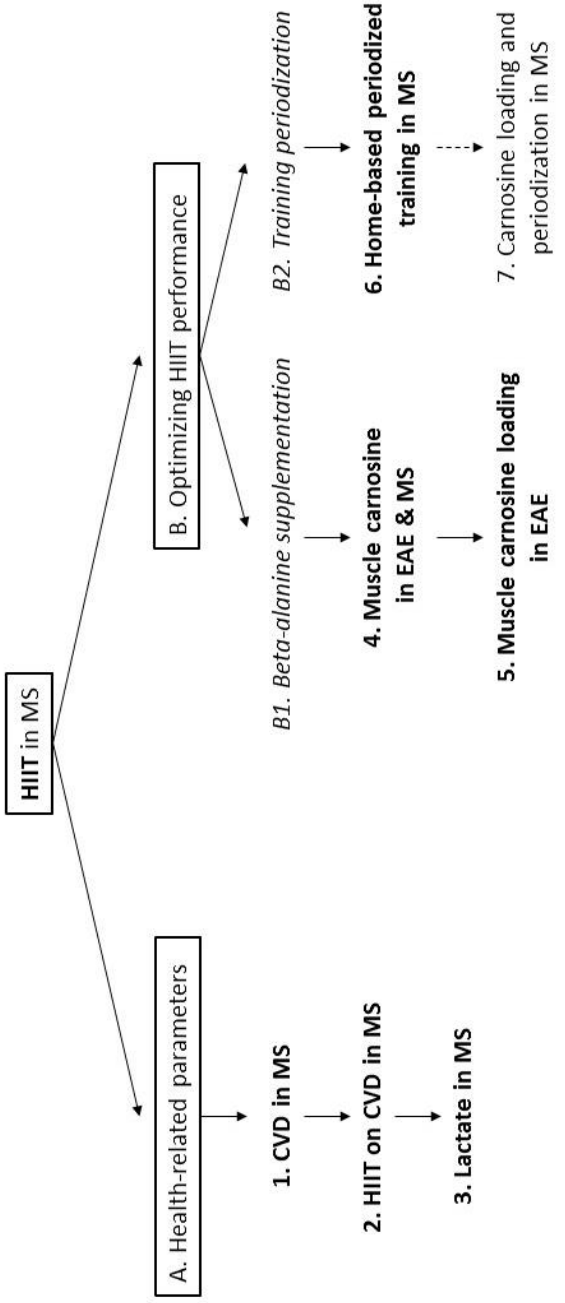

Figure 5. Schematic presentation of the study flow.

Chapter 3

Experimental work and results

Study 1

Elevated cardiovascular risk factors in Multiple Sclerosis.

Based on: Charly Keytsman, Bert O Eijnde, Dominique Hansen, Kenneth Verboven, Inez Wens

Multiple Sclerosis and Related Disorders. 2017 Oct;17:220-223

ABSTRACT

Background. Multiple sclerosis (MS) is associated with elevated cardiovascular mortality. To prevent this a better understanding of their CVD risk factors and interrelations is necessary.

Methods. MS patients (n=52) and healthy controls (HC, n=24) were matched for age, height, weight, body mass index and physical activity. Body composition, resting blood pressure (BP), resting heart rate (HR), glucose tolerance, HbA1c, blood lipids (HDL, LDL, total cholesterol, triglyceride concentrations) and c-reactive protein concentrations were analyzed. Regression analyses identified independent CVD risk factors and their interrelations in MS.

Results. In MS and compared to HC, fat mass (25.1±1.2kg vs. 17.9±1kg), fat percentage (33.8±1.2% vs. 28.4±1.5%), systolic (130±1.8mmHg vs. 120±2.9mmHg) and diastolic (79±1.1mmHg vs. 71±1.9mmHg) BP, resting HR (72±1.4bpm vs. 60±2bpm), blood triglycerides (113.8±8.6mg/dl vs. 98.2±17.4mg/dl), fasting (13.5±2.9mU/l vs. 7.2±0.8mU/l) and 2h insulin (71.9±12.5mU/l vs. 35.8±8.1mU/l), 2h glucose (6.3±0.5mmol/l vs. 4.8±0.5mmol/l) and HOMA index (3.7±1.1 vs. 1.7±0.2) were significantly ($p<0.05$) elevated. Total cholesterol, blood HDL and LDL concentrations did nog differ between groups ($p<0.05$). Regression analyses indicated that MS is independently associated with elevated fat mass/percentage, systolic and diastolic BP and HR and in MS fat mass appears to be an independent contributor of the other measured CVD risk factors in MS.

Conclusion. Persons with MS have an increased risk for CVD and fat mass appears to be an important risk factor. Therefore, normalizing whole body fat should be an essential part of MS treatment.

INTRODUCTION

MS patients display a reduced exercise capacity [125, 126], impaired muscle strength [41, 43] and overall functional capacity [44, 125, 127, 128]. This results in a sedentary lifestyle and thus further deconditioning. The latter leads to progressive worsening of fatigue and muscle weakness, reduced exercise and functional capacity and deterioration of various neurological symptoms, independently of the disease process *per se* [128, 129]. In healthy persons, such a sedentary lifestyle is associated with an increased overall risk to develop cardiovascular diseases (CVD) [47, 48]. In MS the likelihood for cardiovascular mortality is significantly greater compared to the general population (HR: 1.29) [51]. To improve survival in persons with MS, it is thus relevant to explore what CVD factors are primarily affected in this population.

We have previously shown that CVD risk factors may be elevated in MS compared to healthy controls (HC) [49]. However, in MS it is still unclear whether an increased CVD risk is primarily mediated by changes in body composition, blood pressure, blood lipid profile or glycemic control [49]. More recently Moccia et al. [130] investigated the CVD risk in a large cohort of persons with MS and reported that the CVD risk was comparable to healthy controls. This study applied the Framingham Risk Score (FRS) to assess CVD risk, in which sex, age, smoking behavior, blood pressure, blood glucose concentrations and body mass index (BMI) are taken into account [131, 132]. However, it is well known that in persons with MS the use of BMI is not recommended because it underestimates adiposity in this population [133]. Furthermore, the FRS does not take into account various other important CVD risk factors such as body composition and blood lipid profile. Finally, FRS is not validated in MS yet. Thus, whether and how, CVD risk is elevated in MS remains unclear but is of great clinical importance.

Therefore the present study investigates all common CVD risk factors in MS simultaneously and explores their interrelations.

MATERIALS & METHODS

Subjects

Patients with MS (n=52, mean EDSS = 2.9±0.2, range 0 to 5.5) and healthy controls (HC, n=24) were matched for age, height, weight, body mass index (BMI), physical activity, and were included following written informed consent and aged >18 years. Subjects were excluded if they participated in another study, had (in case of MS) an acute exacerbation 6 months prior to the start of the study or had an EDSS score >6. Subjects were recruited following local advertisement. Phenotypes of MS (RR; relapsing remitting, PP; primary progressive, SP; secondary progressive) and the use of disease modifying therapies (DMT) were inventoried. The study was approved by the local Ethical Committee of the Jessa hospital and Hasselt University, and was performed in accordance with the Declaration of Helsinki. This study was registered at ClinicalTrials.gov (NCT02466165).

Study Design

In this cross-sectional study, body composition, resting blood pressure and heart rate, and blood HDL, LDL, total cholesterol, triglyceride concentrations, c-reactive protein, glucose- and insulin concentrations were assessed. Furthermore, all subjects were asked whether they smoked and used medication to regulate blood pressure, cholesterol or glucose tolerance.

Measurements

Body composition:

Whole body fat and lean tissue mass (with exclusion of the head) were assessed using Dual Energy X-ray Absorptiometry scan (DEXA, Hologic Series Delphi-A Fan Beam X-ray Bone Densitometer, Vilvoorde, Belgium)

and a calibrated analogue weight scale (Seca®) was used to measure total body mass. Android fat mass and the ratio of android fat mass to total fat mass were evaluated.

Blood pressure and heart rate:

Resting blood pressure and heart rate were measured with an automatic blood pressure cuff (Omron M4-I, Omron Healthcare Europe B.V., Hoofddorp, The Netherlands) in a supine position (7min rest) immediately following the DEXA scan (to prevent orthostatic effects).

Oral glucose tolerance test:

Glycemic control was investigated using an oral glucose tolerance test (OGTT). Following a 10h overnight fast, all participants received a 1g glucose/kg body weight solution (300ml, [50]. Before and after glucose administration, capillary blood samples were collected from a hyperaemic earlobe at 30min intervals (2h), to measure blood glucose concentrations immediately (Analox®, GM7 Micro-stat, Analox instruments Ltd, London, UK). Blood glucose concentrations were converted to plasma concentrations using a multiplier of 1.11 [134]. To determine serum insulin concentrations, 4cc of venous blood was collected in serum separation tubes (SST, BD Vacutainer®, Becton Dickinson, Erembodegem, Belgium) at 1h intervals. After 30min, allowing blood coagulation, samples were centrifuged during 10min at 3500rpm. The obtained serum was frozen and stored at -80°C for batch analysis of serum insulin concentrations (Mercodia Insulin ELISA®, Uppsala, Sweden).

Cardiovascular risk factors: blood analysis

Following an overnight fasting period (10h), a venous blood sample was collected and centrifuged at 2000 rpm for 10min. Plasma was frozen immediately in liquid nitrogen and stored at -80°C until further analysis. Plasma samples were analyzed for blood insulin, total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglyceride concentrations, C-reactive protein (CRP, Beckman Synchron LX 20 Analyzer®, Beckman Coulter Inc., Diamond Diagnostics, USA), and glycosylated haemoglobin (HbA1c) (Hi-Auto A1c Analyzer®, Menarini Diagnostics Inc., Florence, Italy) concentrations. The Homeostasis Model Assessment (HOMA-IR: fasting blood glucose [mmmol/l] x fasting serum insulin [mU/l]/22.5, [135] was used to estimate/calculate insulin resistance (IR) and β-cell function from fasting glucose and insulin concentrations. This assessment model indicates a normal insulin sensitivity with a score between 1.7 and 2, whereas a score of >2 suggests altered insulin sensitivity.

Physical activity

Participants were asked to report their physical activity level (Physical Activity Scale for Individuals with Physical Disabilities, PASIPD,[136]. The PASIPD is a 13-item physical activity questionnaire involving participation in household, recreational and occupational activities over the past 7 days. The scores are the average hours of participation daily multiplied with a metabolic equivalent value.

Statistical Analysis

All data were analyzed using SPPS v. 22.0 (IBM) and checked for normality (Shapiro-Wilk test). Data was not normally distributed and differences between groups were analyzed using an unpaired student's t-test (Mann Whitney U test). Fat mass, lean body mass, total mass, fat percentage, systolic and diastolic blood pressure, resting heart rate, blood total cholesterol, HDL, LDL and triglyceride concentrations, CRP, fasting and 2h blood glucose concentrations, HbA1c, fasting and 2h blood insulin concentrations and HOMA index were inserted into a multivariate regression model to examine relations between CVD risk factors and the presence of MS, taking age, gender, physical activity, smoking behavior and medication for blood pressure, cholesterol and glucose tolerance into account. Next, multivariate regression analyses were applied to examine interrelations between CVD risk factors, in which for every deviant CVD risk factor a model was created that examined relations with all other CVD risk factors, in MS only. All data are presented as means ± SEM and considered statistically significant when $p<0.05$ (2-tailed).

RESULTS

Subject characteristics

Gender differences and differences in smoking behavior were present between groups ($p<0.05$, Table 1). Medication intake for blood pressure, cholesterol and glucose tolerance were higher in MS. Age, height, weight, BMI and physical activity did not differ between groups. Relapsing-remitting MS was diagnosed in 37 patients, whilst 2 patients suffered from primary-progressive MS and 13 patients from the secondary-progressive form. Forty-five MS patients were on disease modifying therapy, of which 7 on interferon-beta, 6 on glatiramer acetate, 14 on natalizumab, 4 on dimethyl fumarate, 5 on teriflunomide and 9 on fingolimod.

Table 1. Subject characteristics

	HC (n=24)	MS (n=52)
Age (y)	48.6 ± 2.5	51.2 ± 1.5
Height (m)	1.7 ± 0.0	1.7 ± 0.0
Weight (kg)	69.9 ± 2.9	72.6 ± 1.9
BMI	24.2 ± 0.5	25.7 ± 0.6
PASIPD	21.7 ± 2.2	17.9 ± 1.8
Gender (f/m)	15/9	36/16*
EDSS	-	2.9 ± 0.2
Med. BP (n=)	2	14*
Med. Cholesterol (n=)	2	10*
Med. glucose tolerance (n=)	0	2*
Smoking (n=)	2	7*
Interferon-beta	-	7
Glatiramer acetate	-	6
Natalizumab	-	14
Dimethylfumaraat	-	4
Teriflunomide	-	5
Fingolimod	-	9
RRMS	-	37
PPMS	-	2
SPMS	-	13

*Data are expressed as means ± SEM and represent subject characteristics of healthy controls and MS patients. Abbreviations: f, female; m, male; BMI, Body Mass Index; EDSS, Expanded Disability Status Scale; PASIPD, Physical Activity Scale for Individuals with Physical Disabilities. Med, medication. DMT; Disease Modifying Therapy. RRMS; Relapsing Remitting MS. PPMS; Primary Progressive MS. SPMS; Secondary Progressive MS. *p<0.05 compared to HC.*

CVD risk factors

In MS patients, body composition variables such as fat mass (+40%) and body fat percentage (+19%) were significantly elevated compared to HC (Table 2) with significantly greater ($p<0.05$) android (21.8±1.2 kg vs. 14,7±1.2 kg) fat mass and a significantly ($p<0.05$) higher ratio of android to total fat mass (0.96±0.0 vs. 0.80±0.0)in MS (data not shown). Furthermore, systolic (+8%) and diastolic blood pressure (+11%), resting

heart rate (+20%), blood triglyceride concentrations (+16%), 2h glucose (+31%), fasting (+87%) and 2h insulin (+101%) concentrations and HOMA index (+117%) were significantly elevated in MS patients compared to HC. Blood total cholesterol, blood HDL and LDL concentrations were not different ($p>0.05$) between groups.

Table 2. Cardiovascular risk factors in multiple sclerosis and healthy controls

	HC	MS	p-value
Fat mass (kg)	17.9 ± 1	22.6 ± 1.2*	0.01
Lean body mass (kg)	45.9 ± 2.4	43.6 ± 1.4	0.37
Total mass (kg)	63.7 ± 2.6	66.3 ± 1.9	0.42
Fat percentage (%)	28.4 ± 1.5	33.8 ± 1.2*	0.01
Systolic BP (mmHg)	120 ± 2.9	130 ± 1.8*	0.001
Diastolic PB (mmHg)	71 ± 1.9	79 ± 1.1*	0.001
HR_{rest} (bpm)	60 ± 2	72 ± 1.4*	0.000
$Cholesterol_{Total}$ (mg/dl)	190.1 ± 7.6	199.7 ± 4.8	0.18
LDL (mg/dl)	110.5 ± 6.2	112.1 ± 4.2	0.95
HDL (mg/dl)	62.3 ± 3.6	65.6 ± 2.6	0.69
Triglycerides (mg/dl)	98.2 ± 17.4	113.8 ± 8.6*	0.02
CRP (mg/dl)	2.4 ± 0.7	5.4 ± 1.8	0.27
Fasting glucose (mmol/l)	5.3 ± 0.2	5.5 ± 0.1	0.80
2h glucose (mmol/l)	4.8 ± 0.5	6.3 ± 0.5*	0.01
HbA1c (%)	5.2 ± 0.1	5.3 ± 0.1	0.61
Fasting insulin (mU/l)	7.2 ± 0.8	13.5 ± 2.9*	0.000
2h insulin (mU/l)	35.8 ± 8.1	71.9 ± 12.5*	0.03
HOMA index	1.7 ± 0.2	3.7 ± 1.1*	0.001

*Data are expressed as means ± SEM and represent various cardiovascular risk factors of healthy controls (HC) and MS patients (MS). Body composition was determined with exclusion of the head. Abbreviations: BP, blood pressure; HR, heart rate; bpm, beats per minute. *p<0.05 compared to HC.*

Regression analysis

When adjusted for age, gender, physical activity, smoking behavior and medication for blood pressure, cholesterol and glucose tolerance, MS was independently related ($p<0.05$) to elevated fat percentage and fat mass, systolic and diastolic blood pressure and resting heart rate.

Furthermore, after investigating the interrelations between CVD risk factors, in which for every deviant CVD risk factor a model was created that examined relations with all other CVD risk factors, fat mass appeared to be independently related ($p<0.05$) to fat percentage, total mass, lean tissue mass, diastolic blood pressure, blood triglyceride and CRP concentrations , resting heart rate, blood HbA1c, 2h blood glucose and HDL concentrations and a trend for systolic blood pressure ($P=0.05$).

DISCUSSION

This study shows that MS is independently associated with several abnormal CVD risk factors. This probably increases their risk to develop CVD. Interestingly, following regression analyses fat mass appears to be an important contributing factor for this.

To our knowledge the present study is the first to investigate all common CVD risk factors in MS [49] simultaneously. Following inter-group comparisons we now show that MS patients exhibit greater whole body fat mass and fat percentage, higher blood pressure (systolic and diastolic), resting heart rates, blood triglyceride concentrations, a disturbed whole body glucose tolerance and disturbed insulin concentrations compared to HC. Consequently, MS patients are probably more prone to develop future (preventable) cardiovascular complications and/or comorbidities. The present findings contrast with a recently performed study of Moccia et al. [130] investigating the risk to develop CVD in MS using the FRS [131, 132]. This risk score comprises an algorithm that estimates the 10-year likelihood to develop CVD including risk factors such as age, gender, smoking status,

body mass index, systolic blood pressure, glucose tolerance and use of antihypertensive medication. Moccia et al. [130] concluded that, based on the FRS, CVD risk was normal in MS patients. The discrepancy with our findings probably has several reasons. First, we have included a wider range of CVD risk factors such as fat mass, fat free mass and blood cholesterol. These risk factors have been shown to correlate better with CVD incidence than the Framingham Score factors [48] and therefore they are probably more predictive. Second, an important component of the FRS algorithm is the BMI [131, 132]. However in MS, Pilutti [133] et al. clearly showed that BMI significantly underestimates the amount of adipose tissue. Consequently, we have matched MS patients and HC for BMI and clearly demonstrate significantly higher total body fat mass and fat percentage compared to HC. This shows that screening MS patients using BMI or predictive calculations that include BMI probably underestimates their actual risk to develop CVD. Under the conditions of the present study in MS blood lipid profile (total cholesterol, HDL, LDL) was not altered compared to HC and may thus be an inferior CVD predictor in this population.

It is important to note that age, smoking, gender, medication for blood pressure, cholesterol and glucose tolerance [137] may importantly contribute to the above described findings. In the current study more persons with MS smoked, used medication for blood pressure, cholesterol and glucose tolerance and gender differences were present between groups. To exclude the effect of these confounding factors and investigate the sole impact of MS on CVD risk we performed regression analyses and interestingly demonstrate that irrespective of age, gender, physical activity, smoking behavior, medication for blood pressure, cholesterol and glucose tolerance, a higher fat percentage, fat mass, systolic and diastolic blood pressure as well as resting heart rate are independently related to MS. In a second series of regression analyses, we then examined the interrelations between these CVD risk factors, in which for every deviant CVD risk factor (fat mass, fat percentage, diastolic and systolic blood pressure and resting heart rate) a model was created that examined relations with all other CVD risk factors.

We now demonstrate that in MS, fat mass is independently related to systolic and diastolic blood pressure, resting heart rate but also to blood HDL, CRP, 2h glucose, HbA1c, CRP and triglyceride concentrations. This suggests that fat mass is a very important CVD risk factor in MS. Furthermore, it is important to notice that persons with MS in this study presented significantly more android fat mass, as well as higher ratios of android to total fat mass ratios. This indicates the presence of more visceral adipose tissue in MS, which is known to be associated with increased cardiovascular risk factors [138]. In recent years, the role of adipose tissue in the pathogenesis of MS has become subject of great interest [139]. It is known that elevated fat tissue causes an increase in the production of adipokines, which are involved in the regulation of various physiological processes, such as energy balance and insulin sensitization, and the immune response. As such, it is believed that increased fat mass, and thus elevated levels of adipokines, may be involved in the altered immune response and inflammatory processes in MS. Therefore, increased fat mass may not only affect cardiovascular risk in MS but may also negatively influence MS progress and treatment response as reported elsewhere [139].

Our findings may thus indicate that multidisciplinary programs directed to prevent/treat (cardiovascular) complications and mortality in MS should also include strategies that lower lowering fat mass.

Limitations

Although not significant, it is important to note that a 1.5-unit difference in BMI between MS and HC was observed in this study. This may cause the potential for residual confounding.

CONCLUSION

Persons with MS are probably more prone to an increased risk for CVD and fat mass appears to be a very important mediating risk factor. Therefore, MS treatment should also include interventions that restore/optimize whole body fat mass.

Acknowledgements

We thank the MS Liga Flanders for partly funding this project.

Conflicts of interest

The authors declare that they have no conflict of interest.

Study 2

Impact of high intensity concurrent training on cardiovascular risk factors in persons with Multiple Sclerosis – pilot study

Based on: Charly Keytsman, Dominique Hansen, Inez Wens and Bert O. Eijnde

Disability and Rehabilitation. 2017 Oct: 1-6

ABSTRACT

Purpose. High intensity concurrent training positively affects cardiovascular risk factors. Because this was never investigated in multiple sclerosis the present pilot study explored the impact of this training on cardiovascular risk factors in this population.

Methods. Before and after 12 weeks of high intense concurrent training (interval and strength training, 5 sessions per 2 weeks, n=16) body composition, resting blood pressure and heart rate, 2h oral glucose tolerance (insulin sensitivity, glycosylated hemoglobin, blood glucose and insulin concentrations), blood lipids (high and low density lipoprotein, total cholesterol, triglyceride levels) and C-reactive protein were analyzed.

Results. Twelve weeks of high intense concurrent training significantly improved resting heart rate (-6%) , 2h blood glucose concentrations (-13%) and insulin sensitivity (-24%). Blood pressure, body composition, blood lipids and C-reactive protein did not seem to be affected.

