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Effect of exercise therapy on muscle contractile properties in experimental autoimmune encephalomyelitis and multiple sclerosis

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List of Abbreviations

ACSM:	American College of Sports Medicine
ANOVA:	Analysis of Variance
BDNF:	Brain Derived Neurotrophic Factor
BBB:	Blood Brain Barrier
CON:	Control group
CON_{EX}:	Exercising Control group
CON_{SED}:	Sedentary Control group
CNS:	Central Nervous System
CSA:	Cross Sectional Area
EAE:	Experimental Autoimmune Encephalomyelitis
EAE_{EX}:	Exercising EAE group
EAE_{SED}:	Sedentary EAE group
EAE-H:	High intense EAE group
EAE-L:	Light intense EAE group
EAE-M:	Moderate intense EAE group
EAE-S:	Sedentary EAE group
EDL:	m. Extensor Digitorum Longus
EX:	Exercising group
MBP:	Myelin Basic Protein
MS:	Multiple Sclerosis
ns:	not significant
PBS:	Phosphate Buffered Saline
PwMS:	Persons with Multiple Sclerosis
RES_E:	Resistance Training group with additional Electro-stimulation
RES_O:	Resistance Training group
IL:	Interleukin
SED:	Sedentary subgroup
SEM:	standard error of the mean
TA:	m. Tibialis Anterior
TNFα:	Tumor Necrosis Factor alfa
WBV:	Whole Body Vibration

Chapter I:

General Introduction

Multiple Sclerosis

Multiple Sclerosis (MS) is a progressive neurodegenerative disease that can be considered as a prototype inflammatory autoimmune disorder of the central nervous system (CNS). It is characterized by demyelization and axonal loss (1,2). Usually, disease onset is between the age of 20 and 40 years. The exact etiology is still unknown but multiple factors seem to affect its onset. First, a genetic component probably contributes to the development of MS (3). First-, second- and third degree relatives of persons with MS are at higher risk to develop the disease and it has been suggested that multiple genes are involved (4-7). Second, several environmental factors and/or lifestyle related aspects such as vitamin D (related with hours of sunlight) and smoking possibly affect the susceptibility to MS as well (8,9). Interestingly, the prevalence of MS also has a geographical component (10) with the highest prevalence in the north Americas (USA and Canada), northern Europe, southern Australia and New Zealand (0.05-0.15%) (2,11,12). Third, several viral candidates such as Epstein Barr virus (13) and human herpes virus 6 (14) may also trigger MS. However, for the latter there is no conclusive evidence (15).

Although many pharmacological therapies that have been developed during the last decade decrease disease progression, so far, MS is still not curable. Consequently, other treatment strategies that remediate or decrease MS symptoms are important. These therapies often include different (para)medical experts such as general practitioners, physiotherapists or occupational therapists to reduce the impact of the disease on a patient's quality of life. To reach this objective therapists often function in multidisciplinary teams (neurologist, physical therapist, occupational therapist,…) to treat a wide range of MS related primary symptoms and secondary consequences. Secondary consequences of MS such as decreased aerobic capacity and reduced muscle strength often are inactivity-related. Because they clearly affect a patient's quality of life and are partly reversible through exercise, exercise therapy probably is an appropriate strategy to remediate these inactivity related secondary MS consequences. Interestingly, to date it has been suggested that exercise may also have a potential anti-inflammatory effect in MS.

1.1. Pathogenesis

The inflammation process and formation of inflammatory MS lesions are considered as the pathological substrates of the early phase of the disease. In the following progressive phase, neurodegeneration is believed to be the prevailing and most important disease mechanism. We have described these diseases phases more in detail below (see Figure 1.1.).

Although immunology, neuroscience, epidemiology and genetics today provide new insights, many important aspects of the MS cause and pathogenesis remain unknown.

Inflammation

MS is an immune-mediated disease. In the early disease phase, the inflammatory processes within the immune system initiates MS-associated nerve-tissue injuries (16,17). According to the general accepted hypothesis and in contrast to healthy individuals, autoreactive T cells become activated in the systemic circulation. Normally, the blood brain barrier (BBB) separates the CNS from the circulation which keeps immune cells out of the CNS. In MS, up regulated expressions of adhesion molecules in the periphery allows the migration of the autoreactive T cells throughout the BBB. As indicated in figure 1.1., in the CNS the autoimmune T cells (Th1 and Th17) are reactivated by antigen presenting cells such as microglia, which trigger an immunological cascade leading to recruitment of macrophages, B cells and cytotoxic CD8+ T cells in the CNS. (2,18-20). In contrast to Th1 and Th17 cells which are probably the main inducers of the disease, the CD8+ T cells may be more relevant for tissue damage because these cells can recognize antigens of the major histocompatibility complex (MHC) class I which are expressed during inflammation on neurons and oligodendrocytes (19) and consequently kill the neurons. Other effector mechanisms involve free radicals, glutamate-mediated excitotoxicity and cytokines. In general, cytokines coordinate all phases of the immune response and act in highly complex networks which could be influenced by paracrine and/or autocrine stimuli. To maintain homeostasis, a dynamic balance between pro- and anti-inflammatory cytokines in the CNS and in the periphery is required. In the CNS of a person with MS, antigen-presenting cells and activated T cells secrete several cytokines that may orchestrate the (detrimental) immune response. Hence, in MS it has been suggested that the balance between Th2 (anti-inflammatory) and Th1 (pro-inflammatory) response is disturbed (21). Generally, IL-12, INF-γ and TNF-α induces Th1-type immune response, while IL-6 and IL-10 induce a TH2-type immune response (18). Briefly, higher levels of IL-6 which is a cytokine with pro- and anti-inflammatory capacities, have been observed (22,23). Furthermore, inconsistent data on IFN-γ levels (pro-inflammatory cytokine with a real influence on the course of MS) are reported. Some studies indicate increased serum levels (24) or elevated numbers of IFN-γ mRNA blood mononuclear cells (25), while other studies did not report alterations to either the percentage of IFN-γ expressing blood cells (26) nor the blood level of IFN-γ mRNA (27,28). Another important pro-inflammatory cytokine in MS is TNF-α. Increased serum concentrations (24) and higher levels of TNF-α-secreting blood mononuclear cells (29) are

reported. It is also documented that TNF-α stimulates the production of IL-12 (30) which in turn induces IFN-γ production. Nevertheless, confusing results regarding IL-12 levels have been found. Serum levels of IL-12 did not differ between patients with MS and controls (31,32) while an increase in the number of IL-12 mRNA-expressing blood mononuclear cells (33) and higher IL-12 mRNA blood levels (34) have been described. Elevated concentrations of pro-inflammatory cytokines must be kept under control by the anti-inflammatory cytokines which have been considered as beneficial (35). IL-10 can be considered as the most important anti-inflammatory cytokine because it has inhibitory effects on IL-2, IL-12, TNF-α and IFN-γ (18). However, the role of IL-10 in MS is not very clear because elevated numbers of IL-10 mRNA-expressing blood mononuclear cells are described (36) while others did observe lower serum levels and decreased numbers of IL-10-secreting blood mononuclear cells (27, 29,37).

More recently, the discovery of IL-17 producing Th17 cells which can also trigger autoimmune inflammation in rodent brain provided some new insights (38). To date, it is clear that both Th1 and Th17 cells are involved in the pathogenesis of MS because these cells and their corresponding cytokines (Th1: IL-12, TNF-γ: Th17: IL17, IL-23) are found at the MS-related inflammation site (39,40). Furthermore, these cytokines exhibit an important role in autoimmunity (41). In addition, Th1 and Th17 cells express chemokine receptors on their surfaces possessing the potential of migrating across the BBB (42).

Neurodegeneration

In addition to nerve-tissue damage triggered by the inflammatory processes, as described above, many studies also indicate neurodegeneration as a major cause of MS related disability. Observations in MS lesions suggest that in some cases oligodendrocytes apoptosis may occur without immune infiltration (43). Furthermore, axonal pathology has also been described in MS lesions. In particular, axonal swelling and transection were found in active lesions sites and at the border of chronic MS lesions (44). In addition, it has also become clear that MS is not only characterized by white matter demyelination, but also by extensive axonal loss and gray matter pathology (45). All these different pathways are responsible for the persistent MS symptoms. In the early disease course, axonal damage is only associated with little clinical disability due to brain plasticity which compensates the neuronal loss. Unfortunately, during the progressive disease phase neural plasticity is no longer able to compensate, resulting in irreversible neurological decline (45).

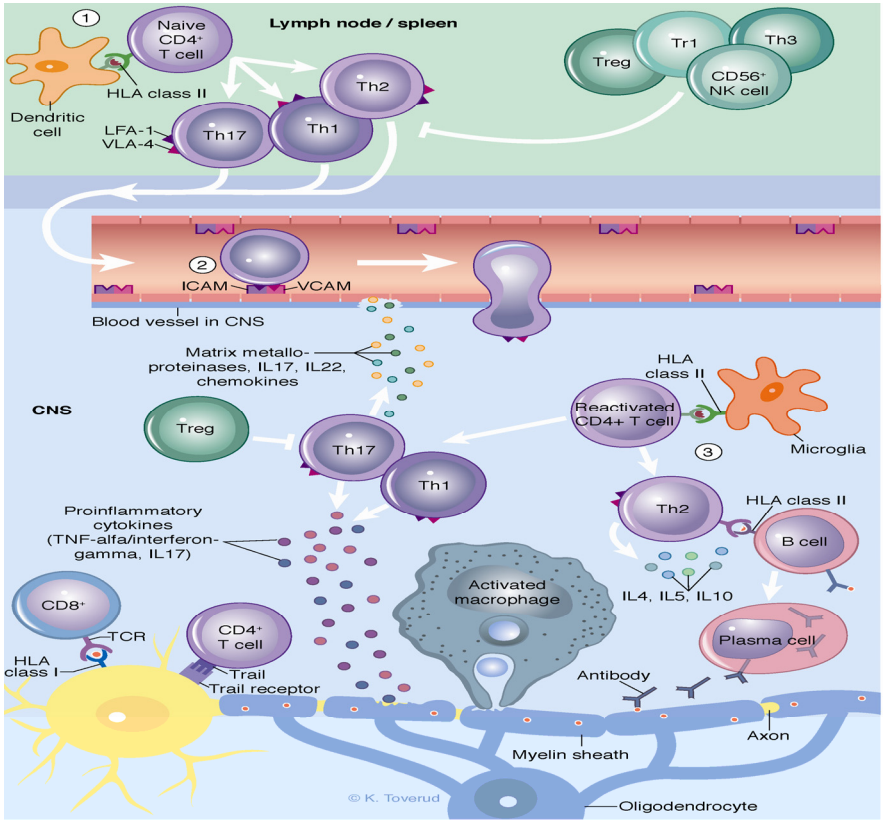

Figure 1.1. Immunopathogenesis of MS.

Naive autoreactive CD4+ T cells recognize antigen presented by HLA class II molecules on dendritic cells in secondary lymphoid organs (1). The T-cell antigen is not known but could be an infectious derived agent. Depending on the cytokine milieu, the activated CD4+ T cells develop into different T helper (Th) cells (Th1, Th2, Th17). Th1 and Th17 T cells are probably the main inducers of disease, whereas CD8+cytotoxic T cells may be more relevant for tissue damage. Autoreactive T cells are usually controlled by regulatory T cells (Tregs), which include Tregs, Tr1, Th3 and CD56 natural killer (NK) cells. Activated T cells express the adhesion molecules very late activation antigen 4 (VLA-4) and lymphocyte function-associated antigen 1 (LFA-1), which are required to penetrate the blood–brain barrier (2). After reactivation within the central nervous system, pro-inflammatory Th1 and Th17 cells activate macrophages/microglia and cytotoxic CD8+ T cells. CD8+ T cells can recognize antigens presented by HLA class I molecules expressed on neurons and other central nervous system cells, whereas CD4+ T cells express the death molecule TRAIL and may kill neurons by attaching to their TRAIL receptor. Th2 cells activate B cells into plasma blasts and contribute to antibody-mediated and complement-mediated cell damage. Other effector mechanisms involve free radicals, cytotoxic cytokines and glutamate-mediated excitotoxicity. Release of cytokines and matrix metalloproteinases disrupts the blood–brain barrier, and chemokines further attract immune competent cells. CNS, central nervous system; HLA, human leukocyte antigen; IL, interleukin; TNF, tumor necrosis factor. Reprinted with permission from Holmoy and Hestvik (19).

1.2.Diagnosis

To date, MS diagnosis is based on the internationally accepted criteria that were first formulated by Poser et al in 1983 (46). They were later revised by McDonald et al (47). The diagnostic procedure is performed by a neurologist and includes clinical as well as paraclinical (MRI, spinal fluid measurements and evoked potentials) measurements to quantify white matter disease activity disseminated in space and time (Figure 1.2., 47).

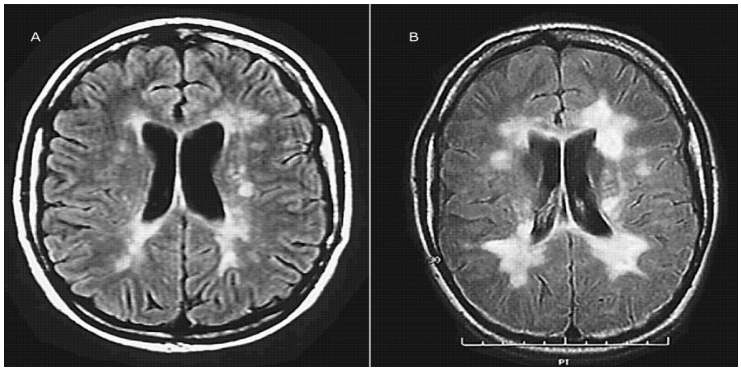

Figure 1.2. MRI scans of the brain of a 25-year-old woman with relapsing-remitting MS (A) and nine months later (B) .

1.3.Clinical course

In 1996 Lublin described five (Figure 1.3.) different types of MS. The most common MS type (85-90%) is the relapsing-remitting form with a female predominance of approximately 2/1. This disease type is defined as a relapsing disease course because periods between relapses (with full or partial recovery) are characterized by a lack of disease progression (48). The second most common MS type (10-15%) has equal prevalence amongst both sexes and is defined as primary progressive MS. It is characterized by a gradual increase of symptoms or clinical signs over time (1,2). Very often relapsing-remitting MS evolves to secondary progressive MS. This third MS type (80%) is characterized by an initial relapsing-remitting disease course followed by progression with or without occasional relapses, minor remissions and plateaus (48). A fourth MS type (<5%) is defined as a progressive-relapsing disease course from an onset with clear acute relapses, with or without full recovery. Periods between relapses are characterized by continuous progression of the level of disability (48). Finally, a benign MS type (<1%) also exists (not on Figure 1.3.). In this case MS patients remain fully functional in all neurologic systems fifteen years after the onset of the disease (48).

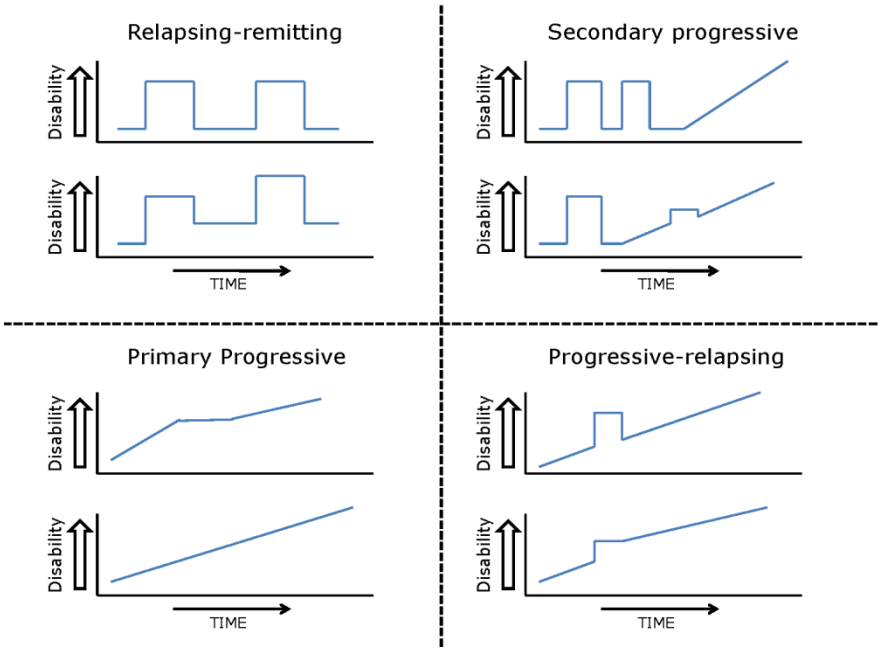

Figure 1.3. Different MS disease courses

1.4.Symptoms

The inflammatory process in the CNS does not cause clinical symptoms *per se* but induces regions with manifest nerve demyelination. Because the resulting demyelinating lesions are scattered in the CNS (see Figure 1.2.), a variety of clinical symptoms may occur. These symptoms are often defined as 'primary MS symptoms'. Clinically, relapsing-remitting MS typically induces unilateral optic neuritis, diplopia, sensory disturbances, clumsiness, ataxia, bladder and bowel problems, Lhermittes's sign (neck flexion that evokes trunk and limb paresthesias) and reduced muscle strength. Prominent extra-pyramidal symptoms (rigidity and chorea) and clinical manifestations of other cortical lesions such as aphasia, apraxia or early dementia only very rarely dominate the clinical picture. Progressive MS is characterized by slow evolving upper-motor neuron symptoms such as quadriparesis, cognitive decline (memory loss, impaired attention), visual loss (optic neuritis), brainstem syndromes, cerebral, sexual, bowel and bladder dysfunctions (1,2,11).

The above described primary symptoms often cause a variety of secondary consequences. Interestingly, many secondary problems such as cardiovascular disease, obesity, insulin resistance and type-II diabetes, osteoporosis, additional muscle weakness and fatigue and muscle atrophy mirror those symptoms observed in an inactive healthy population (49,50).

As summarized in Table 1.1., persons with MS have an inactivity related physiological profile that, compared to healthy subjects, is characterized by decreased aerobic capacity and reduced muscle strength (51). In MS, decreased maximal muscle strength of e.g. the lower limbs is frequently reported (52-61) and from the MS patient's point of view it is often considerate as one of the most disabling aspects of the disease (62). Reduced muscle strength, of course, results from increased central conduction times (63), reduced motor unit recruitment and firing rates (primary symptoms) (60). The reported impact of MS on muscle fiber characteristics, however, is not fully clear. Some authors demonstrated no alterations in body/muscle mass (64) and muscle cross sectional areas (53,59) whereas others reported a lower muscle mass combined with reduced muscle fiber cross sectional areas (55,57).

Table 1.1. Comparison of aerobic capacity and muscle contractile characteristics between person with MS and healthy subjects

	MS patients versus healthy subjects	Reference numbers
Aerobic capacity		
VO_2Max		(65,66)
Blood pressure (rest)		
Systolic	= or ↑	(67,68)
Diastolic	= or ↑	(67,68)
Heart rate (rest)	= or ↑	(65,67,68,69)
CVD risk	↑	(70)
Muscle contractile properties		
Isometric strength	↓	(54,59,71)
Isokinetic strength	↓	(52,53,58,72)
Rate of force development	= or ↓	(54,59,71)
Muscle mass (%FFM)	= or ↓	(64,73)
Muscle fiber CSA	= or ↓	(53,56,59)
Muscle activation	↓	(54,59,60)
Other		
Bone mineral density	↓	(73,74,75)
Daily activity level and function	↓	(76,77,78,79)

CVD: cardiovascular diseases, FFM: fat free mass, CSA: cross sectional area

1.5.Treatment

Given the fact that, so far, the pharmacological treatment of MS can only slow down disease progression, recent MS treatment strategies consist of a multi-disciplinary approach directed towards maintaining a MS patients' functional status. In general they comprise pharmacological, rehabilitation and exercise therapy.

1.5.1. Pharmacological therapy

To date, the pharmacological treatment of MS includes treatment of (i) acute relapses, and (ii) disease-modifying treatments.

(i) Glucocorticosteroids are used to treat acute relapses by shortening the duration of the relapse and the relapse-related symptoms. However, long-term effects on disease activity are not clear and in some patients the clinical response to steroid treatments is not effective (80).

(ii) To date, six FDA-approved disease-modifying medications are available (81). Glatiramer acetate (GA, Copaxone®) and interferon beta (INF-β; Avonex®, Rebif® and Betaseron®) are frequently applied immunomodulatory therapies which reduce the relapse rate. Patients with relapsing-remitting MS who do not respond to GA or INF-β are treated with natalizumab (Tysabri®). However, it is important to note that in the post marketing phase natalizumab was associated with an increased frequency of progressive multifocal leukoencenphalopathy and continuous clinical observation is mandatory in patients receiving natalizumab (82). Immunosuppressive agents for example mitoxantrone (Novantrone®) are administered to patients who deteriorate on the application of established therapy, mostly patients with secondary progressive MS (83). Although this treatment has a strong potential, its use is characterized by an increased risk of toxic cardiomyopathy and leukemia. The maximum cumulative dose therefore is limited.

New promising oral therapies (FTY720 and cladribine) emerge and are tested now in phase III clinical trials (84,85). Furthermore, a comparative study between alemtuzumab and INF-β1a, was performed in a phase II randomized blinded trial with encouraging results (86).

Unfortunately, current pharmacological treatments only slow the inflammatory disease process down and are only effective for a subgroup of MS patients and their side effects can be substantial. As such, the symptomatic treatment very often includes rehabilitation and/or exercise therapy to minimize the impact of MS on daily life activities.

1.5.2. Rehabilitation

Rehabilitation comprises several aspects directed to maximize an individual's physical, emotional, social and vocational independence and to improve quality of life for them and their caregivers. The most frequently used in an inpatient as well as outpatient rehabilitation setting is (i) physiotherapy, followed by (ii) occupational therapy and (iii) psychology, (87).

(i) Physiotherapy aims to enhance and restore a patients' functional

ability and quality of life. Specific neurological physiotherapy contains balance, coordination, gait, transfer, endurance training and mobilization therapies (88,89). For ambulatory MS patients the exercise training part of physical therapy (see below) ranges from endurance and resistance exercises to hippotherapy (90), yoga (91,92) as well as aquatic exercise (93,94). In more severely disabled people, other treatment techniques such as mobilization and coordination therapy and a multidisciplinary rehabilitation program may provide better results than exercise alone (87).

(ii) Occupational therapy promotes health by enabling patients/people to perform meaningful and purposeful occupations. These include (but are not limited to) work, leisure, self care, domestic and community activities. In MS, occupational therapy can help the patients to meet their potential for independence. Different principles are used that focus on a patient's physical, emotional and social independence, especially in inpatient settings (95,96) and have been extensively described in various articles reviewed by Erickson et al (97).

In general, (iii) psychological therapy for example aims to increase an individual's sense of well-being. In MS, it mainly focuses on cognitive deficits which may originate from either a primary (neurologic) symptom or as a secondary consequence (i.e. depression, drug effect, 87). Furthermore, psychological therapy may also help patients and their families address MS-associated behavioral problems (87).

1.5.3. Exercise therapy

As already indicated above, from diagnosis onwards MS often have a decreased activity level leading to inactivity related 'secondary problems'. In fact, many MS patients indicate that these secondary MS consequences such as reduced lower limb function are amongst the most important rehabilitation topics (62). They affect gait capacity which is in turn responsible for a person's independency and thus its quality of life (62). Because exercise therapy is an efficient approach to counteract inactivity associated symptoms such as muscle weakness in the healthy (98) and in other pathologies (99,100), it is more and more considered as an effective tool in MS treatment as well. Basically, exercise therapy includes (i) endurance training that mainly stimulates the cardiorespiratory system, (ii) resistance training that mainly affects skeletal muscular strength (101,102) and (iii) other potential (strength) training modes such as resistance training with additional electro-stimulation or whole body vibration.

(i) Endurance or cardiorespiratory training mainly stimulates the cardiorespiratory system. As such, it increases heart size, blood volume, stroke volume, cardiac output, VO_2max and some respiratory volumes, while it decreases blood pressure (if high) and resting and submaximal exercise heart rates. It improves aerobic and anaerobic muscle energy metabolism

including improved oxidative phosphorylation, myoglobine and triglyceride storage and it enhances the number and size of mitochondria. Other body systems affected by endurance training are body mass/fat and blood composition such as improved blood lipid profiles. Furthermore, it is suggested that endurance training may affect the systemic pro- and anti-inflammatory cytokine balance (103-105).

(ii) Resistance training induces neural, morphological, biochemical and physiological changes of the musculoskeletal system. Neurological changes include increased motor unit activation and recruitment, improved discharge frequency of motor neurons and a lower neural inhibition. Morphologically, it promotes muscle hypertrophy due to a gradual increase in contractile proteins, number and size of myofibrils, connective tissue and size of type II fibers. Furthermore, bone density and muscle capillary density also improve. Besides these histological changes, several biochemical aspects in the muscle can be altered such as, lower mitochondrial volume density, minor improvements in adenosine triphosphate and creatine phosphate storage and minor enhancements in creatine phosphokinase, myosine ATPase and myokinase activity. Furthermore, some additional factors are altered after resistance training such as improved bone health and fat-free mass (102). Similar to endurance training, it is documented that muscle fibers can produce, express and release cytokines (106) and might induce peripheral cytokine alterations.

(iii) Besides the 'classical' training modes described above, a variety of other exercises modes exists ranging from hippotherapy to a gymnastic exercise program. In this regard, whole body vibration or superimposed electro-stimulation also focus on enhancing the physiological profile of the subject and have (in)direct effect on the musculoskeletal system which fits in the scope of the purpose of this project. Furthermore, these methods are often applied in patient groups.

In general, training outcome and efficiency is highly correlated to training mode (training specificity) but also exercise intensity, duration, frequency and progression is important to induce improvements. Of course, the latter four parameters vary depending on the health status, program goals and the related functional capability of the target population (102). Briefly, exercise intensity and duration determines the specific physiological changes during exercise training and are inversely related; the higher the intensity, the shorter the duration. Exercise frequency typically refers to the total number of weekly exercise sessions and is related with intensity as well as duration. Finally, as the exercise program progresses, an individual will be able to perform more work (progression). Thus besides selecting the right training mode, training volume and frequency are also important parameters.

1.5.3.1. Endurance training in MS

The effects of endurance training in MS have been studied extensively. So far, a wide range of aerobic exercise modalities have been used to explore its effects on a wide range of functional and physiological parameters. These included (bi)cycle ergometry (66,92,107-110), arm-leg ergometry (111-113), aquatic training (114-116) and treadmill running (117). It must be noted that these studies have a moderate to high risk of bias (118) because their general methodological quality is relatively low (51), training regimes are often insufficiently described and training intensity has been poorly controlled (51). Nevertheless, endurance training seems to induce a variety of psychological and physiological improvements such as improved VO_2max, lower blood lactate levels and higher aerobic thresholds (51). In contrast, diverse findings of altered functional capacity are reported, for example the effect on gait velocity range from reduced to improved (107,109,116,117). Endurance training seems a safe and tolerable exercise mode for patients with MS.

Only a few endurance training studies explored the effect on muscle strength (112,115,116) and documented that there were no or only modest (+10%) improvements. In healthy subjects high-intensity training probably poses the potential to further enhance muscle contractile properties (119). The impact of high intensity endurance training on muscle strength and muscle architecture in persons with MS is not investigated yet. Furthermore, the impact of endurance exercise on acute (107) and chronic cytokine levels so far has only been studied by two authors (107,120). These studies did not demonstrate a pro- or anti-inflammatory deviation after endurance training but both concluded that additional studies are needed to provide a more comprehensive of the dynamic cytokine alterations after exercise training.

Based on the existing endurance studies therapists are recommended to instruct their patients to train at an initial intensity of 50-70% of VO_2max, corresponding to 60-80% of maximal heart rate, for 10 to 60 minutes two to three times per week (51,118). Training progression should be enhanced by increasing the training volume during the first 2-6 months (51). As reported in healthy subjects, after this initial training period a higher training intensity, which may exceed the anaerobic threshold, could be recommended to further ensure an optimal training stimulus. However, it is not known if persons with MS can tolerate higher endurance training intensities (51) and because not all endurance training intensities are investigated, the optimal training intensity for MS patients and the effect on muscle fiber characteristics remains unclear.

1.5.3.2. Resistance training in MS

The existing literature covering the impact of resistance training on MS (121-128) generally has a poor methodological quality (51). In particular, most of these studies did not include a control group (121-127) and used very heterogeneous training regimes (training intensity / duration) leading to very inconclusive and uncomparable findings. More recent other studies (129-131) have also investigated the effect of resistance training in persons with MS but with the exception of the study performed by Dalgas et al. (130), the methodological quality remains poor (129,131). The applied training periods were relatively short (range 3-8 weeks) (122-126,128-131) whilst only one study included a 12-week intervention period (130). Therefore, it seems fair to conclude that the effects of long-term resistance training have not been investigated thoroughly yet. The former studies aimed to increase maximal muscle strength and measured this using various measurement techniques such as dynamometry (121-130), RM tests (124) and/or less standardized techniques such as 'the leg extensor power rig' (128). Furthermore, many of them included functional measures ranging from spasticity to walking performance tests and very often no to only moderate functional improvements were reported. Kent-Braun and coworkers examined the skeletal muscle composition in persons with MS and suggested that the morphology and biochemical profile of skeletal muscle altered in the direction of disuse (57). The impact of strength training on muscle fiber composition and distribution was only explored using MRI techniques (122,129) or by Dalgas et al who determined muscle fiber cross sectional areas (CSA) with an immunohistochemical staining protocol (132). In addition and as observed by Chung et al many MS patients also develop asymmetric leg strength (133). Because in a healthy population resistance training induces greater neuromuscular adaptations in weaker versus stronger muscles (134), progressive unilateral resistance training already has been applied in stroke patients to optimize training stimulus. This approach improved both paretic and non-paretic maximal lower limb muscle strength and reduced the stroke-associated functional limitations and overall disability (135,136). However, given the underlying disease mechanisms of MS such as increased central conduction time (137) and reduced motor unit recruitment and firing rates (60), it is unclear if unilateral strength training in MS has similar effects. To the authors' knowledge the reported resistance training studies in MS all use 'classical' bilateral training methods (122,124,130). Unilateral resistance training applying relative workloads to investigate strength gains in weaker versus stronger legs has not been applied in a MS population yet. Finally, only one study explored the effects of a resistance training on cytokine levels (121) and Golzari and co-workers explored the impact of combined exercise training (endurance and resistance training) on cytokine levels (138).

In summary, from the existing literature it is clear that exercise

therapy has the potential to improve aspects of endurance capacity and muscle strength. However, general guidelines concerning resistance training in MS are difficult to formulate because the used exercise protocols were very diverse which complicates comparison of the results. Therefore, standardized resistance training programs and muscle strength evaluations as well as comparisons to a control group are necessary in a study design to enhance the methodological quality in this exercise therapy field. Furthermore, longer-term training effects have not been performed yet. Finally, in healthy subjects improved muscle function results in augmented functionality (98). In MS patients this is less clear.

1.5.3.3. Other potential strength training modes in MS

As indicated in the MS symptoms section (see 1.4.) persons with MS often have a variety of sensory and motor disorders. Frequently, these problems result in disturbed gait and balance that affect the capability to execute transfers such as moving between and on exercise equipment safely. Given the fact that more classical endurance and/or resistance training always requires reasonable transfer capacities in order to perform the exercise program properly. Many persons with MS have tried alternative physical activity modes such as electrical stimulation and whole body vibration.

In this context, (i) superimposed neuromuscular electrical stimulation may have the potential to further enhance muscular strength compared to voluntary contraction alone. The Henneman size principle of voluntary motor unit recruitment described the progressive size-depended recruitment of motor units (139). Briefly, this principle indicated that an action potential produced by normal physiological mechanisms initially recruits the smallest-diameter neurons prior to recruitment of the larger-diameter fibers (139). In contrast, neuromuscular electrical stimulation follows the principle of "reverse recruitment order" wherein the nerve stimulus threshold is inversely proportional to the neuron (140). Thus large-diameter nerve fibers, which innervate larger motor units, are recruited preferentially (140). In post-immobilized patients, significantly higher isometric strength after electrical superimposed stimulation compared to voluntary muscle contraction alone, has already been observed (141). So far, only one study explored the effect of electrical neuromuscular stimulation in combination with active exercises in persons with MS and indicated an increase in muscle strength (+26%, 142). The methodological quality of the latter study however was (too) low (143) to make solid conclusions.

(ii) Because many severely affected MS patients are unable to train safely on specific, more classic, strength training equipment Whole Body

Vibration (WBV) may offer good strength training alternatives (144,145). In contrast to endurance and resistance training the exact physiological mechanism of WBV to affect body structures such as muscle strength is not fully clarified. It is hypothesized that provoked vibrations stimulate muscle spindles and alpha-motorneurons that initiate a muscle contraction (146). This vibration stimulus can be initiated by two general principles. The synchronous mode where the platform displaces vertically and the side-alternating mode where the platform displacement is a tilting movement (145). The mechanical parameters determining the intensity of vibrations are frequency (Hz) and amplitude (mm) from which velocity and acceleration can be derived. Given the fact that static and dynamic exercises performed on a vibration platform have therapeutic potentials (increased muscle strength) in a geriatric population (147-150) and even in patients with a neurodegenerative disorder (151-153), its use and effects in MS should be investigated. So far, solid evidence based conclusions in a MS samples, however, cannot yet be made because only acute (154) and short-term (155,156,157) WBV effects in persons with MS have been explored.

Finally, given the fact that skeletal muscle can produce cytokines these alternative strength training modes might also have the potential to affect the systemic cytokine as reported in the conventional exercise therapy modes. However, this has not been investigated yet.

1.5.3.4. Optimizing exercise therapy in MS

As already mentioned (see 1.5.3.1.), it is reported that **endurance training** enhances aerobic capacity. However, given the fact that the impact of anaerobic intensities on physiological changes in persons with MS is not studied yet, it is still unknown if high exercise intensity can be tolerated in this population. Furthermore, the impact on muscle strength and muscle fiber characteristics still remains unknown. Finally, cytokine responses have only been explored and very diverse findings on daily life functional capacities are reported. Based on these lacunas, the effects of high intense endurance training on muscle contractile properties, functional benefits and cytokine responses are interesting research topics.

The second major exercise mode is **resistance training**. Here, further improvements with respect to the methodological quality of the performed studies, that are described in 1.5.3.2., needed to improve in order to draw more solid evidence based conclusions (51,118). Furthermore, long-term training effects of resistance training remain unexplored.

Finally, unilateral resistance training applying relative workloads to investigate strength improvements in weaker versus stronger legs has not been applied in a MS population yet

The **other potential strength training** modes such as superimposed electro-stimulation and WBV, similar shortcomings regarding the methodological quality can be made. Therefore, the same remarks given for the resistance training mode must be taken into account in order to improve the study design towards an acceptable quality for making scientifically funded conclusions.

In summary, to date the effects of exercise training on muscle contractile properties, functional benefits and systemic cytokine response are often only explored. However, improved knowledge of the latter can provide fundamental information to further optimize training strategies for persons with MS.

1.*5.3.4.1. The International Classification of Functioning, Disability and Health model*

The International Classification of Functioning, Disability and Health (ICF) model (see Figure 1.4.) classifies health (disease or disorder) in different levels. The (i) body functions and structures level, (ii) activity level and (iii) participation level that can be influenced by contextual factors such as environmental and personal factors (158). To date, this model is often used in rehabilitation settings and research to design (standardize) rehabilitation strategies such as applied during exercise therapy.

Optimizing exercise therapy in MS, ideally, fits in (some levels of) the ICF model. In general, exercise therapy affects a very broad range of physiological parameters such as muscle contractile properties (ICF body function & structure level) that of course can be considered as a primary as well as secondary consequence of MS. According to the ICF model, muscle strength (ICF body function) and the related muscle fiber morphology (ICF body structure) determine a person's activity level (159-161). Consequently, improved muscle contractile properties have the potential to affect walking and balance abilities of persons with MS. Finally, cytokines produced by skeletal muscle fibers (ICF body structure, 106) can influence systemic and local cytokine levels that might have the potential to affect MS associated relapses (ICF health condition).

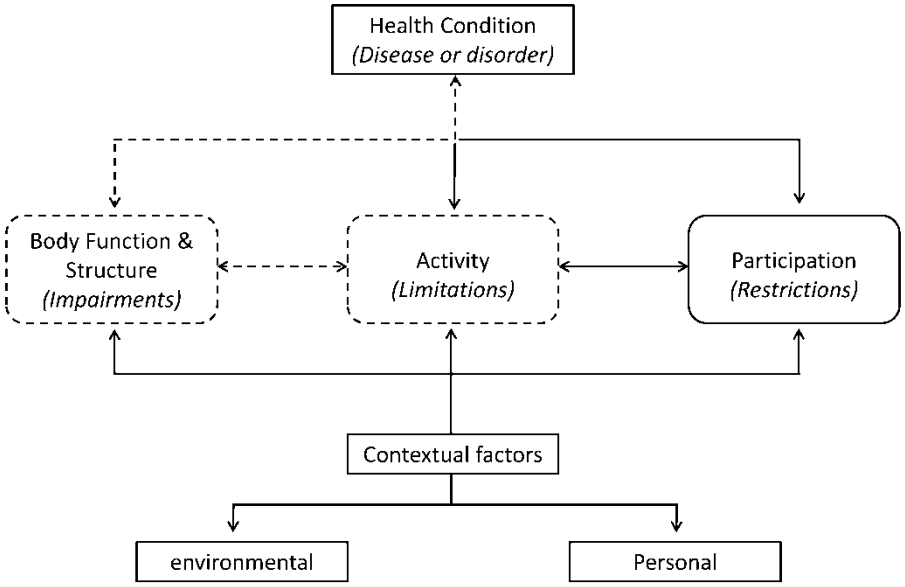

Figure 1.4 ICF model (dotted line - scope of this Ph.D. thesis)

1.5.3.4.2. An animal MS model: experimental autoimmune encephalomyelitis

Because the impact of (very) high intensity endurance training on functional capacity in persons with MS is largely unknown, it seems appropriate to use an animal MS model (EAE) to investigate its impact on the disease symptoms and muscle fiber characteristics. During EAE, which is induced by an injection of a myelin antigen frequently combined with an oil like adjuvant, an MS-like inflammation process is described. Briefly, following this injection into the animals, myelin reactive T cells are activated in the periphery which then could cross the BBB and will be reactivated in the CNS leading to an inflammation process (162-164). Of course, no single MS animal model mimics all aspects of MS but each model reflects one or more specific (immunological) aspects of the MS disease. The myelin protein induced (EAE) model is used and can be induced in mice (165), rats (166), hamsters (167), marmosets (168), rabbits (169), goats (170), sheep (170) and dogs (171). Depending on the myelin protein and animal strain (genetic background) used, the disease course follows an acute monophasic or a more chronic relapsing-remitting course. Clinically, exacerbations are characterized by hindquarter paralysis that starts at the tip of the tail progressing to the diaphragm possibly leading to death. The degree of

disease severity, notably paralysis, is evaluated on a scale (172) ranging from 0 (no signs) to 5 (death).

This model has already led to the development of several approved medicinal therapies for patients with MS (173). Nevertheless, given the fact that acute EAE only mimics the inflammatory process of MS, it is difficult to extrapolate EAE results. In this respect, there are numerous examples of different treatment strategies that are effective in EAE but fail when testing in MS (173).

To date, exercise training in EAE rodents already showed promising results on disease course of the rodents (174-176). Here, the disease onset of hindquarter paralysis associated with chronic EAE was delayed (174-176) and after voluntary wheel running a less pronounced spinal loss in the striated neurons has been reported (176). Unfortunately, none of these studies determined muscle fiber characteristics. So far, only de Haan and coworkers reported that all muscle fibers of the medial gastrocnemius were reduced in the experimental autoimmune encephalomyelitis (EAE) animal model (177).

Chapter II:

Experimental work and results

Objectives

The studies performed in this Ph.D. thesis involved both animal (rat) and human experiments and are described in detail, in this chapter. The studies carried out are presented in the form of six original research papers that have been published, submitted or are in preparation for publication to peer-reviewed journals with a focus on clinical and/or neurological rehabilitation in a MS population. These six different studies fit into three objectives representing the basis of this thesis (see Figure 2.). As described in Chapter 1, this Ph.D. project aimed to investigate the effects of exercise therapy on muscle contractile properties such as muscle strength and muscle fiber characteristics ***(first objective)***. Furthermore, the effects of exercise therapy on walking and balance capacity of persons with MS and EAE disease scores (animal) were examined ***(second objective)***. Finally, this thesis also explored the impact of exercise therapy on systemic serum cytokine levels that are important in the MS inflammation process *(third objective)*. These three objectives are investigated in EAE as well as in MS (Figure 2.).

General outline

As described in the general introduction, the effect of low to moderate aerobic endurance training in MS has already been investigated and often resulted in a variety of improved physiological parameters. The effects of higher exercise intensities on muscle fiber characteristics and disease progression, however, have not yet been studied. Because research investigating the impact of high-intensity training on disease course and muscle contractile properties (e.g. muscle fiber typing) is invasive, in the first part of this thesis an animal model for MS was used. As such, in Study 1 we hypothesized that very high (above the anaerobic threshold) intensity exercise training, such as swimming with an external load of 5.5% of body weight, further optimized muscle contractile properties *(first objective)* and possibly improved EAE disease course *(second objective)*. In order to further optimize training intensity, the impact of light, moderate and intense exercise (near the anaerobic threshold) on muscle contractile properties *(first objective)* and disease progression *(second objective)* was investigated using treadmill running in Study 2. Given the fact that systemic cytokine production is exercise intensity related, its impact on chronic cytokine levels *(third objective)* was also explored in this study.

Because the effect of resistance training on muscle functional capacity in MS was not clear at the start of this Ph.D. project, a series of experiments in a human MS population was performed to further elucidate the effects of (i) long-term training, (ii) unilateral training and (iii) whole body vibration training. In Study 3 we investigated the impact of different resistance training modes. Here, a long-term randomized controlled trial with the objective to improve health related muscle strength in persons with MS

was performed using a moderate intense standardized unilateral resistance training program *(first objective)*. In this study, resistance training with simultaneous electro-stimulation was also included. We hypothesized that this additional motor stimulus directly applied on the muscle belly might further enhance muscle strength. Because some MS patients may experience difficulties in performing conventional resistance training, either or not in combination with additional electro-stimulation as applied in Study 3, we explored the use of whole body vibration strength training. This is a promising exercise strategy for patients with a chronic neurological disease such as stroke and MS. (Study 4, *first objective*). To evaluate the effects of resistance training and/or whole body vibration training, we assessed some functional capacity measures in both Study 3 and 4 (second objective). To further explore the possible role of exercise therapy on systemic cytokines, chronic cytokine levels of the MS subjects that participated in study 3 and 4 were determined (Study 5, third objective). Finally, and because improved muscle strength might also enhance functional mobility, we investigated the relationship between upper leg muscle strength and walking capacity (study 6, *second objective*).

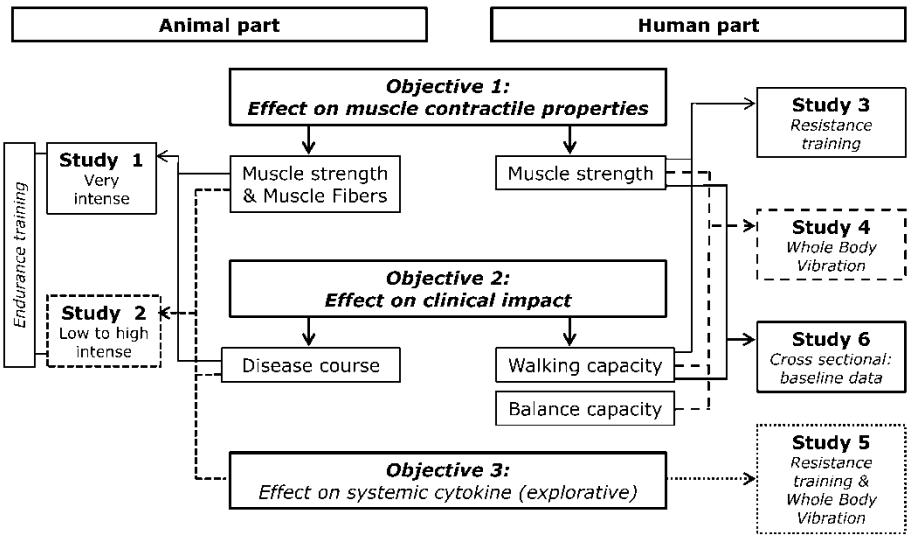

Figure 2. *Schematic presentation of the objectives of this Ph.D. thesis and the performed studies*

STUDY 1

Exploring the impact of intense exercise on disease progression and muscle contractile properties during experimental autoimmune encephalomyelitis.

Based on: Broekmans T, Alders G, Hendriks JJ, Savelberg HH, Hesselink MK, Eijnde BO.
Submitted (Acta Physiologica)

2.1.1. Abstract

Objective. The current study investigated the effect of intense exercise on the onset of the experimental autoimmune encephalomyelitis (EAE) hindquarter paralysis and muscle contractile properties.

Methods. Following EAE induction control- (CON) and EAE groups were subdivided in a sedentary and swimming subgroup (1h/d). Approximately 4 days after development of maximal hindquarter paralysis, isokinetic (50°/s) foot extensor strength was measured (115 consecutive contractions) and tibialis anterior (TA) and extensor digitorum longus muscle fiber characteristics were analyzed.

Results. Swimming worsened overall hindquarter paralysis ($p<0.05$). In CON, isokinetic muscle work peaked during the first 30 contractions while this peak was absent ($p<0.05$) in EAE. TA type IIb CSA in EAE was ~57% smaller ($p<0.05$) without an exercise effect.

Discussion. This study suggests that intense exercise training worsens EAE-induced hindquarter paralysis and that EAE probably reduces TA type IIb CSA. This might explain the absence of peak muscle strength during repetitive contractions.

2.1.2. Introduction

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) that, amongst others, is characterized by decreased functional muscle strength/capacity (76). Although reduced muscle strength clearly impedes quality of daily life (178,179), adequate rehabilitation/exercise therapy can partly restore muscle capacity (51,118 130,180). Despite the fact that functional muscle weakness and fatigue in MS probably are related to reduced physical activity (2,51,76), the impact of MS on muscle fiber composition and contractility remains unclear. In fact, to date reported data are rather inconsistent. Some studies in patients with MS did not detect alterations in body mass (64) and in muscle fiber cross sectional area (CSA) (53,59), whilst others reported a loss of muscle mass combined with reduced muscle fiber size (55,57). The latter was also reported by De Haan et al. in acute experimental autoimmune encephalomyelitis (EAE) which serves as an animal model for MS (177). Apart from reduced body and muscle weight these investigators also reported reductions of 40-50% in all muscle fiber types of the medial gastrocnemius muscle. Given the correlation between muscle fiber CSA and force production (181), muscle strength deficits were also reported in persons with MS (52-54,58,72) and in EAE rats (177). In the latter study, De Haan et al. found ~35% lower relative maximal muscle power in EAE that was accompanied by similar linear fatigue rates between control (30±6%) and EAE (32±4%) animals during a series of 20 consecutive fused titanic isometric contractions (150 Hz, 150ms, 1 contraction every 500ms).

It has become evident that low to moderately intense exercise training improves muscle weakness (51) and quality of life in persons with MS (112,182,183). This improvement is paralleled by significantly increased (~11%) cross sectional areas (CSA) of all muscle fibers as reported by Dalgas et al. following 12 weeks of moderately intense progressive resistance training (132). Because high-intensity training further enhances muscle fiber size at least in healthy subjects (119), it is important to investigate the impact of high-intensity exercise on training outcome and muscle architecture in persons with MS (51). A growing body of evidence indicates that adequate endurance exercise training can, at least in part, reverse some of the clinical symptoms (51,118). However, it is still unknown whether high intensity endurance exercise training affects the MS-related inflammatory exacerbation process. So far, Le Page and co-workers demonstrated that treadmill running after immunization, delayed the onset and duration of hindquarter paralysis that is associated with chronic EAE. However, maximal clinical scores were not affected (174) and these investigators did not examine if these adaptations were associated with the anticipated changes in muscle CSA. More recently, in mice with chronic EAE less severe neurological deficits and a less pronounced spinal loss in the striated neurons after voluntary wheel running have been reported (176).

In keeping with the above line of reasoning, the current study aims to investigate the impact of intense physical exercise on hindquarter paralysis and muscle fiber size and distribution during and after acute EAE. We hypothesized that intense exercise training could further delay the onset of hindquarter paralysis and improve muscle contractile properties.

2.1.3. Methods

2.1.3.1. Animals

Twenty-six female Lewis rats (age: 6-7 weeks, body weight: 120-170g) from the Harlan (Harlan CPB, Zeist, The Nederlands) were maintained on a constant light/dark cycle (12:12) and temperature (22°C), a relative humidity of 22-24% in our animal breeding center and were fed ad libitum with normal rat pellets (Carfil RN-01-K12, Harlan). The animal Ethics Committee of Hasselt University approved the study protocol in accordance with the Helsinki Declaration.

2.1.3.2. Study design

Following animal breeding center familiarization (days -14 to -7, adaptation phase) a control (CON, n=12) and experimental autoimmune encephalomyelitis experimental group (EAE, n=14) were created 7 days prior to the start of the experiments. Each group comprised of a sedentary (SED, n=6) and an exercise (EX, n=7) subgroup, randomized by their body weight. Hereafter, (days -7 to -1, habituation phase) exercised rats were gradually (-d7: 10min, -d6: 10min, -d5: 20min, -d4:30min, -d3: 40min, -d2: 50min, -d1: 60min) familiarized to swim training in 32°-34°C water (184). At day 0 experimental autoimmune encephalomyelitis was induced in the EAE subgroups by a single percutaneous injection in both footpads that consisted of purified myelin basic protein (MBP) in combination with *mycobacterium tuberculosis* (37 Hra, Difco, Detroit, MI) and complete Freunds adjuvant (CFA, Difco) under urethane (1.5g/kg, i.p.) anesthesia (172). Typically, maximal clinical hindquarter paralysis develops 12-14d after induction, where after these clinical signs partly recover. From the induction phase (days 0 to 2) onwards exercised rats swam 1h/d with weights (5.5% of body weight) attached to their tails until progressive hindquarter paralysis prevented swimming (exercise phase, days 3 to 10) and rats entered the follow-up phase (days 11-17). Sedentary rats remained sedentary throughout the study period. Following the adaptation phase, daily food intake and body weight were registered and rats were examined for the development of neurological signs. Eighteen days following immunization, 4 days after the peak of the disease, rats were anaesthetized by an intraperitoneal injection of pentobarbital sodium (5 $mg \cdot 100\ g^{-1}$ BW) to determine dorsiflexor contractile properties. Hereafter, the m. extensor digitorum longus (EDL) and the m. tibialis anterior (TA), were dissected and freed of connective tissue and visible blood. The mid-part of each muscle was mounted in embedding tissue (Tissue-Tek®, Miles Laboratories), frozen in isopentane cooled in liquid N_2, and stored at -80°C until analysis were performed. Finally, rats were sacrificed by an intracardial injection of pentobarbital sodium.

2.1.3.3. In vivo testing

Hindquarter paralysis. Following EAE induction, hindquarter paralysis was scored (not blinded) daily (9 a.m.) on a scale from 0 to 5: 0, no signs; 0.5: partial loss of tail tonus; 1.0: complete loss of tail tonus; 2.0: hind limb paresis; 3.0: hind limb paralysis; 4.0: moribund; 5.0: death due to EAE (172). Overall hindquarter paralysis was then expressed as the average of the summated daily scores.

Isokinetic foot extensor performance. After being anaesthetized as described above, left hind limb repetitive foot extensor work (mJ) was assessed isokinetically as described elsewhere (185,186). Briefly, this involved the application of percutaneous needle electrodes placed on the common peroneal nerve fusing 115 consecutive concentric isokinetic foot dorsiflexions (50°/s, 1 mA, 250ms, 3s rest intervals) after standardized knee and ankle fixations on a custom build Ashton-Miller (187) like rat dynamometer. To express work fatigue, the highest work performed in the first 30 consecutive contractions was set at 100% and the other values were expressed as a percentage from this peak.

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

2.1.3.4. Fiber CSA and distribution

To quantify type I, IIa and IIb muscle fiber CSA and distribution, transverse sections (8µm) from the obtained muscle samples were cut at -20°C. Air-dried (30min) cryosections were washed (5min) in 0.5% Triton-100 added to phosphate-buffered saline (PBS) and then rinsed (5min) in PBS. Hereafter, sections were incubated for 60min at room temperature with a mix of 2 mouse monoclonal antibodies against myosin heavy chain I (1:25; A4.840 supernatant, Developmental Studies Hybridoma Bank, Iowa, USA) and IIA (1:25; N2.261 supernatant, Developmental Studies Hybridoma Bank, Iowa, USA) and 1 rabbit polyclonal laminin antibody (1:100; L-9393, Sigma, Zwijndrecht, The Netherlands). Then the slides were washed 3 times for 5 minutes with PBS, followed by an incubation period of 45min at room temperature with a mixture of secondary antibodies (1:500, Goat anti-Mouse IgM AlexaFluor 555; 1:200 Goat anti-Mouse IgG_1 AlexaFluor 488 and 1:130 Goat anti-Rabbit IgG AlexaFluor 350; Molecular probes, Invitrogen, Breda, The Netherlands) diluted in PBS. Hereafter the sections were washed 3 times for 5min with PBS and mounted in Fluorescent Mounting Medium (Dako, North America, California, USA). Muscle fibers were examined and recorded using a Nikon E800 fluorescence microscope (Nikon, Boerhavedorp, Germany). The fluorescence signals were recorded using a TRITC and FITC

filter for type I and IIa muscle fibers and DAPI filter for cell membrane. Digital images (x20 magnification, exposure time for TRITC and FITC 400ms, DAPI 800ms) were analyzed using Lucia G software (LIM, Prague, Czech Republic). Furthermore, all muscle samples of one rat were analyzed in one assay.

2.1.3.5. Statistical analyses

All data were analyzed using SAS software (SAS Institute Inc, Cary, USA). Hindquarter paralysis scores were analyzed by a (Subgroup [EAE_{SED}, EAE_{EX}] x Time [Days 0-17]) mixed model ANOVA. Group overall symptom intensity was analyzed by a student's t-test. Isokinetic foot extensor data were analyzed using a (Group [EAE, CON] x Activity [SED, EX] x Contraction [115 dynamic muscle contractions]) mixed model ANOVA and muscle fiber type area and distribution were analyzed using a one-way ANOVA. Finally, body weight and food intake were analyzed using a (Group [EAE, CON] x Activity [SED, EX] x Study phase [Habituation phase; Induction phase; Exercise phase; Follow-up phase]) mixed model ANOVA. When appropriate, post hoc pre-planned contrast tests were applied. Post hoc power calculations were performed using G power 3 software (253). All data are presented as means ± SE and $p<0.05$ was chosen as the threshold for statistical significance.

2.1.4. Results

2.1.4.1. Hindquarter paralysis

This hindquarter paralysis is a representation of the seriousness of disease activity. As shown in Figure 2.1.1. swim training did not modify disease onset, peak and remission. However, intense physical exercise training worsened ($p<0.05$) the overall hindquarter paralysis by ~45% compared to EAE_{SED}.

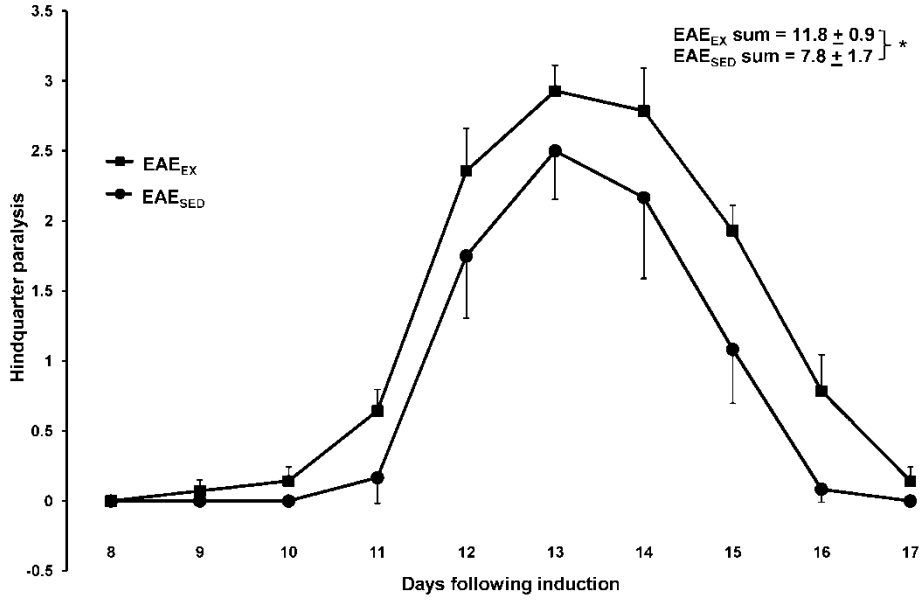

Figure 2.1.1. Effects of experimental autoimmune encephalomyelitis and intense physical exercise on hindquarter paralysis.
*Values are means ± SE and express the level of hindquarter paralysis on a scale from 0 to 5 (172), 8 to 17 days after EAE induction in sedentary (SED) and exercised (EX, swimming 1h/day) EAE rats. Furthermore, the average sum of the hindquarter paralysis score for each EAE subgroup is indicated. *p<0.05 compared to the corresponding sedentary value. See methods for further details.*

2.1.4.2. Muscle fiber type area and distribution

To investigate the impact of very intense exercise therapy on muscle fiber morphology, *muscle fiber cross sectional area (CSA) and distribution* (, Table 2.1.1.) was studied. EAE significantly reduced ($p<0.05$) the CSA of TA type IIb muscle fibers (686±83µm²) compared to CON (1599±77µm²). More particular, in EAE_{EX} (-65%) and in EAE_{SED} (-49%) lower ($p<0.05$) type IIb fibers of TA were found compared to the corresponding CON. Furthermore,

no exercise effect was found in CON and EAE. In EDL fiber CSA was not affected by EAE nor intense exercise training.

Fiber type distribution. As shown in Table 2.1.1., muscle fiber type distribution in EDL and TA did not differ between CON and EAE. Furthermore, intense swim exercise did not affect distribution in neither EDL nor TA.

Table 2.1.1. Effects of experimental autoimmune encephalomyelitis and intense physical exercise on muscle fiber cross sectional area and distribution.