Conclusion. Under the conditions of this pilot study, 12 weeks of concurrent high intense interval and strength training improved resting heart rate, 2h glucose and insulin sensitivity in multiple sclerosis, but did not affect blood C-reactive protein levels, blood pressure, body composition and blood lipid profiles. Further, larger and controlled research investigating the effects of high intense concurrent training on cardiovascular risk factors in multiple sclerosis is warranted.

INTRODUCTION

Decreased exercise capacity, post-exercise fatigue and reduced muscle contractile functioning are frequently occurring comorbidities in Multiple Sclerosis (MS) that lead to an inactivity-related physiological profile and thus more associated (secondary) health risks than provided by the disease *per se* [43, 128, 140, 141]. Furthermore, and similar to other populations, such sedentary lifestyle is associated with an increased risk for the development of cardiovascular diseases [47] such as hypertension, obesity, type 2 diabetes mellitus and dyslipidemia [47, 49].

Low-to-moderate intensity exercise therapy (cardiovascular and resistance training) has become an important part of overall symptom management in MS [123] leading to improved exercise capacity, muscle strength and various functional measures [66]. In healthy controls, moderate intensity exercise training clearly affects various cardiovascular risk factors such as whole body glycemic control [142]. Consequently, we previously investigated its effectiveness on glucose tolerance in persons with MS [37] but, surprisingly, were unable to show any effects [37]. It is difficult to explain this discrepancy. Possibly, in persons with MS higher exercise intensities may be required to induce positive effects. This was already demonstrated in healthy controls, obese subjects and in stroke/cardiac patients were a wide range of high intensity interval training modes (1-5 high intensity exercise bouts ranging from 6s-4min interspersed by recovery periods of 30s-4min, intensities of 90-100% maximal heart rate) substantially improve exercise performance and muscle strength [98] but also various cardiovascular risk factors such as body composition (subcutaneous and abdominal fat) [99], blood glucose and insulin sensitivity [101], functional recovery (improved cardiorespiratory fitness, reduced effort of walking) [143], intrinsic heart pump activity [102] and systemic cytokine balance. In keeping with this, we recently evaluated the impact of both moderate (24w, 5 sessions per 2 weeks, 50-70% maximal heart rate) and high intensity training (12w, 5 sessions per 2 weeks, 90-100% maximal heart rate) on various cardiovascular risk factors in MS [38] and reported substantially greater improvements in exercise capacity,

muscle contractile characteristics and in glucose tolerance following 12 weeks of high intensity interval training (HIIT) compared to 24 weeks of moderate intensity training [37, 38]. Interestingly, in healthy subjects and compared to HIIT alone high intensity concurrent training (HICT) that combines high intense interval and strength training has been shown to further improve body composition, muscle strength, overall health and cardiovascular fitness [74-76]. Consequently, in this population the combined effects of HIIT and resistance training on important cardiovascular risk factors appear to be worthwhile investigating. The effects of high intensity concurrent training on various cardiovascular risk factors in MS however have not been investigated yet.

In keeping with the above line of reasoning, the present pilot trial therefore explores the effects of a 12-week HICT intervention on various cardiovascular risk factors, such as body composition, blood pressure and heart rate, blood lipid profiles, whole body glucose disposal and C-reactive protein (CRP) levels in a small sample of persons with MS. We hypothesize that HICT improves these cardiovascular parameters in persons with MS.

METHODS

Subjects

Following local advertisement and written informed consent, sixteen persons with MS (mean Expanded Disability Status Scale; EDSS 2.6±0.2) were included. Subjects were excluded if they were pregnant, aged <18 years, participated in another study, experienced an acute exacerbation 6 months prior to the start of the study, had contraindications to perform physical exercise, or had an EDSS score >6. Use of disease-modifying therapy and other medication intake was inventoried. Subjects were asked to maintain their usual medication intake constant throughout the study course. All data was collected at the Rehabilitation Research Centre of Hasselt University. The study was approved by the local Ethical Committee

of the Jessa hospital and Hasselt University, and was performed in accordance with the Declaration of Helsinki of 1975. This study was registered at ClinicalTrials.gov (NCT02466165).

Study Design

At baseline, body composition, resting blood pressure and heart rate, blood sample analysis (detection of high- and low density lipoprotein, total cholesterol, triglyceride levels, CRP, glucose- and insulin concentrations), exercise capacity (maximal graded exercise test) and muscle strength (isometric/isokinetic dynamometry) were assessed. Furthermore, previous cardiovascular problems, smoking and any antihypertensive medication were registered. Following baseline screening persons with MS were enrolled in a 12-week high intensity concurrent training (HICT) program under a one on one supervision of a physiotherapist to increase compliance and adherence. Hereafter, baseline measurements were repeated.

Measurements

Body composition:

Whole body fat and lean tissue mass were obtained using Dual Energy X-ray Absorptiometry scan (DEXA) (Hologic Series Delphi-A Fan Beam X-ray Bone Densitometer, Vilvoorde, Belgium). A calibrated analogue weight scale (Seca®) was used to measure total body mass.

Blood pressure and heart rate:

Resting blood pressure and heart rate were measured with an automatic blood pressure cuff (Omron M4-I, Omron Healthcare Europe B.V., Hoofddorp, The Netherlands) in a supine position (7 min) immediately following the DEXA scan (to prevent orthostatic influence).

Oral glucose tolerance test:

Glycemic control of persons with MS was investigated using an oral glucose tolerance test (OGTT). Following a 10h overnight fasting period, all participants received a 1g glucose/kg body weight solution. Before and after glucose administration, capillary blood samples were collected from a hyperaemic earlobe at 30min 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 [134]. 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 30min, allowing blood coagulation, samples were centrifuged during 10min at 3500 rpm. The obtained serum was frozen and stored at -80°C for batch analysis of serum insulin levels (Mercodia Insulin ELISA, Uppsala, Sweden).

Blood analysis

Following an overnight fasting period (10h), a venous blood sample was collected and centrifuged at 2000 rpm for 10min. Plasma was frozen immediately in liquid nitrogen and stored at -80°C until further analysis. Plasma samples were analyzed for total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), plasma triglycerides, CRP, (Beckman Synchron LX 20 Analyzer®, Beckman Coulter Inc., Diamond Diagnostics, USA) and glycosylated hemoglobin (HbA1c) (Hi-Auto A1c Analyzer®, Menarini Diagnostics Inc., Florence, Italy). Whole-body insulin sensitivity was calculated by the homeostasis model assessment (HOMA: [fasting plasma glucose (mmmol/l) x fasting serum insulin (mU/l)]/22,5) [135].

Physical activity

Participants were asked to report their physical activity level (Physical Activity Scale for Individuals with Physical Disabilities, PASIPD) of the previous 7 days [136].

Maximal exercise test

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 48 hours separated from the isometric muscle strength test to exclude interference of muscle fatigue. Female and male persons with MS started at 20 watt (W) and 30W, respectively, during the first minute. Hereafter, workload increased, respectively, 10W and 15W per minute [65]. Oxygen uptake (VO_2), expiratory volume (VE), and respiratory exchange ratio (RER) were collected breath-by-breath and averaged every minute. 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 whether the test was maximal. In addition, maximal cycling resistance (W_{max}), maximal heart rate (HR_{max}), test duration and maximal oxygen uptake (VO_{2max}), defined as the corresponding load, heart rate, minutes and oxygen uptake measured at the level of exhaustion, were reported.

Isometric/isokinetic strength test

After five minutes of warming-up on a cycle ergometer maximal voluntary isometric muscle strength of the knee extensors and flexors (45° and 90° knee angle) was measured, 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 the weakest or strongest leg. This subdivision was maintained in further analysis, replacing a conventional left-right classification. The isokinetic test was initiated following 3 submaximal trial contractions. Subjects performed 20 maximal dynamic knee-extensions/flexions at a velocity of 180°/s to assess strength endurance of the knee muscles. Extension of the knee was initiated at a joint angle of 90° to an angle of 160°. Following each extension the leg was returned actively to the starting position from which the next contraction was immediately initiated. Throughout the range of movement, workload was kept constant during both extension and flexion. To determine muscle strength endurance, the average work (J) of the first 6 contractions was compared with the last 6 contractions and expressed as a percentage decrease.

High intensity concurrent training (HICT)

HICT consisted of combined high intense interval and resistance training. High intensity interval training was performed on a cycle ergometer under close supervision at a frequency of 5 sessions per 2 weeks, throughout the 12-week program. During the first 6 weeks of training, cycle exercise duration gradually increased from 5x1min bouts of high intense exercise followed by 1min rest intervals, to 5x2min exercise bouts with 1min rest intervals. Exercise intensity was 100% of the maximal workload (obtained during the exercise test), corresponding to 85-90% of the maximal heart rate. During the second 6 week training cycle, duration remained stable at 5x2min exercise bouts with 1min rest intervals and the workload increased to 100% of maximal heart rate. The second part of the training session consisted of resistance training for upper (vertical traction, arm curl and chest press) and lower (leg press, leg extension and leg curl) limbs. Training intensity and volume were adjusted throughout the intervention program from 1x10 repetitions to 2x20 repetitions at an individual maximal attainable load for each subject. To exercise at similar relative workloads and to compensate for frequently occurring bilateral strength differences between the legs of persons with MS , resistance training of the lower limbs was performed unilaterally.

Statistical Analysis

All data were analyzed using SPPS v. 22.0 (IBM). Pre-post differences within groups were analyzed using paired t-tests. All data are presented as means ± SD's or mean difference (MD) with accompanying confidence interval (CI) and the threshold for statistical significance was set at $p<0.05$.

RESULTS

Subject characteristics

Subjects characteristics are displayed in Table 1. Thirteen persons with MS were on disease-modifying therapy of which 2 on teriflunomide, 6 on interferon-beta, 1 on dimethyl fumarate, 1 on glatiramer acetate and 3 on natalizumab. Furthermore, 1 person was on ACE-inhibitors, 1 on beta-blockers, 1 on statins and 1 on biguanides. Subjects scored 18.3±11,7 on the PASIPD (data not shown).

Table 1. Baseline subject characteristics.

Age (years)	52.8 (7.2)
Weight (kg)	68.7 (13.2)
Height (m)	1.71 (0.1)
Sex (f/m)	9/7 (56/44%)
Smoking (y/n)	4/12 (25/75%)
Medication (y/n)	3/13 (19/81%)
BMI (kg/m²)	23.5 (3.3)
EDSS	2.6 (1.5)

Data are expressed as means (SD's) and represent baseline characteristics of the MS subjects (n=16). Abbreviations: f, female; m, male; y, yes; n, no; Medication, antihypertensive medication; BMI, Body Mass Index; EDSS, Expanded Disability Status Scale.

Adherence and adverse events

No drop-outs or adverse events were reported during the course of the study.

Cardiovascular risk factors

In MS, resting heart rate (MD 4bpm, 95% CI 2 to 7), 2h glucose (MD 1 mmol/l, 95% CI -1 to 2) and HOMA index (MD 0.5, 95% CI 0 to 1) improved significantly ($p<0.05$) during the study course (Table 2). Other cardiovascular risk factors did not differ.

Table 2. Cardiovascular risk factors and impact of high intensity concurrent training.

	PRE	POST
Total Mass (kg)	62.3 (12.5)	61.2 (11.4)
Fat Mass (kg)	17.8 (4.7)	16.9 (4.9)
Lean Body Mass (kg)	44.2 (10.7)	44.2 (10.2)
Fat percentage (%)	29 (6.7)	28.2 (7.5)
Systolic BP (mmHg)	130 (13.5)	129 (14.7)
Diastolic BP (mmHg)	81 (8.6)	82 (9.4)
HR rest (bpm)	68 (8.9)	64 (8.7)*
Total Cholesterol (mg/dl)	193.7 (29.8)	188.2 (28.5)
LDL (mg/dl)	110 (24.9)	106.9 (19.6)
HDL (mg/dl)	62.7 (21.5)	59.9 (18.8)
Triglycerides (mg/dl)	103.6 (54.9)	106.9 (46.5)
CRP (mg/l)	2.1 (2.7)	1.25 (1.1)
HbA1c (%)	5.2 (0.3)	5.3 (0.3)
Fasting glucose (mmol/l)	5.5 (1.0)	5.2 (0.6)
2h glucose (mmol/l)	7.8 (2.8)	6.8 (2.1)*
Fasting insulin (mU/l)	8.6 (4.2)	7.1 (2.4)
2h insulin (mU/l)	44.1 (33.1)	44.1 (25.1)
HOMA	2.1 (1.2)	1.6 (0.5)*

*Data are expressed as means (SD's) an represent cardiovascular risk factors before (PRE) and after (POST) 12 weeks of high intensity concurrent training (n=16). Abbreviations: BP, blood pressure; HR, heart rate. *p<0.05: significant difference between PRE and POST.*

Maximal cardiopulmonary exercise test and isometric/isokinetic dynamometry

Following 12 weeks of HICT workload capacity (MD 25W, 95% CI -34 to -16), time to exhaustion (MD 2min, 95% CI -3 to -1), VE max (MD 15l/min, 95% CI -23 to -7) significantly increased (Table 3). Furthermore, isometric and isokinetic muscle strength of the quadriceps and hamstrings of both legs improved (e.g. +14% extension 45° weakest leg, MD 14 Nm, 95% CI -23 to -6) significantly after the 12-week HICT intervention (Table 4).

Table 3. Cardiopulmonary exercise capacity and impact of high intensity concurrent training.

	PRE	POST
Workload (watt)	142.2 (66.4)	167.5 (75.4)*
Time to exhaustion	10.3 (4.3)	12.3 (4.7)*
$VO2_{max}$ (ml/min/kg)	26.8 (8.6)	32.7 (11.4)*
VE_{max} (l/min)	82.3 (30.6)	99.4 (37.4)*
RER_{max}	1.24 (0.1)	1.2 (0.2)*
HR_{max} (bpm)	161 (13.6)	162 (18.5)
$Lactate_{max}$ (mmol/l)	5.8 (1.8)	5.7 (1.4)
$HR_{recovery}$ (bpm)	117.6 (19.3)	122.9 (18.7)
$Lactate_{peak}$ (mmol/l)	8.2 (2.8)	10.3 (2.2)*

*Data are expressed as means (SD's) and represent parameters of the maximal exercise test before (PRE) and after (POST) 12 weeks of high intensity concurrent training (n=16). Abbreviations: VE, expiratory volume; RER, respiratory exchange ratio; HR, heart rate.. * p<0.05: significant difference between PRE and POST.*

Table 4. Isometric/isokinetic strength and impact of high intensity concurrent training.

	PRE	POST
Isometric strongest leg		
Ext 45°	111.6 (35.1)	124 (42.9)*
Flex 45°	80.3 (26.6)	88.9 (31.2)
Ext 90°	142.4 (48.5)	144.8 (43.3)
Flex 90°	62.1 (19.7)	68.7 (22.1)*
Isokinetic strongest leg		
Ext 180°	83.9 (28.9)	89.9 (34.8)*
Flex 180°	52.7 (22.4)	56.9 (24.7)*
Isometric weaker leg		
Ext 45°	101.1 (45)	115.4 (45.3)*
Flex 45°	63.7 (26.1)	76.4 (27.1)*
Ext 90°	111.8 (49.4)	127.6 (46)*
Flex 90°	50.4 (18.1)	60.9 (22.9)*
Isokinetic weaker leg		
Ext 180°	74.1 (35.5)	80.6 (36.1)*
Flex 180°	42.3 (24.7)	44.7 (22.6)

*Data are expressed as means (SD's) and represent isometric and isokinetic flexion (Flex) and extension (Ext) strength (in Nm) before (PRE) and after (POST) 12 weeks of high intensity concurrent training (n=16). Abbreviations: VE, expiratory volume; RER, respiratory exchange ratio; HR, heart rate.. * p<0.05: significant difference between PRE and POST.*

DISCUSSION

Twelve weeks of high intensity concurrent training (HICT) in MS substantially improved exercise capacity and leg muscle strength as well as resting heart rate and 2h blood glucose and HOMA index. In contrast to larger (n=8-84) controlled studies applying high intensity exercise in healthy controls [98, 99, 101], older subjects, persons with overweight, type 1 diabetes [99, 144] and heart disease [92, 102, 143], the present pilot study, not including a control group, did not demonstrate comparable effects on most cardiovascular risk factors in persons with MS.

To date exercise therapy has become an important part of MS rehabilitation, leading to improvements in various health related parameters [123]. To optimize therapy adherence and avoid complications, exercise interventions in this population so far have mainly focused on low to moderate intensity rehabilitation training. In other populations however higher intensity exercise therapy clearly further improves therapy outcome. In keeping with this, our research group was the first to perform a well-tolerated HIIT program in this population, presenting substantial improvements in endurance capacity and muscle contractile characteristics [38] as well as whole body glucose disposal [65]. The present pilot study confirms this showing improved exercise capacity (oxygen uptake, time to exhaustion, workload, Table 3) and muscle strength (Table 4) as well as reduced 2h blood glucose and HOMA index (Table 2). Similar to our previous work, subjects did not report exercise-induce side effects or discomfort and safely tolerated HIIT.

HICT has been shown to be an efficient strategy to improve cardiovascular fitness, aerobic capacity, body composition and muscle strength in healthy (older) adults [74-76]. Because persons with MS also present elevated cardiovascular risk factors [141] the present pilot study investigated the impact of an HICT intervention on several of these cardiovascular risk factors in MS. As described above and similar to our previous HIIT study 2h blood glucose, HOMA index and resting heart rate improved. In contrast to other comparable studies (1-5 high intensity exercise bouts ranging from 6s-4min interspersed by recovery periods of 30s-4min, at intensities of 90-100% heart rate or VO_{2max}, 8 to 12 weeks of training, n=8 to 84) in other populations [92, 98, 99, 101, 143, 144] blood lipid profile (total cholesterol, HDL, LDL), body composition (fat and lean tissue mass) and hemodynamic parameters (systolic and diastolic blood pressure) however were not affected. It is difficult to explain the latter but despite the fact that we and others previously successfully performed HIIT protocols in MS to improve exercise capacity and glucose tolerance, it is possible that persons with MS cannot reach the required maximal exercise intensities to affect other

cardiovascular parameters. Some recently published findings support this hypothesis. First, MS is associated with cardiovascular autonomic dysfunction leading to impaired carotid baroreflex control [145], attenuated elevations in blood pressure [146] and disturbed increases in heart rate [147] during exercise. As a result, subjects probably did not reach near maximal heart rates during HIIT. In other populations [148], cardiovascular autonomic dysfunction often induces chronotropic incompetence (inability of the heart rate to increase proportionally to an increase in activity or metabolic demand). Whether persons with MS also exhibit such abnormality is presently unknown but the obtained maximal heart rates of the HIIT group during the exercise test in the current study may indirectly confirm this hypothesis (PRE: 161±3.5 bpm vs. POST:162±4.8 bpm). Second, abnormal muscular energy metabolism in MS has been demonstrated involving reduced Krebs cycle and complex I and II activities [149], overproduction of reactive oxygen species [115], increased basal AMP-activated protein kinase phosphorylation [150] and delayed phosphocreatine resynthesis after exercise [149-151]. This suggests higher basal and exercise related energy expenditure and increased exercise-induced intramyocellular lactate accumulation, and thus greater muscle fatigue, as recently evidenced by increased basal [89] serum lactate concentrations. Therefore impaired energy supply may attenuate adequate exercise therapy response in MS. Although Amorini et al [89] only reported elevated serum lactate levels under resting conditions this again may indicate that, in the present pilot study, maximal exercise intensity was not reached as evidenced by the lower maximal blood lactate concentrations (Table 3), despite high post exercise muscle fatigue and overall perceived exertion rates (BORG: 14.7±1.5).

Study limitations

The present study warrants future research but it is clear that to better differentiate between high intensity interval therapy alone and high intense concurrent training, future intervention studies in this population should include a control group performing other exercise intervention types. Furthermore, under the conditions of the present pilot study effects on cardiovascular parameters seem limited. Despite the fact that similar training regimens in our previous training intervention study [38, 65] affected exercise capacity, muscle strength and glucose tolerance, longer training program duration may be required to improve other cardiovascular effects. Finally, this pilot study explored the impact of HICT on cardiovascular risk factors in a small sample size of persons with MS. Consequently, these data need confirmation in a larger scale study.

CONCLUSION

Under the conditions of the present pilot study, 12 weeks of high intensity concurrent training improved exercise capacity and muscle strength but does not appear to affect important cardiovascular risk factors such as blood pressure, body composition, blood lipid profiles and CRP levels in MS. The present findings warrant further, larger and controlled studies.

Declaration of interest

The authors report no conflicts of interest.

Study 3

Exercise-induced lactate responses in Multiple Sclerosis.

Based on: Charly Keytsman, Dominique Hansen, Inez Wens, Bert O Eijnde

Under review: Multiple Sclerosis and Related Disorders

ABSTRACT

Background. Multiple Sclerosis (MS) patients show elevated resting serum lactate concentrations compared to healthy controls (HC). Whether they also exhibit higher lactate concentrations during acute exercise and/or following training (repeated exercise) is unknown.

Methods. In this retrospective study, blood lactate concentrations (mmol/l) during submaximal (HC, n=11; MS, n=32) and maximal (HC, n=20; MS, n=24) exercise testing (ergospirometry) were analyzed. Submaximal ($lactate_{rest}$, $lactate_{bout1}$, $lactate_{bout2}$) and maximal ($lactate_{start}$, $lactate_{max}$) lactate concentrations were compared between groups. Hereafter, a portion of both MS groups were enrolled in a 24 week mild to moderate intensity (MS_{submax}, n=12) or 12 week high intensity interval (MS_{max}, n=13) exercise intervention program and hereafter performed POST exercise submaximal and maximal exercise tests.

Results. Under submaximal exercise conditions in MS and compared to HC, $lactate_{rest}$ (MS: 2.7±0.6 vs HC: 2.3±0.7) was significantly ($p<0.05$) elevated in MS. After 24 weeks of mild-to-moderate-intensity exercise training and compared to PRE values, $lactate_{bout2}$ (2.5±0.7 vs 3.4±1.1) was significantly ($p<0.05$) decreased during submaximal testing in MS_{submax}. Under maximal conditions, $lactate_{start}$ (2.3±1.0 vs 1.7±0.9) was significantly ($p>0.05$) elevated in MS. Twelve weeks of high intensity interval training did not improve this ($p>0.05$).

Conclusions. Under the conditions of the present study we conclude that MS patients have higher resting lactate concentrations but during acute submaximal and maximal exercise, lactate responses appear to be similar compared to healthy controls. Moderate intensity exercise therapy (24w) probably improves lactate accumulation but high intensity exercise therapy (12w) did not.

INTRODUCTION

In Multiple Sclerosis (MS) both central and peripheral mechanisms induce neuromuscular dysfunctions [152]. In skeletal muscle peripheral components include impaired excitation-contraction coupling and muscle energy metabolism [152]. With respect to the latter Kent-Braun et al. [149] already demonstrated lower succinic dehydrogenase (SDH) activity and higher α-glycerol-phosphate dehydrogenase (GPDH). We have shown impaired muscle signalling for mitochondrial and myofibrillar biogenesis, with upregulated phosphor-AMPK (oxidative stress) in MS [150]. Furthermore, higher proportions of type II at the expense of type I fibers, together with a decrease of cross-sectional area of all fibers in MS were found [43]. In this population these data point towards a higher involvement of anaerobic energy supply and therefore higher lactate production as recently shown by Amorini et al. [89], showing elevated resting serum lactate concentrations in MS compared to healthy controls (HC). Interestingly, they also demonstrated a linear correlation between serum lactate levels and the expanded disability status scale (EDSS), which might lead to new insights for objective measurable parameters to evaluate disease progression [89].

Exercise interventions of moderate- to high intensity training have become an important part of overall rehabilitation in MS [38, 123]. They improve muscle strength, exercise capacity and thus various functional measures [66], with superior effects of high intensity training compared to moderate intensity exercise [38, 65]. However, during/following such exercise training, subjects with MS report higher perceived fatigue perceptions compared to healthy controls [37, 38]. Under submaximal exercise conditions lactate is typically removed by muscular gluconeogenesis and oxidation, or transported to the blood and subsequently filtered by other cells [153, 154]. Under maximal exercise conditions, however, lactate increases rapidly as a result of anaerobic/glycolytic energy metabolism and, if not removed adequately, eventually accumulates. Lactate accumulation is one of the contributing factors causing higher perceived fatigue [155] during exercise, which might lead to cessation of the activity. However, whether patients with MS also

exhibit higher lactate concentrations during exercise, possibly causing greater fatigue perceptions and perhaps poorer exercise adherence, is not known.

In HC [156, 157] as well as in obese subjects [158] 8 to 12 weeks of sub- and maximal exercise training effectively improves exercise-induced lactate clearance. Whether patients with MS also profit from moderate to high intensity training (i.e. repeated exercise sessions) to gradually improve whole body lactate accumulation [89] is not clear.

In keeping with the above lines of reasoning, the first aim of the present study is to analyze blood lactate accumulation during both acute (i.e. one exercise session) submaximal and maximal exercise bouts in MS patients. Second, we explore whether submaximal or maximal intensity exercise therapy (i.e. repeated exercise sessions) can affect whole body lactate response. We hypothesize that lactate accumulation during submaximal and maximal exercise testing is higher in MS patients and that exercise therapy will decrease this.

METHODS

The present study retrospectively investigates exercise-induced lactate accumulation during sub- and maximal exercise testing in MS and HC including data originating from previously performed studies of our research group that were registered at ClinicalTrials.gov (NTC01718392 - NCT02466165) and published elsewhere [37].

Participants

Subjects were excluded if they were pregnant, participated in another study, had (in case of MS) an acute exacerbation 6 months prior to the start of the study or had an EDSS score >6. Participants were informed about the nature and risks of the experimental procedures before their written

informed consent was obtained. The submaximal testing group involved 32 MS patients (EDSS 3.1±0.2) and 11 HC, whereas the maximal testing group involved 24 MS patients (EDSS 2.9±0.2) and 20 HC. Both studies were approved by the medical ethical committee of Hasselt University.

Study design

At baseline all MS patients and HC underwent a DEXA scan to establish whole body lean tissue mass and performed either a submaximal ($baseline_{submax}$, HC, n=11; MS, n=32) or maximal ($baseline_{max}$, HC, n=20; MS, n=24) exercise test on a cycle ergometer including ergospirometry and blood lactate measurements. Hereafter, a portion of both MS groups were enrolled in a 24 week mild to moderate intensity (after submaximal exercise test, MS_{submax}, n=12) or 12 week high intensity interval (after maximal exercise test, MS_{max}, n=13) exercise intervention program. HC and the remaining MS patients did not further participate in the interventions. Following 24 or 12 weeks of exercise intervention, DEXA scans and submaximal (MS_{submax}) and maximal (MS_{max}) graded exercise tests were repeated in the respective MS exercise groups.

Primary outcomes measurements

Exercise testing

Cardiopulmonary graded exercise tests were performed on an electronically braked cycle ergometer (eBike Basic, General Electric GmbH, Bitz, Germany). Pulmonary gas exchange was continuously measured breath-by-breath with a mass spectrometer and volume turbine system (Jaeger Oxycon, Erich Jaeger GmbH, Germany). During the exercise test, oxygen uptake (VO_2 ml/min) was assessed breath-by-breath, after which this data were averaged every 10s. Heart rate was continuously monitored by a 12-lead electrocardiograph device. During maximal exercise testing RER values

were evaluated to verify maximal exhaustion. In addition, maximal cycling resistance (W_{max}), maximal heart rate (HR_{max}), test duration and VO_{2max}, defined as the corresponding load, heart rate, minutes and oxygen uptake measured at the level of exhaustion, were reported. Capillary blood samples were obtained from the earlobe to analyze blood lactate concentrations (mmol/l).

Submaximal exercise testing

Subjects were seated on the bike for 3min to obtain resting data. Next, subjects were instructed to cycle at 70rpm against a resistance corresponding to 25% (MS patients) or 35% (HC) of predicted cycling power output (W_{max}), for 6min (first submaximal exercise bout). Hereafter, subjects remained seated on the bike for 6min (recovery phase), after which a second 6min submaximal exercise bout and a 6min recovery phase were performed. Predicted W_{max} was based on gender, age, body weight, height, and calculated by previously published formulae [160]. In HC a higher resistance was selected to obtain comparable relative exercise intensities. Immediately after the 3min resting period ($lactate_{rest}$) and each 6min exercise bout ($lactate_{bout1}$, $lactate_{bout2}$), capillary blood samples were obtained from the fingertip to analyze blood lactate concentrations.

Maximal graded exercise testing

Maximal exercise capacity (until volitional exhaustion) was tested to evaluate maximal workload (W_{max}), and time to exhaustion. Following a 10min standardized warm up, participants started at a low workload that gradually increases after each completed minute (♂: 30W+15W/min, ♀: 20W+10W/min). No resting lactate concentrations were collected. Immediately after the first test minute ($lactate_{start}$) and subsequently every 2min until volitional cessation ($lactate_{max}$), capillary blood samples were taken from the earlobe to analyse blood lactate concentrations.

Secondary outcome measurements

Body composition

Lean tissue mass was obtained using Dual Energy X-ray Absorptiometry scan (DEXA) (Hologic Series Delphi-A Fan Beam X-ray Bone Densitometer, Vilvoorde, Belgium). A calibrated analogue weight scale (Seca®) was used to measure total body mass.

Exercise intervention programs

Submaximal exercise conditions

Part of the MS subjects (MS_{submax}), that were tested submaximal, participated in a supervised 24-week combined training program, at a frequency of 5 sessions per two 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 1x6min/session to 3x10 min/session, interspersed by 2min of rest. The second part consisted of resistance training (leg press, leg curl, leg extension, vertical traction, arm curl, and chest press; Technogym®). To improve muscle fitness, sets of repetitions gradually increased during intervention, from1x10 repetitions to 4x15 repetitions, with maximal attainable load, interspersed by 2min of rest. All exercises were performed at a mild to moderate workload corresponding to 12-14 ratings of perceived exertion (RPE) on a 20-point Borg scale.

Maximal exercise conditions

Part of the MS subjects (MS_{max}) that were exercise tested maximally, performed a 12-week HIIT training program on a cycle ergometer and moderate-to-high intensity resistance training under close supervision. During the first 6-week training cycle, exercise duration gradually increased from 5x1min (1min rest intervals) to 5x2min. Exercise intensity was 100% of the maximal workload (highest power output at the point of exhaustion obtained during the exercise test), corresponding to 85-90% of the maximal heart rate. During the second 6-week training cycle, duration remained stable at 5x2min and the workload (Watt) increased to 100% of maximal heart rate. The second part consisted of intensity resistance training. Here, training intensity and volume were adjusted throughout the intervention program from 1x10 repetitions to 2x20 repetitions at a maximal attainable load.

Prior to the first training session, a familiarization visit was performed to determine starting loads for the resistance exercises. Because MS patients often experience bilateral strength differences between legs [161], exercise training was performed at similar relative workloads. Consequently, resistance training of the lower limbs was performed unilaterally during both training interventions.

Statistical Analysis

All data were analysed using SPSS v.22.0 (IBM). Normality was analysed using Shapiro-Wilk test. Baseline differences between HC and MS were analyzed using the Mann Whitney U test (unpaired). Pre-post differences within the MS exercise groups were analyzed using Wilcoxon Signed Ranks tests (paired). All data are presented as means ± SD's and the threshold for statistical significance was set at $p<0.05$.

RESULTS

Subject characteristics

Baseline characteristics did not differ between HC and MS (Table 1 & 2). Characteristics of the MS exercise intervention groups (MS_{submax} and MS_{max}) did not differ.

Table 1. Submaximal testing and exercise intervention: subject characteristics.

	$Baseline_{submax}$		**MS_{submax}**
	HC	**MS**	**EX**
Age (years)	52.6± 7.5	48.1 ± 10.4	46.3 ± 12
Length (m)	1.72 ± 9.8	1.70 ± 7.8	1.70 ± 9.2
Weight (kg)	74.1 ± 12.9	73.4 ± 14.8	72 ± 16.1
BMI	24.8 ± 1.7	24.4 ± 4.62	24.6 ± 4.5
EDSS	/	3.1 ± 1.29	3.3 ± 1.5

Data are expressed as means ± SD and represent baseline subject characteristics (BMI: body mass index, EDSS: Physical Activity Scale for Individuals with Physical Disabilities) of healthy controls (HC, n=11) and MS (n=33), and a subgroup of MS patients before (PRE) 24 weeks of mild-to-moderate-intensity exercise therapy (MS_{submax}, n=12, see methods).

Table 2. Maximal testing and exercise intervention: subject characteristics.

	$Baseline_{max}$		**MS_{max}**
	HC	**MS**	**EX**
Age (years)	47.8 ± 11.8	50.7 ± 8.6	51.5 ± 7.1
Length (m)	1.69 ± 0.1	1.70 ± 0.1	1.71 ± 9.1
Weight (kg)	69.9 ± 14.3	72.1 ± 13.6	68 ± 12.9
BMI	24.2 ± 2.8	24.7 ± 4.4	23.5 ± 3.1
EDSS	/	2.9 ± 1.5	2.6 ± 1.5

Data are expressed as means ± SD and represent baseline subject characteristics (BMI: body mass index, EDSS: Physical Activity Scale for Individuals with Physical Disabilities) of healthy controls (HC, n=20) and MS patients (n=24), and a subgroup of MS patients before (PRE) 12 weeks of high intensity exercise therapy (MS_{max}, n=13, see methods).

Lactate concentrations

Submaximal conditions

In MS $lactate_{rest}$ (+17%) was significantly elevated compared to HC, whereas $lactate_{bout1}$ and $lactate_{bout2}$ did not differ between groups (p>0.05)(Table3). Following 24 weeks of mild-to-moderate-intensity exercise training and compared to PRE exercise intervention values $lactate_{rest}$ (2.6±0.4 mmol/L vs. 2.2±0.7 mmol/L, p=0.1) and $lactate_{bout1}$ (3.3±0.7 mmol/L vs. 2.8±0.9 mmol/L, p=0.07) tended to decrease. Twenty four weeks of submaximal exercise significantly (p<0.05) reduced blood lactate during the second exercise bout (Table 4).

Table 3. Blood lactate concentrations during submaximal and maximal exercise testing.

	HC	MS
Submax. test		
$Lactate_{rest}$	2.3 ± 0.7	2.7 ± 0.6*
$Lactate_{bout1}$	3.1 ± 1.2	3.1 ± 0.8
$Lactate_{bout2}$	2.7 ± 1.5	3.0 ± 0.7
Max. test		
$Lactate_{start}$	1.7 ± 0.9	2.3 ± 1.0*
$Lactate_{max}$	5.6 ± 1.7	5.4 ± 1.8

*Data are expressed as means ± SD and represent both baseline blood lactate concentrations (mmol/L) of healthy controls (HC) and MS patients during submaximal ($baseline_{submax}$: HC, n=11; MS, n=33) and maximal ($baseline_{max}$: HC, n=20; MS, n=24) exercise testing. *p<0.05 compared to baseline HC.*

Maximal conditions

Compared to HC, $lactate_{start}$ (+35%) was significantly elevated in MS, whereas $lactate_{max}$ did not differ between groups (Table 3). Twelve weeks of high intensity interval training did not improve this in MS_{max} (Table 4).

Table 4. Exercise test blood lactate concentrations following exercise therapy

	PRE	POST	p-value
MS$_{submax}$			
Lactate$_{rest}$	2.6 ± 0.4	2.2 ± 0.7	0.10
Lactate$_{bout1}$	3.3 ± 0.7	2.8 ± 0.9	0.07
Lactate$_{bout2}$	3.4 ± 1.1	2.5 ± 0.7*	0.00
MS$_{max}$			
Lactate$_{start}$	2.2 ± 1.1	2.1 ± 0.7	0.71
Lactate$_{max}$	5.8 ± 1.7	5.5 ± 1.3	0.50

*Data are expressed as means ± SD and represent blood lactate concentrations (mmol/L) of a subgroup of MS patients before (PRE) and after (POST) 24 weeks of mild-to-moderate-intensity exercise (MS$_{submax}$: n=12) or 12 weeks of high intensity exercise therapy (MS$_{max}$: n=13) respectively. *p<0.05 compared to corresponding PRE MS.*

DISCUSSION

This study confirms higher resting blood lactate levels in MS compared to healthy controls. During acute submaximal and maximal exercise, these differences in blood lactate concentrations disappear. Twenty-four weeks of moderate intensity exercise therapy tended to improve resting blood lactate levels and reduced blood lactate during submaximal exercise. Surprisingly, 12 weeks of high intensity exercise therapy in this population did not have comparable effects during maximal testing.

We here confirm increased resting/start blood lactate levels in MS patients compared to HC as recently reported by Amorini et al. [89]. Amorini and coworkers were the first to show elevated resting serum lactate concentrations in MS and they assumed that this originated from either neural or muscular tissue. To further explore this we used a submaximal and maximal exercise test to induce muscle based blood lactate accumulation. Surprisingly and with the exception of the second submaximal exercise bout, the exercise-induced lactate responses during both acute submaximal and maximal exercise were similar in MS and HC.

Therefore, increased subjective fatigue perceptions during exercise in persons with MS [37, 38] does not seem to originate from higher lactate concentrations.

In HC [156, 157] and other populations [158] exercise training at sub- or maximal intensity is known to effectively reduce exercise-induced lactate accumulation. In the present study, a 24-week moderate intensity exercise intervention tended to reduce resting blood lactate levels and blood lactate following a first bout of submaximal effort. Following a second bout of aerobic muscle work blood lactate was effectively lower. Twelve weeks of high intense exercise therapy did not have such effect on both resting and exercise-induced blood lactate concentrations either or not corrected for lean tissue mass. This may suggest an abnormal lactate response in MS under anaerobic/glycolytic circumstances such as during high intensity training which confirms earlier reported skeletal muscle anaerobic metabolism malfunctions in MS [149, 150, 152]. High intensity exercise therapy in MS, however, elicits superior effects for exercise capacity, muscle contractile characteristics [38] and glucose tolerance [65] compared to moderate intensity training. As such, a combination of high intense exercise training with moderate intensity physical therapy seems ideal to maximally benefit from the advantages of both training modalities.

It is important to note that an exercise test performed under both submaximal and maximal conditions following moderate and after high-intensity exercise training would be ideal. Unfortunately, the available retrospective data did not allow this. Therefore, and because of the different lactate responses during moderate and high intensity training exercise presented in this study, future research seems warranted.

The results of this retrospective study may implicate that muscular activation under both submaximal and maximal exercise conditions might be a valid strategy to reduce elevated resting lactate concentrations in MS. Furthermore, any strategy that may improve exercise-induced lactate response during high intensity exercise therapy may be worthwhile

investigating, since this may improve exercise adherence and performance and thus possibly lead to better clinical outcomes in MS.

Finally, it is important to note that interpretation of the presented retrospective results is however difficult, as potential bias for exercise testing/intervention may include different MS phenotypes and varying levels of cardiorespiratory fitness in the investigated subjects. These factors may indeed influence (exercise-induced) lactate accumulation. Future, controlled, research regarding lactate responses in MS should therefore clearly take factors such as MS phenotype, level of disability, medication intake and cardiorespiratory levels into account.

CONCLUSION

Under the conditions of the present study we conclude that MS patients indeed have higher resting blood lactate concentrations. During acute submaximal and maximal exercise, however, lactate responses appear to be similar between MS and healthy controls. Furthermore, moderate intensity exercise therapy probably improves lactate concentrations but high intensity exercise therapy did not.

Conflict of Interest

The authors declare no conflicts of interest.

Study 4

Muscle Carnosine in Experimental Autoimmune Encephalomyelitis and Multiple Sclerosis

Based on: Charly Keytsman, Laura Blancquaert, Inez Wens, Maarten Missine, Pieter Van Noten, Frank Vandenabeele, Wim Derave, Bert O Eijnde

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ABSTRACT

Background. Muscle carnosine is related to contractile function (Ca^{++} handling) and buffering of exercise–induced acidosis. As these muscular functions are altered in Multiple Sclerosis (MS) it is relevant to understand muscle carnosine levels in MS.

Methods. Tibialis anterior muscle carnosine was measured in an animal MS model (EAE, experimental autoimmune encephalomyelitis, n=40) and controls (CON, n=40) before and after exercise training (EAE_{EX}, CON_{EX}, 10d, 1h/d, 24m/min treadmill running) or sedentary conditions (EAE_{SED}, CON_{SED}). Human m. vastus lateralis carnosine of healthy controls (HC, n=22) and MS patients (n=24) were measured.

Results. EAE muscle carnosine levels were decreased ($p<0.0001$) by ~40% to ~64% at 10d and 17d following EAE induction (respectively) regardless of exercise ($p=0.823$). Similarly, human MS muscle carnosine levels were decreased (-25%, $p=0.03$).

Conclusion. Muscle carnosine concentrations in an animal MS model and MS patients are substantially reduced. In EAE exercise therapy does not restore this.

INTRODUCTION

Multiple sclerosis (MS) is a neurodegenerative autoimmune disease of the central nervous system [162], in which inflammatory and demyelination processes also result in physical inactivity, decreased exercise capacity, excessive (post-exercise) fatigue and reduced muscle contractile function [39, 44]. These frequently occurring secondary symptoms substantially affect various daily life activities, ultimately leading to an impaired quality of life [128]. Central mechanisms [40, 149, 152, 163, 164] such as reduced motor firing rates, impaired motor unit recruitment and increased central motor conduction time clearly contribute to the above described disuse-related physiological profile [140]. A portion of the neuromuscular dysfunction in MS however, probably also resides within the skeletal muscle [40, 42, 43, 149, 151, 152, 163].

We, and others, already reported altered muscle fiber composition (shift to glycolytic fibers), disturbed muscle contractile characteristics and cross-bridge (Ca^{2+} handling) abnormalities in muscles of MS patients [40, 42, 43]. Furthermore, reduced Krebs cycle and complex I and II activities [149, 165], overproduction of reactive oxygen species (ROS) [115], increased basal AMP-activated protein kinase phosphorylation [150] and delayed phosphocreatine resynthesis after exercise [149-151, 165, 166] suggest muscular mitochondrial dysfunction in MS and higher basal and exercise-related energy expenditure. In keeping with this, increased intramyocellular lactate accumulation, leading to increased basal serum lactate concentrations [89], have been shown to cause greater (perceived) muscle fatigue in MS patients. It thus seems that MS patients do not only exhibit central neurological abnormalities but also impairments in both muscular contraction mechanisms and energy supply. In keeping with this, exercise rehabilitation therapy, that amongst others induces muscular acidosis, has become an important part of overall MS treatment [123].

So far, small but significant improvements in exercise capacity and muscle contractile characteristics following various types (modality, frequency) of short to longer term regular exercise therapy have been reported in both a frequently used animal MS model, notably Experimental Autoimmune Encephalomyelitis (EAE)[28] and in MS [66, 71, 73, 129, 167, 168]. In an attempt to further improve muscle contractile and energy metabolic responses to exercise, our laboratory recently performed high(er) intensity training studies in MS showing substantially improved (+25-50%) muscle strength and exercise capacity following 8-12w of exercise [28, 38]. Although promising, most MS subjects however reported higher post-exercise muscle fatigue and overall perceived exertion rates (BORG: 14.7±1.5 vs. 12.7±1.3) compared to regular intensity training [43]. Consequently, any strategy that attenuates this may further improve therapy outcome.