	Type I				Type IIa				Type IIb			
	EDL				EDL				EDL			
	Area (µm²)	p	Distr (%)	p	Area (µm²)	p	Distr (%)	p	Area (µm²)	p	Distr (%)	p
CON	478 ± 52		5.2 ± 0.5		465 ± 55		22.1 ± 2.8		1233 ± 160		72.7 ± 3.0	
EAE	556 ± 70		4.7 ± 1.4		484 ± 60		26.0 ± 2.7		969 ± 108		69.3 ± 3.5	
CON_{SED}	498 ± 63	0.99	5.2 ± 0.9	0.15	502 ± 76	0.75	22.9 ± 4.7	0.56	1258 ± 223	0.43	71.9 ± 5.1	0.16
CON_{EX}	475 ± 89		5.1 ± 0.6		428 ± 83		21.4 ± 3.4		1210 ± 251		73.5 ± 3.4	
EAE_{SED}	579 ± 70		4.4 ± 0.5		552 ± 79		25.1 ± 3.4		1160 ± 114		70.5 ± 3.6	
EAE_{EX}	536 ± 118		5.0 ± 2.7		426 ± 83		26.8 ± 4.2		806 ± 150		68.2 ± 5.8	
	TA				TA				TA			
	Area (µm²)	p	Distr (%)	p	Area (µm²)	p	Distr (%)	p	Area (µm²)	p	Distr (%)	p
CON	698 ± 91		1.6 ± 0.3		649 ± 85		20.9 ± 2.1		1599 ± 208		77.4 ± 2.1	
EAE	601 ± 108		1.2 ± 0.2		562 ± 93		16.1 ± 1.1		686 ± 115*		83.0 ± 1.1	
CON_{SED}	678 ± 119	0.44	2.2 ± 0.4	0.52	645 ± 114	0.59	21.6 ± 2.4	0.91	1579 ± 266	0.03	76.3 ± 2.5	0.80
CON_{EX}	714 ± 144		1.2 ± 0.3		653 ± 132		20.4 ± 3.1		1616 ± 334		78.4 ± 3.1	
EAE_{SED}	707 ± 197		1.4 ± 0.2		637 ± 151		17.7 ± 1.8		811 ± 163*		80.9 ± 1.9	
EAE_{EX}	509 ± 112		1.0 ± 0.3		497 ± 121		14.8 ± 1.2		579 ± 162*		84.2 ± 1.0	

Values are means ± SE and express muscle fiber cross sectional area (µm²) and fiber type distribution (Distr; %) in Extensor Digitorum longus (EDL) and Tibialis Anterior (TA) of sedentary (Sed) and exercised (Ex) control and EAE rats. p represents p-value of group (EAE, CON) x activity effect * $p<0.05$ compared to corresponding control value.

2.1.4.3. Isokinetic muscle performance

To determine the exercise effect on muscle strength isokinetic muscle power was measured. A significant ([group] x [activity] x [contraction number]) interaction effect was found (Figure 2.1.2.). Here, post hoc contrast analyses indicated a significant within-group difference (*CON: -26±5% decline versus EAE: -4±2% decline*) during repetitive contractions. Furthermore, over this contraction period a within-subgroup activity effect in CON (*CON_{SED}: -18±7% decline versus CON_{EX}: -34±7% decline*) was found. In summary, during contractions CON muscle work progressively decreased over the 115 consecutive contractions whereas muscle work of EAE animals remained stable.

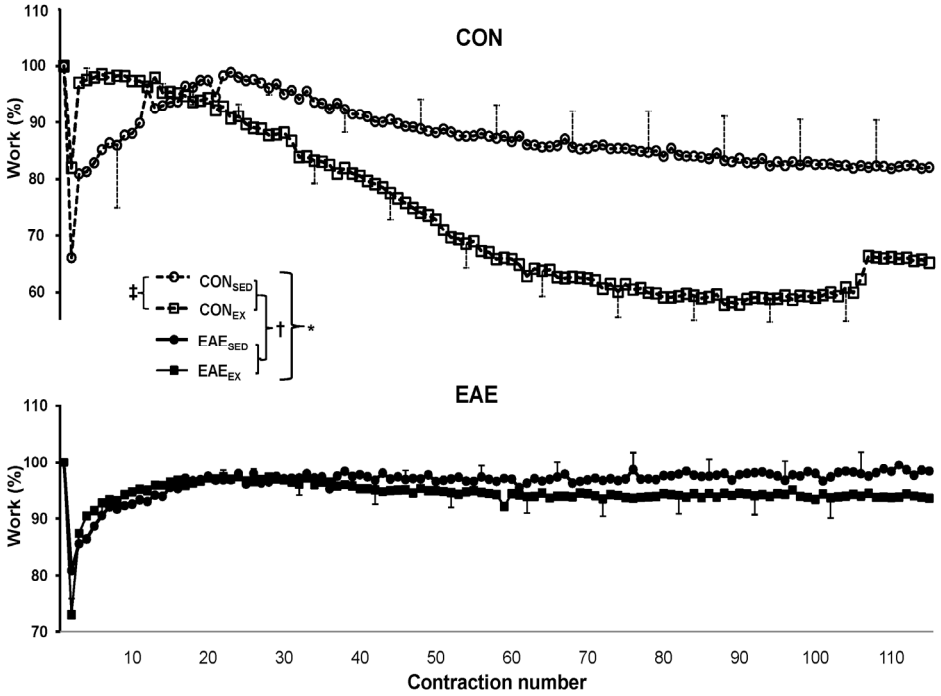

Figure 2.1.2. Effects of experimental autoimmune encephalomyelitis and intense physical exercise on isokinetic muscle work.

*Values are means ± SE and express muscle work (%) during 115 consecutive maximal muscle contractions (1mA, 150Hz, 250ms) in sedentary (SED) and exercised (EX, swimming 1h/day) control (CON) and EAE rats. *p<0.05, indicates an overall Group [CON, EAE] x Activity [SED,EX] x Contraction interaction effect and †p<0.05, represents a post hoc group (CON, EAE) effect. ‡p<0.05, represents post hoc contrast analyses activity (SED, EX) effect between groups. See methods for further details.*

2.1.4.4. Body weight & food intake

To study the disease and exercise impact on the rodents body weight and food intake were measured. An overall interaction effect (group [EAE, CON] x activity [SED, EX] x study phase [Habituation phase; Induction phase; Exercise phase; Follow-up phase]) in body weight data was found ($p<0.05$, Figure 2.1.3.). In the exercise and follow-up phase EAE decreased ($p<0.05$) body weight compared to CON with a significant exercise effect in the follow-up phase between CON_{EX} and CON_{SED}. Similar significant outcomes for food intake were found (data not shown). In addition, during the induction phase EAE rats ate less ($p<0.05$) compared to CON, without an exercise effect.

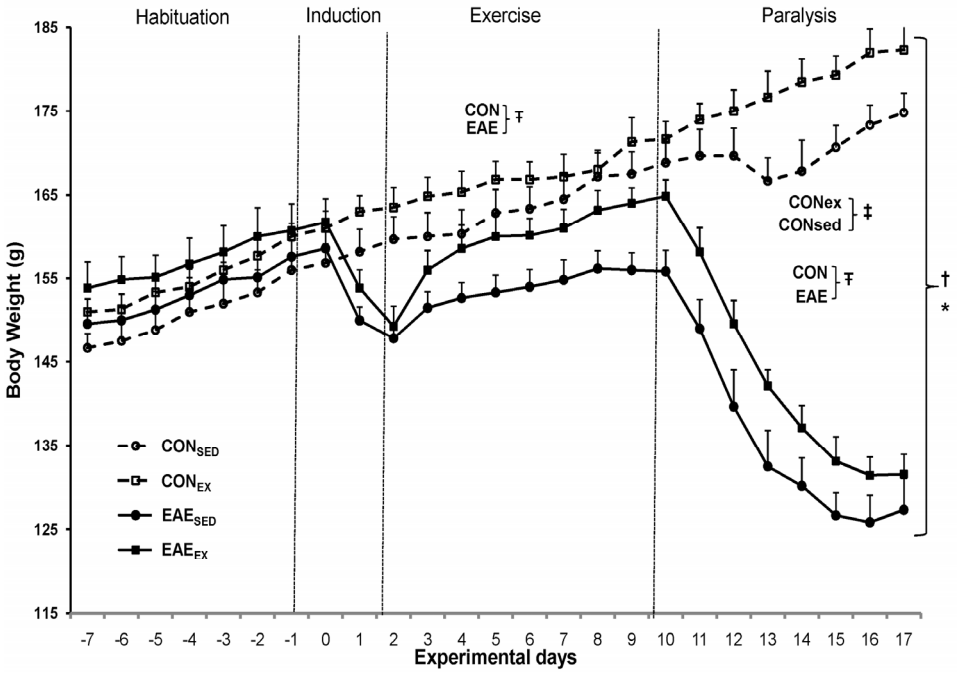

Figure 2.1.3. Effects of experimental autoimmune encephalomyelitis and intense physical exercise on rat body weight

*Values are means ± SE and express body weight (g) in sedentary (SED) and exercised (EX, swimming 1h/day) control (CON) and EAE rats. *p<0.05, indicates a Group [CON,EAE] x Activity [SED, EX] x Study phase [Habituation phase; Induction phase; Exercise phase; Follow-up phase] effect and † represents a Group [CON, EAE] x study phase [Habituation phase; Induction phase; Exercise phase; Follow-up phase] effect (p<0.05). ǂp<0.05, indicates post hoc contrast analyses for SED compared to EX in CON and EAE for each study phase and ∓p<0.05, represents post hoc contrast analyses for each Time Window between CON and EAE rats. See methods for further details.*

2.1.5. Discussion

The current study is the first to explore the impact of intense physical exercise on acute EAE, muscle fiber characteristics and consecutive isokinetic muscle work. Intense exercise worsened overall hindquarter paralysis but did not affect the onset, peak or remission of the disease. Furthermore, in EAE significantly lower cross sectional areas of type IIb tibialis anterior fibers were found. Consequently, peak muscle work during a series of isokinetic muscle contractions was absent in EAE.

The data of the present study indicate that intense swimming exercise during EAE did not affect hindquarter paralysis onset, peak or remission. However, the overall degree of hindquarter paralysis, expressed as the average sum of these scores, was significantly higher ($p < 0.05$) in EAE_{EX} compared to EAE_{SED}. The present findings contrast with previous treadmill studies of Le Page and co-workers showing a delayed onset of clinical signs yet similar maximal clinical scores in EAE rats following standardized running (174,175). In accordance with the latter, Rossi et al. reported reduced neurological deficits after voluntary wheel running in chronic EAE mice(176). It is difficult to explain these dissimilarities but they may be caused by the use of different training modalities (swimming versus treadmill running) or exercise intensities (lower versus higher training intensities). Unfortunately, it is impossible to compare between studies because the exercise protocol applied by Le Page et al (174) was not described in detail and Rossi and coworkers used a voluntary and not a controlled exercise program. The training intensity used in the present study, however, was probably higher compared to the work of Le Page and Rossi. In the current study, an external load of 5.5% of the bodyweight was used to increase exercise intensity. As indicated in previous studies using loads of 5 to 6% of the body mass during incremental load swimming (total swimming duration: 30 min), the present load probably raises training intensity to the anaerobic threshold (188-190). Moreover, loads of 5-6% of the body weight and swim durations of 30 to 60 minutes, as applied in the current study further increases training intensity above the anaerobic threshold (190). Finally, it is important to note that swimming induces higher ($p<0.05$) stress hormone levels (ACTH and corticosterone) compared to treadmill running (191). In keeping with the above line of reasoning and despite the fact that in the present study stress hormones were not measured, the applied training regimen might be too intense to temper EAE symptoms.

The present study also examined the impact of acute EAE on muscle contractile characteristics either or not in combination with exercise training. First, CON muscle fiber characteristics of the present study are comparable with previously reported cross sectional areas of TA (mean: 1469µm²) and muscle fiber distributions of EDL (type I: 5.5%; type IIa: 18.8%; type IIb: 75.7%)(192,193). Here, type IIb fiber CSA's of EAE rats are substantially

lower compared to CON (EDL EAE: 969±108µm², EDL CON: 1233±160µm², n.s.; TA EAE: 686±115µm², TA CON: 1599±208µm², $p<0.05$). However, muscle fiber distribution of both muscles did not change. De Haan et al. already investigated the impact of acute EAE on muscle characteristics and also indicated no muscle fiber shifts but reported significantly reduced CSA of type I (~37%) and IIa (~42%) muscle fibers (177). Surprisingly, intense swimming exercise did not affect muscle fiber CSA and muscle fiber distribution of the foot extensor muscles in CON or EAE as previously reported by Faria and co-workers following 8 days of swim training (45 min/day, 194). Possibly, the absence of an exercise effect on muscle fiber CSA in the current study can be explained by the fact that after a relatively short training period (11 days) the exercising rodents did not swim for 6 days before muscle biopsies were taken (hindlimb paralysis period in EAE). As depicted in Figure 2.1.2. and as previously reported by Wakatsuki et al (195) in healthy rats, muscle work in both CON subgroups peaked during the first 30 contractions and then progressively declined. This was also reported in control and EAE rodents by De Haan et al. during a series of 20 repeated maximal isometric contractions of m. gastrocnemius (177). In both EAE subgroups of the present study muscle work did not peak. This is in contrast with the results obtained by De Haan. The discrepancy between the present study and De Haan et al. might be caused by the fact that different muscle groups were analyzed (TA and EDL with predominately glycolitic muscle fibers (192) versus medial gastrocnemius with a proximal region containing all muscle fibers and a distal region containing only type IIx and IIb fibers (177) and a different strength measurement protocol (isokinetic strength versus isometric strength).

Given the exploratory nature of the present study, it contains some limitations. First, given the fact that literature investigating the effect of exercise on EAE disease course is scarce it is difficult to calculate a proper effect size. Therefore, no a priori power analysis have been performed. As such, the used sample size may have been too small and may therefore have reduced statistical power. However, post hoc power analyses, using the actual effect size of the current study indicated a statistical power of 0.9 for the hindquarter paralysis data. Power for muscle strength and muscle fiber determination was only just below 0.80 (0.74-0.75). Second, we did not measure the exact exercise intensity during swimming. However, we have estimate the applied swimming intensity based on literature (188-190).

In summary, the present study suggests that intense swim training increases the overall severity of EAE induced hindquarter paralysis but does not change its onset, peak or remission. Furthermore, the CSA of type IIb tibialis anterior fibers is decreased in EAE rats. This probably explains the absence of peak muscle work during the first 30 contractions of a series of isokinetic muscle performance in EAE. Exercise did not affect muscle fiber contractile properties in EAE.

STUDY 2

Disease progression, muscle contractile properties and cytokine response during experimental autoimmune encephalomyelitis: impact of different running intensities

Based on: Broekmans T, Hellings N, Biesmans S, Stinissen P, Eijnde BO.
Submitted (Acta Physiologica)

2.2.1. Abstract

Objective. Disease progression, muscle contractile properties and basal cytokine concentrations in combination with different endurance training intensities were investigated during acute EAE in Lewis rats.

Methods. A control (CON) and EAE group, were divided in a sedentary (S), a light (L, 5m/min), a moderate (M, 11m/min, 15° inclination) and a high (H 18m/min, 25° inclination) intensity exercise (1h/day, treadmill running) subgroup. After recovery from EAE, tibialis anterior (TA) and extensor digitorum longus (EDL) muscle fiber cross sectional area (CSA) and distribution were assessed.

Results. Compared to EAE-S (hindquarter paralysis score > 0.5; day 11.0±0.1), the onset of hindquarter paralysis was delayed ($p<0.05$) in EAE-H (hindquarter paralysis score > 0.5; day 11.7±0.3). In all EAE groups lower ($p<0.05$) type IIb CSA were found. This possibly explains the absence of peak muscle strength during a series of repetitive contractions in EAE-H. Moderately and high intense treadmill running might have the potential to increase type IIa muscle fibers of TA in EAE rodents. No differences between subgroups in basal IL-6, IL-10 and TNF-α serum concentrations were found.

Conclusions. The impact of exercise on clinical symptoms and type IIa muscle fibers seems to be intensity related, especially after high intense treadmill running.

2.2.2. Introduction

Multiple sclerosis (MS) is a chronic neuroinflammatory and neurodegenerative disease (2) of the central nervous system that affects many aspects of daily life functional activity (76,179,196,197). A growing body of evidence indicates that adequate endurance exercise can, at least in part, reverse some of the clinical symptoms but the optimal endurance training intensity is unclear. Furthermore, given the potential anti-inflammatory effects of exercise training in other chronic pathologies (198,199), the role of cytokines in MS has been explored following an acute exercise bout (107,120) and longer-term (120) aerobic exercise training, following psychological stress (200) and longer-term resistance training (121) but never following different endurance training intensities.

To date persons with MS are recommended to train at moderate exercise intensities in order to improve their physical health profile (51,118). Usually, the applied training frequencies and intensities range between 2-3/week and 50-70% of VO_2max (51,118). However, if higher exercise intensities are tolerable for MS sufferers enhanced training/rehabilitation outcome could be expected as reported in healthy subjects (119,201), in persons with coronary disease (202,203) and diabetes (204). Given the explorative nature of this hypothesis the use of an animal MS model such as experimental autoimmune encephalomyelitis (EAE) seems appropriate. Acute EAE mimics several inflammatory aspects of MS. More particular, analogous to MS, EAE is a T-cell mediated autoimmune demyelinating disease of the CNS where lymphocytic and mononuclear cell infiltrate into the CNS due to an increase in permeability of the blood brain barrier (173).

So far, literature investigating the impact of exercise training on disease progression in EAE is limited but results are promising. Le Page and co-workers demonstrated that treadmill running after immunization, delayed the onset and duration of paralysis in chronic EAE (174). Furthermore, mice with chronic EAE also exhibited less severe neurological deficits and a smaller amount of dendritic spinal loss in the striated neurons after voluntary wheel running (176). In contrast, very intense swimming exercise worsened hindquarter paralysis (Broekmans et al., submitted). The impact of EAE on muscle contractile properties has also been explored. Here, De Haan et al. reported reduced (-40-50%) fiber size of all medial gastrocnemius muscle fibers following acute EAE (177). We reported smaller type IIb fibers of tibialis anterior in EAE rodents and no training effect on muscle fiber cross sectional area (CSA) or distribution (Broekmans et al., submitted).

During the last decade a multitude of studies have investigated the potential anti-inflammatory effects of regular exercise in healthy subjects (205-210), in healthy rodents (211,212) and in animal models for arthritis (213), stroke (214) and parkinsonism (215). So far it is clear that exercise training improves CNS functioning probably via neurotrophic factors (216) and/or immuno-modulation (104). Because in MS immune function is

dysregulated and characterized by an excessive inflammatory component (34), some evidence already suggests that exercise training (107,120,121, 138) may also have a potential anti-inflammatory effect in MS. The effects of physical exercise on cytokine responses in EAE have never been investigated.

The current study aims to investigate the impact of low, moderate and high intense endurance training on the disease progression, muscle contractile properties and cytokine responses during acute EAE. We hypothesize that exercise have the potential to delay the onset of hindquarter paralysis, modifies muscle contractile properties and affects pro- and anti-inflammatory cytokine balance.

2.2.3. Methods

2.2.3.1. Animals

Sixty-four female Lewis rats (age, 6-7 weeks, body weight 120-170g), were obtained from the Harlan (Harlan CPB, Zeist, The Nederlands) animal breeding center, maintained on a constant light:dark cycle (12:12), temperature (22°C) and a relative humidity of 22-24%. Rodents were fed *ad libitum* with normal rat pellets (Carfil RN-01-K12, Harlan). The animal Ethics Committee of Hasselt University approved the study protocol in accordance with the Helsinki Declaration.

2.2.3.2. Study design

Following animal breeding center acclimatization (days [d] -21 to -15) rats were randomized according to their body weight in a control (CON, n=32) and an experimental autoimmune encephalomyelitis (EAE, n=32) group. Hereafter the habituation period (d -14 to -1) started. Both groups were subdivided in a sedentary (S, n=8), light- (L, n=8), moderate- (M, n=8) and high (H, n=8) training intensity subgroup and rats were familiarized to treadmill running. Briefly, in the first three running sessions (d-14, d-12, d-10) all groups ran at a treadmill speed (4 m/min) and inclination (0°) for respectively 15 (d-14), 20 (d-12) and 30 (d-10) minutes. Hereafter, treadmill inclination was set (d-7) at 0° (L), 15° (M) and 25° (H), training duration gradually increased 45 (d-7), 50 (d-6), 55 (d-5) and 60 (d-4) minutes and running speed was progressively increased (*d-7*: L: 5 m/min, M: 8 m/min, H: 12 m/min; *d-6*: L: 5 m/min, M: 8 m/min, H: 13 m/min; *d-5*: L: 5 m/min, M: 9 m/min, H: 14 m/min; *d-4*: L: 5 m/min, M: 11 m/min, H: 16 m/min; *d-3*: L: 5 m/min, M: 8 m/min, H: 18 m/min). In order to induce similar rodent manipulation circumstances, S rats were daily placed on the treadmill. From habituation period onwards, daily food intake and body weight were registered. At the start of the training period (d 0 to +11), experimental autoimmune encephalomyelitis was induced in the EAE subgroups by a single percutaneous injection in both footpads and consisted of purified myelin basic protein (MBP) in combination with *mycobacterium tuberculosis* (37 Hra, Difco, Detroit, MI) and complete Freunds adjuvant (CFA, Difco) under urethane (1.5g/kg, i.p.) anesthesia (172). Typically, hindquarter paralysis developed 12-14d after induction, where after rats partly recovered. Following induction L, M and H rats exercised daily (1h/d; L: 5 m/min, 0° inclination; M: 11 m/min, 15° inclination; H: 18 m/min 25° inclination). From the training period onwards, all EAE rats were examined daily for the development of hindquarter paralysis. At day 1 and 10, a pre and post exercise capillary blood sample (30-50 µl) of 5 animals of each subgroup was collected in a capillary tube (Analox Instruments®, London) to

determine blood lactate concentration. Finally, rats entered the paralysis period (d12-17) with no training. To determine serum cytokine levels in a part of each subgroup (n=6) tail arterial blood samples (1ml, Multivette® 600 serum, Sarstedt, Germany) were collected under gas anesthesia (Isoflurance®), centrifuged (4800rpm, 6 minutes) and immediately stored at -80°C at days 0, 9, 13 and 17. At day 17 rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (5 mg·100 g^{-1} BW). Following determination of repetitive isokinetic foot extensor performance of the left hind limb, the m. extensor digitorum longus (EDL) and the m. tibialis anterior (TA) of the right hind limb were dissected and freed of connective tissue and visible blood. Hereafter, the mid-part of each muscle was mounted in embedding tissue (Tissue-Tek®, Miles Laboratories), frozen in isopentane cooled in liquid N_2, and stored at -80°C until analysis were performed. Finally, rats were sacrificed by an intracardial injection of pentobarbital sodium.

2.2.3.3. In vivo testing

Hindquarter paralysis. At 8.30 a.m. during the training and paralysis period hindquarter paralysis were scored on a scale ranging from 0 to 5: 0, no signs; 0.5: partial loss of tail tonus (is defined as the disease onset); 1.0:complete loss of tail tonus; 2.0: hind limb paresis; 3.0: hind limb paralysis; 4.0: moribund; 5.0: death due to EAE (172).

Isokinetic foot extensor performance. After general anesthesia as described above, left hind limb foot extensor muscle strength during fatiguing isokinetic muscle contractions was assessed as described elsewhere (185,186). Briefly, this involved the application of percutaneous needle electrodes placed on the common peroneal nerve fusing 120 consecutive concentric isokinetic foot extensions (50°/s, 1 mA, 250ms, 3s rest intervals) after standardized knee and ankle fixations on a custom build Ashton-Miller (187) like rat dynamometer. To express work fatigue, the highest work performed in the first 30 consecutive contractions was set at 100% and the other values were expressed as a percentage from this peak.

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

Lactate concentrations. Whole blood lactate concentrations were determined by a GM7 Micro-Stat® (Analox Instruments®, London) analyzer and each subgroup contained 5 animals.

2.2.3.4. Fiber CSA and distribution

To quantify type I, IIa and IIb muscle fiber cross sectional area (CSA) and distribution, serial transverse sections (8µm) from the obtained muscle samples were cut at -20°C and stained with triple-coloring. Briefly, air-dried (30min) cryosections were washed (0.5% Triton-X, 5 minutes), then sections were incubated 60 minutes at room temperature with a mix of 2 mouse monoclonal antibodies against myosin heavy chain I (1:25; A4.840 supernatant, Developmental Studies Hybridoma Bank, Iowa, USA) and IIA (1:25; N2.261 supernatant, Developmental Studies Hybridoma Bank, Iowa, USA) and 1 rabbit polyclonal laminin antibody (1:100; L-9393, Sigma, Zwijndrecht, The Netherlands). Then the slides were washed 3 times for 5 minutes with PBS, followed by an incubation period of 45 minutes at room temperature with a mixture of secondary antibodies (1:500, Goat anti-Mouse IgM AlexaFluor 555; 1:200 Goat anti-Mouse IgG_1 AlexaFluor 488 and 1:130 Goat anti-Rabbit IgG AlexaFluor 350; Molecular probes, Invitrogen, Breda, The Netherlands) diluted in PBS. Hereafter the sections were washed 3 times for 5 minutes with PBS and mounted in Fluorescent Mounting Medium (Dako, North America, California, USA). Muscle fibers were examined and recorded using a Nikon Eclipse 90i fluorescence microscope (Nikon, Boerhavedorp, Germany). The fluorescence signals were recorded using a TRITC and FITC filter for type I and IIa muscle fibers and DAPI filter for cell membrane. Digital images (x20 magnification, exposure time for TRITC and FITC 400ms, DAPI 800ms) were then analyzed using NIS Elements® BR 3.0 software (LIM, Prague, Czech Republic).

2.2.3.5. Cytokine determination

To determine serum interleukin 6 (IL-6), Tumor Necrosis Factor alfa (TNF-α) and interleukin 10 (IL-10) concentrations in all subgroups (n=6) flow cytometry with a commercial available Cytometric Bead Array (CBA) Flex Set (Becton-Dickson, Franklin Lakes, USA; IL-6, Bead A9; IL-10, Bead A8; TNF-α, Bead C8) was used. The intra-assay coefficients of variability for IL-6, IL-10 and TNF-α were 6%, 6% and 7%, respectively. The theoretical sensitivities ($pg \cdot ml^{-1}$) of IL-6, IL-10 and TNF-α were 9.9, 19.4 and 27.7, respectively.

2.2.3.6. Statistical analyses

All data were analyzed using SAS software (SAS Institute Inc, Cary, USA). First normality was checked using the Shapiro-Wilk test for all

variables. EAE dropout rate was analyzed by means of a survival analysis. Furthermore, to analyze the course of hindquarter paralysis a 4x17 (EAE Group [EAE-S; EAE-L; EAE-M; EAE-H] x Time [Days 1-17]) mixed model ANOVA with body weight as confounding factor was used. To analyze disease onset which was defined as a hindquarter paralysis score equal to 0.5, a time to event analysis was used for all EAE groups. Body weight was analyzed by a 2x4x3/9/6 (Group [CON; EAE] x Activity [S; L; M; H] x study phase [induction phase: day 0-2; exercise phase: day 3-11; paralysis phase: day 12-17]) mixed model ANOVA. Muscle fiber type area and distribution were analyzed using a 2x4 (Group [CON; EAE] x Activity [S; L; M; H]) mixed model ANOVA and muscle fatigue contractile data were analyzed using a (Group [CON; EAE] x Activity [S; L; M; H] x Contraction [120 dynamic muscle contractions]). Cytokine data were analyzed using a 2x4x4 mixed model ANOVA (Group [CON; EAE] x Activity [S; L; M; H] x Time [day 0; day 9; day 13 and 17] with left censored data (to take detection limits into account). Finally, post blood lactate concentrations were analyzed using a 2x4x2 mixed model ANOVA (Group [CON; EAE] x Activity [S; L; M; H] x Time [day 1and 10]. When appropriate, post hoc pre-planned contrast tests were applied. Post hoc power calculations were performed using G power 3 software (253). All data are presented as means ± SE with a p-value of 0.05 for acceptance of statistical significance.

2.2.4. Results

2.2.4.1. Drop out

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

2.2.4.2. Hindquarter paralysis, body weight and food intake

This hindquarter paralysis is a representation of the seriousness of EAE disease activity while body weight and food intake were taken into account to study the impact of disease and exercise on the rodents. Disease peak and remission did not differ between groups. However, in EAE-H disease onset, was significantly delayed compared to EAE-S (Figure 2.2.1.). This is mirrored by EAE-H body weight (Figure 2.2.1.) and food intake (data not shown) during the paralysis phase (activity effect between EAE-H and EAE-S).