Carnosine, a dipeptide composed of β-alanine and L-histidine, is found in high concentrations in mammalian skeletal muscle [105]. Together with anserine or ophidine/balenine, the methylated analogs that possess the same bioactivity and are exclusively found in animals, carnosine forms the histidine-containing dipeptides (HCD). The physiological role of carnosine is related to contractile function in general and more specific to Ca^{++} handling [106]. Interestingly, carnosine has also been shown to buffer exercise–induced acidosis [106], to affect mitochondrial respiration [107] and to protect against exercise-induced oxidative stress [108]. The functional role of carnosine in skeletal muscle is thus closely related to several of the above described muscle alterations seen in MS. So far, altered tissue carnosine concentrations were shown in other neurological diseases such as Amyotrophic Lateral Sclerosis (ALS) [116] and Parkinson's Disease [105, 117]. Moreover, reduced serum carnosinase activity was already found in patients with MS [118], indicating that several of the above described muscle alterations like impaired exercise capacity, excessive (post-exercise) fatigue and reduced muscle contractile function in MS [39, 43, 44] may, in part, be related to reduced muscle carnosine levels. This, however, has not been investigated in animal MS models and/or MS yet. Finally, it is important to

note that although the overall impact of exercise on muscle carnosine content in healthy controls and disease is contradictory [169-174], increased muscle carnosine has been shown to reduce exercise-induced fatigue and hereby improve exercise performance during high-intensity intermittent exercise (1-4min) [106, 109, 170]. Therefore, the first aim of this study is to investigate muscle carnosine levels in both EAE animals and MS patients. We hypothesize that muscle carnosine concentrations in EAE and MS are reduced compared to healthy controls. Secondly, the effect of exercise on muscle carnosine levels in EAE rats is explored.

METHODS

Muscle carnosine content was measured in both an animal MS model, notably Experimental Autoimmune Encephalomyelitis (EAE, Part I), and MS patients (Part II). The analyzed muscle samples originate from studies that were previously performed and published by our laboratories [28, 141].

Part I

In a first phase, rodent muscle carnosine and anserine levels were investigated using the EAE animal MS model [25]. Briefly, this model is characterized by the induction of EAE on day 0, an inflammatory period with no clinical symptoms (day 0 to day 10) and gradual hindquarter paralysis (day 11 to day 14) followed by almost full recovery (day 15 to day 17).

Animals

Eighty female Lewis rats (age 6-7 weeks, body weight 100-120 g, Harlan CPB, Zeist, The Netherlands) were individually housed (12h/12h light/dark cycle; temperature of 22°C; relative humidity of 22-24%) in the animal facilities at Hasselt University. Rats were fed *ad libitum* with water and

normal rat pellets (Carfil RN-01-K12, Harlan). The animal Ethics Committee of Hasselt University approved the study protocol that complied with the national/European legislation and the National Research Council's guide for the care and use of laboratory animals.

Study design

Following acclimatization and adaptation (day -21 to -15), animals were randomized into two subgroups: a sedentary group (SED, n=40) and an exercise group (EX, n=40). In order to induce comparable levels of stress, SED animals were seated on the stationary treadmill (1 hour) on a daily basis (day -14 to 0). Daily food intake and body weight were registered. At day 0, SED and EX groups were subdivided into a healthy control group (CON_{SED} n=20; CON_{EX} n=20) and an EAE group (EAE_{SED} n=20; EAE_{EX} n=20). Briefly, EAE was induced by a single percutaneous injection in both footpads (100µl/foot) under isoflurane anesthesia 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), 120ùl complete Freunds adjuvant (CFA, Difco) and 31µl phosphate-buffered saline (PBS) [27]. Hereafter, EX rats exercised daily for 1 hour/day, during 10 consecutive days, until progressive hindquarter paralysis prevented this. Treadmill performance/intensity was visually monitored daily by the researchers and did not appear to be different between the control and EAE groups.

At day 10 (D10), just before onset of hindquarter paralysis, treadmill training was terminated and 10 animals of each group were evaluated. The remaining rats (n = 10 per group) endured hindquarter paralyses (EAE group, day 11 – 14) and were evaluated after (almost full) recovery on day 17 (D17). All animals were anaesthetized using an intraperitoneal injection of pentobarbital sodium (5 mg 100 g-1 BW) and m. tibialis anterior (TA) of the right hind limb was dissected and freed of connective tissue and visible blood. The mid-part of each muscle was snap-frozen using liquid nitrogen,

and stored at -80°C until further analysis were performed. All animals were sacrificed after muscle sampling.

Throughout the study course, animals were carefully monitored on daily basis. Animals did not display severe discomfort immediately following immunization. Paralysis and typical signs of EAE occurred 11 days post-immunization, which is the typical time frame for this animal model. If animals exhibited signs of severe pain (high clinical score for 2 consecutive days), distress, were unable to reach food or when humane endpoints were reached, euthanasia to relief pain was performed. However, none of the animals included in the study underwent euthanasia for one of those reasons.

Part II

MS subjects

Twenty-two healthy controls (HC) and twenty-four patients with MS (aged >18 years; mean EDSS 3.1±1.5, range 0→6, median 2.5) were included following written informed consent. Subjects were excluded if they participated in other studies, had (in case of MS) an acute exacerbation 6 months prior to the start of the study or had an EDSS score >6. Phenotypes of MS (RR, relapsing remitting; PP, primary progressive; SP, secondary progressive) and the duration of the disease were inventoried. The study was approved by the local Ethical Committee of the Jessa hospital and Hasselt University and complied with the Declaration of Helsinki. This study was registered at ClinicalTrials.gov (NCT02466165).

Muscle biopsies

Muscle biopsies were obtained from MS patients (n= 24) and HC (n= 22) from the middle part of the m. vastus lateralis (Bergström needle technique), by an experienced medical doctor. Biopsies were sampled 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, since muscle strength associated with each leg (left vs. right or dominant vs. nondominant) is equal in healthy persons [175, 176]. The sample was freed from connective tissue and snap-frozen with liquid nitrogen and stored at -80°C, until further analysis.

Muscle dipeptide and free amino acid content

Dipeptide and free amino acid concentrations were determined by high-performance liquid chromatography (HPLC), as previously described [177]. Muscle samples (15mg wet weight, WW) were deproteinized using 35% sulfosalicylic acid and centrifuged (5min, 16,000g). Deproteinized supernatant (5µl sample) was mixed with AccQ Fluor Borate buffer (75µl) and reconstituted Fluor Reagent (20µl) from the AccQTag chemistry kit (Waters). The derivatized samples were applied to a Waters HPLC system comprised of an XBridge BEH column (4.6 x 150 mm, 2.5µm) and fluorescence detector (excitation/emission wavelength: 250/395nm). Carnosine and anserine were assessed in Part I (animals) and carnosine, taurine, serine, glutamine and histidine were measured in Part II (human subjects). To compare muscle biopsy quality between healthy controls and MS, some reference muscle amino acid (serine, histidine and glutamine, in Part II) concentrations, expressed as total areas under the curve (tAUC), were compared between groups. In case of normal serine, histidine and glutamine concentrations, low carnosine concentrations could not be due to poor biopsy quality .

Statistical Analysis

All data were analyzed using SPPS v. 22.0 (IBM). Normality of data distribution was evaluated using the Shapiro-Wilk test. In animals, ANOVA was used to evaluate the (main and interaction) effect of 'group' (CON vs. EAE), 'activity' (SED vs. EX) and 'time' (D10 vs. D17) on carnosine and anserine concentrations and. For human analysis, group comparison was performed using unpaired student's t-tests in case of normality and non-parametric independent t-tests (Wilcoxon) otherwise. All data are presented as means ± SD and the threshold for statistical significance was set at $p<0.05$.

RESULTS

Part I.

Rat muscle carnosine and anserine concentrations

Muscle carnosine concentrations were significantly lower (main group effect, CON vs. EAE, $p<0.0001$ range: -40% → -64%) in EAE compared to CON and D10 to D17(main time effect, D10 vs. D17, $p=0.005$, range: -4% → -46%)(Table 1). However, exercise had no influence on carnosine concentrations (main activity effect, SED vs. EX, $p=0.823$). No interaction effects were found. Muscle anserine was significantly higher in EAE compared to CON (main group effect, CON vs. EAE, p=0000, range: +36% → +40%). No main activity and time effects as well as interaction effects were detected.

Table 1. Rat muscle carnosine and anserine concentrations.

	CON_{SED}		CON_{EX}		EAE_{SED}		EAE_{EX}	
	D10	D17	D10	D17	D10	D17	D10	D17
Carnosine	2.5 ± 0.6	2.2 ± 0.4	2.5 ±0.6	2.4 ± 1.1	1.5 ± 0.6	0.8 ± 0.4	1.4 ± 0.3	0.9 ± 0.5
Anserine	2.2 ± 0.7	2.5 ± 0.4	2 ± 0.5	2.3 ± 1.0	3 ± 0.7	3.4 ± 1.0	3.5 ± 0.7	2.9 ± 0.6

Data are expressed as means ± SD and represent rat m. tibialis anterior carnosine and anserine concentrations (mmol/kg WW) 10 (D10, n=10/group) and 17 (D17, n=10/group) days after acute experimental autoimmune encephalomyelitis induction (EAE) or healthy control (CON) under sedentary (CON_{SED}, EAE_{SED}) or exercise (treadmill running, 1h/d, 18m/min, CON_{EX}, EAE_{EX}) conditions. Main effects for group (CON vs. EAE, p=0000) and time (D10 vs. D17, p=0.005) were present for carnosine concentrations. For anserine concentrations, a main group effect (CON vs. EAE, p=0000) was found.

Part II.

Subject characteristics

Subject characteristics (Table 2) did not differ between HC and MS. Relapsing-remitting MS was diagnosed in 16 patients, whilst 6 patients suffered from primary-progressive MS and 2 patients from the secondary-progressive form. Mean disease duration was 13 ± 8 years (range 1→26y).

Table 2. Baseline subject characteristics.

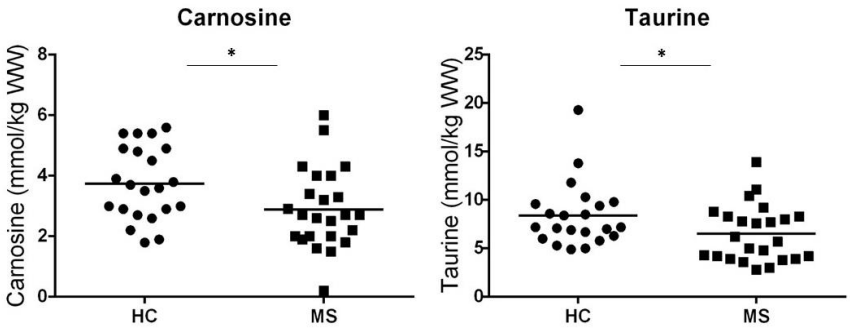
	HC	MS
Age (years)	48.7 ± 11.8	52.7 ± 8.9
Gender (f/m)	14/8	13/11
Weight (kg)	69.5 ± 13.8	71.6 ± 12.4
Height (m)	1.69 ± 0.1	1.71 ± 0.1
BMI	24.1 ± 2.4	24.5 ± 3.5
Disease duration (years)	/	13 ± 8
EDSS	/	3.1 ± 1.5

Data are expressed as means ± SD and represent subject characteristics (BMI: body mass index) of healthy controls (HC, n=22) and MS patients (n=24). EDSS, Expanded Disability Status Scale.

Muscle carnosine and taurine content

In MS, muscle carnosine (3.8±1.2 mmol/kg WW vs. 2.9±1.3 mmol/kg WW, p=0.03) and muscle taurine (8.4±3.3 mmol/kg WW vs. 6.5±2.9 mmol/kg WW, p=0.04) levels were lower compared to HC (Figure1). Total areas under the curve of muscle serine (5.1±2.7 vs. 4.3±1.1, p=0.12), histidine (6.2±1.6 vs. 5.5±1.5, p=0.07) and glutamine (59.7±30.3 vs. 61.1±28.6, p=0.43) did not differ between groups (data not shown).

Fig 1 Muscle carnosine and taurine content of healthy controls and multiple sclerosis



Data represent m. vastus lateralis carnosine and taurine concentrations (mmol/kg WW) of *healthy controls (HC, n= 22) and MS patients (n=24). *p<0.05 between HC and MS.*

DISCUSSION

Compared to healthy controls the present study clearly shows reduced muscle carnosine levels in both human MS and a frequently used animal MS model. Furthermore, in animals, exercise did not restore carnosine concentrations. In MS, lower muscle carnosine was paralleled by reduced taurine.

Literature indicates that carnosine metabolism could be altered in neuromuscular diseases. Wassif et al. [118] already demonstrated reduced serum carnosinase activity (-49%) in fresh blood samples from MS patients compared to healthy controls. Carnosinase causes hydrolysis of carnosine into β-alanine and L-histidine. This suggests alterations in carnosine metabolism in patients with MS. Stuerenburg [116] investigated the carnosine content of skeletal muscles from patients with suggestive neuromuscular diseases and rats of various ages. Following stepwise regression modelling they reported that another neurological disease such as ALS was negatively correlated with muscle carnosine content and they suggested that this was caused by progressive denervation processes, as also seen in MS. Although altered tissue carnosine concentrations in other neuromuscular diseases such as ALS [116] and Parkinson Disease [105, 117] and reduced serum carnosinase activity in MS [118] were already described, we are now the first to report substantially reduced muscle carnosine levels in an animal MS model and confirm this in MS patients.

Apart from reduced muscle carnosine in EAE we also detected increased muscle anserine concentrations. Interestingly anserine, the methylated form of carnosine, is exclusively found in animals where it possesses the same bioactivity as carnosine [105]. Hence, when carnosine decreases and anserine tends to increase (Table 1), the total amount of histidine-containing dipeptides (HCD) remains unchanged. In order to keep the total HCD concentration constant, it is possible that carnosine methyltransferase (CMT) is upregulated following EAE induction, leading to enhanced muscle anserine levels. Interestingly, Drozak et al. (2015) recently molecularly identified UPF0586 protein C9orf41 as the mammalian carnosine-N-methyltransferase, responsible for anserine formation in rat muscle [178]. However, to date, no activity assay for CMT is available. Quantitative PCR could be an alternative to measure the effect of EAE on CMT gene expression, but this is not always in agreement with the amount of mRNA that is effectively translated into protein. In human subjects however, the methylated analog anserine is absent. Therefore, in humans a decrease in

muscle carnosine content implicates an effective reduction of the total histidine-containing dipeptide store.

Although the exact underlying mechanisms remain unclear, several contributing factors may explain reduced carnosine stores in MS muscles. In MS, central mechanisms [40, 149, 152, 163, 164], such as impaired motor unit recruitment and delayed conduction/reaction times induce a disuse-related physiological profile [140]. In sedentary populations and in chronic disease (e.g. ALS, osteoarthritis), lower muscle carnosine levels [116, 179] have been reported that might result from an inactivity related reduction in muscle protein content. However, in the present study muscle histidine, glutamine and serine, that are also prone to inactivity, were not affected in MS muscle samples. As such, we assume that the presented decrease in muscle carnosine content was not due to poor biopsy or muscle quality. Possibly, several intramyocellular dysfunctions that are associated with MS and relate to the physiological role(s) of carnosine in muscle [105], could partly be related to the present findings. However, the exact effects of muscle carnosine on, amongst others, disturbed muscle contractile characteristics and cross-bridge (Ca^{2+} handling) abnormalities [40, 42, 43], excessive exercise-induced acidosis [40, 149, 152] and mitochondrial dysfunction [149-152, 165, 166] in MS have not been investigated yet.

The effect of exercise therapy on muscle carnosine content is not fully clear. With the exception of Suzuki et al. [169], who detected increased carnosine concentrations after an 8 week sprint interval program, most studies concerning this matter did not demonstrate positive effects after a 4-16 week isokinetic resistance training program [172-174] or a 5 week sprint-training intervention [171] on muscle carnosine concentrations in healthy subjects. The present study explored this in an animal MS model and confirmed that exercise therapy did not prevent reduction of muscle carnosine. Possibly, nutritional interventions (e.g. β-alanine supplementation) are required to exert such effects.

Muscular carnosine concentrations are a good marker of the total body carnosine stores, as >99% of the compound is found in skeletal muscle cells [105]. A reduction of total carnosine stores is possibly also related to the increased oxidative stress and the resulting accumulation of cytotoxic compounds, such as the reactive carbonyls acrolein and hydroxynonenal (HNE). Carnosine serves as a sacrificial sequestering agent for acrolein and HNE by forming unreactive adducts [180] and as such provides an endogenous protective mechanism against protein and ultimately tissue damage induced by these reactive carbonyls [181, 182]. The increased urinary elimination of carnosine-carbonyl conjugates has been demonstrated for certain metabolic conditions (metabolic syndrome, obesity, type-2 diabetes) that are characterized by increased carbonyl stress [183]. Interestingly, acrolein has also been implicated in the pathogenesis of MS, as it directly damages myelin, through reaction with protein and lipid components of myelin, leading to demyelination. Furthermore, acrolein has recently been identified as a promising and effective therapeutic target [184, 185] in this population. In fact, when Leung [185] and co-workers treated EAE mice with the acrolein scavenger hydralazine, myelin integrity appeared to be largely preserved indicating that acrolein removal may offer neuroprotection. Possibly, reduced muscle carnosine content in MS results from the incapacity of endogenous carnosine synthesis to compensate for the increased 'consumption' of carnosine to detoxify and eliminate acrolein. According to this line of reasoning, nutritional strategies to improve muscle carnosine content in persons with MS are worthwhile to explore. Carnosine supplementation (~1.5g/d β-alanine) has already been shown to improve a number of neurological symptoms [186] in Alzheimer's [187] and Parkinson's [105] disease. Clinically, nutritional interventions that normalize or even elevate the reduced muscle carnosine content could therefore be a valid new approach to improve muscle contractile properties, myocellular energy supply and/or possibly neurological symptoms in MS. Moreover, MS patients would be able to exercise more efficiently at higher intensities leading to a better clinical rehabilitation therapy outcome in these patients.

Limitations

The present study holds certain limitations that should be taken into account regarding future research. We show that EAE and MS reduce muscle carnosine, though because no nerve tissue was collected it was not possible to investigate whether this was due to neuroaxonal injury and/or muscle denervation. It is therefore warranted that in future EAE research nerve tissue is sampled to investigate peripheral nerves, motor end-plates and the extent of both neuronal and axonal damage in the spinal cord. In MS, future studies should also investigate spinal cord lesion load.

The current paper describes a reduction in muscle carnosine concentrations in an EAE group, compared to control animals. However, in order to determine whether reduced muscle carnosine levels were actually related to EAE itself, a control group with injection of Complete Freund's Adjuvant alone, causing inflammatory processes, would be appropriate. Indeed, the injection of CFA may have caused local inflammation and thus influenced m. tibialis anterior carnosine concentrations. This issue may also be addressed by unilateral immunization and comparison of muscle carnosine of the two hind limbs. Furthermore, for future carnosine related research in MS, assessment of fatigue levels and dietary habits is worthwhile.

In summary, under the conditions of the present study EAE and MS reduce skeletal muscle carnosine levels. Exercise therapy alone could not prevent this in the animal MS model. This warrants further research investigating the effect of nutritional interventions that restore muscle carnosine levels either or not in combination with exercise therapy.

Study 5

Carnosine supplementation in Experimental Autoimmune Encephalomyelitis: impact on muscle carnosine

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Manuscript in preparation

ABSTRACT

Background. Muscle carnosine levels are reduced in animals and humans with Multiple Sclerosis (MS). This may contribute to the muscular abnormalities observed in MS and possibly attenuate rehabilitation outcome during/following exercise therapy interventions. So far, to restore/increase muscle carnosine levels carnosine supplementation has been successfully applied in healthy subjects and various chronic diseases but not in MS. Before using muscle carnosine supplementation in MS we first apply this in an animal MS model.

Methods. Tibialis anterior and soleus muscle carnosine were measured in an animal MS model (EAE, experimental autoimmune encephalomyelitis) after 24 days of carnosine supplementation (CAR, 1.5% in drinking water, n=13), β-alanine supplementation (βA, 0.6% in drinking water, n=13) or placebo (PLA, n=13). Furthermore, carnosine concentrations of the bulbus olfactorius (brain) were assessed.

Results. Muscle carnosine concentrations were significantly elevated in CAR (2.2±0.5 mmol/kg WW, p=0.001) and βA (2.1±0.5 mmol/kg WW, p=0.009) compared to PLA (1.4±0.4 mmol/kg WW). Compared to PLA (9.8±0.7 days & 11.8±0.4 days), in CAR disease onset (10.4±0.5 days, p=0.02) and disease peak (12.5±0.7 days, p=0.01) were significantly delayed whereas time until recovery was significantly lower (3.2±0.9 vs. 4.9±0.9 days, p<0.001). In βA, disease peak was lower compared to PLA (p=0.03). Furthermore, disease peak and cumulative disease scores were significantly (p<0.05) lower in CAR (3.1±0.3 and 10.1±0.6 respectively) compared to PLA (3.8±0.7 and 14.4±3.6) and βA (3.8±0.9 and 14.0±3.9). Bulbus carnosine concentrations tended to differ between groups (p=0.09).

Conclusion. Carnosine and β-alanine supplementation in EAE increase/restore muscle carnosine values. Additionally, carnosine intake can attenuate/delay progression of the disease. This warrants further research investigating the impact of carnosine supplementation on the EAE disease course.

INTRODUCTION

Carnosine, a dipeptide composed of β-alanine and L-histidine, is found in high concentrations in mammalian skeletal muscle[105]. The physiological role of carnosine is related to contractile functioning (Ca^{++} handling), buffering of (exercise-induced) acidosis and protection against oxidative stress [106, 107]. Variants of carnosine are anserine and ophidine, the methylated analogs with the same bioactivity, which are exclusively found in animals. Together with anserine, carnosine forms the histidine-containing dipeptides (HCD). Interestingly, the functional role of skeletal muscle carnosine is closely related to various muscular alterations (altered Ca^{++} handling, overproduction of reactive oxygen species and increased lactate accumulation) previously described in persons with Multiple Sclerosis (MS)[40, 42, 89, 115, 149, 151, 152, 163].

We previously demonstrated reduced muscle carnosine concentrations in an animal MS model (EAE, Experimental Autoimmune Encephalomyelitis, ~40% to ~64% reduction), and confirmed this in MS patients (-25%)[188]. In this population this may impair rehabilitation outcome during/following exercise therapy intervention programs that induce muscular fatigue and acidosis such as during e.g. high intense exercise interventions/therapy that are currently applied in MS rehabilitation. Therefore and because part of the above described neuromuscular dysfunctions in MS might be related to reduced muscle carnosine, any strategy that restores muscle carnosine levels in MS is worthwhile investigating.