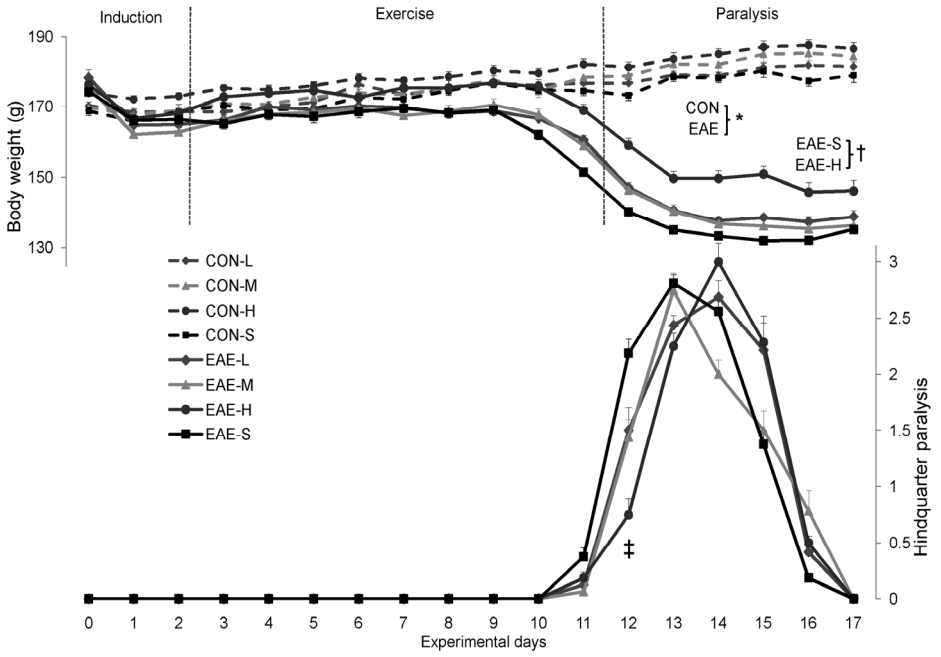

Figure 2.2.1. Effects of experimental autoimmune encephalomyelitis (EAE) and exercise intensity on body weight and hindquarter paralysis.
*Values are means ± SE and express body weight of all rats (CON: control; L: low; M: moderate; H: high intense running exercise) and hindquarter paralysis in EAE animals. *p<0.05 body weight of EAE compared to CON. †p<0.05 body weight of EAE-S compared to EAE-H. ‡p<0.05 delayed onset of hindquarter paralysis of EAE-H compared to EAE-S. See methods for further details.*

2.2.4.3. Muscle fiber type area and distribution

To investigate the impact of very intense exercise therapy on muscle fiber morphology, *muscle fiber cross sectional area (CSA)* and *distribution* (, Table 2.2.1.) was investigated. As depicted in Table 2.2.1., in EAE CSA of EDL (-23%) and TA (-38%) type IIb muscle fibers were significantly reduced ($p<0.05$) compared to CON. A statistical trend ($p=0.08$) was found in the group x activity analysis with a significant group effect. Thus, in EAE CSA of TA type IIa fibers increased (+8%). Furthermore in EAE-M and EAE-H TA CSA was 16% and 23% higher ($p<0.05$) compared to EAE-S (Table 2.2.1.). However, no exercise effect on type IIb and I fibers size was found between the different intensity levels neither in CON nor in EAE.

Fiber type distribution. Fiber type distribution in EDL and TA did not differ between CON and EAE (Table 2.2.1.). Exercise did not affect fiber distribution in EDL or TA.

Table 2.2.1. Cross sectional area and muscle fiber composition of Extensor Digitorum Longus (EDL) and Tibialis anterior (TA)

EDL

	Type I				Type IIa				Type IIb			
	Area (µm²)	p	Distr (%)	p	Area (µm²)	p	Distr (%)	p	Area (µm²)	p	Distr (%)	p
CON	524 ± 23.8		5.8 0.4		677 ± 24.1		24.8 1.4		1032 ± 31.0		69.5 ± 1.4	
EAE	571 ± 23.5		5.9 0.5		682 ± 25.5		24.4 1.5		793 ± 34.9*		69.9 ± 1.4	
CON-S	511 ± 46.6		5.7 ± 0.9		699 ± 55.7		26.1 ± 2.4		996 ± 59.2		68.1 ± 2.4	
CON-L	516 ± 42.7		5.0 ± 0.8		693 ± 44.3		26.9 ± 2.4		1137 ± 63.4		68.2 ± 2.8	
CON-M	514 ± 46.9	0.64	6.4 ± 0.8	0.18	654 ± 39.7	0.65	20.0 ± 3.1	0.70	988 ± 57.5	0.04	73.7 ± 3.3	0.94
CON-H	559 ± 63.0		6.0 ± 0.8		658 ± 60.5		26.3 ± 2.9		1003 ± 62.7		67.8 ± 3.0	
EAE-S	552 ± 24.8		5.9 ± 0.9		680 ± 55.6		24.9 ± 2.0		772 ± 51.2*		69.2 ± 1.8	
EAE-L	562 ± 95.0		7.2 ± 1.2		685 ± 24.4		23.6 ± 3.6		800 ± 109.8*		69.2 ± 3.9	
EAE-M	624 ± 43.2		4.6 ± 0.5		735 ± 60.9		23.4 ± 3.6		832 ± 74.2		72.0 ± 3.6	
EAE-H	539 ± 35.0		6.3 ± 1.4		619 ± 35.8		25.5 ± 3.2		767 ± 72.1*		69.2 ± 2.7	

TA

	Type I				Type IIa				Type IIb			
	Area (µm²)	p	Distr (%)	p	Area (µm²)	p	Distr (%)	p	Area (µm²)	p	Distr (%)	p
CON	763 ± 25.0		2.0 ± 0.4		767 ± 17.1		16.8 ± 1.1		1269 29.4		81.1 ± 1.1	
EAE	783 ± 35.7		1.9 ± 0.3		827 ± 24.0*		18.8 ± 1.3		786 31.5*		79.4 ± 1.3	
CON-S	682 ± 62.6		1.3 ± 0.3		797 ± 40.1		16.8 ± 2.9		1384 ± 84.2		81.9 ± 2.9	
CON-L	724 ± 29.8		1.3 ± 0.2		723 ± 36.1		15.5 ± 1.4		1198 ± 37.2		83.2 ± 1.4	
CON-M	875 ± 40.8	0.45	3.2 ± 1.2	0.15	774 ± 39.3	0.08	15.3 ± 1.7	0.88	1267 ± 35.4	0.03	81.5 ± 1.5	0.87
CON-H	746 ± 43.5		2.0 ± 0.3		774 ± 22.1		19.5 ± 2.4		1244 ± 64.8		78.5 ± 2.5	
EAE-S	807 ± 56.8		2.6 ± 1.0		751 ± 41.2		18.2 ± 2.9		762 ± 47.4*		79.2 ± 3.1	
EAE-L	740 ± 89.4		1.7 ± 0.5		778 ± 37.3		18.7 ± 2.8		786 ± 73.2*		79.6 ± 2.6	
EAE-M	804 ± 95.2		1.4 ± 0.3		872 ± 42.3†		18.9 ± 2.4		786 ± 78.9*		79.8 ± 2.4	
EAE-H	769 ± 54.5		1.6 ± 0.2		922 ± 46.3*†		19.5 ± 2.7		818 ± 72.4*		78.9 ± 2.7	

Values are means ± SE and express muscle fiber cross sectional area (µm²) and fiber type composition (Distr %) in Extensor Digitorum longus (EDL) and Tibialis Anterior (TA) of sedentary (S), low- (L), moderate- (M) and High- (H) training intensity control (CON) and experimental autoimmune encephalomyelitis (EAE) rats. p represent p-value of group (CON,EAE) x activity (S, L, M, H) effect. * p<0,05 compared to corresponding control value. † p<0.05 compared to EAE-S

2.2.4.4. Isokinetic muscle performance

To determine the exercise effect on muscle strength isokinetic muscle power was measured. A significant group x activity x contraction effect was not found (Figure 2.2.2). However, group x contraction sub-analysis indicated that EAE rodents significantly differed compared to CON. More particular, during the first 30 contractions in CON muscle work peaked and then declined (decline: -36±1%) while in EAE work did not peak but remained stable (decline: -5±0%) over the 120 dynamic muscle contractions.

2.2.4.5. Cytokine levels

To determine the impact of different exercise intensities on systemic inflammatory markers, several serum cytokine concentration were measured. IL-6 (Table 2.2.2.) and IL-10 (Table 2.2.4.) did not differ between groups throughout the study. Compared to CON, in EAE TNFα concentration was higher ($p<0.05$) at each time point (Table 2.2.3.). Furthermore, exercise did not affect IL-6, IL-10 and TNFα serum concentrations neither in CON- or EAE groups.

Table 2.2.2. IL-6 resting levels of all the experimental groups

	IL-6							
	n	Day 0	n	Day 9	n	Day 13	n	Day 17
CON	7	286.1 ± 65.2	12	145.4 ± 35.7	10	182.4 ± 56.3	5	183.7 ± 51.4
EAE	15	197.1 ± 60.0	13	258.6 ± 50.2	11	226.0 ± 52.5	9	216.3 ± 58.8
CON-S	3	261.7 ± 91.4	2	57.6 ± 21.1	1	106.0 ± /	1	149.4 ± /
CON-L	2	194.5 ± 42.7	3	200.1 ± 97.1	2	219.8 ± 130.5	1	96.0 ± /
CON-M	0	ud	3	118.3 ± 76.3	2	106.8 ± 56.8	0	ud
CON-H	2	414.1 ± 191.5	4	168.8 ± 62.6	5	213.8 ± 104.6	3	224.3 ± 80.6
EAE-S	4	96.9 ± 48.2	3	114.7 ± 59.8	2	123.5 ± 78.1	1	161.1 ± /
EAE-L	5	219.5 ± 87.0	4	349.1 ± 130.8	3	255.4 ± 130.4	3	231.5 ± 113.6
EAE-M	3	426.3 ± 225.9	3	266.2 ± 60.5	4	135.3 ± 38.2	3	110.2 ± 35.9
EAE-Z	3	64.4 ± 51.4	3	273.6 ± 89.3	2	466.1 ± 0.7	2	380.1 ± 190.6

Values are means ± SE and represent the blood serum concentrations of Interleukine 6 (IL-6) at four different experimental time points (Day 0; Day 9; Day 13; Day 17) of the control (CON), experimental autoimmune encephalomyelitis (EAE) group and the sedentary (S), low- (L), moderate- (M) and high- (H) training intensity subgroups.

Table 2.2.3. TNF-α resting levels of all the experimental groups

	TNF-α							
	n	Day 0	n	Day 9	n	Day 13	n	Day 17
CON	8	10.8 ± 1.3	4	7.2 ± 2.4	4	10.3 ± 1.7	3	7.8 ± 1.1
EAE	12	20.7 ± 3.6*	19	24.6 ± 2.2*	17	21.7 ± 2.1*	17	25.3 ± 2.0*
CON-S	1	17.5 ± /	2	49.4 ± 4.9	1	15.4 ± /	3	7.8 ± 1.1
CON-L	3	9.7 ± 1.5	0	ud	2	9.2 ± 0.7	0	ud
CON-M	1	7.9 ± /	1	8.4 ± /	1	7.5 ± /	0	ud
CON-H	3	10.9 ± 2.4	1	10.4 ± /	0	ud	0	ud
EAE-S	2	19.4 ± 7.8	4	23.9 ± 6.4	5	21.6 ± 4.9	4	24.4 ± 4.3
EAE-L	3	20.9 ± 7.6	5	27.6 ± 6.0	3	22.3 ± 4.5	4	22.5 ± 3.4
EAE-M	3	27.8 ± 8.7	5	22.5 ± 3.3	4	20.8 ± 5.8	4	27.3 ± 4.8
EAE-Z	4	16.0 ± 6.6	5	24.1 ± 3.1	5	22.2 ± 3.0	5	26.8 ± 4.6

Values are means ± SE and represent the blood serum concentrations of Tumor Necrosis Factor alfa (TNF-α) at four different experimental time points (Day 0; Day 9; Day 13; Day 17) of the control (CON), experimental autoimmune encephalomyelitis (EAE) group and the sedentary (S), low- (L), moderate- (M) and high- (H) training intensity subgroups.* $p<0,05$ compared to corresponding control value.

Table 2.2.4. IL-10 resting levels of all the experimental groups

	IL-10							
	n	Day 0	n	Day 9	n	Day 13	n	Day 17
CON	15	34.3 ± 11.9	16	27.7 ± 7.9	14	27.0 ± 7.3	13	16.4 ± 4.5
EAE	18	48.6 ± 16.2	22	50.4 ± 9.8	13	41.9 ± 11.6	14	37.7 ± 14.4
CON-S	2	21.8 ± 7.6	2	24.4 ± 3.5	2	12.5 ± 0.2	2	8.1 ± 7.0
CON-L	4	35.7 ± 11.7	4	36.0 ± 18.7	4	32.0 ± 22.5	4	13.4 ± 5.3
CON-M	3	16.4 ± 5.1	5	12.4 ± 2.6	3	22.2 ± 10.7	3	16.3 ± 6.6
CON-H	6	46.4 ± 29.2	5	38.6 ± 20.8	5	31.6 ± 10.7	4	23.7 ± 13.1
EAE-S	6	23.8 ± 14.3	5	29.7 ± 13.8	1	55.4 ± /	2	28.8 ± 19.4
EAE-L	4	46.1 ± 4.3	5	70.3 ± 24.0	3	33.0 ± 17.1	4	21.6 ± 8.6
EAE-M	5	94.8 ± 53.5	6	54.6 ± 22.3	5	29.3 ± 9.6	5	25.3 ± 4.1
EAE-Z	3	24.2 ± 6.8	6	46.8 ± 18.3	4	60.9 ± 35.2	3	85.8 ± 66.7

Values are means ± SE and represent the blood serum concentrations of Interleukine 10 (IL-10) at four different experimental time points (Day 0; Day 9; Day 13; Day 17) of the control (CON), experimental autoimmune encephalomyelitis (EAE) group and the sedentary (S), low- (L), moderate- (M) and high- (H) training intensity subgroups.

2.2.4.6. Lactate concentrations

Lactate concentrations were measured to determine the exercise intensity in the different groups. Pre-exercise values (data not shown) did not differ over time and between groups but a significant group x activity x time effect for post-exercise lactate concentrations was found (Table 2.2.5.). At day 1 CON subgroups had significantly lower post exercise lactate concentrations compared to the corresponding EAE subgroup and all EAE subgroups at day 1 had significant higher lactate levels compared to day 10.

Table 2.2.5. Lactate post exercise

	n	Day 1	n	Day 10
CON-S	5	1.9 ± 0.2	5	1.8 ± 0.1
CON-L	5	1.6 ± 0.1	5	1.7 ± 0.0
CON-M	5	1.4 ± 0.1	5	1.6 ± 0.1
CON-H	5	1.5 ± 0.2	5	1.8 ± 0.1
EAE-S*	5	2.4 ± 0.2†	5	1.8 ± 0.3
EAE-L*	5	3.1 ± 0.3†‡	5	2.1 ± 0.2†‡
EAE-M*	5	3.9 ± 0.2†‡	5	2.3 ± 0.2
EAE-H*	5	4.6 ± 0.2†‡∓	5	2.2 ± 0.2†‡

Values are means ± SE and represent post exercise blood lactate concentration of the control (CON) and experimental autoimmune encephalomyelitis (EAE) group in combination with light- (L), moderate- (M) and high (H) intens or no (S) exercise. *$p<0.05$ day 1 compared to day 10. †$p<0.05$ EAE S, L, M and H compared to CON S, L, M, and H, respectively. ‡$p<0.05$ CON L, M, H or EAE L, M, H compared to CON S or EAE S. ∓$p<0.05$ in CON S, M, H or EAE S, M, H compared to CON L or EAE L.

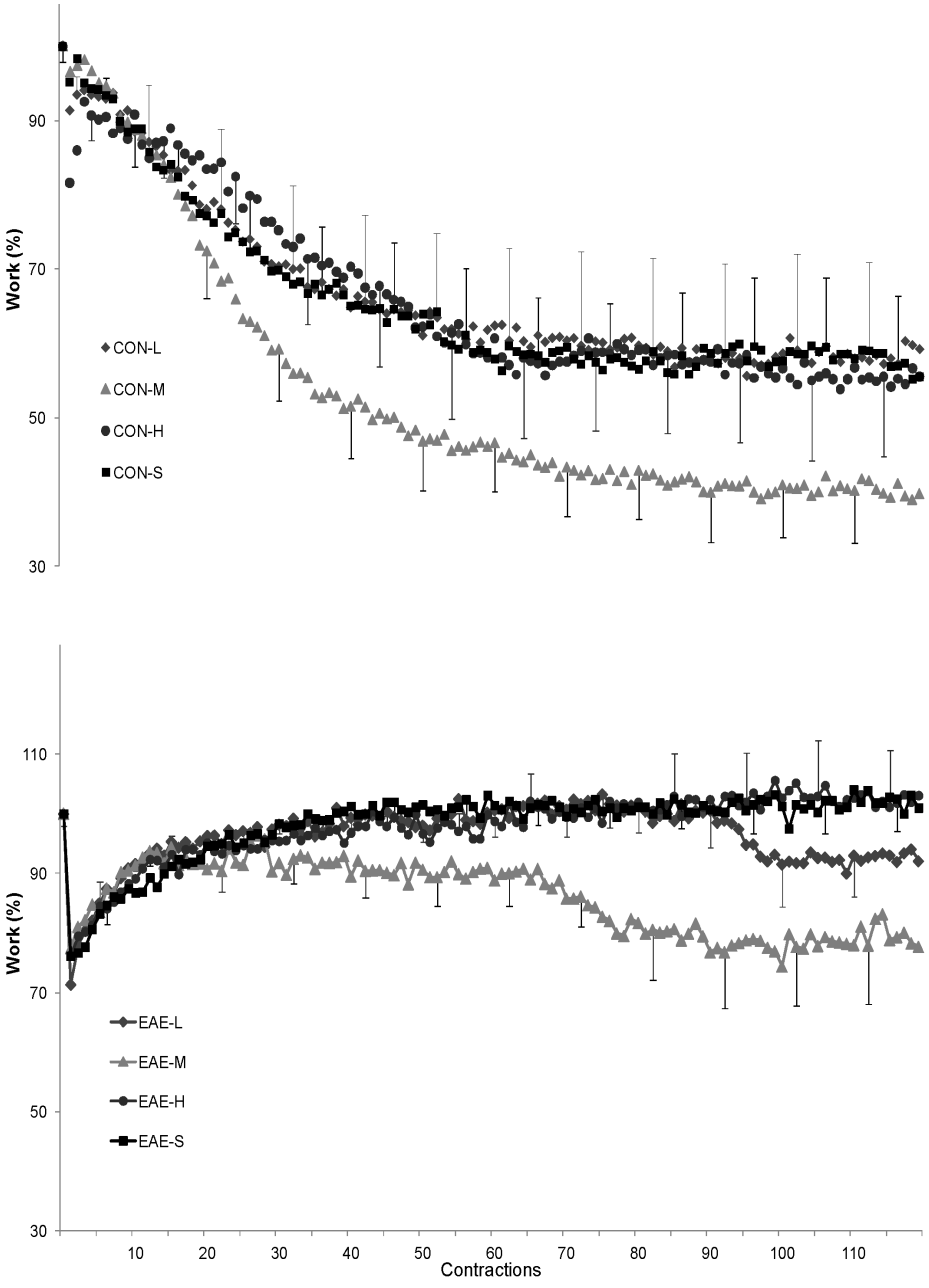

Figure 2.2.2. Effects of experimental autoimmune encephalomyelitis (EAE) and exercise intensity on muscle work.

Values are means ± SE and express muscle work (%) during 115 consecutive maximal muscle contractions (1mA, 150Hz, 250ms) in control (CON) and EAE rats, both subdivided in different exercise intensity groups (L: low; M: moderate; H: high intense running exercise.

2.2.5. Discussion

The current study is the first that investigated the impact of a range of endurance training intensities on acute EAE, muscle fiber characteristics, isokinetic foot extensor performance and basal cytokine responses. Regular intense running exercise delayed ($p<0.05$) the onset of hindquarter paralysis but did not change disease peak or remission. In EAE rodents a lower ($p<0.05$) cross sectional area (CSA) of type IIb muscle fibers in TA and EDL was found whereas in TA type IIa CSA tended to increase ($p=0.08$). Reduced type IIb muscle fiber CSA probably explains the absence of peak force during a series of 120 isokinetic contractions in EAE. Basal IL-6, IL-10 and TNFα serum concentrations did not change by EAE or by exercise training.

Intense treadmill running (18m/min, 25° inclination, 1h/d) delayed ($p<0.05$) the onset of EAE-induced hindquarter paralysis. Furthermore, compared to EAE-S a smaller amount of body weight loss ($p<0.05$) was found in EAE-H during the paralysis period. At the same time, low to moderately intense endurance training did not change EAE disease course suggesting that the exercise load, indeed, seems to determine its effect on the EAE disease course. In fact, in the present study EAE-H exercise intensity is probably near the anaerobic threshold because 4.6±0.2 (day 1) and 2.2±0.2 (day 10) mmol/l post exercise lactate concentrations were measured indicating a training effect (217). Other authors also reported delayed onset of clinical EAE signs following voluntary wheel running in mice (176) and treadmill running in rats (174,175). Given the fact that Rossi et al applied voluntary exercise in mice and did not quantify training load and intensity, it is difficult to compare between studies. Le page and coworkers, however, did report running speed progression (15-30m/min) with a fixed treadmill inclination of 8% (~4°) but did not measure lactate concentrations. Nevertheless, the applied exercise intensity also appears to be close to the anaerobic threshold. Interestingly, we previously reported that very intense exercise training worsened overall EAE related hindquarter paralysis (summated hindquarter paralysis scores, Broekmans et al, submitted). In the latter we applied swimming exercise (1h/day) with an attached external tail load of 5.5% body weight to ensure that the training intensity was above the anaerobic threshold (188-190). Thus, the present findings and the above described work of others suggest that exercise intensity determines training outcome in EAE and that the optimal endurance training intensity in EAE is probably near the anaerobic threshold. Given the fact that the effects of exercise therapy on a variety of functional parameters in MS is at present under investigation, it might be worthwhile to investigate this hypothesis in MS patients. In this respect, very recently Collet et al already explored the most effective exercise intensity in MS using three cycling intensities (218) and concluded that compared to the lower-intensity training group, higher-intensity exercise increased the 2 minutes walking distance. These authors also reported a higher dropout rate in the higher intensity training group

(218).

The impact of acute EAE on muscle contractile properties in combination with different exercise intensities was also examined. Apart from smaller type IIb fibers of the TA (-38%) as previously reported by our laboratories (Broekmans et al., submitted Multiple Sclerosis Journal) the current study also showed decreased CSA of EDL type IIb fibers (-23%) in EAE. Because fast glycolitic type IIb muscle fibers largely contribute to peak force production (219,220) this probably explains the absence of peak muscle work during the first 30 repetitive isokinetic contractions in the present study. De Haan and co-workers also investigated the impact of EAE on the medial gastrocnemius and demonstrated reduced CSA of all fiber types that in a decreasing order was correlated with their oxidative capacity (-47% vs. type IIx: -43%, type IIa: -41% and type: I -37%) (177). The fact that immobilization in rats mainly affects slow anti-gravity muscle fibers and extensors (gastrocnemius) more than flexors (TA, EDL) (221) probably indicates that in EAE muscle fiber atrophy is induced by inactivity. In rats it is documented that exercise training can remediate this (222-224). In the present study running exercise tended to reduce muscle fiber atrophy (training effect) in EAE but only in TA type IIa muscle fibers of EAE-H rats, suggesting a minor training effect. It is already reported that running exercise can increase ($p<0.05$) the CSA of these muscle fibers in healthy and aged rats (225). However, in our CON group no alterations in muscle fiber characteristics were found. This might be explained by the fact that one week of inactivity, as applied during the paralysis period of EAE rats in the present study, reduces rat muscle mass with 37% (226). Because muscle fiber CSA tended to increase in EAE rodents, this might suggest that they are more susceptible for endurance training. To clarify this future research should sample muscle biopsies immediately after the training intervention. Given the fact that EAE reduced CSA of type IIb muscle fibers and these fibers are more susceptible to strength training (227-229) we also recommend to investigate its effect on muscle fiber characteristics following resistance training.

This is the first study that investigated the effect of different endurance training intensities on basal serum cytokine levels of IL-6, IL-10 and TNFα in EAE rodents. EAE or endurance exercise did not alter basal cytokine levels. It is clear that the applied research design does not measure acute cytokine responses following exercise (105,230). Hereby we might have missed a possible anti-inflammatory effect (104) as documented in MS (120). Furthermore, local cytokine changes at tissue levels have been frequently reported without affecting serum cytokine concentrations. Here, in treadmill running rats (7 weeks) Chennaoui et al (211) reported lower ($p<0.05$) IL-6 concentrations in the cerebellum while circulating IL-6 levels were not modified. Although specific literature about TNFα and IL-10 blood concentrations after exercise is lacking in EAE, a muscle TNFα/IL-10 ratio shift towards an anti-inflammatory IL-10 profile has been reported (231-

233). This warrants further research to examine the acute and local tissue related immunological responses of exercise therapy during EAE which could be important to optimize potential anti-inflammatory exercise strategies in MS. Another limitation of the present study is that the used sample size may have been too small and therefore may have reduced statistical power However, to the author's knowledge, literature investigating the effect of exercise intensities on the EAE disease course does not exists and thus it was not possible to calculate a proper effect size. Therefore, no a priori power analyses has been performed. However, post hoc power analyses, using the actual effect size of the current study indicated a statistical power above 0.80 for muscle strength, TNFα and IL-10. Power of hindquarter paralysis, muscle fiber determination and IL-6 were below 0.80.

In summary, the present study suggests that only intense treadmill running delays the onset of hindquarter paralysis in EAE without affecting the peak or remission of the disease course. However, basal cytokine serum levels were not affected. Furthermore, in EAE rodents CSA type IIb fibers of TA and EDL decreased. This probably explains the absence of peak muscle work during the first 30 contractions of a series of isokinetic muscle performance in EAE. High and moderately intense exercise training tended to increase size of type IIa fibers of TA while the other muscle fibers were not enhanced by running exercise.

Grant

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STUDY 3

Effects of long-term resistance training and simultaneous electro-stimulation on muscle strength and functional mobility in multiple sclerosis

Based on: Broekmans T, Roelants M, Feys P, Alders G, Gijbels D, Hanssen I, Stinissen P, Eijnde BO.
Mult. Scler. J. 2011; 17 (4): 468-477

2.3.1. Abstract

Background. Resistance training studies in multiple sclerosis (MS) often use short intervention periods. Furthermore, training efficiency could be optimized by unilateral training and/or electrical stimulation.

Objective. To examine the effect(s) of unilateral long-term (20 weeks) standardized resistance training with and without simultaneous electro-stimulation on leg muscle strength and overall functional mobility.

Methods. A randomized controlled trial involving 36 persons with MS. At baseline (PRE) and after 10 (MID) and 20 (POST) weeks of standardized (ACSM) light to moderately intense unilateral leg resistance training (RES_o, n=11) only or resistance training with simultaneous electro-stimulation (RES_E, n=11, 100Hz, biphasic symmetrical wave, 400µs), maximal isometric strength of the knee extensors and flexors (45°, 90° knee angle) and dynamic (60-180°/s) knee extensor strength was measured and compared to a control group (CON, n=14). Functional mobility was evaluated using the Timed get Up and Go, Timed 25 Foot Walk, 2 Minutes Walk Test, Functional Reach and Rivermead Mobility Index.

Results. Maximal isometric knee extensor (90°; MID: +10±3%; POST: +10±4%) in RES_o and knee-flexor (45°, POST: +7±4%; 90°, POST: +9±5%) in RES_E strength increased ($p< 0.05$) compared to CON but RES_o and RES_E did not differ. Also, impaired legs responded positively to resistance training (unilateral leg strength analysis) and functional reaching increased significantly in RES_o (+18%) compared to CON. Dynamic muscle strength and the remaining functional mobility tests did not change.

Conclusion. Long-term light to moderately intense resistance training improves muscle strength in persons with MS but simultaneous electro-stimulation does not further improve training outcome.

2.3.2. Introduction

Although characterized by sensation, balance, bladder, bowel, cognitive, visual and affective deficits, Multiple Sclerosis (MS) also affects motor pathways leading to muscle weakness and muscle fatigue (54) and thus impaired functioning (234). Until recently MS patients were advised not to participate in intense physical activity to remediate this (235,236). Consequently, many MS patients show reduced physical activity levels and suffer from inactivity-induced muscle atrophy and loss of muscle strength, reducing daily life physical functioning as indicated by Motl et al (237). These investigators also showed that worsening of MS symptoms over a 3-5-year period was associated with lower levels of self-reported physical activity independent of neurological disability and MS-disease course (238). Because an active lifestyle reduces these deficits in healthy persons, the impact of aerobic exercise therapy and resistance training on a broad range of functional parameters such as muscle contractile properties, functional mobility and quality of life in MS has been explored during the last decade (51,118,182). To date, it is clear that regular aerobic exercise of moderate intensity does not induce MS exacerbations and improves functioning as well as quality of life (239). Resistance training may also improve contractile characteristics, cellular respiration, quality of life and walking speed and distance that have been reported to be deficient in MS (51,54-56,118,122, 124,126,130,236,237). However, so far reported effects are small and conflicting, the number of intervention studies is rather limited and with the exception of the work of Dalgas et al. who used a 12-week intervention period (130), the applied intervention periods are relatively short (2-8 weeks). Furthermore, a wide variety of exercise interventions ranging from anti-gravity gymnastics to specific resistance training with standardized equipment is used (51). Therefore, it is difficult to compare between studies and to draw solid conclusions (51,118). As suggested by Garret (118), exercise therapy studies in healthy persons and other patient populations use standardized exercise protocols such as provided by the American College of Sports Medicine (ACSM). These protocols apply relative workloads, longer intervention periods and individual training progression (101).