In 2006, Harris et al.[189] were the first to report the potential of chronic oral β-alanine supplementation (4 weeks, 3.2-6.4g/day), the rate-limiting precursor of carnosine, to increase muscle carnosine concentrations (carnosine loading) in humans. Ever since, various β-alanine supplementation protocols have been applied that successfully increase muscle carnosine concentrations in both humans (3.2-6.4g/day, 23-46 days)[106, 109] and animals (0.6-1.8% β-alanine in drinking water, 2-12 weeks)[177, 190]. Interestingly, carnosine supplementation in animals is not

only able to increase muscle carnosine levels (0.1-1.8% carnosine in drinking water, 8-12 weeks), but furthermore also induces elevated plasma carnosine concentrations. This is possible because of the absence of serum carnosinase (CN1) activity, which is responsible for the hydrolysis of carnosine[177]. As such, supplemented carnosine is not degraded into β-alanine and L-histidine, leading to both increased concentrations of plasma carnosine and elevated muscle carnosine levels in animals. However following carnosine supplementation in humans, the high serum carnosinase activity will immediately lead to degradation into β-alanine and L-histidine, and thus will not induce increased plasma carnosine concentrations. The subsequent increase in plasma β-alanine and L-histidine however can be used to form intramuscular carnosine and thus increase muscle carnosine concentrations in humans. Whether reduced muscle carnosine concentrations in an animal model for MS or humans with MS can be restored through supplementation with β-alanine or carnosine is currently unknown.

In keeping with this, in the present study we first aimed to investigate whether reduced muscle carnosine concentrations in an animal MS model (EAE) can be restored by carnosine and/or β-alanine supplementation. We hypothesized that carnosine and/or β-alanine supplementation increases muscle carnosine concentrations in EAE animals.

METHODS

Animals

Thirty-nine female Lewis rats (age 6-7 weeks, body weight 100-120 g, Harlan CPB, Venray, The Netherlands) were individually housed on a constant light:dark cycle (12h:12h), a temperature of 22°C and a relative humidity of 40-60% in the animal facilities at 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

that complied with the national/European legislation and the National Research Council's guide for the care and use of laboratory animals.

Study design

Following acclimatization and adaptation (days -15 to -8), animals were randomized into three groups: a placebo group (PLA, receiving no plain water, n=13), a β-alanine group (βA, receiving β-alanine supplementation, Sigma Aldrich® Overijse/Belgium; 6g per liter pure tap water[177], n=13) and a carnosine group (CAR, receiving carnosine supplementation, CARNOPURE Flamma® Bergamo/Italy; 15g per liter pure tap water [177], n=13). The β-alanine and carnosine doses were chosen to be equimolar, at 67 mM in drinking water. All animals received standard rodent chow (without supplements). At day -7, carnosine and β-alanine supplementation started in the experimental groups. At day 0, EAE was induced in all groups (PLA, CAR, βA), whilst supplementation proceeded until progressive hindquarter paralysis (+/- day 10) and recovery of EAE (+/- day 17) occurred. The clinical severity of EAE (clinical disease score) was evaluated daily by a blinded assessor, as well as body weight (g) and drinking volume (ml). Afterwards, total ingested dose of carnosine and β-alanine supplements was calculated, by taking total drinking volume and dose concentration of the supplement into account. On day 17, after recovery of EAE, rats were anaesthetized, m. tibialis anterior and m. soleus were dissected and eventually rats were euthanatized. In case animals deceased because of the disease model, no clinical scores were further monitored from that time on. Throughout the study course, animals were carefully monitored on daily basis. Animals did not display severe discomfort immediately following immunization. Paralysis and typical signs of EAE occurred ~11 days post-immunization, which is the typical time frame for this animal model. If animals exhibited signs of severe pain (high clinical score for 2 consecutive days), distress, were unable to reach food or when humane endpoints were reached, euthanasia to relief pain was performed. However, none of the animals included in the study underwent euthanasia for one of those reasons.

EAE induction

EAE was induced by a single subcutaneous injection on both sides of the lower back (100µl/side) under isoflurane anesthesia 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) [27].

Muscle sampling

On day 17 m. tibialis anterior and m. soleus were collected, and animals were sacrificed. All animals were anaesthetized using an intraperitoneal injection of pentobarbital sodium (5 mg100 g-1 BW) and m. tibialis anterior (TA) and m. soleus (SOL) were dissected and freed of connective tissue and visible blood. The mid-part of each muscle was snap-frozen using liquid nitrogen and stored at -80°C until further analysis were performed.

Muscle dipeptide and free amino acid content

Carnosine, anserine, taurine, β-alanine concentrations and total histidine containing dipeptides (HCD, sum of carnosine and anserine) were determined by high-performance liquid chromatography (HPLC), as previously described [177]. Muscle samples (15mg wet weight, WW) were deproteinized using 35% sulfosalicylic acid and centrifuged (5min, 16,000g). Deproteinized supernatant (5µl sample) was mixed with AccQ Fluor Borate buffer (75µl) and reconstituted Fluor Reagent (20µl) from the AccQTag chemistry kit (Waters). The derivatized samples were applied to a Waters HPLC system comprised of an XBridge BEH column (4.6x150mm, 2.5µm) and fluorescence detector (excitation/emission wavelength: 250/395nm). The column was equilibrated with buffer A (10% eluent A

(Waters) – 90% H_2O), buffer B (100% acetonitrile) and buffer C (100% H_2O) at a flow rate of 1ml/min at room temperature.

Clinical evaluation

Clinical severity of EAE was evaluated on a 0 to 5 scale: 0, no clinical symptoms; 0.5, partial tail weakness; 1, complete tail weakness; 2, limp tail weakness and hind-limb weakness (waddling gait); 2.5, hind-limb weakness with movement, but no support; 3, complete hind-limb weakness; 4, complete hind-limb weakness until diaphragm; 5, death caused by EAE. Daily average clinical score of each group throughout the disease course was plotted to investigate the impact of carnosine and β-alanine supplements on neurological symptoms (paralysis). Furthermore, and per group, average peak score, days until the onset, peak disease and recovery, as well as the cumulative disease index [191] (CDI; calculated by area under the curve) were determined.

Metabolite concentrations of bulbus olfactorius

Throughout the study course we observed a positive impact of carnosine, and to a lesser extent β-alanine, supplementation on the EAE disease course. Therefore and given the possible underlying neural mechanisms of these observations we also collected the bulbus olfactorius of all animals on day 17. It is known that the highest carnosine concentrations in the brain are found in the olfactory system. Here, carnosine concentrations are comparable to those usually found in skeletal muscle[105]. Carnosine, anserine, taurine and β-alanine concentrations of the bulbus olfactorius were assessed as described above and we hypothesized that carnosine concentrations in the bulbus olfactorius were elevated in the supplemented groups.

Statistical Analysis

All data were analyzed using SPPS v. 22.0 (IBM). Normality of data distribution was evaluated using the Shapiro-Wilk test. One-way ANOVA and Tukey's Post Hoc Test were used to evaluate differences between the three groups (PLA, CAR, βA) for muscle and bulbus olfactorius dipeptide concentrations and parameters of the clinical disease score (peak score, time to onset, peak and recovery, and CDI). All data are presented as means ± SD and the threshold for statistical significance was set at $p<0.05$.

RESULTS

Drinking volume, body weight and total ingested supplemental dose

Body weight at the start of the intervention and changes in body weight throughout the disease course (Table 1) did not differ between groups ($p=0.41$ and $p=0.52$ respectively). Total drinking volume was significantly higher in CAR (644.4±68.5 ml) compared to PLA (543.9±70.9, $p=0.008$) and βA (564.7±97.1, $p=0.04$). Total ingested doses of the supplements were 9.7±1.0g carnosine in CAR and 3.4±1.6g β-alanine in βA.

Table 1. Drinking volume, body weight and total ingested supplemental dose of the animals.

	PLA	CAR	βA
BW start (g)	140.9 ± 6.8	140.2 ± 6.1	143.3 ± 5.2
ΔBW (g)	-1.3 ± 8.7	-1.2 ± 8.3	-4.7 ± 9
Total DV (ml)	543.9 ± 70.9	644.4 ± 68.3* †	564.7 ± 97.1
Total dose (g)	/	9.7 ± 1.0	3.4 ± 0.6

*Data are expressed as means ± SD and represent initial body weight (BW start), difference in body weight throughout the disease course (ΔBW), total drinking volume (total DV) and total ingested dose of the supplement (total dose) following control (PLA, n=10), carnosine (CAR, n=13) or β-alanine supplements (βA, n=9). *p<0.05 for CAR compared to PLA. †p<0.05 for CAR compared to βA.*

M. tibialis anterior dipeptide and free amino acid content

Compared to PLA, muscle carnosine concentrations were significantly elevated in CAR (+57%, p=0.001) and βA (+50%, p=0.009, Table 2). Furthermore, intramuscular β-alanine concentrations were significantly higher in both CAR (+33%, p=0.01) and βA (+33%, p=0.01) compared to PLA. Total HCD was significantly elevated in βA (+31%, p=0.025) compared to PLA. Muscle anserine and taurine concentrations were not different between groups (p>0.05).

Table 2. M. tibialis anterior dipeptide concentrations following EAE induction, combined with carnosine or β-alanine supplementation.

	PLA	CAR	βA
Carnosine	1.40 ± 0.4	2.22 ± 0.5*	2.11 ± 0.5†
Anserine	6.69 ± 1.5	6.89 ± 1.7	8.48 ± 1.6
Taurine	23.37 ± 4.5	19.09 ± 5.1	24.1 ± 4.4
β-alanine	0.25 ± 0.1	0.37 ± 0.1*	0.38 ± 0.1†
HCD	8.09 ± 1.8	9.10 ± 2.1	10.59 ± 1.9†

*Data are expressed as means ± SD and represent carnosine, anserine, taurine, β-alanine concentrations and histidine containing dipeptides (HCD, sum of carnosine and anserine) in m. tibialis anterior following control (PLA, n=10), carnosine (CAR, n=13) or β-alanine supplements (βA, n=9). *p<0.05 for CAR compared to PLA. †p<0.05 for βA compared to PLA.*

M. soleus dipeptide and free amino acid content

Muscle taurine concentrations were significantly decreased in CAR (-19%, p=0.042) compared to PLA (Table 3). Furthermore, β-alanine concentrations were significantly elevated in CAR (+96%, p=0.007) and βA (+96% 0.022) compared to PLA. Muscle carnosine, anserine and total HCD were not different between groups.

Table 3. M. soleus dipeptide concentrations following EAE induction, combined with carnosine or β-alanine supplementation.

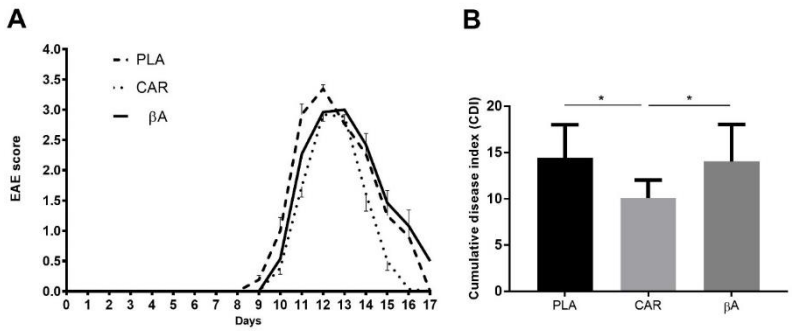
	PLA	CAR	βA
Carnosine	1.15 ± 0.4	1.48 ± 0.4	1.31 ± 0.4
Anserine	2.38 ± 0.5	2.62 ± 0.4	2.31 ± 0.9
Taurine	17.70 ± 3.7	14.40 ± 2.2*	15.27 ± 3.3
β-alanine	0.28 ± 0.1	0.55 ± 0.2*	0.55 ± 0.3†
HCD	3.53 ± 0.9	4.09 ± 0.7	3.62 ± 0.9

*Data are expressed as means ± SD and represent carnosine, anserine, taurine, β-alanine concentrations and histidine containing dipeptides (HCD, sum of carnosine and anserine) in m. soleus following control (PLA, n=10), carnosine (CAR, n=13) or β-alanine supplements (βA, n=9). *p<0.05 for CAR compared to PLA. †p<0.05 for βA compared to PLA.*

Clinical evaluation

In CAR, disease onset (10.4±0.5 days, p=0.02) and disease peak (12.5±0.7 days, p=0.01) were significantly delayed compared to PLA (9.8±0.7 days & 11.8±0.4 days respectively), whereas time until recovery was significantly lower (3.2±0.9 vs. 4.9±0.9 days, p=0.000, Table 4, Figure 1A). In βA, disease peak was significantly postponed compared to PLA (p=0.03). Furthermore, disease peak and cumulative disease index (CDI) scores were significantly (p<0.05) lower in CAR compared to PLA (-18% and -30% respectively) and βA (-18% and -28% respectively).

Figure 1. Clinical disease course following carnosine or β-alanine supplementation.

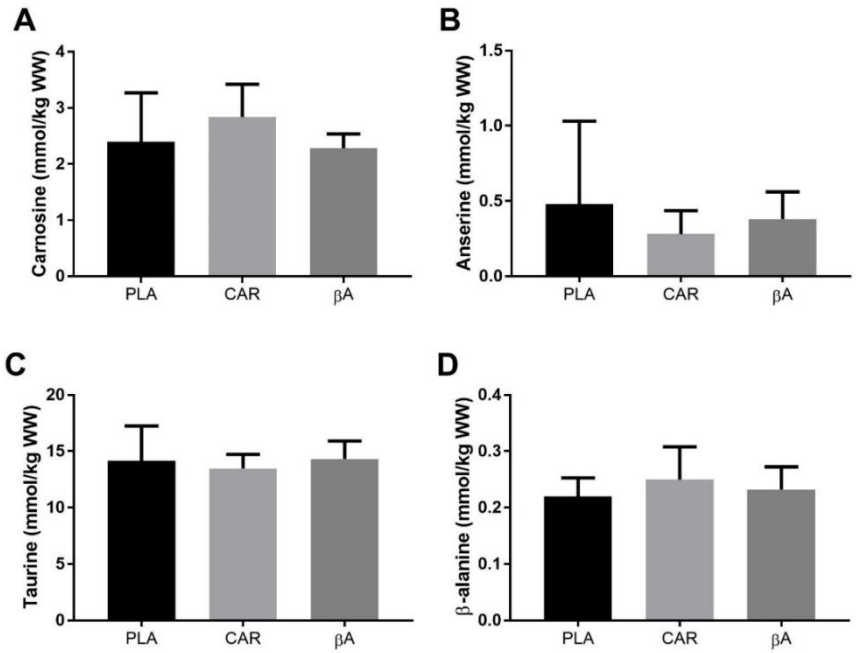


*Data represent mean ± SD daily clinical disease scores (A) and cumulative disease index (B) of placebo (PLA), carnosine (CAR) and β-alanine (βA) animals. *p<0.05 for CAR compared to PLA and βA.*

Metabolite concentrations of bulbus olfactorius

Carnosine (p=0.089), anserine (p=0.409), taurine (p=0.591) and β-alanine (p=0.405) concentrations in the bulbus olfactorius did not differ (group effect) between groups (Figure 1B).

Figure 2. Metabolite concentrations of bulbus olfactorius following carnosine or β-alanine supplementation.



Data are expressed as mean ± SD and represent concentrations (mmol/kg WW) of carnosine (A), anserine (B), taurine (C) and β-alanine (D) in the bulbus olfactorius following placebo (PLA, n=10), carnosine (CAR, n=13) or β-alanine supplements (βA, n=9).

DISCUSSION

The present study demonstrates that carnosine and β-alanine supplementation substantially increase muscle carnosine concentrations in an animal MS model. Furthermore, carnosine supplementation positively affects the clinical EAE disease course.

Carnosine concentrations of m. tibialis anterior in PLA (1.4±0.4 mmol/kg WW) of the present study are similar to the reduced muscle carnosine levels that we previously measured in EAE animals (0.8 to 1.5mmol/kg)[188]. This confirms that, similar to MS, EAE also reduces muscle carnosine concentrations. Interestingly, in an animal MS model we are now the first to show that both carnosine and β-alanine intake are able to substantially increase muscle carnosine (~+50%) to normal healthy control levels (2.2±0.4 mmol/kg WW)[188].

This warrants the application of muscle carnosine loading in MS. As previously described, increased muscle carnosine loading improves exercise performance, especially during high intense intermittent exercise bouts[109] probably due to better Ca^{++} handling and buffering of exercise-induced lactate acidosis[40, 42, 89, 115]. This is interesting because in persons with MS higher intensity exercise therapy regimens have superior effects on various functional measures such as exercise capacity and muscle contractile properties[38, 65, 192] compared to lower intensity exercise. Therefore, increasing muscle carnosine concentrations in MS may further optimize (high intense) rehabilitation interventions and thus provide better clinical outcomes.

Surprisingly, throughout the course of this study we observed substantial improvements in the clinical disease course of the supplemented animals (CAR and βA). This suggests a neuroprotective effect of carnosine in this experimental disease model. Interestingly, the neuroprotective potential of carnosine was already explored in various other animal models of neurological disorders such as Parkinson's and Alzheimer's disease (AD). In 1998, Preston et al.[187] reported inhibitory effects of carnosine against loss

of cerebellar granule cell neurons viability (caused by β-amyloid peptide) in AD rat brain. Corona et al.[193] showed that carnosine supplementation (10mM carnosine in standard tap water, 11-13m) had a strong effect on the restoration of mitochondrial functioning in AD mice, indicating that carnosine could be a useful therapeutic agent in this disease. More recently, Herculano et al.[194] showed that supplementation of carnosine (5mg/d in drinking water, 6w) prevented the cognitive decline in AD mice. Finally, Boldyrev et al.[195] were able to improve neurological deficits, such as rigidity and movement of hands and legs, by adding 1.5g/d of carnosine to the standard L-DOPA therapy in patients with Parkinson's disease. In keeping with this, the present study is the first to show the potential of carnosine supplementation to affect the clinical disease course (hind limb paralysis) in an animal MS model. Compared to PLA, carnosine in drinking water of EAE animals (CAR) substantially postponed the onset of hind quarter paralysis, reduced disease peak and allowed earlier recovery. In contrast to carnosine, β-alanine supplementation induced only a minor clinical impact. This may be due to the elevated plasma carnosine concentrations in the carnosine supplemented group compared to the β-alanine group. In animals, serum carnosinase activity, causing the hydrolysis of carnosine, is absent compared to humans. Supplementing carnosine in animals will not lead to the hydrolysis of carnosine into β-alanine and L-histidine, thus causing increased plasma carnosine concentrations. As such, and in contrast to β-alanine supplementation, the carnosine molecule in the plasma stays intact. This is important, as carnosine is able to cross the blood brain barrier (BBB)[196, 197] to elicit neuroprotective effects in the brain and central nervous system, including disinhibition-induced excitatory effects on CA1 pyramidal neurons[197], ameliorated neurological functioning[198] and anticonvulsive effects on the brain[199]. As such, this may explain the superior clinical effects of carnosine supplementation compared to β-alanine in the present study. It is important to note however that elevated plasma carnosine concentrations, and thus the potentially accompanied neuroprotective benefits, are more difficult to acquire in humans because of the high carnosinase activity. Interestingly though, Wassif et al.[118]

showed that persons with MS actually have reduced serum carnosinase activity, compared to healthy subjects, which might favor direct carnosine supplementation in this population.

Because carnosine is known to induce neuroprotection in the CNS[197-199] we explored the neuroprotective potential of carnosine supplementation in EAE and measured carnosine concentrations in the CNS and more specifically the bulbus olfactorius. The olfactory system is the site in the CNS with the highest carnosine concentrations, comparable to the skeletal muscle whereas other CNS regions have far lower, and often undetectable, carnosine concentrations[105]. If carnosine concentrations increased compared to placebo, this then could indicate that carnosine intake is able to increase carnosine concentrations in the CNS. However, under the conditions of the present study and compared to PLA bulbus carnosine concentrations only tended (p=0,09) to be higher in CAR and were not altered in βA. Although this corresponds with the observed clinical disease scores (lower EAE scores in CAR vs. minor effect in βA) it is difficult to draw solid conclusion. Therefore, further research is warranted.

Another potential mechanism that might explain the observed clinical improvements in this study involves the cytotoxic compound acrolein, that causes oxidative stress and directly damages myelin. As such, acrolein is believed to play part in the pathogenesis of MS[180-182]. Carnosine however serves as a sacrificial sequestering agent for acrolein[180-183], and might thus provide neuroprotection to these mechanisms. Indeed, when scavenging acrolein in EAE mice by hydralazine, Leung et al.[185] found that myelin integrity appeared to be preserved, indicating that acrolein removal may offer neuroprotection, which may contribute to the findings on clinical disease course in this study. Furthermore, after 12 weeks of carnosine supplementation (2g/day) in overweight subjects, Regazzoni et al.[183] were able to detect adducts of carnosine in urine, but not in plasma, of these persons. These adducts derive from a reaction of carnosine with acrolein. Here, a positive correlation between urinary excretion of adducts and carnosine was found, together with an increase of urinary excretion of both

carnosine and carnosine-acrolein adducts. As such, the findings of Regazzoni et al.[183] thus implicate that acrolein is entrapped in vivo by carnosine and excreted through urine. This mechanism may explain the presented findings in this study. However, as no urine was collected, this was not possible to investigate.

Other underlying mechanisms may include altered expression of inflammatory markers, reduced activation and infiltration of leukocytes and dendritic cells into the central nervous system, and thus reduced demyelination and/or axonal loss. Nerve tissue samples at disease peak however are required to investigate these hypotheses. However, because in the present study all animals were sacrificed at day 17 (full recovery), it was not possible to investigate this.

CONCLUSION

In summary, carnosine and β-alanine supplementation in EAE are able to increase muscle carnosine concentrations to normal values (2.2±0.4 mmol/kg WW, see **Study 4**). Furthermore, carnosine supplementation positively affects the clinical disease course of these animals. This warrants future research investigating carnosine supplementation in MS patients.

ACKNOWLEDGEMENTS

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Study 6

Home-based periodized exercise in Multiple Sclerosis.

Based on: Charly Keytsman, Pieter Van Noten, Jan Spaas, Ine Nieste, Paul Van Asch, Bert O Eijnde

Manuscript in preparation

ABSTRACT

Introduction. Despite substantial functional effects of high intensity interval therapy (HIIT) in Multiple Sclerosis (MS), longer term HITT implementation in MS rehabilitation appears to be difficult. Therefore exploration of new exercise therapy approaches that further optimize rehabilitation, improve exercise adherence and promote physical exercise in MS is mandatory.

Methods. Exercise capacity (maximal exercise test) and body composition (DEXA) were assessed at baseline (PRE). Next, all participants were enrolled in a 6m home-based periodized cycling program with remote (Polar® M200 activity tracker) supervision. POST measurements were performed similar to baseline. Hereafter, subjects climbed the Mont Ventoux (France) in the context of an awareness project.

Results. Six months of home-based training induced improvements in body weight (-3%, p=0.008), BMI (-3%, p=0.01), total mass (-2%, p=0.023), VO_{2max} (+5%, p=0.016), workload (+11%, p=0.001), time until exhaustion (+14%, p=0.001), recovery heart rate (+4%, p=0.04), lactate peak (+16%, p=0.03) and RER (+4%, p=0.04) in MS. Furthermore, all persons with MS safely reached the top of the Mont Ventoux, except for two. No injuries or adverse events occurred during the challenge.

Conclusion. The applied 6m home-based, periodized and HIIT-oriented cycling program provided good adherence, showed significant improvements in exercise capacity and body composition, and trained persons with MS sufficiently to climb the Mont Ventoux. These exercise therapy strategies now warrant further investigation in larger-scale controlled studies.

INTRODUCTION

Exercise therapy and increased physical activity in persons with Multiple Sclerosis (MS) improves mobility, muscular strength, physical fitness and fatigue [39, 200] without increasing relapse rate [201]. As such, physical activity and more particular exercise therapy have become an important part of MS rehabilitation. Despite the fact that the positive effects of exercise therapy in MS are obvious [39, 200], only 43 percent of the MS community reports to participate in an exercise program[83]. Therefore, new exercise therapy approaches that further optimize rehabilitation, improve exercise adherence and promote participation in physical exercise in MS are interesting to explore.

High intensity interval training/rehabilitation (HIIT) in MS has gained more and more attention because it substantially enhances exercise capacity (+22%) and muscle strength (+44%)[38, 192]. Furthermore, in this population it is associated with improvements of various functional and quality of life[92, 96] measures as well as cognitive performance (+8%)[64]. Despite these positive findings and the fact that this exercise mode appears to be save and well-tolerated in MS[38, 64, 65, 192], the effective (longer-term) implementation of such high intense exercise interventions in actual rehabilitation programs appears to be difficult. Possibly, rehabilitation regimens consisting of regular high intensity exercise only are too demanding (e.g. 1-5 maximal exercise bouts ranging from 6s to 4min interspersed by recovery periods of 30s–4min, 90-100% maximum heart rate, 2-3/w) and thus require self-guidance and external motivational factors to adhere to longer term HIIT periods. Indeed, attenuated clinical outcomes resulting from reduced compliance following higher intense exercise has also been reported[104]. As such, any exercise therapy strategy that (amongst others) involves HIIT, improves longer term training adherence and thus ensures prolonged significant clinical benefits, such as health-related parameters, in this population is worthwhile investigating.

Such strategies may involve exercise therapy periodization. In high performance sports, conditioning and strength training programs are periodized into sequential phases and cyclical periods to achieve specific performance goals and maximize optimal long-term training/performance stimuli while minimizing overtraining and/or injuries. Here, training does not involve single progressive linear training stimuli for a prolonged period but is divided in periodically alternating blocks of e.g. 1-4 weeks[121] that include different training goals, varying exercise modalities (e.g. intensity, duration) and periods of rest/recovery[119, 120, 202-204]. Many rehabilitation programs in various populations however still mainly use continuous linear progression models that use single stimuli and training modalities. This probably results in suboptimal training/rehabilitation outcomes[119, 120, 204] and may lead to overtraining. Therefore, applying high-performance periodization principles in a rehabilitation setting including high- and lower training intensities as well as adequate recovery periods may further enhance clinical outcome. Such an exercise intervention approach however has not been implemented in the rehabilitation of persons with MS yet.

Barriers for persons with MS to engage in exercise interventions include lack of time, distance, transportation, neurological disability, specialist availability and insurance coverage[83, 84, 205]. Consequently, rehabilitation programs tend to be short (4-8 weeks), are often purely reactive to exacerbations and very often only address acute disability/symptoms[205], without focussing on long-term benefits of consistent adequate physical activity and life-style changes. Possibly, addressing some of these barriers such as lack of time (by low-volume, high intense training sessions) and both distance and transportation difficulties using home-based exercise programs, may improve longer-term increased physical activity. In this respect it is important to note that home-based rehabilitation has already been shown to be safe and effective to improve functionality and symptoms in MS[77, 206]. However, unsupervised training programs in a home- or community setting are reported to be less effective compared to (remotely) supervised home-based training[122]. Consequently, home-based exercise

programs that also include remote supervision and motivational strategies could increase therapy adherence and thus improve long-term clinical outcomes. Furthermore, and to our knowledge, exercise therapy including high intensity exercise has not yet been investigated in a home-based setting in MS patients.

Finally, improving awareness in the MS (patient) population regarding the positive impact of physical therapy is of great importance regarding long-term therapy adherence and exercise therapy participation. In this respect, we already successfully conducted several awareness projects in the MS community (*Machu Picchu*[207] and *MSRUN*[208] project). These projects, including challenging physical/sport performances, seem essential in the promotion of physical therapy in the MS population. As such, awareness project may contribute to improved exercise therapy participation and adherence in this population.

Therefore, the present study investigates whether a newly composed, periodized, home-based exercise intervention is able to improve health-related parameters such as exercise capacity and body composition in MS patients. Furthermore, we investigate whether this exercise intervention enables these subjects to perform a challenging sport performance (climbing the Mont Ventoux, France) in the context of an awareness project to promote physical activity in this population.

METHODS & MATERIALS

Participants

In the present study, healthy controls (HC) were included in the study to investigate whether our new exercise intervention presented similar effects on MS patients compared to HC, but furthermore to accompany the MS patients during the challenge that was part of the awareness project (climbing the Mont Ventoux, see Methods). Persons with MS (all phenotypes, EDSS<4) and healthy controls (aged>18y), were recruited

through local advertisement and announcements via the Rehabilitation Research Center of Hasselt University (REVAL) and MovetoSport foundation (Kontich, Belgium) networks, following written informed consent. Subjects were excluded if they participated in another study, had (in case of MS) an acute exacerbation in the preceding 6 months prior to the start of the project, experienced contraindications to participate in moderate to high intensity exercise, or in the presence of cardiovascular/orthopaedic diseases. Furthermore, subjects were asked to provide/use their own bicycle to participate in this project. The study was approved by the Ethical Committee of the Jessa hospital and Hasselt University, was performed in accordance with the Declaration of Helsinki and was registered at ClinicalTrials.gov (NCT03418376).

Study design

Exercise capacity (maximal graded exercise test) and body composition (DEXA scan) were assessed at baseline (PRE) and the end of the intervention (POST) to evaluate whether our exercise program was able to improve some health- and performance related outcomes. Prior to the maximal exercise test, heart function was evaluated by an experienced medical doctor. Next, all participants received an activity tracker (Polar® M200) and were enrolled in a 6-month home-based exercise program. Each training cycle (3w, see below), participants received personalized training instructions by mail. Training involved cycling on their own personal bicycle and remote supervision using the Polar® Coach module. This allowed online monitoring of each training session including training adherence, number of completed training sessions, training duration, total cycling distance, percentage in target heart rate training zones and caloric expenditure. Participants were contacted by phone if they had been inactive or showed bad compliance to the training instructions. Furthermore, participants continuously had the possibility to communicate with the researchers by phone or email in case of problems. After the 2nd and 5th training cycle, a

group cycling tour was organized to improve group dynamics and motivation. Following 8 training cycles (~6m) POST measurements were performed similar to baseline and one week later subjects climbed the Mont Ventoux by bicycle (Mont Ventoux Challenge).

Throughout the study course part of participants in both groups used an ergogenic supplement (beta-alanine). This did not induce additional effects and is not further detailed in the present study.

Body composition

Whole body fat mass and percentage, body mass index (BMI), total and fat free mass were obtained using Dual Energy X-ray Absorptiometry scan (DEXA) (Hologic Series Delphi-A Fan Beam X-ray Bone Densitometer, Vilvoorde, Belgium). A calibrated analogue scale (Seca®) was used to measure total body weight and height.

Exercise 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 (Metalyzer II® 3B Cortex, Leipzig, Germany) was used. Female and male persons with MS started at 20W and 30W, respectively, during the first minute. Hereafter, workload increased, respectively, 10W and 15W per minute[65] and were asked to bicycle at >70rpm throughout the test. Oxygen uptake (VO_2), expiratory volume (VE), and respiratory exchange ratio (RER) were collected breath-by-breath and averaged every minute. 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 whether 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, minutes and oxygen uptake

measured at the level of exhaustion, were reported. Capillary blood samples were obtained from the earlobe to analyze blood lactate concentrations (mmol/l) at maximal exhaustion (lactate max) and after recovery (lactate peak), using a portable lactate analyzer (Accutrend Plus, Roche Diagnostics Limited, Sussex, UK), which has previously shown to be accurate and reliable[159].

Exercise program

The exercise training program (6 months) involved 8 recurrent 3-week cycles (week I-III) involving Polar® M200 exercise intensity monitoring (% of HR_{max}). During week I, subjects performed moderate to high intensity bicycle training (3/w). Twice a week, subjects performed longer training sessions (2→3h, 60-80% HR_{max}) and once a week a more intense, shorter (1→1.5h, 75-90% HR_{max}) training session was executed. During week II, subjects performed maximum intensity interval cycle training (3/w). High intensity interval cycle training (HIIT) consisted of 3-5 maximal sprints (90-100% HR_{max}) of 60-90s, interspersed by 1-3min rest intervals. A 10min standardized warming up and 10min cooling down was performed before and after each training session. Week III involved a recovery week where subjects performed one short high intense interval training session and one optional endurance session of 2-3h (70-90% HR_{max}). During the last week of each 3-week cycle, subjects received training instructions for the next training cycle.

Mont Ventoux Challenge

On September 16, 2017, the group performed the Mont Ventoux Challenge starting from Sault, France. The Challenge existed of climbing the Mont Ventoux by bicycle, involving a distance of 25.70 km, whilst climbing 1152m (peak altitude: 1912m) with an average gradient of 4.5% (range 1%→10.5%). For safety reasons, every person with MS was accompanied by a HC buddy throughout the challenge. Carbohydrate and water provision was offered after eight and 16 km. Performance of persons with MS was monitored using their Polar® M200 activity tracker.

Statistical analysis

All data were analyzed using SPPS v. 22.0 (IBM). Normality of data distribution was evaluated using the Shapiro-Wilk test. Baseline differences and parameters of training volume (number of completed sessions, total training duration, cycling distance, percentage in target heart rate training zones and caloric expenditure) between groups were analysed using an unpaired student's t-test (Mann Whitney U test). Parameters of body composition and exercise capacity within groups were analysed using a paired student's t-test (Wilcoxon signed-rank test). Difference scores (delta's, POST-PRE) were calculated for both groups and analysed using an unpaired student's t-test (Mann Whitney U test). All data are presented as means ± SD's and the threshold for statistical significance was set at $p<0.05$.

RESULTS

Subject characteristics

Thirty-three persons with MS and an equal amount of HC attended an information session at Hasselt University, after which ten persons with MS and 11 HC declined to participate in the project for reasons not related to the study (e.g. no support from social environment). The remaining twenty-three persons with MS (mean EDSS 1.9 ± 1.1, range 0→3.5) and twenty-two HC were included in present study. Throughout the 6-month home-based training intervention, five persons with MS and three HC dropped out. Reasons were musculoskeletal injuries not related to the intervention program (2 persons with MS, 1 HC), motivational issues (1 persons with MS, 1 HC), one exacerbation in the MS group, and personal reasons (1 person with MS, 1 HC). Eighteen persons with MS and nineteen HC underwent the full clinical analysis (PRE-POST measurement). Eventually, 17 persons with MS and 17 HC participated in the Mont Ventoux Challenge. Baseline subject characteristics are shown in Table 2 including all subjects that performed the full clinical analysis (MS, n=18; HC, n=19). No significant differences were found between groups.

Table 2. Baseline subject characteristics

	MS	HC
Age (years)	41.7 ± 8.5	41.5 ± 9.9
Height (m)	1,73 ± 0.1	1.75 ± 0.1
Gender (f/m)	6/12	5/14
EDSS	1.9 ± 1.1	/

Data are expressed as means (SD's) and represent baseline characteristics healthy controls (HC, n=19) and multiple sclerosis subjects (MS, n=18). Abbreviations: f, female; m, male; BMI, Body Mass Index; EDSS, Expanded Disability Status Scale.

Training adherence

All subjects who completed the 6-month training program showed high adherence. Persons with MS completed 61/64 of the prescribed training sessions (95%) and HC performed 57/64 sessions (89%) with no difference between groups (p=0.225). Missed trainings sessions were due to personal reasons, musculoskeletal injury, holidays or one MS-related exacerbation, not related to the training program. Furthermore, no differences were found between groups for total training duration (p=0.970), total cycling distance (p=0.451), caloric expenditure (p=0.696) and percentage (time) in target heart rate training zones (p>0.05, data not shown).

Body composition

Six months of home-based training (Table 3) induced significant reductions in body weight (-3%, p=0.008), BMI (-3%, p=0.01) and total mass (-2%, p=0.023) in MS. Fat mass, fat percentage and fat free mass did not differ over time (p>0.05) in MS. In HC, parameters of body composition did not significantly differ following the home-based training intervention (p>0.05). No differences were found for PRE-POST changes between groups (p>0.05).

Table 3. Body composition

	MS		HC	
	PRE	POST	PRE	POST
Weight (kg)	74.2 ± 11.2	71.9 ± 10.9*	75.2 ± 11.2	74.1 ± 10.2
BMI	24.8 ± 3.9	24.1 ±3.9*	24.6 ± 2.8	24.3 ± 2.7
Fat mass (kg)	16.6 ± 8.3	15.7 ± 8.5	15.6 ± 5.3	14.9 ± 5.4
Fat percentage (%)	23.8 ± 9.6	23.1 ± 10.1	22.7 ± 7.5	22.1 ±8.1
Fat free mass (kg)	51.1 ± 7.4	50.5 ± 7.9	53.3 ± 9.8	53.1 ± 9.8
Total mass (kg)	67.7 ± 10.9	66.2 ± 10.7*	68.9 ± 10.6	68 ± 9.6

*Data are expressed as means (SD's) and represent parameters of body composition before (PRE) and after (POST) a 6-month home-based cycling intervention of healthy controls (HC, n=19) and multiple sclerosis subjects (MS, n=18). *p<0.05 compared to PRE within group.*

Exercise capacity

In MS, significant elevations were found for VO_{2max} (+5%, p=0.016), workload (+11%, p=0.001), time until exhaustion (+14%, p=0.001), recovery heart rate (+4%, p= 0.0017), lactate peak (+16%, p=0.03) and RER (+4%, p=0.04) following six months of home-based exercise (Table 4). In HC, the home-based intervention elicited increments in VO_{2max} (+6%, p=0.03), workload (+11%, p<0.0001), time until exhaustion (+11%, p=0.001), lactate max (+30%, p=0.005) and a reduction in maximal heart rate (-2%, p=0.03). Besides recovery heart rate (p=0.03), no differences were found for PRE-POST changes between groups (p>0.05).

Table 4. Exercise capacity

	MS		HC	
	PRE	POST	PRE	POST
VO2 max	40.8 ± 6.6	42.9 ± 7.1*	44 ±8.2	46.8 ±7.6*
Workload	213.3 ± 43.2	237.2 ± 47.4*	244.7 ± 53.2	270.8 ± 53.1*
Time (min)	15 ± 2.5	17.1 ± 2.9*	17.3 ± 2.7	19.2 ± 2.6*
HR max (bpm)	180 ± 9	179.1 ± 9.8	181.4 ± 10.8	178.2 ± 11.8*
HR recovery (bpm)	128 ± 17	133.3 ± 15.1*	131.1 ± 13.7	129.5 ± 14.7
Lac max (mmol/l)	4.5 ± 1.6	5.9 ± 1.8	5.3 ± 1.3	6.9 ± 2.1*
Lac peak (mmol/l)	8.9 ± 2.3	10.3 ± 3.1*	9.7 ± 2.4	10.7 ± 2.2
RER	1.13 ± 0.1	1.18 ± 0.1*	1.12 ± 0.1	1.15 ± 0.1

*Data are expressed as means (SD's) and represent parameters of exercise capacity before (PRE) and after (POST) a 6-month home-based cycling intervention of healthy controls (HC, n=19) and multiple sclerosis subjects (MS, n=18). *p<0.05 compared to PRE within group.*

Mont Ventoux Challenge

All persons with MS (and HC) safely reached the top of the Mont Ventoux, (25.70 km, average slope: 4.5%) except for two (termination at 2km and 1km from the top because of exhaustion). The group reached the top of the mountain in a mean time of 2h48min, an average speed of 9.8 km/h, a mean heart rate of 133bpm and maximal heart rate of 175 bpm. No injuries or adverse events (physical complications) occurred during the challenge. Healthy controls were asked to accompany one MS patient throughout the challenge, for safety reasons.

DISCUSSION

The present study investigated the impact of a new, periodized, home-based exercise intervention in mildly affected persons with MS. The exercise program was well-tolerated, provided good adherence and showed significant improvements in health-related parameters such as exercise capacity and body composition. Furthermore, this exercise intervention was able to train persons with MS to climb the Mont Ventoux by bicycle safely, in the context of an awareness project to promote physical activity in this population.

Recent studies clearly show that HIIT is a very efficient rehabilitation strategy to enhance exercise capacity[192], muscle strength[38] and cognitive performance[64]. Hereby, it also improves functionality and quality of life of persons with MS. Next to these clinical improvements[37, 65] HIIT is less time-consuming (total session duration ~20-30min) compared to classic moderate intensity endurance training (total session duration ~30-60min)[37] and is therefore better integrable in to everyday life. Indeed, the latter is often a barrier for engagement in (long term) physical activity[84]. Such high intense training sessions however are very demanding and may hereby reduce training adherence in patients leading to reduced exercise output and eventually reduced clinical outcome[104]. In an attempt to improve long

term training compliance and reduce overtraining, we composed a HIIT-oriented exercise program using training periodization with week by week (week I-III) variations in exercise intensity (high vs. low), session duration (long vs. short) and rest/recovery. This periodized exercise program allowed the application of short demanding HIIT sessions and adequate recovery periods as well as moderate intensity longer duration training sessions[119, 120, 202-204]. In mildly affected person with MS, this program appeared to be feasible during longer training periods and significantly improved exercise capacity ($VO2_{max}$ +5%, workload +11%) and body composition (weight -3%, BMI -3%, total mass -2%). More importantly no differences were found in improvements (difference scores) between MS and HC, indicating that this training program induces similar training effects in mildly affected persons with MS (EDSS: 1.8 ± 1.1) compared to HC. It thus seems that periodizing exercise interventions in MS may be a useful strategy to optimize long-term, HIIT-oriented therapy and performance.

Home-based exercise therapy appears to be a valid strategy to remove known barriers for engagement in physical activity such as lack of time and distance to rehabilitation centres and hereby it improves therapy adherence in persons with MS[83, 84, 205]. As such, home-based exercise could lead to persistent increased levels of physical activity and improved functioning in this population. Indeed, performing home-based exercise programs in MS (3/w, strength/aerobic training, 12w to 23w) improved walking speed, aerobic capacity, functional mobility and cognitive parameters[77, 208]. The results of the present study are consistent with these findings, although on other health-related paremeters. Here and compared to healthy controls, a 6-month home-based cycling program induced similar improvements in exercise capacity and body composition in mildly affected persons with MS. Interestingly and similar to Feys et al[208] the applied training regimen allowed high therapy adherence in persons with MS (95% and 94% training sessions completed, respectively) during a long-term home-based exercise intervention. Probably, this may be attributed to enhanced motivation by goal setting at the end of the study, implemented in both projects (‘Mont

Ventoux Challenge' and 'Antwerp 10 Miles' respectively). Therefore, ambitious goal setting and awareness projects should to be organised to increase adherence and participation to training and/or rehabilitation programs in persons with MS.

Finally, it is important to note that the present project included mildly affected persons with MS (EDSS: 1.8 ± 1.1). In keeping with the above described effects (good long-term training adherence and significant improvements in exercise capacity and body composition) this warrants future studies investigating this training program in more severely affected persons with MS. Furthermore, these strategies should now be investigated separately in large controlled trials and be compared to more conventional, linear progressive training programs.

CONCLUSION

We conclude that a 6-month home-based, periodized bicycling training program provided good adherence and showed significant improvements in exercise capacity and body composition in persons with MS and HC. Furthermore, this exercise program enabled MS patients to perform a challenging sport performance (climbing the Mont Ventoux) in the context of an awareness project to promote physical activity.

Chapter 4

General discussion

General discussion

High intensity interval training (HIIT) is rapidly becoming a popular rehabilitation (exercise therapy) strategy in various (chronic) disease populations, such as Multiple Sclerosis (MS). The impact of HIIT on various functional and health-related parameters (**objective A**) as well as strategies to further optimize these high intensity exercise protocols in MS (**objective B**) are therefore interesting to further investigate. The six different studies of this PhD project that are described in detail in Chapter 2 fit into these objectives. In the following general discussion, we have integrated all study results and compared them with the work of others.

Objective A. We demonstrated that various CVD risk factors, with elevated body fat mass as the most important contributor, are responsible for the increased risk to develop cardiovascular diseases in MS. Surprisingly however and in contrast to other populations, HIIT did not remediate these elevated cardiovascular risk factors. We hypothesized that increased lactate accumulation during exercise in MS may reduce adequate exercise intensity during HIIT, leading to inadequate therapy responses on cardiovascular risk factors. However, because lactate accumulation during exercise did not appear to be different from healthy controls we were unable to confirm our hypotheses. Consequently next to HIIT, other strategies to improve cardiovascular risk factors in MS need to be explored in the future.