The application of a superimposed electrical current during resistance training may further improve training outcome because this is in contrast to the size principle of voluntary motor unit recruitment, it recruits larger-diameter neurons prior to the smallest (240-242). This was confirmed by Delitto et al. (141) who reported significant isometric strength increase after electrical superimposed stimulation compared to voluntary muscle contraction in post-immobilization patients (n=20). So far, only one study explored the use of electro-stimulation combined with active anti-gravity assisted exercise in MS (143). In this study a significant treatment effect (+26% increase from initial muscle strength) was reported, but there was no control group, so it is difficult to conclude that electrical stimulation in MS

improves rehabilitation outcome.

Chronic mild to moderate stroke patients often have a non-paretic and paretic body side caused by upper motor lesions resulting in asymmetric muscle strength (135,136). Because in a healthy population resistance training induces greater neuromuscular adaptations in weaker versus stronger muscles (134), progressive unilateral resistance training already has been applied in stroke patients to optimize training stimulus. This approach improved both paretic and non-paretic maximal lower limb muscle strength and reduced the stroke-associated functional limitations and overall disability (135,136). As observed by Chung et al. many MS patients also develop asymmetric leg strength (133). However, given the underlying disease mechanisms such as increased central conduction time (137) and reduced motor unit recruitment and firing rates (60), it is unclear if unilateral strength training in MS has similar effects. To the authors' knowledge the reported resistance training studies in MS all use 'classical' bilateral training methods (122,124,130). Unilateral resistance training applying relative workloads to investigate strength gains in weaker versus stronger legs has not been applied in this population yet.

We hypothesized that a 20-week ACSM-based standardized resistance training program increases muscle strength and that unilateral leg training and simultaneous electro-stimulation enhances training efficiency.

2.3.3. Methods

2.3.3.1. Subjects

After being informed of all the experimental procedures to be undertaken, 38 ambulatory community-based Multiple Sclerosis (MS) patients residing in the Hasselt region volunteered (written informed consent) to participate in this study. Exclusion criteria on admission were: (a) >3 relapses in the preceding 1 year or >1.0 EDSS (243) increase in the preceding 1 year, (b) corticoid treatments 28 days before the study start, (c) pregnancy, (d) severe psychiatric disorders and (e) any contra-indication for light to moderately intense physical exercise. Thirty six patients (age 47.8±10.6 yrs) with an EDSS score (4.3±0.2, (243) ranging from 2.0 to 6.5, were included in the study and they were asked to maintain their normal living habits except for the physical exercise training program prescribed by the study protocol and not to participate in any other study (See Table 2.3.1 for a detailed description of the subjects). This study was approved by the Hasselt University Ethics Committee according to the Helsinki declaration.

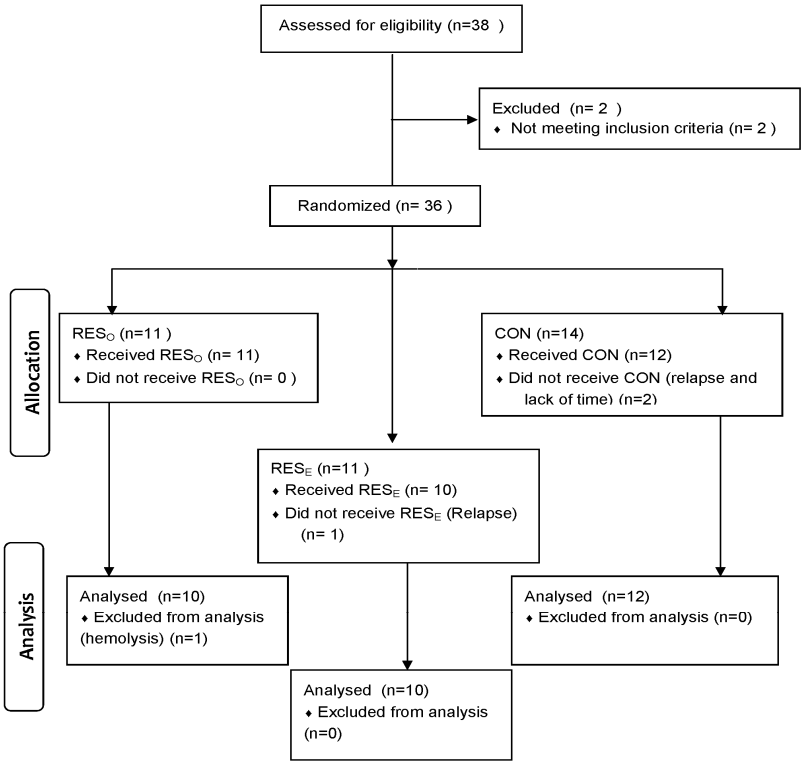

Figure 2.3.1. *Flow diagram of patient eligibility of study 3*

2.3.3.2. Study design

A randomized controlled trial was performed over a 20-week period. Following EDSS determination and study inclusion, baseline (PRE) measurements were performed on 3 separate days, interspersed by at least 48-hours recovery/rest intervals. Measurements involved unilateral skeletal muscle performance testing on an isokinetic dynamometer (day 1, Biodex Medical Systems®, system 3, Inc, Shirley, New York) and evaluation of functional capacity (day 2). Finally, on day 3 routine neurological consultations and registration of perceived fatigue. At baseline, knee-extensor and knee flexor muscle spasticity (Modified Ashworth Scale, MAS, (244) and cognitive functioning (Paced Auditory Serial Addition Task, PASAT, (245) were tested. The latter, to assure adequate cognitive/auditory functioning with respect to the exercise instructions. Following baseline measurements, an independent investigator distributed subjects into groups that, in a decreasing order of importance, were matched for EDSS, age and gender. Hence, 14 subjects were assigned to the control group (CON) and maintained their normal living habits. The remaining subjects were divided in two resistance training groups undergoing ACSM-based resistance training either (RES_E, n=11) or not (RES_o, n=11) in combination with simultaneous electro-stimulation. After the first (MID) and second (POST) training period that consisted of 10 weeks each and at least 72 hours after the last training session baseline measurements were repeated by the same investigator on the same time of the day. The two training periods were separated by a two-week measurement period. The research neurologist that determined the EDSS scores and disease course was blinded. With the exception of the baseline measurements, the other investigators were not blinded. Results were not disclosed to the subjects and investigators until study termination.

Table 2.3.1. Subject characheristics at baseline.

	RES_o	RES_E	CON	p
n	11	11	14	0.18
♀/♂	6 / 5	6 / 5	11 / 3	0.20
EDSS (arbitrary units)	4.5 ± 1.3	4.4 ± 0.9	4.1 ± 1.1	0.72
Age (years)	44.9 ± 11.6	48.7 ± 8.6	49.7 ± 11.3	0.53
Body Weight	70.4 ± 4.2	64.3 ± 3.5	72.1 ± 4.7	0.42
MS Type RR/ SP/ PP	5/3/3	2/4/4	6/3/3	0.81
PASAT (arbitrary units)	41.3 ± 4.8	45.0 ± 3.9	38.8 ± 2.6	0.35
MAS Right (arbitrary units)	0.3 ± 0.1	0.6 ± 0.2	0.5 ± 0.2	0.59
MAS Left (arbitrary units)	0.5 ± 0.3	0.6 ± 0.3	0.6 ± 0.3	0.91
RMI (arbitrary units)	13.7 ± 0.8	13.9 ± 0.4	13.8 ± 0.8	0.69
T25FW (s)	6.2 ± 0.7	5.4 ± 0.4	5.8 ± 0.4	0.89
FR (cm)	31.7 ± 1.5	29.7 ± 1.9	30.5 ± 1.2	0.64
TUG (s)	8.2 ± 0.7	7.1 ± 0.5	7.7 ± 0.5	0.66
2MWT (m)	166.8 ± 8.5	178.2 ± 12.7	165.7 ± 6.9	0.77

Values are numbers or means ± SE and represent baseline subject characteristics (EDSS: Expanded Disability Status Scale; RR: Relaps-Remitting; SP: Secondary Progressive; PP: Primary Progressive; PASAT: Paced Auditory Serial Addition Task; MAS: Modified Ashworth Scale of the right and left quadriceps; RMI: Rivermead Mobility Index; T25FW: timed 25 foot walking test; FR: Functional Reach; TUG: Timed get Up and Go; 2MWT: 2 Minutes Walk Test) of the control (CON) group and the resistance training groups either (RESE) or not (RESo) in combination with simultaneous electro-stimulation. P values represent baseline differences. See Methods for further details.

Resistance training program and simultaneous electro-stimulation

The resistance training protocol consisted of two 10-week training periods and was based on the ACSM guidelines for healthy older adults (101) applying relative workloads to improve muscular fitness. Because standardized strength training guidelines for persons with MS do not exist and elderly also have reduced physical activity levels that can contribute to loss of independence (98,101), this training regimen was chosen to minimize injury risk and maximize training adherence. Throughout the study, subjects were instructed to participate in 5 training sessions (~60min) per fortnight. Each training session started with a standardized warm up on a cycle ergometer (5min, 30watt, 50-70 rpm). Hereafter, RES_o and RES_E subjects performed unilateral leg training (leg-press, leg-extension, leg-curl) on Technogym® resistance training equipment consisting of 1-2 repetition sets ranging from 50% of 1RM to 10 RM that were interspersed by 2-min rest intervals. After the first two training weeks, aiming to familiarize the participating subjects with training procedures, the initial training load was determined by a physiotherapist and hereafter training volume and intensity were gradually increased. As shown in Table 2.3.2., training intensity was light during the first training period. During the second training period a moderate intensity was used. Furthermore, from training session to training

session subjects were instructed to increase the resistance systematically in the following session if they were able to perform the current workload for two or more repetitions over the prescribed number (246). To monitor the average training session workload, subjects were instructed to register the resistance used (kg) in their training diary for the 2 series of each exercise. Each training session was ended by a cool down involving muscle stretching. Throughout the training program, subjects were encouraged and supervised by the same experienced fitness instructors (1/3 therapist - patient ratio). Following each 10-week training period subjects were allowed to compensate for maximum 3 missed training sessions if necessary. To document individual training progression after 3, 10 and 20 weeks of training in RES_o and RES_E on the leg press, leg extension and leg curl unilateral one repetition maximum (1RM) tests were executed. One RM was defined as the heaviest weight that can be lifted only once using good form (101). Briefly, first the subjects performed a light warm-up of five to ten repetitions at 40 to 60% of their perceived maximum. Following a one minute rest period, three to five repetitions at 60 to 80% of the perceived maximum were executed. Then, a small amount of weight was added and a lift was attempted. If the lift was successful a recovery period of three to five minutes was provided. The goal was to find 1RM within three to five maximal efforts (101). One repetition maxima were expressed as the average of the right and left leg.

RES_E patients performed the same training program in combination with simultaneous electro-stimulation. Because subjects trained in a sitting position, standardized/adequate knee flexor electrode fixation was not possible due to sweat production and leg/seat frictions. Therefore electrical stimulation was applied on the m. quadriceps during the leg extension and leg press only. Self adhesive (Dura-stick II Chattanooga Group Inc®; Hixson, USA) electrodes 7x12.7cm were placed on the m. vastus medialis and the proximal m. vastus lateralis. During the first two training weeks subjects were familiarized with simultaneous electro-stimulation by the application of sensorial stimulation (EN-stim 4 Enraf Nonius®; Delft, The Netherlands, constant current at 100Hz, biphasic symmetrical wave, 200µs, 0,5s ramp, 3s hold, 4s rest). Hereafter, electro-stimulation of the knee extensor muscle (EN-stim 4 Enraf Nonius®; Delft, The Netherlands, constant current at 100Hz, biphasic symmetrical wave, 400µs, 3s hold, 4s rest, (247) was applied during resistance training to induce greater muscular activation as shown by Paillard and co-workers (242). We also applied simultaneous electro-stimulation during contractions to standardize concentric and eccentric contraction velocity. To compensate for day to day variation motor stimulation intensities of each training session were set at the threshold at which a subjects' free moving lower leg reached a knee angle of 45°.

Table 2.3.2.Volume and intensity of the resistance training program

Weeks	Goal	Volume	Intensity
-3-0	Baseline testing		
1-2	Familiarization	1x10	Minimal resistance
3-6	increase in intensity	1x10	50-60% 1RM
7-8	increase in volume	2x10	60% 1RM
9-10	increase in volume	2x12	60% 1RM
2 weeks	MID testing		
11		2x12	60%1RM
12-14	increase in volume	2x15	15RM
15-17	increase in intensity	2x12	12RM
18-20	increase in intensity	2x10	10RM
2 weeks	POST testing		

2.3.3.3. Muscle strength tests

Dynamometry. Maximal voluntary unilateral knee-extensor and knee-flexor strength was evaluated on an isokinetic dynamometer (Biodex Medical Systems®, system 3, Inc, Shirley, New York). After a 5-min standardized warm-up on a quadriceps bench, left and right side unilateral strength tests were performed in a semi-supine (5°) sitting position. The rotational axis of the dynamometer was aligned with the transverse knee-joint axis and connected to the distal end of tibia by means of a length adjustable rigid lever arm. The upper legs, hips and shoulders were stabilized with safety belts. To evaluate maximal isometric muscle strength, subjects performed two maximal isometric knee extensions (3s) and flexions (3s) at knee angles of 45° and 90° following one sub maximal trial contraction. Maximal contractions were interspersed by 90-s rest intervals. The highest isometric extension and flexion torques (Nm) of the manually smoothed curves at each knee angle were selected as maximal isometric peak torque. To measure maximal isokinetic muscle strength subjects performed four maximal consecutive isokinetic knee-extensions at a velocity of 60°/s after three sub maximal trial contractions. The knee extensions were initiated at a joint angle of 90° to an angle of 160°. Following each extension the leg was returned passively to the starting position from which the next contraction was immediately initiated. Hereafter, the highest of four isokinetic extension torques (Nm) was selected as maximal isokinetic (60°/s) torque. Finally, and again following three sub maximal trial contractions, subjects performed twenty maximal isokinetic knee extensions at a velocity of 180°/s to assess muscle strength endurance. The knee extensions were initiated at a joint angle of 90° to an angle of 160°. Following each extension the leg was returned passively to the starting position from which the next contraction was immediately initiated. To determine muscle strength endurance, the average work (J) of the first three and last three contractions were compared and work fatigue, expressed as a percentage decrease, was

calculated. Torque was measured during each contraction at a sampling rate of 100Hz and muscle strength was expressed as the average of the right and left leg.

2.3.3.4. Functional mobility

The overall functional mobility was assessed using a variety of functional mobility tests such as the Timed Up and Go (TUG, 248), the Timed 25 Foot Walk (T25FW, 249), the Two Minutes Walk Test (2MWT, 250), the Functional Reach (FR, 251) and the Rivermead Mobility Index (RMI, 252), which is a self reported scale.

2.3.3.5. Statistical analyses

Statistical analyses were performed using SAS® software (Version 9.2; SAS Institute, Inc., Cary, NC). Normal distribution was checked using the Shapiro-Wilk test. Baseline differences between groups were analyzed using one-way ANOVA's. Training effects on muscle strength and functional mobility were analyzed by means of a 3x3 ([CON, RES_o, RES_E] x [PRE, MID, POST]) mixed model ANOVA. One repetition maxima and unilateral training session workloads were analyzed using 2x3 ([RES_o, RES_E] x [week3, week10, week20]) mixed model ANOVA and a student t-test, respectively. When appropriate, pre-planned post hoc contrast analyses were used to determine time or group effects.

To explore the impact of resistance training on mild and severely impaired muscle strength, unilateral isometric and isokinetic peak torque data were used. To distinguish between impairment levels, pooled RES_o and RES_E legs were divided into mildly (RES_{Mi}) and severely (RES_s) affected legs. Leg impairment levels were calculated based on the mean of each isometric and isokinetic baseline value of RES_o and RES_E subjects (mild: ≥ average; severe: < average). Hereafter, a 2X3 (group [RES_{mi}, RES_s] x 3 [PRE, MID, POST]) mixed model ANOVA and pre-planned post hoc contrast analysis were used to determine time and group effects when appropriate. Relative changes during the training period were calculated as the mean of the individual percentages. Post hoc power calculations were performed using G power 3 software (253). The level of statistical significance was set at $p<0.05$ and data are presented as mean ± SE.

2.3.4. Results

2.3.4.1. Drop out, compliance and training load

During the study, two CON and one RES_E patients retreated due to either a severe relapse, perceived lack of time to continue the study and a mild stroke unrelated to this study, respectively. In total, 1050 resistance training sessions were planned. Twelve of which were not executed resulting in a ~99% compliance. Training progression was evaluated by *One repetition maximum (1RM) at 3, 10 and 20 weeks and training load registration of each training session.* Throughout the study period no differences between RES_o and RES_E (group x time) were found in 1RM. However, as indicated in Table 2.3.3, 1RM gradually increased in both intervention groups. During the first 10 training weeks, RES_o and RES_E weights increased with 18±10% and 33±16% (leg press), 14±6% and 17±5% (leg extension) and 6±8% and 16±4% (leg curl), respectively. Compared to baseline, 10 and 20 weeks of training increased 1RM weights by 50±8% and 51±16% (leg press), 32±4% and 39±12% (leg extension) and 38±9% and 46±6% (leg curl) in RES_o and RES_E, respectively. Furthermore, unilateral training load registration (data not shown) indicated that the average training session work load of RES_E (leg press: 29.4±0.8kg, leg extension: 13.0±0.3kg) subjects was significantly ($p<0.05$) higher compared to RES_o (leg press: 23.4±0.6kg, leg extension: 12.0±0.2kg). Leg curl training work load (RES_E: 12.6±0.3kg, RES_o: 12.3±0.2kg) did not differ between groups.

Table 2.3.3. One repetition maxima (1RM in Kg).

	week	RES_o (n=9)	RES_E (n=9)
Leg Press	3	30.6 ± 4.3	48.2 ± 9.0
	10	35.7 ± 6.0	57.2 ± 7.2
	20	44.8 ± 5.5 †,‡	65.6 ± 7.4 †,‡
Leg Ext	3	19.3 ± 2.7	21.4 ± 2.8
	10	21.6 ± 3.2 †	23.6 ± 2.4 †
	20	25.2 ± 3.1 †,‡	27.9 ± 2.6 †,‡
Leg Curl	3	16.9 ± 2.0	17.9 ± 2.2
	10	18.1 ± 2.5	21.0 ± 2.8 †
	20	22.2 ± 2.0 †,‡	25.7 ± 2.7 †,‡

Values are numbers or means ± SE and represent 1RM the resistance training groups either (RES_E) or not (RES_o) in combination with simultaneous electro-stimulation after 3, 10 and 20 weeks of training. P values represent † P < 0.05 compared to the corresponding baseline value, ‡ P < 0.05 POST compared to MID value. See Methods for further details.

2.3.4.2. Muscle strength

Maximal isometric muscle strength. As indicated in Figure 2.3.2 and Table 2.3.4., maximal isometric muscle strength did not differ ($p>0.05$) between groups at baseline and significant interaction effects (group x time) were found. Whereas CON knee-extensor and knee-flexor maximal isometric peak torque remained stable or decreased ($p<0.05$) throughout the study period, isometric muscle strength increased in RES_o and RES_E. At a knee angle of 90° post hoc contrast analysis in RES_o indicated a higher isometric knee-extension torque compared to CON following 10 and 20 weeks of resistance training. At knee angles of 45° and 90° knee flexion torques following 20 weeks of RES_E were 17% higher compared to CON. Within group effects are indicated in Table 2.3.4.

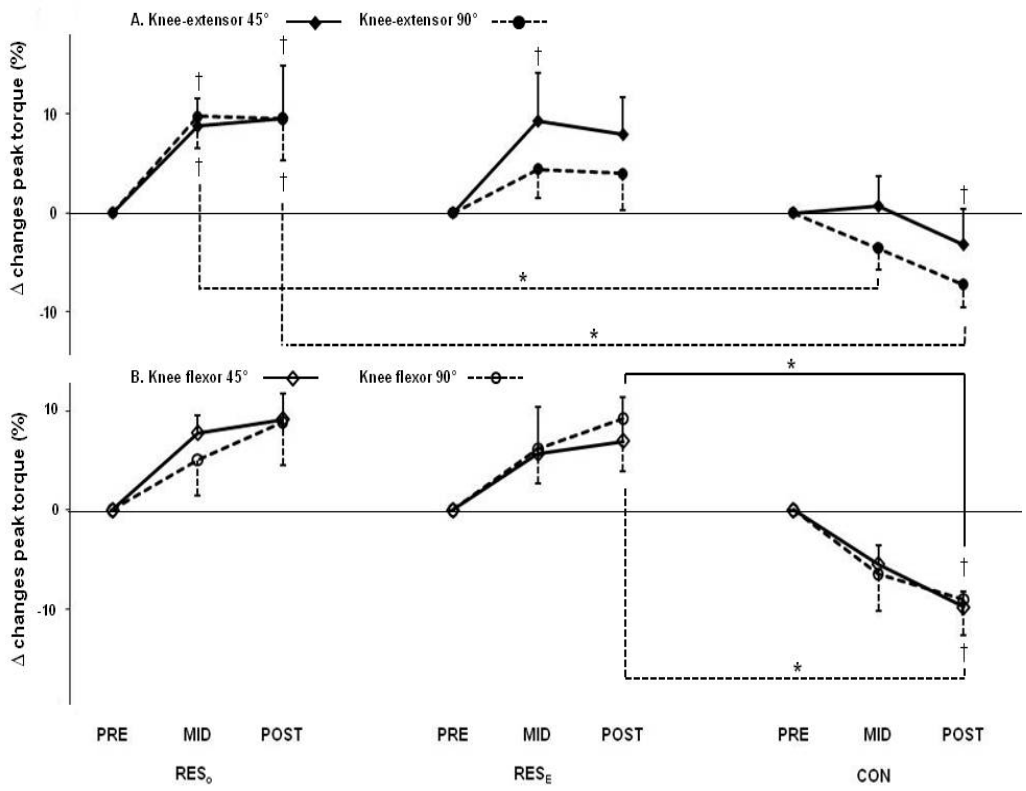

Figure 2.3.2. *Peak torque changes (%) of maximal isometric knee-extensor (A) and knee flexor (B) strength at a knee angle of 45° and 90° following 10 (MID) and 20 (POST) weeks of control conditions (CON) or resistance training either (RES_E) or not (RES_o) in combination with simultaneous electro-stimulation. † P < 0.05 compared to the corresponding baseline value and * p < 0.05 compared to the corresponding CON value. See Methods for further details.*

Maximal isokinetic muscle strength & strength endurance. At baseline, all muscle strength variables did not differ (p>0.05) between groups. Furthermore, no significant interaction effect (group x time) was found (Table 2.3.4.).

Table 2.3.4. Muscle contractile data.

ISOMETRIC (Nm)			p	RES_o (n=11)	RES_E (n=10)	CON (n=12)
Extensor	45	PRE		128.1 ± 12,4	116.8 ± 10.6	97.7 ± 9.5
		MID	0.19	138.8 ± 13.5 †	124.1 ± 8.5 †	97.0 ± 9.2
		POST		138.9 ± 12.8 †	123.5 ± 9.0	93.0 ± 9.1
	90	PRE		107.5 ± 9.1	103.8 ± 9.9	93.3 ± 9.8
		MID	0.01	118.1 ± 11.0 †,‡	107.2 ± 9.3	90.8 ± 11.0
		POST		117.5 ± 10.4 †,‡	106.3 ± 8.7	86.9 ± 9.6 †
Flexor	45	PRE		56.3 ± 6.2	59.0 ± 6.6	49.9 ± 5.0
		MID	0.03	60.0 ± 6.6	62.1 ± 7.3	46.3 ± 5.0
		POST		60.3 ± 6.3	62.7 ± 7.1 ‡	44.6 ± 4.5 †
	90	PRE		44.6 ± 5.2	49.2 ± 5.9	41.7 ± 3.5
		MID	0.04	46.9 ± 5.6	52.2 ± 6.4	39.1 ± 4.0
		POST		46.9 ± 5.1	53.2 ± 6.2 ‡	37.8 ± 3.1 †
ISOKINETIC (Nm)						
Extensor 60°/s		PRE		101.3 ± 10.6	94.1 ± 10.3	87.9 ± 11.5
		MID	0.16	110.6 ± 11.3 †	98.2 ± 9.9	87.9 ± 12.6
		POST		116.6 ± 12.4 †	105.0 ± 8.9 †	87.6 ± 11.6
ENDURANCE (%)						
Work fatigue Extensor 180°/s		PRE		32.7 ± 4.1	25.3 ± 2.8	31.6 ± 4.0
		MID	0.23	27.5 ± 4.6 †	23.7 ± 3.2 †	30.5 ± 2.7 †
		POST		28.5 ± 4.2 †	21.5 ± 2.5 †	27.5 ± 3.7 †

Values are means ± SE and represent peak knee extensor and flexor isometric (ISOM) and isokinetic (ISOK) torque (Nm) and work fatigue (ENDURANCE) of the first compared to the last 3 of a bout of 20 maximal isokinetic (180°/s) knee extensors concentric muscle contractions calculated in terms of percentage (%)before (PRE) and following 10 (MID) and 20 (POST) weeks of control conditions (CON) or guided exercise training either (RES_E) or not (RES_o) in combination with simultaneous electro-stimulation. P values represent time x group interaction effects † P < 0.05 compared to the corresponding baseline value, * p < 0.05 compared to the corresponding CON value. See Methods for further details.

Unilateral strength analysis. To explore the impact of resistance training on mild and severely impaired muscle strength, unilateral isometric and isokinetic peak torque data were evaluated. As indicated, RES_{Mi} baseline isometric knee-extensor (45°:+67%; 90°: +71%), flexor (45°:+91%; 90°: +90%) and isokinetic (+103%) muscle strength is significantly higher compared to RES_S. Whereas isometric knee extensor and flexor strengths were not different (interaction effects ranged from p=0.08 to p=0.15) between RES_{Mi} and RES_S, isokinetic knee extensor strength increased (p<0.05) with 53±18% and 66±19% and following 10 and 20 weeks in RES_S (Table 2.3.5.).

Table 2.3.5. Severely and mildly impaired leg strength progression.

ISOMETRIC (Nm)			n	RES_S	n	RES_{Mi}	p
Extensor	45°	PRE*	20	90.8 ± 4.4	22	151.8 ± 5.5	0.11
		MID		100.8 ± 4.5		160.1 ± 6.4	
		POST		102.0 ± 6.0		158.6 ± 5.7	
	90°	PRE*	21	78.2 ± 3.2	21	133.4 ± 4.4	0.15
		MID		87.8 ± 4.7		138.0 ± 6.0	
		POST		87.5 ± 4.8		136.9 ± 5.4	
Flexor	45°	PRE*	22	40.8 ± 2.3	20	78.0 ± 2.8	0.09
		MID		44.5 ± 2.7		81.0 ± 3.5	
		POST		46.5 ± 3.1		79.6 ± 3.5	
	90°	PRE*	21	32.3 ± 3.0	21	61.4 ± 2.5	0.08
		MID		34.7 ± 3.1		64.2 ± 2.8	
		POST		37.1 ± 3.4		62.8 ± 3.2	
ISOKINETIC (Nm)							
Extensor 60°/s		PRE*		65.5 ± 4.0		133.4 ± 4.8	0.006
		MID		91.2 ± 6.8 †		119.1 ± 8.1	
		POST		98.5 ± 7.9 †		125.1 ± 8.4	

*Values are means ± SE and represent peak knee-extensor and flexor isometric and isokinetic torque (Nm) of severely (RES_S) and mildly impaired leg (RES_{MI}). Measurements were performed before (PRE) and following 10 (MID) and 20 (POST) weeks of guided resistance training. p-values represent time x group interaction effects, * represent baseline difference ($p<0.05$) and † $p<0.05$ compared to corresponding baseline value. See Methods for further details*

2.3.4.3. Functional mobility

At baseline, functional mobility did not differ ($p>0.05$) between groups. In RES_o post hoc contrast analysis showed increased ($p<0.05$, group x time) reaching distance (5.9±1.9 cm) compared to CON following 10 weeks of training. The 2MWT, T25FW, TUG and RMI were not significantly changed in any group (data not shown).

2.3.5. Discussion

Reduced muscle strength and functional mobility are commonly present in MS (76). Recently it has been observed that short (122,124,129) and longer-term higher-intensity (130) resistance training programs can improve muscle strength. Based on the existing literature, however, the optimal training duration (are effects more pronounced after long-term training?) and intensity (is low intensity training also effective?) remain unclear. Here, a standardized 20-week low to moderately intense unilateral resistance training program improved isometric leg muscle strength with 7±4 to 10±4% and the present data suggest that unilateral training may have the potential to improve muscle strength even in severely affected legs. Simultaneous electro-stimulation did not have additional effects while functional mobility did not improve in any of the patient groups.

The low to moderately intense resistance training regimen applied in the present study, improved maximal isometric and isokinetic knee extension torque after 10 weeks on average with 9±3%. Consistent with our findings, White et al. (122) demonstrated comparable knee extensor strength gains (+7%) after an 8-week ACSM-based resistance training protocol. In the present study dynamometric muscle data are paralleled by the obtained 1RM results during the training period. Compared to baseline, 1RM at 10 weeks increased ($p<0.05$; within group effect, Table 2.3.3.) during leg press, leg extension and leg curl exercises. During the second study phase, exercise intensity/volume was augmented (Table 2.3.2.). Hence, it is generally accepted that strength further increases (101). However, whereas in the control group, maximal isometric knee extensor and flexor muscle strength decreased with 3-9% during the second study period, the obtained strength gains following the first 10 training weeks remained stable in the experimental groups during the latter period (Table 2.3.4.). To the authors' knowledge, this is the first training study in MS that applied an intermediary measurement session. It is however unclear whether this observation suggests that patients may reach a training plateau as reported in healthy individuals (254) and suggested by Petajan (255) or that an MS-specific mechanism prevents further improvements as suggested previously by Garner (55).

Notwithstanding the fact that in the present study and in the comparable work of White et al. (122) muscle strength increased significantly, it must be noted that effects are small. Training progressions usually obtained in healthy older or mid-age populations, using similar resistance training programs, range from 15 to 30% (254,256) or from 10-25% in neuromuscular disorders such as spinal muscular dystrophy (257) and post polio (258). Applying a 12-week moderate to high-intensity training protocol, Dalgas et al demonstrated a ~16% increase in maximal voluntary knee-extensor strength in persons with MS (130). The applied training protocol in the current study, however, was designed to maximize training

adherence (~99% compliance) and to minimize drop-out (e.g. 3 drop-outs versus 7 in (130). Similar to White et al. (122), the low to moderately intense training regimen of the present study might have been an insufficient training stimulus to generate larger strength gains. On the one hand, this may be caused by the fact that the magnitude of muscle fiber atrophy in MS subjects is similar (55) or more profound (57) compared to healthy subjects. Hence, a longer rehabilitation period is required. On the other hand, chronically reduced maximal discharge rates (60), delayed transmission velocity (259) and altered or incomplete motor unit activation (56) may have reduced exercise efficiency.

In an attempt to improve resistance training efficiency in MS, we applied simultaneous peripheral electro-stimulation (RES_E) during knee-extensor exercises. As indicated above the average training session load of RES_E was higher (leg press: +26% and leg extension: +8%) compared to RES_o. However, this did not further enhance dynamometric strength in RES_E. Our results match with Paillard and co-workers (242) who, in a review, concluded that superimposed electrical stimulations in a healthy population did not improve training efficiency compared to volitional exercise only. Despite the absence of additional training effects, the applied simultaneous electro-stimulation might be an interesting strategy to assist persons with MS, that often experience cognitive deficits (260), during resistance training. Patients are guided through their strength exercise because contraction velocity as well as the number of executions were indicated on the electrical-stimulation apparatus (personal communications of participants).

Because in many cases MS is characterized with asymmetric leg strength (133), an unilateral strength training regimen was applied. We hypothesized that, compared to bilateral training, training could be optimized by virtue of unilateral relative training loads. The present data (interaction effects ranged from $p=0.006$ to $p=0.15$) suggest that unilateral training may have the potential to improve muscle strength even in severely affected legs (Table 2.3.5.). Clearly, given the fact that the present data are partially significant, this requires further research. However, this new finding is clinically important as it may indicate that weak muscles still have training potential and that muscular adaptations following resistance training in MS are, at least in part, independent of the severity of muscle weakness. This is probably disuse associated but the possible activation of other mechanisms such as changes in cortical mapping following exercise therapy might be worthwhile to investigate.

Muscle strength has been defined as an important predictor of ambulatory function (161,261-263). Given the reported muscle strength gains, improved functional mobility and gait kinematics could be assumed after resistance training as indicated by Seguin in normal healthy subjects (256) and Gutierrez in persons with MS (126). With the exception of functional reach, however, none of the functional mobility measures improved in our MS patient samples. Our results mirror data from White and

co-workers (122) who were unable to detect improved walking speed following 8 weeks of regular resistance training in persons with MS. This could be related to the size of the strength improvement which was lower in the present study, as compared to previous studies reported (124,125,130). This could also be explained by the fact that throughout the study course, participants did not specifically train functional mobility suggesting the need for more specific training and testing (264). Interestingly, in the present study, functional reach improved. This could indicate enhanced lower back or hamstring flexibility following stretching during the cool down of each training session. Because functional reaching involves active hip and knee muscle control this might also be a direct effect of the applied exercise regimen. Given the former precautionary stance towards exercise and MS it is important to note that the applied strength training regimen did not induce spasticity, while no deterioration of the EDSS score (data not shown) was found in any of the experimental groups.

The present study also contains limitations. First, the relative small sample sizes reduced statistical power. Post-hoc power analysis of the main training results using the actual instead of the literature-based effect size, reached a power of 70.3%. However, power analysis using a larger (+15%), literature-based, effect size would have reached a power of 83.4% (265) and post hoc unilateral leg strength analysis already reached a power of 86.4% (265). Another limitation was investigator blinding, which was only the case for the research neurologist and all the investigators during baseline measurements. As such, it could be argued that the investigators were biased and may have influenced the results. Although this limitation is acknowledged, it is also apparent that only modest gains in muscle strength and no gains in functional mobility were reported, so an overestimation of the effects is rather unlikely. Furthermore, subjects were randomized based on EDSS, gender and age and not on baseline muscle strength because it was assumed that similar disability levels would render similar lower limb muscle strength. In fact, mean baseline muscle strength between groups ranged from 98±10 Nm (CON) to 128±12 Nm (RES_o) and groups did not differ significantly.

In conclusion, the current study shows that long-term individualized low to moderately intense resistance training improves muscle strength. Furthermore, unilateral strength training may increase training efficiency in severely impaired legs. Simultaneous electro-stimulation did not have an additional effect and the applied training regimen did not improve functional mobility. These effects were observed after the first 10-week training period and remained stable during the second training period of 10 weeks.

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STUDY 4

Exploring the effects of a 20-week whole body vibration training program on leg muscle performance and function in persons with multiple sclerosis.

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2.4.1. Abstract

Objective. To investigate the acute effects of long-term whole-body vibration on leg muscle performance and functional capacity in persons with multiple sclerosis.

Design. A randomized controlled trial.

Subjects. Twenty-five patients with Multiple Sclerosis (mean age 47.9±1.9yrs; Expanded Disability Status Scale 4.3±0.2) were assigned randomly to whole body vibration training (WBV, n=11) or a control (CON, n=14) group.

Methods. The whole-body vibration group performed static and dynamic leg squats and lunges on a vibration platform (25-45Hz, 2.5mm amplitude) during a 20-week training period (5 training sessions per 2-week cycle), and the control group maintained their usual lifestyle. PRE-, MID- (10wks) and POST (20wks) knee-muscle maximal isometric and dynamic strength, strength endurance and speed of movement were measured using isokinetic dynamometry. Function was determined through the Berg Balance Scale, Timed Up and Go, Two-Minutes Walk and the Timed Twenty-five Foot Walk tests.

Results. Leg muscle performance and functional capacity were not altered following 10 or 20 weeks of WBV.

Conclusion. Under the conditions of the present study, the applied 20-week WBV exercise protocol did not improve leg muscle performance or functional capacity in mild- to moderately impaired persons with MS during and immediately after the training program.

2.4.2. Introduction

Multiple Sclerosis (MS) is a progressive inflammatory and degenerative disease of the central nervous system (2). The most prevalent symptoms are sensory changes, fatigue, balance disorders, spasticity, motor weakness and impaired muscular performance (1,266). As a result, many persons with MS exhibit poor functional capacity and reduced quality of life (59). A considerable part of these symptoms may also result from a disease-related inactive lifestyle which is, at least in part, reversible through physical exercise (76). Contrary to earlier beliefs, aerobic exercise and resistance training to date have been shown to be well tolerated and able to induce beneficial effects in MS patients (51,266). Depending on the degree of disability, however, MS patients may experience difficulties in performing conventional aerobic exercises and resistance training.

Because positive training results in various sports, fitness and geriatric rehabilitation have been demonstrated (144) and because the unloaded static and/or dynamic exercises performed usually require a limited active range of motion, Whole Body Vibration (WBV) exercise may be an interesting alternative exercise mode for upper motor neuron disorders. During WBV the vertical platform vibrations induce involuntary muscle contractions that are initiated by sensory receptors and reduce the recruitment threshold of motor units (146). This probably results in a more rapid activation of high-threshold fast twitch muscle fibers (267,268). In keeping with the above suggested WBV working mechanism, it has already been tested in stroke (152) and cerebral palsy (153). Because MS is, amongst others, associated with an impaired ability to fully activate motor units during voluntary contractions, decreased maximal motor unit firing rates and reduced average muscle fiber cross sectional area (57,60,63), WBV exercise may be a promising strength training method specifically for people with MS. Hence, to date, vibration platforms are increasingly used in MS rehabilitation centers and clinical practices although supporting evidence is still lacking. Minor acute effects on postural control and functional mobility and a trend towards a higher leg muscle peak torque after one single WBV session in MS patients have been reported (154,155), but effects after longer-term training remain largely unexplored. To our knowledge, so far the latter has only been investigated by Schyns and co-workers (156), who recently performed a longer-term 4-week randomized cross-over pilot study in 16 people with MS. They reported that additional WBV training had no additional benefits on muscle strength and functional performance compared to exercise alone, however, patients reported less spasms (156). Four weeks of WBV exercise may have been too short to, on the one hand, induce neural muscle adaptations leading to increased muscle performance and functionality, and on the other hand, progressively increase training volume and training intensity to create an adequate training overload during the intervention period. Furthermore, Schyns et al (156) investigated the

additional effects of WBV compared to the performance of exercises alone, which may obscure minimal effects induced by the vibration to some extent. The effect of WBV compared to no intervention remains unknown.

For above-mentioned reasons and because long-term WBV in sedentary healthy people exerted marked improvements in a wide variety of muscle strength and functional parameters (269-271), the present study investigated the effects of a 20-week WBV training program on leg performance and overall functional capacity in mild- to moderately impaired MS patients.

2.4.3. Methods

2.4.3.1. Subjects

After being informed of all the experimental procedures to be undertaken 29 ambulatory community-based Multiple Sclerosis (MS) patients residing in the Hasselt region volunteered (written informed consent) to participate in this study (see Figure 1). Exclusion criteria on admission were: (a) >3 relapses in the preceding 1 year or >1.0 Expanded Disability Status Scale (EDSS) increase in the preceding 1 year, (b) corticoidsteroids treatments 28 days before the study start, (c) pregnancy, (d) severe psychiatric disorders, (e) internal fracture materials and/or (total) joint replacements and (f) any contra-indication for light to moderately intense physical exercise. After the first screening 25 patients (age 47.9 ±1.9 yrs) with an EDSS score (4.3±0.2, 243) ranging from 1.5 to 6.5 were included in the study and they were asked to maintain their normal living habits and not to participate in any other study. See Table I for a detailed description of the subjects. This study was approved by the Hasselt University Ethics Committee according to the Helsinki declaration.

2.4.3.2. Study design

A randomized controlled trial was performed over a 20-week period. Following study inclusion the baseline (PRE) measurements were performed on 3 separate days, interspersed by at least 48-hours recovery/rest intervals. Measurements involved unilateral skeletal muscle performance testing on an isokinetic dynamometer (day 1, Biodex Medical Systems®, system 3, Inc, Shirley, New York), evaluation of functional capacity (day 2) and routine neurological consultations and registration of perceived fatigue (day 3). At baseline (day 3) only, m. quadriceps spasticity was assessed by the *Modified Ashworth Scale (MAS,* 244) and cognitive functioning was determined by the *Paced Auditory Serial Addition Task (PASAT, 245)* test. Following baseline measurements and to ascertain equality between groups, subjects were coupled into pairs that, in a decreasing order of importance, matched for EDSS, age and gender by an independent investigator. Hence, 14 subjects were assigned to the control group (CON; EDSS: 4.1±0.3, age: 49.7±3.3 yrs, ♀: n=11, ♂: n=3, Table 2.4.1.) and were instructed to maintain their normal living habits. The remaining 11 subjects (EDSS: 4.5±0.4, age: 46±2.1 yrs, ♀: n=7, ♂: n=4, Table 2.4.1.) underwent a Whole Body Vibration (WBV) exercise program. Between group baseline characteristics (Table 1) were not statistically different. Following 10 (MID) and 20 (POST) weeks and at least 72 hours after the last training session, baseline measurements were repeated by the same investigator on the same time of the day in both groups. As such, at MID a 12-day training break interspersed the first and last 10 weeks. With the exception of the

neurologist that determined the EDSS scores, the investigators were not blinded. The results were not disclosed to the subjects and investigators until study termination.

Table 2.4.1. Subject characteristics

	WBV	CON	p
n	11	14	0.83
♀/♂	7 / 4	11 / 3	0.29
MS Type: RR/SP/PP	6/4/1	8/4/2	0.61
EDSS (arbitrary units)	4.5 ± 0.4	4.1 ± 0.3	0.44
Age (y)	46.1 ± 2.1	49.7 ± 3.3	0.36
PASAT (arbitrary units)	44.5 ± 4.5	38.8 ± 2.6	0.29
MAS (arbitrary units)	0.5 ± 0.4	0.5 ± 0.3	0.91

Values are numbers or means ± SE and represent baseline subject characteristics (RR: Relapsing-Remitting; SP: Secondary-Progressive; PP: Primary-Progressive; EDSS: Expanded Disability Status Scale; PASAT: Paced Auditory Serial Addition Task; MAS: Modified Ashworth Scale of the quadriceps muscle) of the control (CON) and the Whole Body Vibration (WBV) training group. p Values represent baseline differences. See Methods for further details.

2.4.3.3. Whole body vibration

The WBV group performed a leg muscle training program consisting of exercises which were executed statically (week 1-20) or dynamically (week 11-20) on a vibration platform (Alpha Vibe® Nijverdal, the Netherlands). This platform was equipped with a supportive horizontal bar/handle offering bilateral standing assistance to MS patients with a high EDSS score. The acceleration of the mainly vertical vibration platform, recorded by means of an accelerometer (ADXL202-SER, Analog Devices, Norwood, USA), was 2.32g and 2.71g at 20 Hz and 40 Hz respectively. The training program included high (knee angle between 120°-130°) and deep squats (knee angle 90°), wide stance squats, lunges and heel rises. These exercises were executed in an 'unloaded' (i.e. without the use of external weights) standing posture. According to the overload principle training volume and intensity was increased systematically over the 20-week training period by increasing the duration and the number of exercise series or the number of different exercises by shortening the rest periods, varying the applied vibration frequency (25 – 40 Hz) and changing the execution form of the exercises from predominantly two-legged to one-legged exercises. See Table 2.4.2. for a detailed description. Between exercises, subjects were allowed to recover (chair sit). The WBV exercise protocol described above was identical to the training protocol for healthy adults previously used by Roelants et al. (269,270). Each WBV exercise session lasted maximal 50 minutes including warming-up performed on a cycle ergometer (Kettler®, 5

min 30 watt, 50-70 rpm, Ense-Parsit, Germany) and cooling-down that involved stretching of the major lower limb muscle groups. Perceived overall and leg fatigue was assessed using a visual analog scale (VAS; range 0 – 10 cm; 0 cm = extremely exhausted; 10 cm = no fatigue) before and after each training session (272). Furthermore subjects completed the BORG scale (range 6 – 20) after each session in their training diary (273). The qualified trainer to subject ratio was 1 to 2.

Table 2.4.2. Training volume and intensity of the whole body vibration program.

	Start	Week 10	Week 20
Volume			
Total duration of vibration in one session (min)	2.5	8.0	16.5
Series of one exercise (N)	1	3	3
Different knee-extensor exercises (N)	2	4	5
Longest duration of vibration loading without rest(s)	30	45	60
Intensity			
Rest period between exercises (s)	120	120	30
Vibration amplitude (mm)	2.5	2.5	2.5
Vibration frequency (Hz)	20	35	45

2.4.3.4. Muscle performance

Dynamometry. Maximal voluntary unilateral knee-extensor and knee flexor strength of the right leg was evaluated on an isokinetic dynamometer (Biodex Medical Systems®, system 3, Inc, Shirley, New York). After a 5-min standardized warm-up on a quadriceps bench, right side unilateral strength tests were performed in a seated position on a backward inclined (5°) chair. The rotational axis of the dynamometer was aligned with the transverse knee-joint axis and connected to distal end of tibia by means of a length adjustable lever arm. The upper leg, hips and shoulders were stabilized with safety belts. The 3-dimensional positions of the rotation axis, position of the chair and length of the lever arm were identical in PRE-, MID- and POST-tests (274,58,130).

Maximal isometric torque. Following one submaximal trial contraction, two maximal isometric knee-extensions and flexions (3s) at knee angles of 45° and 90° were performed. Maximal contractions were interspersed by 90-s rest intervals. The highest isometric extension and flexion torques (Nm) of the manually smoothed curves at each knee angle were selected as maximal isometric torque.

Maximal dynamic torque. Subjects performed four maximal consecutive isokinetic knee-extensions at a velocity of 60°/s after three

submaximal trial contractions. Knee extensions were initiated at a joint angle of 90° to an angle of 160°. Following each extension the leg was returned passively to the starting position from which the next contraction was immediately initiated. Hereafter, the highest of four isokinetic extension torques (Nm) was selected as maximal dynamic torque.

Maximal strength endurance. Following three submaximal trial contractions, subjects performed twenty maximal dynamic knee extensions at a velocity of 180°/s to assess strength endurance. Knee extensions were initiated at a joint angle of 90° to an angle of 160°. Following each extension the leg was returned passively to the starting position from which the next contraction was immediately initiated. To determine muscle strength endurance, the average work (J) of the first six contractions was compared to the last six contractions and expressed as a percentage decrease.

Maximal speed of movement of knee extension. The subjects performed four tests. Subjects were asked to extend their lower leg four times at the highest speed possible from a knee joint angle of 90° to an angle of 160°. Hereby the individual degree of resistance on the lever arm was determined at 1, 20, 40 and 60 percent of the maximal isometric peak torque of the knee extensors at a knee angle of 90°. At each test maximal velocity of the lever arm (°/s) was recorded to determine speed of movement.

2.4.3.5. Functional capacity

Functional capacity was measured using a variety of different tests.

- *Berg Balance Scale (BBS).* This performance-based measure of balance consists of 14 observable tasks involving functional balance control, transfers, turning and stepping. Scoring is based on the patients' ability to perform the tasks independently within certain time and disturbance requirements (275).
- *Timed get Up and Go (TUG).* Here, the time (s) needed to get up off a chair without armrest, walk 3m, turn back to the chair and finally sit down again, was recorded (248).
- *The 2 minutes walk test (2MWT).* Subjects were asked to walk as far (m) as possible within 2 minutes, back and forth along a 30 m long stretch. Subjects were allowed to rest during the 2-minutes time period and use assistive devices. Standardized verbal encouragements were given every 30s (250).
- *The 25 Foot Walk test (T25FW).* Subjects were asked to walk on a demarked 25-foot course (7,62m) as quickly but safely as possible. Assistive devices were permitted if necessary (249).

2.4.3.6. Statistical Analysis

All analyses were executed using SAS 9.2 for windows (SAS Institute Inc, Cary, USA). First normal data distribution was checked using the Shapiro-Wilk test and then baseline differences of all dependent variables between groups were assessed using one-way ANOVA or Kruskal-Wallis test. Hereafter changes in muscle performance, speed of movement, and functional capacity (dependent variables) in WBV and CON (independent variables) were analyzed after 10 and 20 weeks. Statistical analysis were performed with an ANOVA (General Linear Model, GLM) for repeated measures: (2 [group] x 3 [time]) for isometric strength, dynamic strength, strength endurance and functional capacity; (2 [group] x 3 [time] x 4 [resistance]) for speed of movement. The Friedman test was used to analyze changes in the modified Ashworth scale. VAS and BORG data of each training session of the WBV group were summarized for 5 training periods (period 1: week 1-4 , period 2: week 5-8, period 3: week 9-12, period 4: week 13-16 and period 5: week 17-20) and an ANOVA (GLM) for repeated measures was used to evaluate training exertion and when appropriate Tukey-Kramer post-hoc analyses were performed. All values are reported as means ± standard error (SE). Significance level was set on $p < .05$.

2.4.4. Results

2.4.4.1. Training compliance and side effects

Training compliance (Figure 2.4.1.). In total 23 of the 25 subjects completed the study. Two CON patients retreated before study termination due to a severe relapse or perceived lack of time to continue the study measurements. In total 537 of the 550 planned WBV exercise sessions were performed.

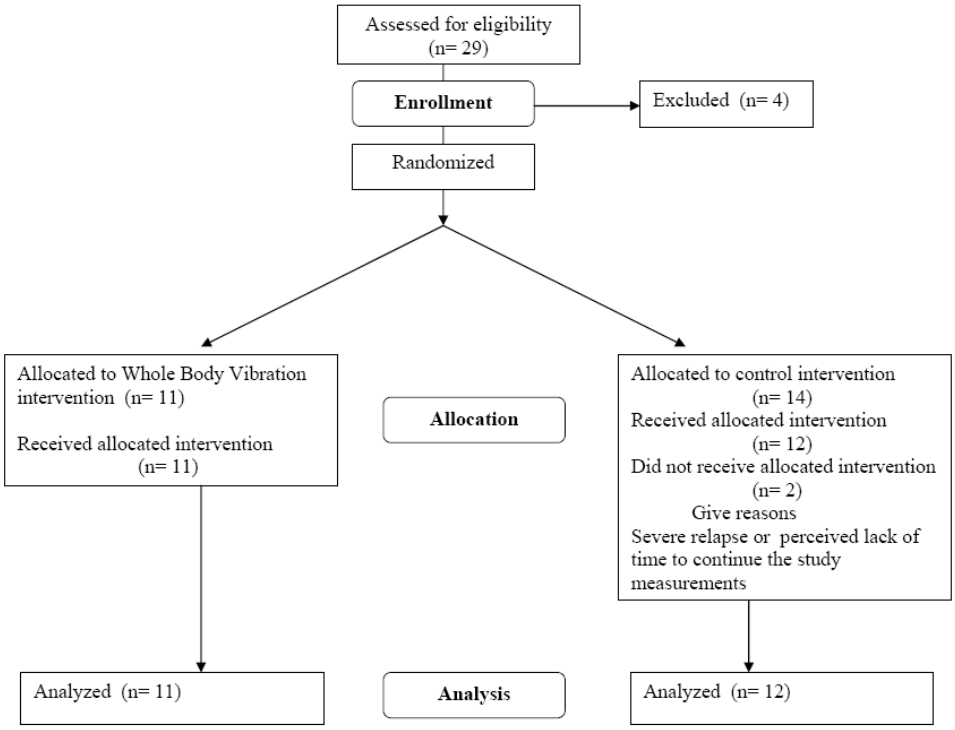

Figure 2.4.1. *Flow diagram of patient eligibility of study 4*

Perceived training exertion. The average VAS measures before and after each training session for the overall and specific lower limb fatigue remained stable throughout the study course. BORG scores increased significantly (p=0.02) over the intervention period and post-hoc Tukey-Kramer analyses indicate a significant increase in period 4 (mean ± SE 12.6±0.28, p=0.01) and period 5 (mean ± SE 12.8 ± 0.34, p=0.02), both compared to period 1 (mean ± SE 10.9 ± 0.25).

2.4.4.2. Muscle Performance

Muscle Strength (Table 2.4.3.). At baseline muscle performance did not statistically differ between groups. Compared to baseline, maximal isometric knee-flexor torque (45° and 90°) at study termination was lower (time effect, $p<0.05$) in CON. Compared to CON, 20 weeks of WBV did not increase maximal isometric knee extensor and knee flexor torque in both knee angles (group x time effect, knee extensors: 45° $p=0.07$, 90° $p=0.23$; knee-flexors: 45° $p=0.64$, 90° $p=0.57$). Maximal dynamic torque and maximal strength endurance of the knee extensors did not change following 20 weeks of WBV in any group (no statistically significant group x time effect).

Table 2.4.3. Maximal isometric, dynamic and endurance muscle strength.

ISOMETRIC (Nm)			p	WBV (n=11)	CON (n=12)
Extensor	45°	PRE	0.07	95.5 ± 9.4	93.6 ± 9.0
		MID		108.8 ± 10.9	92.5 ± 9.5
		POST		99.6 ± 11.4	92.0 ± 8.9
	90°	PRE	0.23	101.8 ± 11.6	94.4 ± 8.9
		MID		103.8 ± 12.5	90.3 ± 9.9
		POST		100.1 ± 13.2	86.3 ± 9.1
Flexor	45°	PRE	0.64	44.1 ± 5.5	53.8 ± 5.9
		MID		42.3 ± 5.0	49.6 ± 6.2
		POST		40.9 ± 5.5	48.0 ± 5.3*
	90°	PRE	0.57	37.3 ± 5.7	44.3 ± 4.6
		MID		36.7 ± 6.4	41.0 ± 4.7
		POST		34.4 ± 6.0	39.1 ± 3.8*
DYNAMIC (Nm)					
60°/s		PRE	0.50	92.1 ± 10.4	89.0 ± 11.8
		MID		95.6 ± 11.1	87.6 ± 13.3
		POST		98.4 ± 12.6	88.8 ± 11.2
ENDURANCE (J)					
180°/s		PRE	0.10	27.1 ± 5.1	25.9 ± 3.8
		MID		23.9 ± 5.0	31.5 ± 2.9
		POST		24.7 ± 4.1	35.4 ± 2.6

*Values are means ± SE and represent maximal isometric knee-extensor and flexor (knee angels of 45° and 90°), maximal dynamic knee-extension and maximal knee-extension strength endurance torques before (PRE) and after 10 (MID) and 20 (POST) weeks of Whole Body Vibration (WBV) training or control (CON) treatment. p-values represent interaction effects and *significant time effect ($p<0.05$) compared to baseline.*

Speed of movement of knee extension. No differences were found in the group x time x resistance analyses (p=0.99). Furthermore, there were no significant (p<0.05) interaction effects (group x time) for the 4 resistance levels (p=0.57 [1%]; 0.98 [20%]; 0.06 [40%] and 0.40 [60%]).

2.4.4.3. Functional capacity

At baseline, there were no statistically differences between groups for the EDSS, Berg Balance Scale, Timed Up and Go, 2 Minutes Walk and timed 25 Foot Walk values. Following the study period, no group x time interaction effects were detected for any functional capacity test (Table 2.4.4.).

Table 2.4.4. Functional capacity

		p	WBV (n= 11)	CON (n= 12)
BBS	PRE	0.15	44.9 ± 4.1	49.6 ± 4.2
	MID		43.6 ± 5.2	51.3 ± 3.3
	POST		41.9 ± 5.9	51.2 ± 3.8
TUG	PRE	0.26	13.7 ± 2.6	9.3 ± 1.7
	MID		14.0 ± 2.8	9.6 ± 1.6
	POST		13.1 ± 2.4	10.3 ± 2.2
2MWT	PRE	0.25	130.5 ± 15.6	154.8 ± 12.6
	MID		137.3 ± 16.0	153.9 ± 12.8
	POST		139.4 ± 15.6	167.5 ± 6.8
T25FW	PRE	0.64	8.7 ± 1.8	6.7 ± 0.9
	MID		8.7 ± 1.8	6.8 ± 1.0
	POST		8.4 ± 1.4	7.2 ± 1.5

Values are means ± SE and represent functional capacity (BBS: Berg Balance Scale; TUG: Timed Get up and go; 2MWT: 2 minutes walk test; T25FW: Timed 25 foot walk test) before (PRE) and after 10 (MID) and 20 (POST) weeks of Whole Body Vibration (WBV) training or control (CON) treatment. p-values represent interaction effects.

2.4.5. Discussion

The current study investigated the effects of 20-week whole body vibration (WBV) training on muscle performance and functional capacity in mild- to moderately-impaired MS patients. The results of this study suggest that 20 weeks of WBV exercise probably does not improve leg muscle isometric and dynamic strength nor strength endurance and speed of movement. Furthermore, under the conditions of the present study WBV appears to have no effects on functional capacity in persons with MS.

To our knowledge this is the first study that investigated the impact of long-term static and dynamic WBV exercise on muscle performance, measured by isometric and isokinetic dynamometry, in persons with MS compared to no intervention at all. Here, WBV exercise did not have any significant effect on knee muscle isometric strength, dynamic strength and strength endurance, nor maximal speed of movement. These are somehow surprising results. On the one hand because the applied WBV exercise program was very similar to previous long-term programs in younger and older healthy people exerting significant strength gains of 7 to 15% (147,148,269,270,276,277). On the other hand because based on the 'initial training status' principle it could be expected that MS patients could realize even greater improvements as they have a significantly lower muscle strength at baseline compared to untrained healthy subjects (134,278). It is difficult to explain the absence of any training effect. One could argue that despite the fact that the training volume and intensity was progressively increased according to the overload principle, the WBV training program used in this study may have been too intense for MS patients inducing overtraining. However, the reported overall (mean ± SE 5.6±0.1) and leg muscle fatigue (mean ± SE 5.8±0.2) VAS and Borg perceived exertion (mean ± SE 12.0±0.1) scores during and after training correspond to moderate-intensity training (278) and subjects described the provided WBV training as non exhausting. Because in MS muscle weakness is probably caused by both the disease process *per se* and by inactivity, the important question then arises as to what extent training stimuli exert similar physiological muscular training responses compared to healthy individuals. So far, a variety of studies have addressed this issue. To date, it is clear that strength and cardiovascular training, either or not combined, improve muscle strength and overall physical fitness in MS (51). Very often, this is associated with improved quality of life (51,179,182) and some functional mobility measures such as walking speed and walking distance, especially when performed in supervised exercise facilities (122,126,279). Compared to the improvements usually acquired in cardiovascular and/or strength training studies with healthy individuals (20 – 30%, 254,256), obtained strength increases in MS are markedly smaller (7 – 16%, 122,130). Possible WBV effects in MS, therefore, may also be limited compared to healthy individuals. In fact,

because reported strength increases resulting from WBV in the healthy are usually small (144), non significant increases in persons with MS could be expected. Finally, it is important to note that the majority of the participating subjects were persons still living in the community. The persons in the intervention group engaged to displace themselves independently to the training facility during 6 months. As such, it may be hypothesized that these subjects were (physically) more active than the common persons with MS and therefore showing no or limited (muscular) deconditioning, (57) limiting the potential for improvement.