Objective B. Besides investigating the impact on health-related parameters, we also attempted to optimize HIIT as a rehabilitation strategy in MS by improving overall HIIT performance and increasing the feasibility/adherence during such high intense rehabilitation therapy. As such, we have explored two strategies that are often used in the exercise/sports community, notably ergogenic supplementation and training periodization. First, we have shown that both animals and persons with MS have substantially lower muscle carnosine levels. This may impede adequate HIIT performance. Muscle carnosine loading in animals with MS was able to restore/increase these reduced concentrations, and

interestingly, showed remarkably positive effects on the clinical disease course. As such, carnosine loading seems an interesting/valid new strategy to improve HIIT performance in MS. Finally, training periodization was able to improve clinical parameters such as exercise capacity in MS and appeared to improve the feasibility and adherence of a HIIT-oriented long-term exercise intervention.

Objective A. HIIT in MS: Impact on health-related parameters?

Cardiovascular risk profile of MS patients

In order to investigate the impact of HIIT on health-related parameters, such as cardiovascular risk factors, in MS, it is first important to understand what cardiovascular risk factors are disturbed in this population. Because so far it was unclear what factors[49, 52] determine the CVD risk profile of MS patients, we have investigated the role of various important CVD risk factors (body composition, hypertension, disturbed lipid profiles and altered glycemic control) in MS simultaneously and explored their interrelations (**Study 1**). We have shown that MS patients present greater whole body fat mass and fat percentage, higher blood pressure (systolic and diastolic), resting heart rate, blood triglyceride concentrations as well as disturbed whole body glucose and insulin disposal. Total cholesterol, blood HDL and LDL concentrations did not differ between MS and HC. Interestingly, we also demonstrated for the first time that MS is independently related to the CVD risk factors whole body fat mass, systolic and diastolic blood pressure and resting heart rate. In this population, we also showed that fat mass is associated/related to almost all other measured risk factors. This implies that irrespective of age, gender and smoking behaviour, MS itself makes these patients more prone to develop future (preventable) cardiovascular comorbidities and that fat mass predominantly mediates the elevated CVD risk. This confirms work of Roshanisefat et al.[52] and Manouchehrinia et al.[51]

who respectively reported an increased risk for venous thromboembolic disorders and ischaemic stroke and a greater likelihood for cardiovascular mortality in MS compared to the general population.

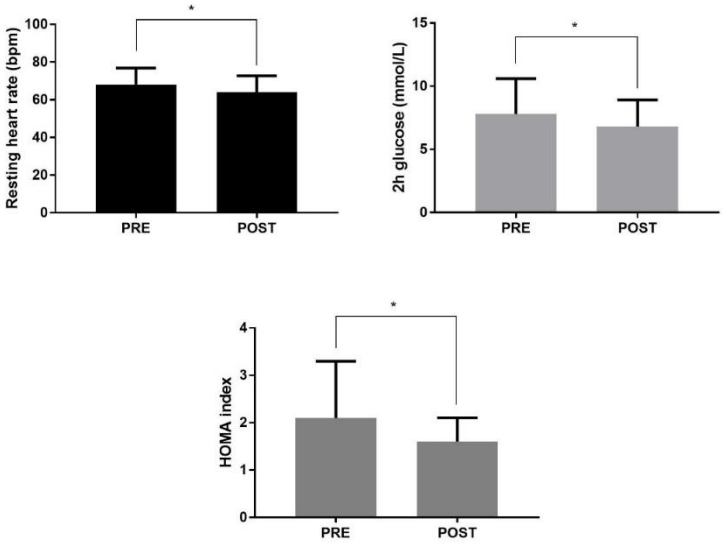
The results of these studies are in contrast to Moccia et al.[130] who concluded that MS patients have a normal CVD risk profile compared to HC. However, in contrast to our study, Moccia et al.[130] did not measure various common cardiovascular risk factors, but instead used the Framingham algorithm that estimates the 10 year-likelihood for CVD development, based on only a limited number of cardiovascular risk factors, such as age, gender, and BMI. However, the BMI seems an invalid parameter to estimate the CVD risk in MS, as it has been shown to severely underestimate adipose tissue in this population[133]. Therefore, using such predictive calculations, including BMI, in MS probably underestimates their actual risk to develop CVD in the future. Wang et al.[209] questioned whether the increased CVD risk in MS is secondary to lifestyle and environmental variables such as physical activity, medication use and dietary factors, or results from common pathobiological features between CVD risk factors and MS. Therefore, they have evaluated the genetic overlap between MS and a number of CVD risk factors, such as systolic blood pressure, total cholesterol, blood LDL and HDL concentrations, triglycerides, type 2 diabetes mellitus, BMI, waist to hip ratio and C-reactive protein. Interestingly, they observed polygenic pleiotropy between MS and several CVD risk factors and furthermore identified various genes that are associated with *both* MS and these CVD risk factors. These findings thus suggest significant genetic overlap between MS and several CVD risk factors indicating that the elevated CVD risk in MS is not solely due to a more sedentary lifestyle, but probably also genetically induced.

From the above it is clear that strategies that alter/reduce these CVD risk factors in MS are of great importance. Here, increased physical activity is a crucial strategy in both the primary and secondary prevention of CVD. Research that investigates exercise interventions to improve the CVD risk in MS needs to further clarify this.

No substantial impact of HIIT on cardiovascular risk factors in MS

In other populations[94, 96, 97, 100] such as healthy controls, elderly, persons with overweight, type 1 diabetes and heart diseases, high intensity exercise therapy improves various of these CVD risk factors including body composition, blood lipid profile and hemodynamic parameters (blood pressure and heart rate). Furthermore, 12 weeks of high intense aerobic and resistance (concurrent) training significantly improves glucose tolerance and insulin sensitivity in MS patients[65]. In keeping with this line of reasoning and because MS patients have an increased risk for CVD (**Study 1**), we explored the effect of a 12-week high intense concurrent training intervention on various CVD risk factors such as body composition, blood pressure and heart rate, blood lipid profile, glycemic control and C-reactive protein levels in MS patients (**Study 2**). However, we could not demonstrate comparable effects on body composition, blood lipid profiles and hemodynamic parameters in this population. The applied 12-week high intense exercise intervention improved resting heart rate, 2h blood glucose and insulin sensitivity (HOMA index) only (see Figure 6). It did not affect all other CVD risk factors. This suggests that next to the superior effects on various functional parameters (exercise capacity, muscle strength) HIIT is not able to directly improve important health-related CVD risk factors in MS.

Figure 6. Cardiovascular risk factors and impact of high intensity concurrent training



Data are expressed as means ± SD and represent improved cardiovascular risk factors before (PRE) and after (POST) 12 weeks of high intensity concurrent training (n=16) in Multiple Sclerosis patients.

These findings are in contrast to studies examining the impact of HIIT on CVD risk factors in other populations. For example, after a meta-analysis, Batacan et al.[100] reported that HIIT interventions of 2-16 weeks were able to reduce body fat levels and waist circumference in persons with elevated body fat mass, and moreover improved blood pressure in an overweight population. This data is supported by Ramos et al.[95] and Boutcher et al.[99], who reported improved waist circumference and body fat percentage in healthy subjects following HIIT. Furthermore, Cassidy et al.[93] found improvements in systolic and diastolic parameters in healthy individuals. However, in our study, no effects of HIIT on these CVD-related parameters were found.

It is difficult to explain this discrepancy. The present high intense exercise intervention was able to improve exercise capacity and parameters of glucose tolerance substantially. This confirms the efficacy of HIIT in MS on functional parameters as well as previous results of our laboratories regarding glucose tolerance[38, 65, 210]. Possibly MS patients may not reach the maximal exercise intensities required to achieve comparable results on other cardiovascular risk factors. This hypothesis is supported by recently published findings. First, MS is associated with disturbed cardiovascular autonomic control[146]. In other populations this involves impaired baroreflex control[145], attenuated elevations in blood pressure[147] and chronotropic incompetence, which is the inability of the heart rate to increase proportionally to an increase in physical activity or metabolic demand, which is often induced by disturbed cardiovascular autonomic control[147, 148]. Whether MS patients also exhibit chronotropic incompetence is currently unknown but, if present, could withhold these patients to reach the required maximal heart rates necessary to impact cardiovascular risk factors during short intense bouts of exercise. We observed rather low maximal heart rates (161±4 bpm) of the MS patients during the maximal exercise test that could confirm this. However, further research to support this hypothesis is needed. Second, abnormal muscle energy metabolism in MS such as reduced Krebs cycle and complex I and II activities[149], overproduction of reactive oxygen species[115] and delayed phosphocreatine resynthesis following exercise[149-151] have been demonstrated previously. These abnormalities suggest higher basal and exercise related energy expenditure, as supported by higher basal serum lactate concentrations[89] that may indicate increased exercise-induced lactate accumulation in MS. Lactate accumulation is an important contributing factor of higher perceived fatigue during exercise[155], that leads to premature cessation of the high intense activity prior to reaching the required maximal intensities. Furthermore, these higher subjective fatigue perceptions may lead to poorer high intense exercise adherence. This may attenuate adequate exercise therapy response to HIIT in MS patients reducing the potential

benefits of high intense exercise therapy on cardiovascular risk factors in this population.

In **Study 3** we thus analysed blood lactate accumulation during both acute (one exercise session) submaximal and maximal exercise in MS. Furthermore, we explored whether submaximal or maximal intensity exercise therapy (repeated exercise sessions) could affect resting blood lactate concentrations and exercise-induced lactate accumulation. We hypothesized that, because resting lactate concentrations are elevated, lactate accumulation during sub- and maximal exercise would also be higher in MS patients, and that an exercise intervention would decrease both basal and exercise-induced lactate accumulation. In this study, we confirmed higher resting blood lactate levels in MS patients compared to healthy controls, which was first reported by Amorini et al.[89]. Here, the authors assumed that these elevated serum lactate concentrations originated from either neural or muscular tissue. To investigate this hypothesis, we have induced muscle-based blood lactate accumulation during submaximal and maximal exercise. Surprisingly, exercise-induced blood lactate responses following acute exercise of both intensities did not differ between MS patients and HC. Furthermore, a moderate intensity exercise intervention tended to improve these resting blood lactate concentrations, and reduced blood lactate accumulation during submaximal exercise. However and in contrast to HC[156, 157] and other populations[158], high intensity training did not affect lactate accumulation during maximal exercise. Taken together, it appears that the higher resting serum lactate concentrations do not originate from muscle tissue, not automatically induce higher exercise-induced lactate accumulation, and thus do not seem to impair high intense exercise performance in MS. However, strategies to reduce resting lactate concentrations in MS are worthwhile investigating in future research.

HIIT in MS: Limitations of our studies and future perspectives

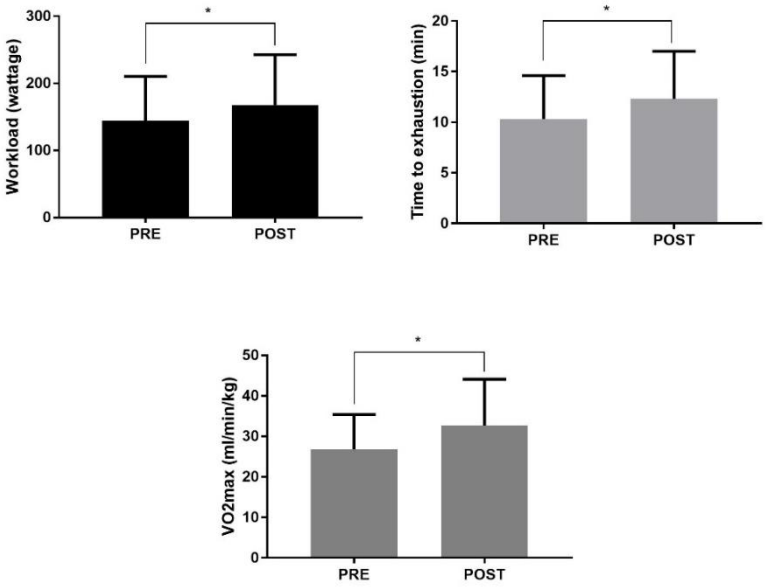
In **Study 1** a 1.5-unit difference in BMI between MS and HC was observed. Although not statistically different, this may cause the potential for residual confounding.

Study 2 was a pilot study in which we explored the impact of high intense concurrent training on cardiovascular risk factors in a small sample size of persons with MS. Consequently, these data need confirmation in a larger scale study. The lack of a sedentary control group in this study makes it difficult to show whether a further deterioration in risk factors would occur in such a group, or whether exercise training managed to impede the deterioration. However, the aim of the present open prospective study was merely to explore whether HIIT is able to modify cardiovascular risk factors in MS. Therefore, a control group was not included in **Study 1**. To better differentiate between the impact of high intensity interval therapy alone and high intense concurrent training on CVD risk factors, future intervention studies in this population should include a control group performing other exercise intervention types. Moreover, as the effects of high intense exercise on cardiovascular parameters in MS seem limited under the conditions of our study, it is possible that longer training program duration may be required to improve other cardiovascular effects. Finally, it should be noted that medication intake for cardiovascular risk factors in both **Study 1 & 2** is indeed a limitation. However, in **Study 1**, regression analyses were used to adjust for parameters such as medication intake. Furthermore, at the end of the experiment in **Study 2**, patients were asked to report whether their medication intake changed over the course of the study, and if so, patients were excluded for further analysis for that specific parameter.

It is important to note that, in **Study 3**, an exercise test performed under both submaximal and maximal exercise conditions following moderate and HIIT training would have given more information regarding the impact of both exercise interventions on exercise-induced lactate responses. Furthermore, investigation of the slope of lactate accumulation during exercise tests under submaximal and maximal conditions may lead to better insights in this matter. However, the available retrospective data did not allow this. Therefore, further research regarding lactate responses during moderate and high intensity exercise in MS seems warranted.

Although we have shown that a high intense exercise intervention did not improve individual cardiovascular risk factors (**Study 2**), it was however able to significantly improve exercise capacity (see Figure 7), reflected by increased $VO2_{max}$ (+22%). This is important to note because $VO2_{max}$ is an important parameter/predictor of the CVD risk, and moreover is the primary measure of the cardiorespiratory fitness (CRF). CRF is the ability of the circulatory and respiratory systems to supply oxygen to the skeletal muscles in order to perform physical activity and is considered an important measure of total body health[211]. Interestingly, a higher CRF was reported to be associated with improved survival rates and a decreased incidence of CVD[94, 96, 97, 103]. Moreover, every increase of 3.5 ml/kg/min VO_2 induces a decrease of 8-17% in CVD-related mortality rate[97]. Therefore, as HIIT has been shown to have superior effects on $VO2_{max}$, and thus on CRF, it may be an efficient strategy to reduce the prevalence of CVD and CVD mortality in MS. Therefore, we recommend the implementation of HIIT to improve CVD-related health in this population. Nevertheless, other strategies should be explored to improve the individual elevated CVD risk factors, such as body fat mass and blood pressure, in MS.

Figure 7. Parameters of cardiopulmonary exercise capacity and impact of high intensity concurrent training.



Data are expressed as means ± SD and represent parameters of the maximal exercise test before (PRE) and after (POST) 12 weeks of high intensity concurrent training (n=16) in Multiple Sclerosis patients.

Objective B. Optimizing high intensity exercise in MS

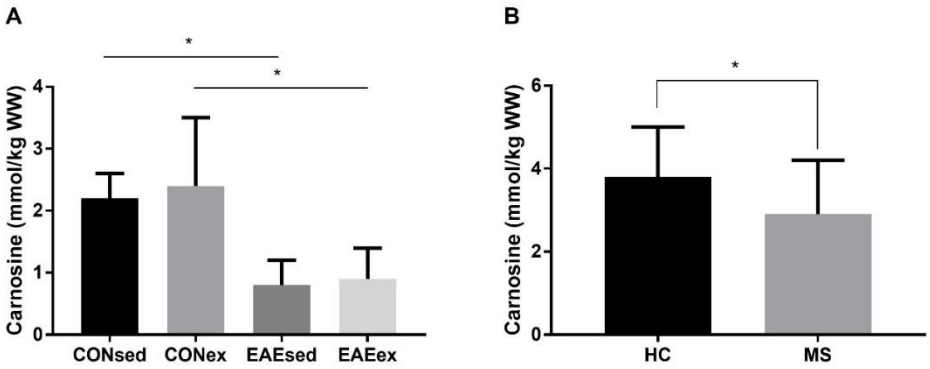
During this PhD project, we also aimed to further optimize HIIT as a rehabilitation strategy in MS. To achieve this, we attempted to optimize overall HIIT performance and functional outcome following HIIT (**B1**), as well as to increase feasibility and adherence to HIIT during rehabilitation in MS (**B2**).

B1. Optimizing high intense exercise performance in MS: muscle carnosine

Muscle carnosine in MS

In the sports and exercise community, high intense intermittent exercise performance is often improved by ergogenic supplementation of β-alanine[109]. This ergogenic substance has been shown to increase muscle carnosine levels and hereby improve high intense exercise performance induced by improved intramyocellular Ca^{2+} handling and buffering of exercise-induced acidosis[109, 170, 212]. Because these properties of muscle carnosine are closely related to the disturbed muscular functions observed in MS, it is relevant to understand muscle carnosine levels in this disease. Therefore, we investigated muscle carnosine levels in both EAE animals and MS patients and furthermore explored the effect of exercise on muscle carnosine levels in EAE (**Study 4**)(see Figure 8).

Figure 8. Muscle carnosine concentrations of rats and humans.



Data are expressed as means ± SD and represent (A) rat m. tibialis anterior carnosine concentrations (mmol/kg WW) after acute experimental autoimmune encephalomyelitis (EAE) or healthy control (CON) under sedentary (CON_{sed}, EAE_{sed}) or exercise conditions (CON_{ex}, EAE_{ex}) and (B) m. vastus lateralis carnosine concentrations (mmol/kg WW) of healthy controls (HC, n=22) and MS patients (n=24).

So far, we were the first to show reduced muscle carnosine levels in an animal MS model (EAE) and confirmed this in MS patients (Figure 8, part A and B respectively). Although the exact mechanisms responsible for the decrease in muscle carnosine remain unknown, several contributing factors may explain these findings.

In MS, central mechanisms[40, 149, 163, 164] such as delayed conduction/reaction times and impaired motor unit recruitment induce a disuse-related physiological profile[140], leading to a more sedentary lifestyle. In sedentary populations, lower muscle carnosine levels might result from an inactivity related reduction in total muscle protein content. In **Study 4** however, we also measured muscle histidine, glutamine and serine, metabolites that are also prone to inactivity and they were not affected. Therefore, we can assume that the specific decrease of muscle carnosine was not due to poor muscle quality or disuse. Possibly, various intramyocellular dysfunctions that are associated to MS and relate to the physiological role of carnosine could explain the present findings. However, the impact of carnosine on disturbed muscle contractile characteristics and cross-bridge (Ca^{2+} handling) abnormalities[40, 42, 43], excessive exercise-induced acidosis[40, 149, 152] and mitochondrial dysfunction[150, 165, 166] in MS have not been investigated yet. As such, further research regarding the exact underlying mechanisms of the decrease in muscle carnosine in MS is needed.

Increased oxidative stress in MS and the subsequent accumulation of cytotoxic compounds, such as the reactive carbonyl acrolein could be another possible explanation for the reduction in muscle carnosine. Carnosine serves as a sacrificial sequestering agent for acrolein by forming unreactive adducts, therefore providing an endogenous protective mechanism against protein and eventually tissue damage, induced by this reactive carbonyl[180-182]. Indeed, increased urinary elimination of carnosine-carbonyl conjugates have been demonstrated in metabolic disorders such as the metabolic syndrome, obesity and type 2 diabetes mellitus that are characterized by increased carbonyl stress[183]. However, the urinary elimination of carnosine-acrolein conjugates in MS has not been

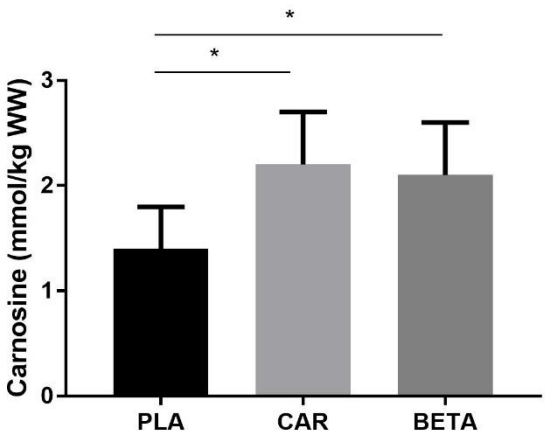
investigated so far. Because acrolein is also believed to be involved in the MS pathogenesis by directly damaging myelin, thus causing demyelination, this may partly explain the reduction of muscle carnosine in this population. Probably, endogenous carnosine synthesis is unable to compensate for the increased 'consumption' of carnosine to eliminate and detoxify the increased levels of acrolein. Indeed, when Leung et al.[185] treated EAE mice with hydralazine, which is an acrolein scavenger, myelin integrity was largely preserved. Therefore, investigating strategies to increase muscle carnosine concentrations in MS are interesting to improve HIIT performance, and thus clinical outcome, but furthermore seem promising regarding the pathological mechanisms of this disease.

A first strategy to increase muscle carnosine levels that we have explored was exercise therapy. However, in **Study 4**, exercise was not able to restore/preserve carnosine concentrations in EAE mice. These findings are in line with current existing literature regarding the impact of exercise therapy on muscle carnosine levels. It was already shown by Kendrick et al.[172, 173] and Mannion et al.[174] that an isokinetic resistance training program of 4-16 weeks was not able to increase muscle carnosine concentrations. Furthermore, Baguet et al.[171] could not demonstrate elevated carnosine levels following a 5-week sprint intervention in healthy subjects. Suzuki et al.[169], did find positive effects of an 8-week sprint interval program on muscle carnosine concentrations in healthy subjects. However, this study was performed on a limited number (n=8) of healthy students, of which the results should carefully be interpreted. Therefore, nutritional interventions, such as β-alanine supplementation, are probably required in order to increase the reduced muscle carnosine concentrations in MS.

Muscle carnosine loading in EAE

In keeping with the above line of reasoning, in **Study 5** and prior to exploring this in human subjects, we have first investigated whether reduced muscle carnosine concentrations in EAE can be restored by carnosine and/or β-alanine supplementation (see Figure 9). Here, we have demonstrated that carnosine and β-alanine supplementation indeed is able to increase/restore muscle carnosine in EAE to levels that are comparable to healthy control animals. These findings warrant carnosine loading via β-alanine supplementation in MS patients in order to further optimize high intense rehabilitation performance and thus provide better clinical outcomes.

Figure 9. Rat m. tibialis anterior carnosine concentrations.



Data are expressed as means ± SD and represent m. tibialis anterior carnosine concentrations following placebo (PLA, n=10), carnosine (CAR, n=13) or β-alanine supplements (BETA, n=9).

Surprisingly, in **Study 5** carnosine supplementation was able to positively affect the clinical disease course of EAE. More specifically, carnosine in the drinking water of EAE animals postponed the onset of the disease symptoms (hind quarter paralysis), reduced disease peak and allowed earlier recovery of the experimental disease model. The neuroprotective role of carnosine was already explored in various animal models of neurological disorders such as Parkinson's and Alzheimer's disease (AD). Here, Preston et al.[187] showed inhibitory effects of carnosine against loss of cerebellar granule cell neurons viability in the brain of AD rats. Furthermore, Corona et al.[193] and Herculano et al.[194] reported restoration of mitochondrial functioning and prevention of cognitive decline in AD mice. Finally, Boldyrev et al.[195] were able to improve neurological deficits, such as rigidity and movement of hands and legs in Parkinson patients, after addition of carnosine to their standard treatment.

However, we were the first to demonstrate the neuroprotective role of carnosine supplementation in an animal MS model with substantial effects on clinical disease scores. Several potential underlying mechanisms may explain these findings. For instance, the above described cytotoxic compound acrolein may be scavenged to a greater extent when carnosine concentrations are elevated through supplementation, leading to neuroprotection and defence against demyelination. Furthermore, the beneficial neuroprotective effect of carnosine may be attributed to the increased plasma carnosine concentrations in animals as, in contrast to humans, carnosine in the circulation of animals is not hydrolysed. To explore the neuroprotective role of carnosine in our EAE animals, we have in **Study 5**, measured carnosine concentrations in the CNS, and more specifically the bulbus olfactorius. The olfactory system is the site in the CNS with the highest carnosine concentrations, comparable to the skeletal muscle whereas other CNS regions have far lower, and often undetectable, carnosine concentrations. If carnosine concentrations increased compared to placebo, this then could indicate that carnosine intake is able to increase carnosine concentrations in the CNS. However, under the conditions of

Study 5 bulbus carnosine concentrations only tended to be higher in the supplemented group. Therefore, it is difficult to draw solid conclusions and further research is warranted. Furthermore, mechanisms such as altered expression of inflammatory markers, reduced activation and infiltration of leukocytes and dendritic cells into the CNS, leading to reduced demyelination and axonal loss, may also explain the findings of this study. Nerve tissue samples at disease peak however are required to investigate these hypotheses.

Because carnosine loading seems to positively influence the disease course in EAE and because various previously described muscular abnormalities, such as disturbed Ca^{++} handling, increased lactate accumulation and overproduction of reactive oxygen species, in MS correspond precisely to the functional role of muscle carnosine, further investigation of carnosine loading in MS is mandatory. Nevertheless, as β-alanine supplementation was shown to increase muscle carnosine levels in EAE, this now validates the use of such supplementation in MS, in order to explore the impact on HIIT performance.

Muscle carnosine in MS: limitations of our studies and future perspectives

Because no nerve tissue was collected, it was not possible to investigate the exact underlying mechanisms responsible for the improved clinical disease course in the carnosine supplemented EAE group in **Study 4**. Future studies should sample nerve tissue at peak disease, to investigate peripheral nerves, motor end-plates, inflammatory markers and the extent of both neuronal and axonal damage in the spinal cord, which could explain the findings of this study.

Furthermore, the injection of Complete Freund's Adjuvant (CFA) itself, causing inflammatory processes, may have influenced the reduced muscle carnosine concentrations in the EAE animals. Ideally, a control group, with injection of CFA alone, should be added in future research in order to determine whether this has an impact on muscle carnosine concentrations, compared to the actual EAE induced animals.

In **Study 1** we have shown that MS patients have an increased risk for the development of CVD, as multiple cardiovascular risk factors such as body composition, blood pressure, blood triglyceride levels and parameters of glucose tolerance are elevated compared to HC. Here, it is interesting to note that carnosine improves various CVD risk factors such as blood pressure and lipid metabolism in animals[213]. De Courten et al.[213] demonstrated that carnosine possesses an anti-hypertensive effect that involves vasorelaxing effects of the autonomic nervous system, leading to reductions in blood pressure. However, in this study that included healthy subjects, muscle carnosine was related to HDL and the atherogenic index, but not to total cholesterol, LDL, triglycerides or blood pressure. As stated by the authors, these findings were probably due to the rather young study population (45±7 years) with normal blood lipid profiles. Because MS patients do present various altered CVD risk factors, the link between these risk factors, muscle carnosine concentrations and carnosine/ β-alanine supplementation is therefore worthwhile investigating in the future.

B2. Optimizing high intense exercise feasibility/adherence in MS: training periodization

Home-based periodization in MS

In an attempt to improve long-term feasibility and adherence of high intense exercise therapy in MS, in **Study 6**, we have explored the impact of a periodized (low-, high intensity exercise and recovery), long-term (6m), home-based cycling exercise program in mildly affected persons with MS. Here, our periodized and HIIT-oriented exercise program appeared to be feasible and provided good adherence.

Despite positive findings of HIIT in MS, the application of high intense exercise in current MS rehabilitation programs is difficult. Probably, rehabilitations regimens consisting of HIIT alone are too demanding, require self-guidance and external motivational factors to adhere to longer term HIIT periods. Indeed, reduced therapy compliance resulting in attenuated clinical outcome, following high intensity exercise has been reported[104]. Moreover, rehabilitation programs that include high intense exercise therapy in MS are often purely reactive to exacerbations and merely address acute disability/symptoms, without focussing on long-term benefits of high intense exercise. Therefore, we have explored several exercise therapy strategies, that involve HIIT, to improve feasibility and adherence to long-term HIIT interventions.

A first strategy was exercise therapy periodization. Current exercise interventions in MS typically use uniform workloads with little to no variation and progressive linear training characteristics[39, 62]. Although improving various health-related and functional parameters[61, 122, 123], long-term adherence to such exercise interventions is rather low, but furthermore has been shown to induce psychological symptoms such as mental fatigue, mood disturbances and reduced motivation, which are directly related to performance and clinical outcomes[124]. However, in high performance sports prolonged training periods do not involve such single progressive linear training stimuli[121]. Here, training programs are divided into periodically

alternating blocks of typically 1-6 weeks, including variations in training stimuli, intensity, duration and frequency[119]. Furthermore, these varying exercise modalities are interspersed with periods of adequate rest and recovery to induce a supercompensation phase, which causes substantial improvements in exercise performance that exceed pre-load levels[120]. Applying such high-performance periodization principles in a rehabilitation setting including the superior, though demanding, high intensity exercise sessions, as well as lower intensity exercise and adequate recovery periods, may further enhance clinical outcome, but more importantly improve long-term adherence and feasibility of HIIT in MS. Indeed, by using weekly variations (week I-III) in exercise intensity (high vs. low) and session duration (long vs. short), alternated with a rest/recovery week, our periodized exercise program appeared to be feasible during a prolonged training period (6m) and significantly improved exercise capacity and body composition in MS patients, at a similar extent to healthy controls. Furthermore, this periodized training program, including short HIIT sessions, is also less time consuming compared to classic moderate intensity endurance training (total session duration ~30-60min) programs. It was already reported by Asano et al.[84] that 'lack of time' is a major barrier for engagement in (long-term) physical activity. As such, our newly composed periodized exercise program predominantly consists of (very) short HIIT sessions (total session duration ~20-30min), which might remove this barrier for participation in long-term physical activity. By also applying high-volume, low-intensity endurance training sessions, we believe that therapy/exercise compliance on longer term in MS can be improved. This is important, as Perri et al.[104] stated that an abundance of high intense exercise may lead to reduced therapy compliance and thus attenuated clinical outcomes. Furthermore, the moderate intensity endurance sessions might even be beneficial to reduce the elevated resting serum lactate concentrations in MS, as discussed above in **Study 3**.

Second, we have explored the efficacy of our periodized intervention in a home-based setting with remote supervision (using a commercially available activity tracker) first. Although physical exercise elicits major benefits in MS, only 43% of the MS patients reported to actively participate in an exercise intervention/training program[83]. Often, another significant barrier for MS patients to engage in a (long-term) physical activity program is distance/transportation to the rehabilitation centre[83, 84]. A possible strategy to remove this barrier is home-based training. In this respect, it is important to note that home-based rehabilitation interventions in MS have already been reported to be safe and to efficiently improve functional parameters such as walking speed, aerobic capacity, mobility and cognitive performance in previous research[77, 208]. Therefore, it appears that home-based interventions in MS could lead to persistent increased levels of physical activity and thus improved clinical outcome. Indeed, the results of **Study 6** are consistent with these findings, as our 6m home-based exercise program induced significant improvements in exercise capacity and body composition in these patients. Furthermore, and more importantly, this home-based, HIIT oriented, exercise program provided high therapy adherence (95% training sessions completed), which is comparable to a previous home-based intervention of Feys et al.[208] in MS. However, this could be due to the remote supervision, using a commercially available activity tracker, in the present study. Indeed, Snook et al.[122] showed that unsupervised training programs in a home- or community based setting were less effective compared to remotely supervised interventions. Therefore, it appears that home-based exercise in MS is an interesting strategy to increase therapy adherence, although further research regarding the influence of remote supervision, using activity trackers in MS, on this matter is warranted.

Taken together, under the conditions of the present study, it appears that a periodized, although HIIT oriented, exercise intervention is able to improve high intense exercise feasibility and adherence in MS patients. As such, integrating periodization principles in current rehabilitation programs, including high intense exercise sessions, seems a valid next step in the rehabilitation strategy of MS patients.

Home-based periodized training: limitations of our studies and future perspectives

Although this applied, home-based, periodized exercise program demonstrated positive findings, it is now important to investigate this program in a more controlled research setting. Furthermore, to investigate the potential of periodized, HIIT-oriented exercise therapy in MS and to see whether this approach is superior to current existing monotonous MS rehabilitation programs, this strategy should be compared to such classic exercise interventions. Furthermore, feasibility of the periodized exercise program was assessed by personal communication with the participants. As such, standardized assessment of feasibility should be implemented in future studies.

Finally, in **Study 6** we have used the same HIIT protocols as in **Study 2**. Although very efficient, these rather long (1-2min) intervals of high intense exercise may still be too demanding. Hence, further optimization of these HIIT protocols, in order to keep improving HIIT feasibility and adherence in MS, is warranted.

Therefore, in a currently ongoing project (**Study 7**), we are now investigating periodized exercise therapy using shorter HIIT protocols (3x20sec) and β-alanine supplementation including muscle carnosine measurements, in a controlled research setting, compared to classic moderate intensity exercise training in MS.

Chapter 5

Nederlandstalige samenvatting

Dit doctoraatsproject omvat twee grote onderzoeksdoelstellingen (**A & B**). Ten eerste richtte dit project zich op het onderzoeken van de impact van hoog-intense interval training (HIIT) als therapeutische bewegingsinterventie op gezondheidsgerelateerde comorbiditeiten (bvb. cardiovasculaire risico's) die veelal het gevolg zijn van MS gerelateerde inactiviteit (**A**). Ten tweede trachtten we in dit project om HIIT als revalidatiestrategie in MS verder te optimaliseren (**B**). Om dit te verwezenlijken hebben we getracht de algemene HIIT prestatie en functionele uitkomst na HIIT te verbeteren (**B1**). Verder hebben we strategieën onderzocht om de haalbaarheid en therapietrouw van HIIT in de revalidatie van personen met MS te optimaliseren (**B2**).

Om de impact van HIIT op enkele belangrijke gezondheidsgerelateerde MS comorbiditeiten zoals cardiovasculaire aandoeningen, die vaak veroorzaakt worden door inactiviteitsgerelateerde secundaire symptomen, is het eerst nodig om te weten welke cardiovasculaire risicofactoren verstoord zijn in personen met MS. Eerder onderzoek heeft uitgewezen dat personen met MS inderdaad een verhoogd risico op cardiovasculaire aandoeningen hebben vergeleken met gezonde controles. Echter is het niet duidelijk of dit verhoogde risico te wijten is aan veranderingen in lichaamssamenstelling, bloeddruk, bloed lipidenprofiel of controle van de suikerregulatie. Daarom hebben we in **Studie 1** (**doelstelling A**) onderzocht welke cardiovasculaire risicofactoren voornamelijk bijdragen aan het verhoogde risico op cardiovasculaire aandoeningen in MS, en hebben we de relatie tussen al deze factoren bekeken.

In andere populaties is het positieve effect van hoog intense oefentherapie op gezondheidsgerelateerde parameters, inclusief cardiovasculaire risicofactoren, reeds bewezen. Op basis van de resultaten uit **Studie 1**, en omdat dit nooit eerder onderzocht werd in MS, hebben we in **Studie 2** onderzocht of een hoog intens oefenprogramma het potentieel heeft om een aantal belangrijke en verstoorde cardiovasculaire risicofactoren in MS patiënten te verbeteren.

Onlangs werd aangetoond dat MS patiënten verhoogde concentraties lactaat in het bloed hebben t.o.v. gezonde controles. Of deze bloedlactaatconcentraties ook verhoogd zijn tijdens inspanning bij MS patiënten is echter niet geweten. Dit is zeer relevante informatie, aangezien verstoorde inspanningsgerelateerde bloedlactaatconcentraties tijdens HIIT kunnen leiden tot grotere subjectieve vermoeidheid en mogelijks snellere opgave van de fysieke activiteit. Indien zo, kan dit leiden tot een ontoereikende hoog intense prikkel en dus een verzwakte en verlaagde klinische uitkomst. Om deze redenen hebben we in **Studie 3** onderzocht of opstapeling van lactaat in het bloed, tijdens sub- en maximale inspanning, verschillend is in MS patiënten t.o.v. gezonde controles.

De tweede doelstelling (**B**) van dit doctoraatsproject omvatte het optimaliseren van de algemene HIIT prestatie/revalidatie in MS (**doelstelling B1**). Hiermee willen we inspanningscapaciteit, spierkracht, functionaliteit en levenskwaliteit in deze populatie verder verbeteren. Bovendien, en dankzij de veelbelovende resultaten van HIIT in MS, is het nodig om de bestaande HIIT protocollen te optimaliseren en zodoende de haalbaarheid en therapietrouw op lange termijn te verbeteren (**doelstelling B2**). Dit is noodzakelijk om de integratie van HIIT in de bestaande revalidatiestrategieën te vergemakkelijken.

In de sportwereld is β-alanine (**doelstelling B1**) een zeer populair prestatiebevorderend supplement, aangezien dit de spiercarnosine concentratie verhoogt en hierdoor een verbetering geeft van de sportprestatie, voornamelijk tijdens hoog intens intermittente inspanningen. De onderliggende mechanismen die hier aan de grondslag liggen zijn een verbeterde Ca^{2+} regulatie en buffering van inspanningsgerelateerd bloedlactaat. Het verhogen van spiercarnosine, door β-alanine supplementatie, leidt dus tot een verbetering van de HIIT prestatie. Echter, vooraleer we de impact van β-alanine supplementatie en verhoogde spiercarnosine op de HIIT prestatie bij MS kunnen onderzoeken,

is het nodig om de impact van MS op deze concentraties van spiercarnosine te begrijpen. In **Studie 4** (**doelstelling B1**) hebben we daarom onderzocht of carnosine concentraties in de spieren van een proefdiermodel voor MS (EAE, Experimentele Auto-immune Encephalomyelitis) en MS patiënten, verschilt van gezonde controles. Omdat zowel EAE als MS de spiercarnosine concentraties lijken te beïnvloeden, hebben we eerst het effect van carnosine/ β-alanine supplementatie in dieren onderzocht (**Studie 5, doelstelling B1**).

In de topsportwereld worden trainingsprogramma's vaak geperiodiseerd in opeenvolgende fases en cycli. Dit om specifieke trainingsdoelen en optimale prestaties op lange termijn te bereiken en ook overbelasting en het ontstaan van blessures te minimaliseren. In de huidige MS revalidatie wordt echter nog steeds gebruik gemaakt van monotone, uniforme belasting en progressief lineaire revalidatie/trainingsprogramma's die vaak leiden tot verminderde motivatie, mentale vermoeidheid, slechte therapietrouw, overbelastingsletsels en suboptimale trainingsstimuli. Bovendien en ondanks substantiële klinische verbeteringen na HIIT in MS, lijkt de effectieve implementatie van dergelijke hoog intense trainingsinterventies in de huidige revalidatie moeilijk. Revalidatieprogramma's die enkel bestaan uit HIIT zijn fysiek zeer veeleisend en dit lijkt de therapietrouw op lange termijn negatief te beïnvloeden. Inderdaad, verminderde klinische uitkomsten, resulterend uit verminderde therapietrouw na hoog intense inspanningen werden reeds gerapporteerd bij sedentaire volwassenen. Daarom hebben we in **Studie 6** (**doelstelling B2**) onderzocht of een geperiodiseerd trainingsprogramma, inclusief veeleisende HIIT sessies, de therapietrouw en haalbaarheid van HIIT in MS kan verbeteren.

Doelstelling A

Tijdens dit doctoraatsproject hebben we kunnen aantonen dat verschillende cardiovasculaire risicofactoren aan de basis liggen van het verhoogde risico op de ontwikkeling van cardiovasculaire aandoeningen in MS patiënten, met een toegenomen vetmassa als primaire risicofactor. Echter, in vergelijking met andere populaties konden we deze verstoorde risicofactoren niet remediëren m.b.v. een hoog intens oefenprogramma. We veronderstelden dat een toegenomen bloedlactaatproductie tijdens inspanning in MS deze patiënten belemmerde om te kunnen trainen aan een voldoende hoge intensiteit, hetgeen zou leiden tot onvoldoende responsen op cardiovasculaire risicofactoren na deze inspanning. Echter, aangezien de opstapeling van bloedlactaat tijdens inspanning niet verschillend bleek te zijn tussen MS patiënten en gezonde controles, konden we deze hypothese niet bevestigen. Hieruit kunnen we dus concluderen dat, naast HIIT, andere strategieën onderzocht moeten worden om de verstoorde cardiovasculaire risicofactoren in MS te kunnen verbeteren.

Doelstelling B

Naast de impact op gezondheidsgerelateerde parameters hebben we ook getracht om HIIT als revalidatiestrategie te optimaliseren in MS. Dit probeerden we te bereiken door het verbeteren van de algemene HIIT prestatie, alsook de haalbaarheid en therapietrouw op lange termijn te optimaliseren. Om dit te bekomen onderzochten we twee strategieën, dewelke veelvuldig in de sportwereld worden toegepast om HIIT prestaties te verbeteren, namelijk ergogene supplementatie en training periodisatie. Ten eerste hebben we aangetoond dat zowel dieren als personen met MS substantieel verlaagde carnosinegehaltes in de spier hebben. Dit kan ervoor zorgen dat de HIIT prestatie verslechtert in deze populatie. Echter, d.m.v. β-alanine supplementatie konden we de carnosine concentratie in de spier terug verhogen/herstellen. Verder toonde deze supplementatie veelbelovende resultaten op het klinisch ziekteverloop bij de dieren. Om

deze redenen lijkt het erop dat verhoging van de carnosine concentraties in MS een interessante strategie is om verder te onderzoeken. Tenslotte bleek training periodisatie een efficiënte strategie om klinische parameters zoals inspanningscapaciteit te verbeteren, alsook de haalbaarheid en therapietrouw van een HIIT interventie te verbeteren. Deze strategieën dienen nu verder onderzocht te worden in grotere, gecontroleerde studies.

Appendices

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Curriculum Vitae

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University degrees

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Career positions

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Intern research fellowship, Hasselt University, 2013-2014

Current position
PhD student: 2014-2018 University Hasselt

Additional skills

Public Speaking – Universiteit Hasselt – 2015

Good Scienfic Conduct & Lab book taking – Universiteit Hasselt – 2015

Project and Time Management – Universiteit Hasselt – 2015

Academic English – Universiteit Hasselt – 2015

Biosafety – Universiteit Hasselt – 2016

Laboratory Animal Science (FELASA B) – Universiteit Hasselt – 2016

Flames, Introduction to JMP Statistics – Universiteit Hasselt – 2016

Effective Image Editing – Universiteit Hasselt – 2017

PhD Management: Succesfully dealing with stakeholders – 2017

Participation in international conferences

2015 – SIG Mobility – Bad Wildbad, Germany: Poster presentation

2017 – RIMS conference – Barcelona, Spain: Poster presentation

2017 – International Congress on Carnosine and Anserine – Kentucky, USA: Poster presentation

2018 – European College of Sports Science – Dublin, Ireland: Poster presentation

2018 – RIMS conference – Amsterdam, The Netherlands: Oral presentation (awarded, 2nd best oral presentation, €300)

Referee assignments on request of editorial board of international journals

Diabetes Research and Clinical Practice 2015

Nigerian Journal of Clinical Practice 2016

Medicine and Science in Sports and Exercise 2017

Neurorehabilitation and Neural Repair 2017

American Journal of Medicine and Rehabilitation 2017

Clinical Interventions in Aging 2017

List of publications from this work (PhD)

Charly Keytsman, Bert O Eijnde, Dominique Hansen, Kenneth Verboven, Inez Wens. Elevated cardiovascular risk factors in Multiple Sclerosis. Multiple Sclerosis and Related Disorders. 2017 Oct;17:220-223 2017 (IF: 2.4)

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Charly Keytsman, Laura Blancquaert, Pieter Van Noten, Tim Vanmierlo, Jeroen Bogie, Jan Spaas, Anneke Volkaert, Wim Derave, Bert O Eijnde. Carnosine supplementation in Experimental Autoimmune Encephalomyelitis. *Manuscript in preparation.*

Charly Keytsman, Pieter Van Noten, Jan Spaas, Ine Nieste, Paul Van Asch, Bert O Eijnde. Home-based periodized exercise in Multiple Sclerosis. *Revision in 'Multiple Sclerosis and Related Disorders'.*

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