Similar to healthy individuals (280), a positive relationship between muscular strength and functional capacity during daily living in persons with MS has been demonstrated (59). Functional capacity can be assessed using a variety of different measures such as gait speed, walking distance and balance (122,126,130,156,262). We applied the Berg Balance Scale, Timed to Get Up and Go, 2 min walk test and 25 foot walk test. In accordance with the muscle strength data and despite the fact that we applied both static and dynamic WBV exercises, functional capacity did not improve following 20 weeks of WBV training in the MS patients of this study. This confirms results of Schyns et al (156) who reported statistically unchanged 10m-walking time and Timed Up and Go performance following additional WBV training performance compared to exercise alone.

Due to its explorative nature the present study contains limitations. The small sample sizes used probably decreased statistical power. This could have clouded a possible statistical significance during isometric knee-extension ($p=0.07$, interaction effect at 45° knee angle). Also, training programs were not individualized. This may have decreased the rate of training progression. No 'placebo' group was included and thus it is uncertain that a sufficient vibration stimulus was ever provided. We applied lower limb open chain strength measures. Hereby, closed chain training effects such as in WBV may be underestimated with our test battery and lower limb training effects remain unexplored. Finally, this study only investigated the acute upper leg strength and function effects following long-term WBV training. Long-term effects and effects on quality of life that may be present remain unknown. Given its weaknesses and considering the high variability (EDSS scores, leg muscle strength asymmetry, functional capacity) occurring in MS compared to healthy subjects the following recommendations should be considered. First, larger scale training studies that include a 'placebo' group, quality of life assessment and follow-up measurements are necessary. Second, a more individualized training progression approach using optimal individual vibration parameters through for example electromyography or calf muscle strength measurements may stimulate the adaptive responses of MS patients. Finally, the use of closed chain strength measurements following WBV is probably more appropriate.

In conclusion, the applied WBV protocol seems safe for MS patients but under the conditions of the present study long-term WBV probably does not improve upper leg muscle strength and functional capacity in mild- to moderately impaired community-based MS patients during and immediately after the training program.

ACKNOWLEDGMENTS

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STUDY 5

Long-term, moderately intense strength training does not alter systemic basal cytokine levels in Multiple Sclerosis

Based on: Broekmans T, Hellings N, Hensen K, Rummens JL, Stinissen P, Eijnde BO.
Under Revision (J Rehabil Med)

2.5.1. Abstract

Objective. This study examined the effects of long-term (20w) standardized resistance training either or not in combination with simultaneous electro-stimulation and Whole Body Vibration (WVB) on pro- and anti-inflammatory cytokine resting levels.

Methods. 47 persons with MS (EDSS 1.5-6.5) were included. At baseline (PRE) and after 10 (MID) and 20 (POST) weeks of standardized leg resistance training (n=11), resistance training with simultaneous electro-stimulation (n=11) or whole body vibration (WBV, n=11) systemic IL-1β, IL-1ra, IL-6, IL-8, IL-10, IL-12p70 and TNFα resting levels were compared to a control group (n=14).

Results & Conclusion. Resistance training with or without simultaneous electro-stimulation or WBV did not alter cytokine concentrations.

2.5.2. Introduction

Multiple Sclerosis (MS) is a progressive degenerative disease of the central nervous system with an immune mediated etiology (16,17). In this respect, it has become evident that MS is associated with a dysregulation between pro-inflammatory T helper 1 (Th-1) and anti-inflammatory (Th-2) cytokines shifting towards a Th-1 profile (34). In fact, changes in some pro-inflammatory cytokine concentrations (IFNγ, TNFα, IL-1) expressed in MS lesions mediate neurodegeneration, and alterations in tumor necrosis factor (TNF)-α concentrations have been linked with changes in the inflammatory disease status of MS (18). Interestingly, recently developed disease modifying MS drugs such as interferon exert their beneficial actions through their ability to induce anti-inflammatory (e.g. IL-10, IL-1ra) activities and/or reduce pro-inflammatory activities (e.g. IL-1) (281). In healthy subjects, physical exercise dramatically increases serum IL-6 concentrations showing strong correlations with exercise intensity. IL-6 promotes IL-1ra en IL-10 secretion and inhibits TNFα en IL-1 expression. Given the fact that in MS-affected persons exercise therapy improves aerobic capacity and muscle strength (51,118,282), similar cytokine responses following physical activity may have the potential to reduce the inflammation process of MS (51,283,216). To date, in MS only a limited number of studies explored the effects of aerobic exercise therapy on acute cytokine responses (107) and chronic (8 weeks) alterations (120,121,138) and only White and coworkers explored the effects of an 8-week resistance training program on cytokine responses (121). Given the fact that muscle fiber can produce and regulate cytokines (106) it is interesting to explore cytokine responses after alternative strength training modes such as superimposed electrical stimulation and whole body vibration (WBV), that are currently under investigation in MS populations. Cytokine responses following longer term aerobic and/or resistance training have, to the author's knowledge, not been investigated.

In accordance with the above line of reasoning, the present study explored the impact of 6 months resistance training on basal serum cytokines responses. We hypothesized that strength training modifies the pro- and anti-inflammatory immune response in ambulatory patients with MS.

2.5.3. Methods

2.5.3.1. Subjects

In the present study 47 ambulatory MS subjects (Table 1) complied with the inclusion criteria: a definite MS diagnosis according to the McDonald criteria and EDSS (47) score between 1.5 and 6.5. Patients were excluded for this study if they were under relapse related corticoid treatment one month prior the start of the study or had any other orthopedic problem interfering with walking. All participants gave their written informed consent.
This longitudinal study is part from a larger controlled randomized clinical trial which was approved by the Hasselt University Ethics Committee according to the Helsinki declaration and is registered on www.controlled-trials.com/ISRCTN60122826.

2.5.3.2. Study design

This randomized trial was performed over a 20-week period. At baseline and following an overnight fast (12h) blood samples (12ml) were collected in endotoxin-free and heparinized tubes (Vacutainer®, Becton-Dickson, Franklin Lakes, USA), centrifuged (2800rpm, 10 minutes) and immediately stored at -80°C for later analysis. After baseline (PRE) subjects were randomized (independent investigator) into groups that were matched, in a decreasing order of importance, for EDSS, age and gender. Hence, 14 subjects were assigned to the control group (CON) and maintained their normal living habits. The remaining subjects were divided in three strength training groups undergoing moderately intense unilateral ACSM-based resistance training with or without (RES_O, n=11) simultaneous electro-stimulation (RES_E, n=11) and one whole body vibration training group (WBV, n=11). Training protocols are described in detail elsewhere (282,284). Briefly, in RES_O and RES_E knee-extensor and knee flexor were trained unilateral at a light (50-60% of 1 RM) to moderate (10 RM) intensity. The WBV group performed static and dynamic leg squats and lunges on a vibration platform (25-45 Hz, 2.5 mm amplitude). CON subjects were instructed to maintain their normal living habits. Exercise frequency was for all groups five training sessions per 2-week cycle. After the first (MID) and second (POST) 10-week training period that was separated by a two-week assessment period, all baseline measurements were repeated by same investigator on the same time of day. A blinded research neurologist determined the EDSS and other measurements were assessed by a qualified nurse and physical therapists which were only blinded during the baseline measurements.

Table 2.5.1 Subject characheristics at baseline.

	RES_O	RES_E	WBV	CON	p
n	11	10	10	11	0.81
♀/♂	6 / 5	6 / 5	7 / 4	11 / 3	0.44
EDSS (arbitrary units)	4.5 ± 0.4	4.4 ± 0.3	4.8 ± 0.3	4.3 ± 0.4	0.63
Age (years)	44.9 ± 3.5	48.7 ± 2.7	47 ± 2.1	50.4 ± 3.5	0.62
MS Type RR/ SP/ PP	2/7/2	3/2/5	3/3/4	7/1/3	0.08

Values are numbers or means ± SE. p-value indicate baseline differences

2.5.3.3. Cytokine determination

Serum cytokines concentrations were determined by flow cytometry (FACSCanto®II) using a commercial available Cytometric Bead Array (CBA) Human Inflammatory Cytokines kit (Becton-Dickson, Franklin Lakes, USA) that contained interleukin 8 (IL-8), interleukin 1beta (IL-1β), interleukin 6 (IL-6), interleukin 10 (IL-10) Tumor Necrosis Factor (TNF-α) and interleukin 12p70 (IL-12p70). The intra-assay coefficients of variability for IL-8, IL-1β, IL-6, IL-10, TNF-α and IL-12p70 were 4%, 7%, 6%, 6%, 9% and 4%, respectively. The individual sensitivities ($pg \bullet ml^{-1}$) of IL-8, IL-1β, IL-6, IL-10, TNF-α and IL-12p70 were 3.6, 7.2, 2.5, 3.3, 3.7 and 1.9, respectively. Furthermore, interleukin-1 receptor antagonist (IL-1ra) was determined by sandwich ELISA (R&D systems Quantikine®, Minneapolis, USA). The intra-assay coefficient of variation for IL-1ra was 5.3% and the detection limit ($pg \bullet ml^{-1}$) was 6.26.

2.5.3.4. Statistical analyses

Statistical analyses were performed using SAS® software (Version 9.2; SAS Institute, Inc., Cary, NC). The normal distribution of all variables was investigated using the Shapiro-Wilk test. Baseline differences were explored by one-way ANOVA or Kruskal-Wallis tests. When data was normally distributed a 4x3 mixed model ANOVA (Group [RES_O, RES_E, WBV, CON] x Time [PRE, MID, POST] was used. If the data was not normally distributed a Friedman test was performed. Post hoc power calculations were performed using G power 3 software (253). The level of statistical significance was set at $p<0.05$ and data are presented as means ± SE.

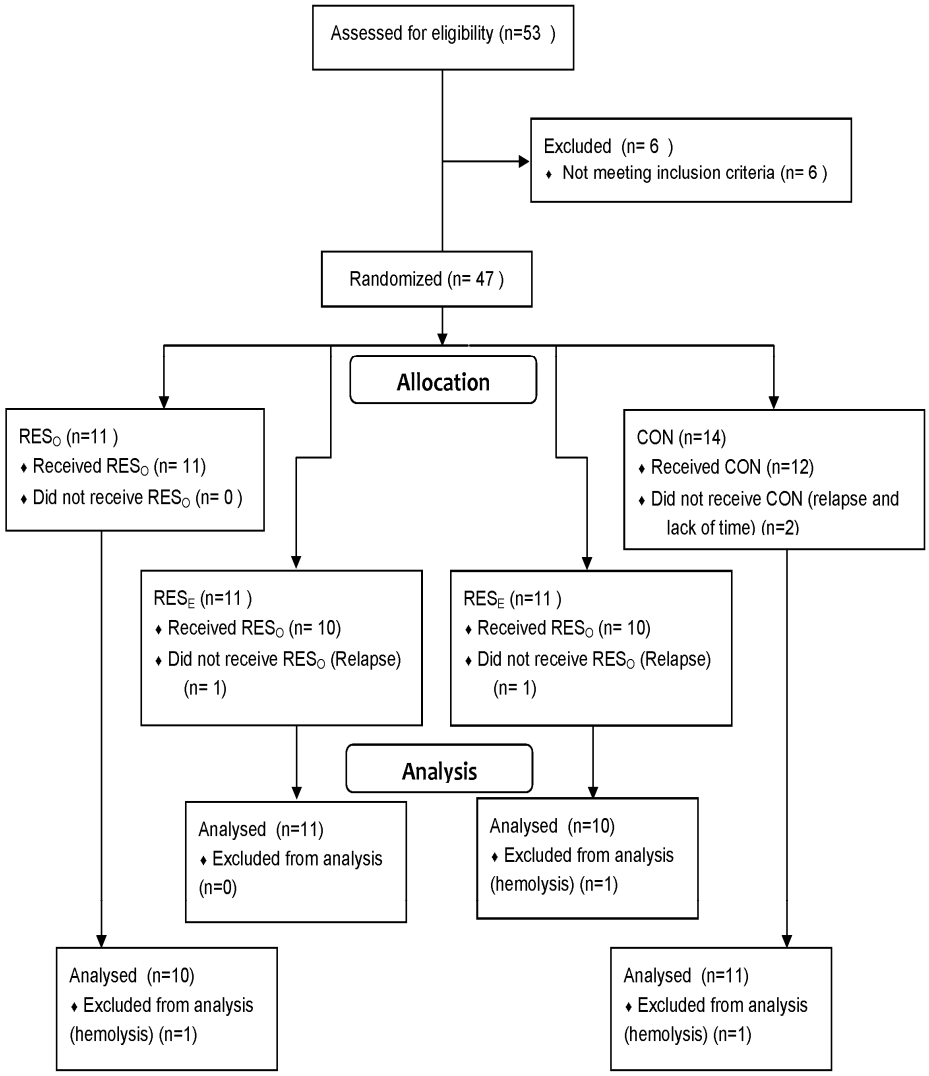

Figure 2.5.1. *Flow diagram of patient eligibility of study 5*

2.5.4. Results

In total 3 subjects retreated before the study finished. Two CON patients and one RES_E subject withdrew due to a severe relapse or perceived lack of time. Furthermore, one subject of the CON and the WBV group was excluded from analysis due to sever blood sample hemolysis. At baseline no differences in patient characteristics (Table 2.5.1.) were found.
As depicted in Table 2.5.2., no baseline differences between groups were detected. Furthermore, cytokine levels were not altered after 10 and 20 weeks of resistance training with or without simultaneous electro-stimulation or WBV compared to the control group.

Table 2.5.2 Pro- and anti-inflammatory cytokines

	RES_0			RES_E			WBV			CON		
	PRE	MID	POST	PRE	MID	POST	PRE	MID	POST	PRE	MID	POST
IL8 (pg/ml)	9.3 ± 1.2	8.0 ± 1.1	8.3 ± 1.0	15.4 ± 6.2	12.5 ± 5.4	11.4 ± 3.2	9.3 ± 1.7	8.8 ± 1.1	7.9 ± 1.0	10.7 ± 1.2	9.7 ± 1.4	9.4 ± 1.3
IL6 (pg/ml)	1.8 ± 0.3	2.0 ± 0.3	1.8 ± 0.3	1.6 ± 0.4	0.7 ± 0.2	1.4 ± 1.2	6.2 ± 2.3	3.4 ± 1.0	4.0 ± 1.2	3.8 ± 2.1	5.3 ± 2.8	1.5 ± 0.3
IL1β (pg/ml)	0.7 ± 0.3	1.2 ± 0.4	0.9 ± 0.3	0.5 ± 0.2	0.3 ± 0.2	0.4 ± 0.1	0.6 ± 0.3	1.1 ± 0.5	0.9 ± 0.5	0.2 ± 0.1	0.4 ± 0.2	0.5 ± 0.2
IL10 (pg/ml)	1.3 ± 0.4	1.4 ± 0.3	1.1 ± 0.3	1.8 ± 1.0	0.6 ± 0.1	1.1 ± 0.3	1.0 ± 0.2	1.0 ± 0.2	1.1 ± 0.3	0.9 ± 0.2	0.8 ± 0.2	0.8 ± 0.2
TNF-α (pg/ml)	0.6 ± 0.2	0.8 ± 0.3	0.8 ± 0.1	0.9 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	0.9 ± 0.2	1.1 ± 0.2	1.2 ± 0.3	0.9 ± 0.3	0.6 ± 0.2	0.7 ± 0.1
IL12p70 (pg/ml)	0.7 ± 0.4	1.3 ± 0.4	1.1 ± 0.4	1.0 ± 0.2	1.0 ± 0.3	1.7 ± 0.4	1.5 ± 0.3	1.6 ± 0.4	1.5 ± 0.5	1.5 ± 0.3	1.1 ± 0.4	1.2 ± 0.5
IL1ra (ng/ml)	595 ± 92	480 ± 53	548 ± 74	575 ± 67	423 ± 43	556 ± 956	558 ± 166	425 ± 47	460 ± 43	626 ± 89	494 ± 65	536 ± 92

Values are means ± SE and represent circulating blood serum cytokine concentrations (IL8: interleukine 8; IL6: interleukine 6; IL1β: interleukine 1 beta; IL10: interleukine 10; TNF-α: Tumor Necrose Factor; IL12p70: interleukine 12p70; IL-1ra: interleukine 1 receptor antagonist.) before (PRE) and after 10 (MID) and 20 (POST) weeks of Resistance training with (RES_0) or without simultaneous electro stimulation (RES_E), Whole Body Vibration (WBV) training or control (CON) treatment.

2.5.5. Discussion

The current study explored the hypothesis that standardized strength training may modify chronic serum pro- and anti-inflammatory cytokine levels in ambulatory patients with MS. As previously reported the applied resistance training protocols (RES_O, RES_E) improved ($p < 0.05$) maximal muscle strength but in RES_E an additional effect on maximal muscle strength was not found (282), while WBV did not affect maximal muscle performance (284). The present study indicates that 10 and 20 weeks of strength training does not modify MS-related pro-inflammatory (IL-6, IL-8, IL-12p70 and TNF-α) and anti-inflammatory chronic serum cytokine concentrations (IL-10, IL-1ra).

In persons with MS and compared to healthy individuals, higher concentrations of resting pro-inflammatory cytokines are reported (18, 24,200). In the current study the serum cytokine levels are situated within this range (121,200,285). In contrast to White et al, who indicated a decrease in some basal pro-inflammatory cytokine concentrations (IFNγ, $p<0.05$; CRP, $p<0.05$; TNFα $p=0.07$) after 8 weeks of resistance training (121), systemic cytokines levels in the current study were not affected by neither of the applied strength training protocols. Our findings confirm former results of Bruungaard et al. (286) indicating unchanged pro-inflammatory TNFα levels in geriatric persons after 12 weeks resistance training and the conclusions made by the review of de Salles and co-workers (287). Furthermore, in MS it is suggested that low IL-10 concentrations may result in excess inflammation (18). In the present study IL-10 and IL-1ra concentrations were unaffected which contrasts with the findings of White et al (121) who documented a reduction (43% $p<0.05$) of IL-10 concentrations after 8 weeks resistance. Given the complex role of cytokines and their inter-individual variability in MS (35) it is clear that solid clinical conclusions on the impact of resistance training and exercise *per se* on cytokine responses cannot be made yet. Other putative mechanisms such as modified acute cytokine responses (107,120) or protective brain-derived neurotrofic factors (288) might also alter the course of MS, but remain speculative. Additional research is needed to elucidate the effects of exercise training on immune activity and disease progression in MS. Nevertheless, based on the current data, showing no changes in serum cytokine levels, it seems fair to conclude that moderately intense strength training probably does not stimulate the inflammatory process as suggested by other standardized resistance training (122,130) and whole body vibration studies (156) reporting no additional exacerbations in persons with MS. Resistance training with higher loads (80-100% of 1RM) and volumes (3-5 sets), however, seems to alter resting levels of pro- and anti inflammatory cytokines in healthy subjects (289). Thus, it may also be worthwhile to investigate chronic cytokine responses after an intense long-term resistance training in a MS population.

The current study also contains some limitations. We did not

standardize drug intake in this study because this probably resembles real rehabilitation settings where patients take different drugs. However, based on the fact that immunomodulatory agents shift the immune response from pro-inflammatory autoimmune conditions towards a more beneficial anti-inflammatory environment (83), we might have missed a possible pro-inflammatory cytokine response. Furthermore, given the fact that only White et al (121) performed a similar study with no clear effects, a proper a priori power calculation was not possible. A post hoc power analyses based on the effect size of the current study indicated that with the exception of IL-6 and IL-10 statistical power was low (<0.5). Therefore, and given the complex role of cytokines, these data should be interpreted with caution.

In conclusion, under the conditions of the current explorative study low to moderately resistance training or WBV probably does not modify basal pro- and anti-inflammatory cytokine responses.

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STUDY 6

The relationship between upper leg muscle strength and walking capacity in persons with Multiple Sclerosis

Based on: Broekmans T, Gijbels D, Eijnde BO, Alders G, Lamers I, Roelants M, Feys P
Submitted (Mult. Scler. J.)

2.6.1. Abstract

Background. In persons with MS (PwMS), resistance training improves muscle strength, but effects on walking capacity are not always present.

Objective. The objective was to determine the relation between different types of upper leg muscle strength measurements and walking capacity in PwMS.

Methods. An observational cross-sectional study design was applied. Upper leg muscle strength of 52 PwMS (Expanded Disability Status Scale, EDSS range 1.5-6.5) was measured using isometric (knee-extensors and flexors) and isokinetic (knee-extensors) dynamometry. Walking capacity was assessed using the Timed 25-Foot Walk, Timed Up and Go and 2-Minute Walk Test. Subgroups with mild (EDSS 1.5-4.0, *n*=31) and moderate (EDSS 4.5-6.5, *n*=21) ambulatory dysfunction were distinguished, as results were hypothesized to differ depending on MS-related disability status. Correlation and regression analyses were performed.

Results. The highest and most significant correlation coefficients were found in the moderate MS subgroup. Within knee-extensor measurements, it was found that isokinetic endurance strength related best to walking capacity. When comparing maximal isometric strength measurements, knee flexors (r~0.3-0.7) related better to walking capacity than knee-extensors (r~= 0.1-0.4). Regression analyses confirmed that endurance knee-extensor strength (~35 %) and isometric knee flexor strength (~46%) as main predictors for walking capacity.

Conclusion. Resistance training protocols may consider inclusion of exercises focusing on endurance knee-extensor and isometric knee flexor strength when aiming to enhance walking capacity in persons with moderate ambulatory dysfunction.

2.6.2. Introduction

Multiple Sclerosis (MS) is an chronic progressive inflammatory and neurodegenerative disease with a variety of motor symptoms (2). Primary motor symptoms include muscle weakness, hypertonia, and coordination problems often manifesting first in the lower limb. Motor impairments may result in a reduced mobility and physical activity level, which on its turn can culminate further impairment, for example loss of muscle strength (i.e. disuse secondary to MS). Persons with MS (PwMS) perceive diminished lower limb function and walking as a major limiting disease characteristic (62) that probably contributes to a reduced quality of life (178,237). In this context, rehabilitation in general and exercise therapy in specific poses a high potential for improving functioning and participation of PwMS (182,290) in addition to disease modifying drugs treatment because they typically prevent or slow down disease progression in a substantial number of MS patients (81).

In MS rehabilitation, part of the studies on exercise therapy have focused on improving the function level of the International Classification of Functioning (ICF), with the aim to improve abilities at activity level such as walking. Muscular strength and fitness is certainly an important factor of walking capacity (291,292) as already documented in stroke patients (159-161). To date, a number of studies in PwMS have consistently been shown that resistance training leads to increased maximal muscle strength (122, 126,128,130,282) but not all of them reported a positive impact on walking or other functional capacity tests (128,282). This can be related to factors as training intensity, duration and level of disability in patients. However, in healthy subjects it was reported that knee extensor muscle strength is best related with walking speed (159), while Thoumie et al. (293) reported in MS patients that the correlation between isokinetic (60°/s) muscle strength and gait velocity was actually highest for knee flexors.

An improved understanding of different isometric and isokinetic muscle performance variables, as well as muscle groups that predict ambulatory walking capacity in MS may assist optimization of future resistance training programs. Therefore, the present study aimed to determine the relationship between muscle strength and walking capacity in PwMS. A first objective was to investigate the relationship between various knee extensor strength outcomes with walking capacity. A second objective was to investigate whether isometric knee extensor or flexor isometric strength related best with walking capacity. MS subgroups with different ambulatory dysfunction were distinguished as relationships were hypothesized to differ depending on the individual's mobility status.

2.6.3. Methods

2.6.3.1. Study design and subjects

The present study was a part of a larger research project (see www.controlled-trials.com/ISRCTN60122826) that examined the effects of resistance and whole body vibration training on muscle strength and functional capacity in PwMS (284,282). Fifty-two ambulatory MS subjects (Table 1) were recruited with the inclusion criteria: a definite MS diagnosis according to the McDonald criteria (47) and EDSS score between 1.5 and 6.5. Patients were excluded if they experienced a relapse or were under relapse related corticoidtreatment, one month prior to the start of the study or had any other orthopedic problem(s) interfering with walking.

Subject were subdivided in subgroups with mild (EDSS ≤ 4.0) and moderate (EDSS > 4.0 and ≤ 6.5) ambulatory dysfunction. This EDSS cut-off score of 4.5 (able to walk without aid or rest some 300 metres) was previously successfully used to discriminate relatively mild ambulatory function versus moderate to severe ambulatory dysfunction (263). All participants signed the informed consent which was approved by the Hasselt University Ethics Committee according to the Helsinki declaration.

2.6.3.2. Experimental Design & Outcome measures

The present study reports baseline values of muscle strength and walking capacity tests of above-mentioned research project, using a cross-sectional study design. Muscle strength and walking tests were applied on two different days interspersed with at least 48 hours rest interval to avoid mutual interference.

Muscle Strength

Maximal voluntary unilateral strength of the right and left leg was evaluated on an isokinetic dynamometer (Biodex Medical Systems®, system 3, Inc, Shirley, New York) at a sampling rate of 100Hz. Here, muscle strength is expressed as average of both legs. After a 5-min standardized warm-up on a quadriceps bench, strength tests were performed in a seated position on a backward inclined (5°) chair. The rotational axis of the dynamometer was aligned with the transverse knee-joint axis and connected to the distal end of tibia by means of a length adjustable lever arm. The upper leg, hips and shoulders were stabilized with safety belts.

Maximal isometric torque. Following one sub maximal trial contraction, two maximal isometric contractions were performed by knee extensors and flexors at knee angles of 45° and 90°. Maximal contractions (during 3s) were interspersed by 90-s rest intervals.

Maximal isokinetic torque. Subjects performed four maximal consecutive isokinetic knee-extensions at a velocity of 60°/s after three sub-maximal trial contractions. Knee extensions were initiated at a joint angle of 90° towards an angle of 160°. Following each extension, the leg was returned passively to the starting position from which the next contraction was immediately initiated. The highest of four isokinetic extension torques (Nm) was selected as maximal dynamic torque.

Maximal isokinetic muscle endurance. Finally and also following three sub-maximal trial contractions, subjects performed twenty maximal isokinetic knee-extensions at a velocity of 180°/s to assess muscle strength endurance. The knee extensions were initiated at a joint angle of 90° towards an angle of 160°. Following each extension the leg was returned passively to the starting position from which the next contraction was immediately initiated. Muscle strength endurance was expressed as the percentual decrease in average work (J) of the first three and last three contractions.

Walking capacity

Assistive devices were permitted if necessary during all walking tests, which were performed in random order.

Timed 25 Foot Walk test (T25FW, 249). Subjects were instructed to walk 25 foot as quickly but safely as possible.

Timed Get Up and Go (TUG, 248) time (s) was recorded while subjects get up of a chair, walk 3 meter, turn around, walk back and sit down again.

Two Minute Walk Test (2MWT, 250). Subjects were instructed to walk as much distance within the two minutes time frame. Outcome on the 2MWT was shown in PwMS, to be strongly related to that on the 6MWT (Gijbels et al. Comparison of the 2- and 6-Minute Walk Test in multiple sclerosis. Accepted Mult Scler J)

2.6.3.3. Statistical analyses

The normal distribution of all variables was investigated using the Shapiro-Wilk test. Student's t-test for independent samples or Mann-Whitney tests were applied to examine differences between subgroups. Further analyses were applied on the total sample as well as on subgroups separately. Pearson correlation coefficients were calculated to examine the relationship between maximal knee muscle strength and functional mobility tests and were interpreted as: poor ($r < 0.30$); low (r: 0.30-0.50); moderate (r: 0.50-0.70); high (r: 0.70-0.90) or very high (r: >0.90). To investigate the predictability of walking capacity tests by different knee-extensor strength

variables, multiple regression models with a backward selection procedure were performed for the maximal isometric, isokinetic knee extensor peak torque and maximal dynamic knee extensor endurance as independent variables while each walking test served as dependent variable. To investigate the predictability of walking capacity tests by isometric knee-extensor versus flexor strength, multiple regression models with a backward selection procedure were performed for the maximal isometric knee-extensor and flexor peak torque as independent variables while each walking test served as dependent variable. Multicolinearity was checked for all models. The level of statistical significance was set at $p<0.05$ and data are presented as mean ± SE. Statistical analyses were performed using SAS® software (Version 9.2; SAS Institute, Inc., Cary, NC). Post hoc power calculations were performed using G power 3 software (253).

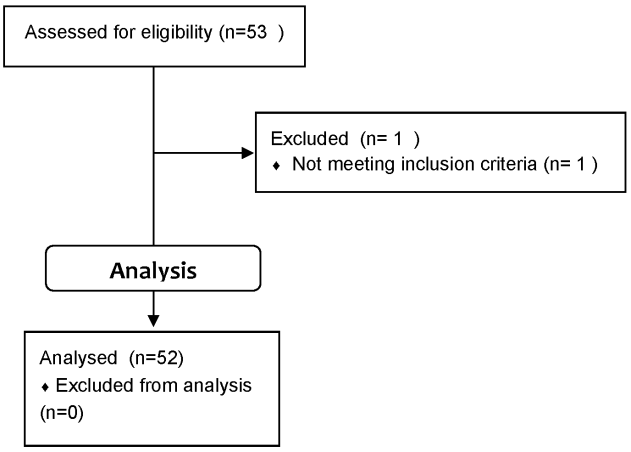

Figure 2.6.1. *Flow diagram of patient eligibility of study 6*

2.6.4. Results

2.6.4.1. Description of the MS subgroups

Table 2.6.1 presents the clinical characteristics and experimental outcome measures of the total MS group and both subgroups. Age and disease duration were not significantly different between subgroups with different EDSS scores. All muscle strength variables and walking tests were significantly ($p<0.05$) worse in the subgroup with moderate compared to mild ambulatory dysfunction with exception of isokinetic knee-extensor endurance strength justifying the use of the EDSS 4.5 cut-off score for differentiation among MS subgroups.

Table 2.6.1 Descriptive and experimental outcome measures of total MS group and subgroups.

	Variable	Total Sample (n=52)	Mild (n=31)	Moderate (n=21)
Age (years)		47.2 ± 1.4 (24-68)	46.1 ± 1.5 (25-62)	48.9 ± 2.5 (24-68)
EDSS*		4.4 ± 0.2 (1.5-6.5)	3.6 ± 0.1 (1.4-4)	5.5 ± 0.1 (4.5-6.5)
Sex ♀/♂ (n)		36/15	20/11	16/5†
MS type RR/SP/PP (n)		22/16/14	19/7/5†	3/9/9
Disease duration (years)		17.9 ± 1.1 (6-42)	18.1 ± 1.6 (6-42)	17.6 ± 1.5 (8-36)
	Muscle strength			
Isometric	Knee flexor 45° (Nm)*	51.3 ± 2.6 (14-92)	57.1 ± 3.3 (14-92)	42.8 ± 3.5 (19-92)
Isometric	Knee flexor 90° (Nm)*	42.7 ± 2.2 (3-80)	49.2 ± 2.3 (23-80)	33.1 ± 3.3 (3-72)
Isometric	Knee-extensor 45° (Nm)*	109.2 ± 5.0 (43-198)	120.0 ± 7.0 (43-198)	93.3 ± 5.5 (48-143)
Isometric	Knee-extensor 90° (Nm)*	98.1 ± 4.3 (47-172)	110.3 ± 5.7 (47-172)	79.3 ± 4.0 (48-118)
Dynamic	Knee-extensor 60°/s (Nm)*	90.3 ± 4.8 (22-188)	104.3 ± 6.1 (22-188)	69.7 ± 5.0 (23-106)
Dynamic	Knee-extensor endurance 180°/s (%)	30.1 ± 1.6 (7-50)	31.9 ± 1.7 (9-50)	27.5 ± 3.1 (7-50)
	Functional mobility			
T25FW (s)*		7.4 ± 0.6 (3.9-25)	5.4 ± 0.2 (3.9-10.8)	10.3 ± 1.4 (4.5-25)
TUG (s)*		10.3 ± 0.9 (4.8-38)	7.7 ± 0.4 (4.8-18.6)	14.2 ± 2.0 (5.4-38)
2MWT (m)*		148.0 ± 6.8 (31-224)	172.9 ± 5.3 (100-224)	111.0 ± 10.9 (31-210)

Values are means ± SE (range) and represents subject charateristics expressed by Expanded Disability Status Scale (EDSS), Timed get Up and Go (TUG), Timed 25 Foot Walk (T25FW) and 2 Minutes Walk Test (2MWT). * $p<0.01$ Mild subgroup versus Moderate subgroup. † $p<0.05$

2.6.4.2. Different types of maximal knee strength in relation to walking capacity.

Table 2.6.2 displays the correlations coefficients between upper leg strength and walking tests. First, the relationships between isometric, isokinetic and endurance knee-extensor strength and the walking tests are discussed. In the total MS sample, all strength measures were significant, ranging from poor to moderate, related to the walking tests. When analyzing results of the subgroup with mild ambulatory dysfunction, no significant correlations between knee-extensor strength and walking capacity tests were found except for a low correlation between isometric knee extensor strength at 90° and the 2MWT. For the subgroup with moderate ambulatory dysfunction, a systematically significant correlation (0.60 up to 0.62) is found between muscle endurance and all walking tests, added with a significant correlation

between isokinetic strength and the 2MWT.
Secondly, the relationships between isometric knee extensor versus knee flexor strength and the walking tests are discussed. For the total MS group as well as both subgroups, isometric strength of knee flexors was systematically better related to walking capacity tests compared to isometric knee-extensor strength. In the subgroup with mild ambulatory dysfunction, isometric muscle strength was significantly correlated with the 2MWT, but not with the shorter walking tests. In the subgroup with moderate ambulatory dysfunction, moderate to high correlations were only found for the isometric knee flexor strength .

Table 2.6.2 Pearson correlation coefficients between maximal muscle strength and walking capacity

		T25FW (s)			TUG (s)			2MWT (m)		
		Total	Mild	Moderate	Total	Mild	Moderate	Total	Mild	Moderate
Knee Flexor										
IM	45°	-0.50 **	-0.34	-0.56 *	-0.49 **	-0.30	-0.55 *	0.63 ***	0.51 *	0.61 *
	90°	-0.62 ***	-0.17	-0.70 **	-0.61 ***	-0.15	-0.70 **	0.71 ***	0.40 *	0.70 **
Knee-extensor										
IM	45°	-0.31 *	-0.23	-0.24	-0.33 *	-0.16	-0.32	0.47 **	0.38 *	0.36
	90°	-0.37 *	-0.06	-0.34	-0.36 *	-0.10	-0.34	0.53 ***	0.32	0.42
IK		-0.40 *	-0.15	-0.37	-0.36 *	-0.05	-0.34	0.55 ***	0.32	0.49 *
ENDUR		-0.52 *	-0.26	-0.62 *	-0.46 **	0.01	-0.60 *	0.43 *	0.10	0.61 *

Values represent correlation coefficients between maximal isometric (IM) knee-extensor (KE) / flexor (KF) strength in a knee angle of 45° and 90°, maximal isokinetic (IK) knee-extensor strength at 60°/s , isokinetic muscle endurance strength (ENDUR) at 180°/s and walking capacity tests (Timed Get up and Go (TUG), Timed 25 Foot Walk (T25FW) and 2 Minutes Walk Test (2MWT)) of the total MS sample and the mild and moderate subgroups. *p<0.05, ** p<0.001, ***p<0.0001.

2.6.4.3. Predictive value of walking capacity by various knee strength variables.

Given the fact that the highest correlation coefficients were found in the moderate subgroup, multiple regression models to predict walking capacity with different knee strength variables were only applied in this subgroup (Table 2.6.3.). First, various knee extensor strength variables were used as independent variables, to predict walking capacity. The only significant predictor (range 33-35%) was endurance strength. Second, isometric knee-extensor and flexor strength variables were used as independent variables, to predict walking capacity. For all walking tests isometric knee flexor strength at 90° was the only significant predictor with a consistent predictive value of 46%.

Table 2.6.3 Predicting walking capacity with different strength variables in the moderate MS subgroup

	Isometric, isokinetic and endurance knee-extensor strength					**Isometric knee-extensor vs flexors strength**				
Dep var		Ret var	β	SE	t		Ret var	β	SE	t
T25FW	R^2 0.39	ENDUR	-0.28	0.08	-3.5 *	R^2 0.49	IM90KF	-0.29	0.07	-4.3 **
	Adj R^2 0.36					Adj R^2 0.46				
TUG	R^2 0.36	ENDUR	-0.39	0.12	-3.3 *	R^2 0.49	IM90KF	-0.42	0.10	-4.3 **
	Adj R^2 0.33					Adj R^2 0.46				
2MWT	R^2 0.37	ENDUR	2.13	0.64	3.3 *	R^2 0.49	IM90KF	36.13	19.31	4.2 **
	Adj R^2 0.34					Adj R^2 0.46				

Data represents predictive value (R^2); Adjusted (Adj) R^2; estimate (β); standard error (SE) and t-value (t) of the different regression models with dependent variables (Dep var): Timed Get up and Go (TUG), Timed 25 Foot Walk (T25FW), 2 Minutes Walk Test (2MWT) and Retained variables (Ret var, endurance strenght: ENDUR; isometric knee flexor strength in 90°: IM90KF. *p<0.05, **p<0.001.

2.6.5. Discussion

To our knowledge, only a few studies have extensively investigated the relationship between muscle strength and walking capacity in neurological conditions, taking different types of strength measures as well as different muscle groups into account. The present study in persons with MS, also taking severity of ambulatory dysfunction into account, revealed muscle endurance strength to be the most predictive knee extensor variable for walking in persons with moderate ambulatory dysfunction only, while isometric muscle strength of knee flexors is more predictive for walking compared to the knee extensors.

Relationships between various knee extensor strength measures and walking capacity

The first objective was to investigate the clinical relevance of different muscle strength measures and walking capacity. Isometric and isokinetic strength of the knee extensors, as well as a measure for muscle endurance, were significantly related to short (T25FW and TUG) and longer (2MWT) walking tests in the total group. In line with a previous study (263), the EDSS cut-off score of 4.5 validly distinguished subgroups with mild and moderate ambulatory dysfunction, given significant differences in walking capacity. As well, muscle strength was different between subgroups except for the endurance measure.

In subjects with mild ambulatory dysfunction, no significant correlations were found except for isometric knee extensor strength measured at 45° with the longer 2MWT. The lack of robust relationships between maximal muscle strength and walking capacity tests may be related to the fact that the mild subgroup almost performed as good as healthy subjects (294), possibly inducing floor effects on short walking tests as already reported in other patient populations (295,296). In addition, levels of knee extensor strength in this subgroup was likely greater than what is strictly required for walking (291,292). In contrast, patients with moderate ambulatory dysfunction showed greater albeit not always significant correlations between walking capacity and knee extensor strength measures. Specifically, knee extensor endurance strength instead of maximal isometric and isokinetic strength was revealed as an important variable related to walking rather than maximal muscle strength, which was also unambiguously confirmed by the regression analyses. This muscle endurance strength variable can be considered as a measure of muscle fatigability during repeated contractions, which is also required during walking. An exploratory study in MS by Schwid et al (71) indicated that fatigability, defined as reduced strength as routine exercise of muscle groups proceeds (297), was

associated with maximal walking distance and time needed to walk 5 feet (71). Intriguingly, muscle endurance, which was not different between subgroups, did not significantly relate with walking capacity in PwMS with mild ambulatory impairment. The latter may indicate that muscle strength during gait remained above a certain threshold in this subgroup, even after repeated contractions.

Relationships between knee extensor and flexor isometric muscle strength and walking capacity

The second objective was to investigate whether relationships between knee extensor and flexor isometric strength with walking capacity differed. Strikingly, maximal isometric knee flexor strength showed systematically greater correlations coefficients compared to isometric knee extensor strength across subgroups. More particular, the greatest correlations were found in the subgroup with moderate ambulatory dysfunction, while in the mild subgroup, correlations of isometric muscle strength and walking tests were low and generally not significant except for the longer 2MWT.
A better association between isokinetic knee flexor compared to knee extensor strength, and walking capacity, has already been reported before in stroke (298,299) and in MS (293). In the walking pattern of healthy subjects, knee flexors act concentrically at mid-swing to augment knee flexion for foot clearance and thus unimpeded limb progression, and eccentrically at terminal swing to decelerate the lower limb in preparation of initial contact while avoiding knee hyperextension (300). These activations influence the gait pattern and related variables as step length (300). MS patients of the moderate subgroup might have importantly shown poor knee flexion during the swing phase, similarly as suggested in stroke patients with a paretic lower limb (299), as isometric muscle strength at 90° knee flexion showed greater correlations and was most predictive for diminished walking capacity in the regression analysis compared to strength at 45°. In support of this view, Olney et al. (301) identified, in patients with hemiplegia, a positive relationship between maximal flexor moments of knee, hip and ankle during swing phase and gait speed. Unfortunately, a limitation of the present study was that it did not include kinematic analysis or recordings of spatio-temporal characteristics (302), making it difficult to define the contribution of knee flexor weakness on the gait pattern and speed with certainty. Also, the impact of other muscle weakness, such as calf muscles or hip extensors, should be taken in account in future research while it is also acknowledged that walking capacity is also influenced by factors such as impaired postural control, sensory dysfunction and increased muscle tone, as previously documented in stroke (298,303-305). In future research, it may be worthwhile to also take into account the impact of

potential muscle weakness at ankle and hips joints, and other impairments on walking capacity of PwMS.
Relationships between muscle strength and walking capacity appeared to be different in the subgroups. In contrast to the present study, Thoumie et al. concluded that knee extensor strength is more related with walking than knee flexors when disability level increased (293). However, the latter study differentiated subgroups without (n=65) and with (n=35) cane assisted-walking importantly documenting that the use of a cane likely alters the relationships between muscle strength and gait speed. In our study, only six subjects in the subgroup with moderate ambulatory dysfunction made use of a cane, which did statistically not allow the formation of a separate subgroup to verify above-mentioned findings. One may also comment that we compare results of isometric muscle strength in the present study with that of isokinetic muscle strength in the study of Thoumie et al (293). However, it is noted that, despite this methodological bias, knee flexor maximal strength explained similarly about 45% of the variance of walking capacity in both studies. Furthermore, the present study only focused on motor impairment caused by muscle weakness, while Thoumie et al (293) included patients with different types of impairment (sensory, cerebellar, pyramidal) still coming to similar conclusions regarding the importance of the knee flexors for non-assisted gait.

Clinical Implementations

The findings of the present study seem relevant to improve clinical evidence-based practice. It is indicated that, in patients with only mild ambulatory dysfunction, no major carry-over effects of increased muscle strength after resistance training on walking capacity tests should be expected. However, it is not excluded that other activities such as stair climbing, requiring higher levels of muscle strength, may improve. In PwMS with moderate ambulatory dysfunction, however, training of especially knee extensor endurance strength and maximal isometric knee flexor strength seems advocated given strong associations with walking capacity. So far, most resistance training programs in healthy individuals (98,246,306), stroke (307-309), and MS patients (122,126,128,130,282) seem to focus on increasing maximal peak strength of commonly the knee extensors. The content of standardized exercise therapy programs in previous studies may explain to some extent the limited effects of resistance training on walking capacity. While effects of resistance training on maximal knee flexor strength has been shown before (130,282), it is rather unclear whether muscle endurance can be improved after specifically designed training protocols. Besides, future research should also investigate whether endurance of knee flexors is equally important as that of knee extensors.

Findings of the present study indicate that knee extensor endurance strength, as well as isometric knee flexor strength are important predictors for walking capacity in PwMS with moderate ambulatory dysfunction.

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Chapter III:

General discussion and conclusions

General discussion

To date, it is clear that exercise therapy is a fundamental part of integrated MS therapy because it can improve some of the clinical symptoms associated with MS. As such, research that investigates the effects of exercise training on muscle contractile properties *(first objective)*, functional benefits *(second objective)* and systemic cytokine responses *(third objective)* can provide additional information to further optimize MS rehabilitation. The six different studies of this Ph.D. project, presented in Chapter II, fit into these three objectives. In the following general discussion, an attempt is made to integrate all the study results and compare them with the work of others.

3.1.EAE

As indicated in the General Introduction, studies that evaluate muscle contractile properties during or following an endurance training intervention are limited (112,115,116). Although the effect of different endurance exercise intensities on muscle functioning, even in animal MS models, has never been investigated, it may increase our understanding of the physiological impact of exercise therapy on MS. To the author's knowledge, however, both animal studies (Studies 1 and 2) of this Ph.D. thesis are the first to examine the effect(s) of EAE either or not combined with different endurance training intensities on muscle contractile characteristics, disease course and systemic cytokine responses. Therefore, comparison with the work of others is difficult. Nevertheless, we have tried to discuss our results in the wake of all the relevant literature.

3.1.1. Muscle contractile properties: muscle strength and fiber type composition

In Study 1 and 2 isokinetic endurance strength of the foot extensors in healthy and EAE rats was evaluated after very intense swim training and after low, moderate and intense run training on a treadmill. Given the fact that isokinetic dynamometry is considered as the most standardized methodology to evaluate muscle strength, a custom built Aston-Miller-like rat dynamometer was used to document isokinetic muscle strength (185-187,310) following training. Our *in vivo* muscle strength measures indicated that peak muscle work usually produced during the initial 20-30 contractions of a series of 115 consecutive muscle contractions was completely absent in all EAE groups of both experiments (Study 1 and 2, see Figures 2.1.2. and 2.2.1.). This may suggest that fast twitch (glycolitic) that are predominantly

responsible for peak strength production muscle fibers were more affected. In order to investigate whether this finding was associated with altered muscle fiber morphology (181) we studied muscle fiber composition and/or distribution. Here, we clearly showed lower CSA of type IIb muscle fibers (TA: in Studies 1 and 2 $p<0.05$; EDL: Study 1: ns; Study 2 $p<0.05$) in all the EAE groups of both experiments. This probably explains the reported absence of peak work during the first 30 isokinetic muscle contractions (219,220). In accordance, de Haan et al. already reported a lower isometric peak force in EAE rodents and significantly smaller muscle fibers of the medial gastrocnemius compared to a control group (177). In summary and based on the above mentioned facts, changes in muscle contractile property were probably induced by EAE related hindquarter paralysis which caused inactivity-like muscle alterations (221).

In rats it is already documented that exercise training can remediate inactivity related consequences (222-224). In our work, only moderate to high intensity treadmill running (study 2) seems to improve ($p<0.05$) the CSA of type IIa fibers of TA in EAE whereas these muscle fibers did not change in the control group(s). This contrasts with the work of Brown et al. who already reported that running exercise can increase (~35%, $p<0.05$) the CSA of type IIa muscle fibers in healthy and aged rats (225). It must be noted, however, that one week of inactivity, as occurred during the paralysis period of EAE rats in both studies, reduces rat muscle mass with ~37% (226). This may have clouded the training effects in our control group. Future research should therefore take muscle biopsies immediately after the training period in order to clarify the impact on the muscle fiber characteristics. Given the fact that EAE reduced CSA of type IIb muscle fibers and these fibers are more susceptible to strength training (227-229) we also recommend to investigate its effect on muscle fiber characteristics following resistance training.

3.1.2. Functional benefits: disease course

To date, it is still unknown whether high intense endurance exercise affects the MS-related inflammatory exacerbation process (51). Given the fact that acute EAE mimics several inflammatory aspects of MS, the utilization of this frequently used animal MS model seems appropriate. However, because the acute EAE animal model only mimics some aspects of the inflammatory process of MS, caution is necessary to extrapolate findings to patients with MS. In an attempt to further investigate the impact of exercise on the EAE disease course we have assessed some clinical parameters such as hindquarter paralysis, body weight and food intake which are frequently used to describe the disease course of the animals. In this Ph.D. thesis, these clinical parameters were used to evaluate the functional benefits.

The very high intensity swimming protocol that we applied increased overall hindquarter paralysis in the rodents. In contrast, high intensity treadmill running (Study 2) slightly delayed (0.7 day, $p < 0.05$) the onset of the hindquarter symptoms. In this respect, it is important to note that swimming with an external weight of 5.5% of the body weight (study 1) involves a very high exercise intensity that is clearly above the anaerobic threshold (189,190). Furthermore, swimming exercise induces higher stress hormone levels compared to treadmill running (191). In contrast, rats submitted to the high intensity treadmill running protocol (18m/min and 25° inclination) of study 2 produced blood lactate concentrations that were probably close to the anaerobic threshold (188,217). Based on these facts, it seems fair to conclude that the exercise intensity applied in our first animal study was higher compared to the second study. Possibly this was too high to improve disease course. Thus, under the conditions of our studies, exercise intensities close to the anaerobic threshold are probably the optimal endurance training intensities to alter the EAE disease course. As indicated in study 2, compared to the sedentary EAE rodents hindquarter paralysis was delayed in the high intensity training group. This was paralleled by the bodyweight and food intake data. Other authors also reported delayed onset of clinical EAE signs following treadmill running in rats (174,175) and voluntary wheel running in mice (176). Although Le page and coworkers reported running speed progression (15-30m/min) with a fixed treadmill inclination of 8% (~4°) she did not measure blood lactate concentrations (174). Nevertheless, based on the reported running parameters the applied exercise intensity also seems to be close to the anaerobic threshold. Unfortunately, it is impossible to compare with the study of Rossi et al. because they applied voluntary exercise in mice and did not control or quantify training load and intensity (176). However, these authors reported that their mice before the motor impairments ran long distances while during the EAE symptoms were less active (176). Based on all these facts, it seems fair to conclude that exercise intensity seems an important factor to induce clinical benefits during EAE. Given the fact that the effects of exercise therapy on a variety of functional parameters in MS are at present under investigation, it might be worthwhile to investigate this hypothesis in MS patients. In this respect, very recently Collet et al explored the most effective exercise intensity in MS using three cycling intensities (218). These authors suggested that compared to the lower-intensity training group, higher-intensity exercise increased the 2 minutes walking distance. They also reported a higher dropout rate in this training group (218).

3.1.3. Cytokines

Based on the corresponding literature and as mentioned above, altered chronic cytokine levels may have contributed to the improved EAE disease course (Study 2) as already suggested by White et al (121). To the author's knowledge, study 2 is the first that investigated the effect of different endurance training intensities on basal serum cytokine levels of IL-6, IL-10 and TNFα in EAE rodents. Unfortunately, no changes in basal serum cytokine levels were observed. It is clear that the applied research design does not measure acute or local cytokine changes at tissue levels. In this regard, a muscle TNFα/IL-10 ratio shift towards an anti-inflammatory IL-10 profile has already been reported (231-233) but specific literature about TNFα and IL-10 blood concentrations after exercise in EAE is lacking. Furthermore, Chennaoui et al (211) reported lower ($p<0.05$) IL-6 concentrations in the cerebellum while systemic IL-6 levels were not modified. This warrants further research to examine the acute and local tissue related immunological responses of exercise therapy during EAE which could be important to optimize potential anti-inflammatory exercise strategies in MS.

3.2.MS

3.2.1. Muscle contractile properties: muscle strength

In an attempt to further improve standardization, studies performed in this Ph.D. thesis used ACSM-based training protocols (studies 3 and 4). These ACSM guidelines are more and more considered as the 'golden standard' in exercise therapy. Unfortunately, to date ACSM-based guidelines specifically developed for persons with MS do not exist. Therefore, the applied resistance training protocol was based on guidelines for older individuals because this population group often has similar reduced physical activity levels (98,101). In the resistance training study a low to moderately intense exercise regime was chosen to improve maximal muscle strength but at the same time to maximize training adherence. This strategy increased isometric muscle strength by ~10%. This is comparable with the work of White et al. (122) who showed a significant muscle strength increase (+8%). Although statistically significant, it must be noted that effects were small. Possibly, a higher-intensity resistance training program might further enhance muscle strength. In this context, Dalgas et al already investigated the effect of an intense resistance training program of 12-weeks and reported isometric knee-extensor strength increases of ~15% (130). On the one hand it must be noted that, compared to our study, (mean EDSS: 4.4, range from 2-6.5) Dalgas included subjects with a better disability status

(mean EDSS: 3.7 range from 3-5.5). On the other hand, training adherence in our study was very high while Dalgas and colleagues reported a dropout rate of 5 subject (~20%) in their exercising group. Furthermore, Chung and coworkers (133) indicated that persons with MS often suffer from asymmetric leg strength. These authors (133) suggested that classical bilateral strength training may not provide an optimal training stimulus for each leg (133). Thus to further optimize the training stimulus for each leg, unilateral strength training was applied in our resistance training study (study 3). In this regard, progressive unilateral resistance training has already been applied in stroke patients which also suffer from asymmetric muscles strength. This approach improved both paretic and non-paretic maximal lower limb muscle strength and reduced the stroke-associated functional limitations and overall disability (135,136). Our results (study 3) also suggested that unilateral muscle strength increased (interaction effects ranged from $p=0.006$ to $p=0.15$). Thus, in MS this 'unilateral training principle' may have the potential to further improve muscle strength even in severely affected legs (Table 2.3.5.) which is a new and important clinical finding. However, only modest strength increases in severely impaired legs were found which may support the fact that motor unit recruitment and firing rates are reduced as already indicated by Rice et al (60). This definitely requires further research because the strength enhancements we reported were only partially significant.

Other potential strength training modes include additional electro-stimulation which can be combined with an ACSM-based resistance training protocol. This may further enhance muscle strength compared to resistance training alone and this hypothesis was examined in Study 3. Unfortunately, our data indicated that maximal muscle strength increases were not different compared to the standardized resistance training protocol alone. However, for the leg extension and leg press exercises the registered training loads during the intervention period were higher ($p<0.05$) (electrical stimulation was only on quadriceps muscle) while exercise loads for the leg curl were similar for both resistance training groups. Our results match with Paillard and co-workers (242) who, in a review, concluded that superimposed electrical stimulations in a healthy population did not improve training efficiency compared to volitional exercise only. Nevertheless, the applied simultaneous electro-stimulation might be an interesting tool to assist persons with MS, that often experience cognitive deficits (260), during resistance training. More particular, patients are guided through their strength exercise because contraction velocity as well as the number of executions were indicated on the electrical-stimulation apparatus (personal communications of participants).

WBV is another strength training mode with clearly described exercise protocols that induced already some (modest) strength effects in healthy subject (144,311). Study 4 explored the effects of 20 weeks of WBV training on muscle performance and functional capacity in mild- to

moderately-impaired MS patients. In WBV research a variety of WBV protocols with different vibration parameters are used (312). A specific long-term WBV program for persons with MS does not exists. Hence, the used exercise protocol (study 4) was adopted from geriatric persons and induced strength increases in this population (269,270). The results of Study 4 suggest that 20 weeks of WBV training did not improve leg muscle isometric and dynamic strength nor strength endurance and speed of movement. To date, in MS patients a trend towards a higher leg muscle peak torque after one single WBV session and only minor acute effects on postural control and functional mobility have been reported (154,155). So far, only Schyns and co-workers (156) recently performed a longer-term 4-week randomized cross-over pilot study in 16 people with MS. They reported that WBV training had no additional benefits on muscle strength and functional performance compared to exercise alone. In their study, patients reported less spasms (156).

Finally, this Ph.D. thesis an attempt was made to study the effects of a standardized (unilateral) strength training protocol on some muscle contractile properties. The expected maximal muscle strength changes induced by the different standardized training protocols were assessed using an isokinetic dynamometer (Biodex®) which is accepted as the golden standard frequently used in healthy subjects and different patient populations (313-316) such as MS (58). Furthermore, in study 3 standardized RM tests were also performed to document strength gains on the training equipment. All these above mentioned methodological considerations are innovative methodological changes compared to previous publications. However, blinded assessors / patients and the (still) relative small sample sizes (studies 3 and 4) are limitations of the conducted experiments.

3.2.2. Functional benefits: clinical impact

Improved muscular strength is important if it also enhances functional aspects of daily life activities such as balance and walking capacity. A positive relationship between muscular strength and functional capacity during daily live in persons with MS has already been reported (59). Consequently, the clinical impact of strength training on many functionally important measures were also examined in this Ph.D. project.

EDSS is a general measure of the overall status of functioning of the MS patients which was included in our human studies. Although the EDSS measure is probably not sensitive enough, it is still used for daily routine practice and frequently reported in clinical research (317). Under the conditions of the present studies and in accordance to previous exercise studies (51,156), the EDSS scores of the subjects did not change during and following different strength training interventions therapy. However, given

the fact that training adherence was very high (Study 3: ~99% and Study 4:~98%) it seems fair to suggest that the applied intervention strategies appear to be very tolerable and safe.

More reliable and objective measures such as walking and balance tests were also performed by the participating subjects (study 3 and 4). With exception of the functional reach (study 3), resistance training or WBV did not affect the walking or balance capacity (Study 3 and 4). It is difficult to interpret these data but probably the improved reaching distance is a consequence of the stretching exercises performed during the cooling down of each resistance training session. Others authors often reported overall improved functional capacities following resistance training. Compared to our work this was associated with higher strength increases (124,125,130) As such, the strength increases (~+10%) we reported were probably too small to affect for example walking capacity. Another explanation might be that the walking capacity tests used in both strength training studies may not be sensitive enough for a subgroup with mild MS (study 6). In addition, the walking capacity tests probably reside more on muscle endurance strength whereas the different strength training protocols that we applied mainly focused on improving maximal muscle strength (study 6). In this regard, Dalgas et al included tests were the role of knee extensor strength was more pronounced such as a chair stand and a stair climb assessment (130) as compared to our walking and balance capacity tests. In accordance with the effects of WBV on muscle contractile properties, functional capacity did also not improve following 20 weeks of training in the MS patients of this study. This confirms the findings of Schyns et al (156) who reported unchanged 10m-walking time and Timed Up and Go performance following WBV training in MS.

3.2.3. Cytokine response

Given the pivotal role of systemic cytokines in MS, the last objective of this thesis was to explore the effect of strength training on chronic systemic cytokines in persons with MS. Given the fact that skeletal muscle can produce cytokines (106) and that these immunoregulatory glycoproteins are also important in the inflammation process of MS (24-29,34,36,37) we expected that strength training would change (long-term) peripheral concentrations (209) that, in turn, might affect the MS inflammation process (318).

Unfortunately, the three different strength training interventions of this thesis did not affect chronic cytokine levels. In contrast, White et al reported a decrease in some basal pro-inflammatory cytokine concentrations (IFNγ, $p<0.05$; CRP, $p<0.05$; TNFα $p=0.07$) after 8 weeks of resistance training (121). Our findings confirm former results of Bruungaard et al (286)

indicating unchanged pro-inflammatory TNFα levels in geriatric persons after 12 weeks of resistance training and the conclusions made by the review of de Salles and co-workers (287). Furthermore, in MS it is suggested that low IL-10 concentrations may result in excess inflammation (18). In the present study IL-10 and IL-1ra concentrations were unaffected which contrasts with the findings of White et al (121) who documented a reduction (43% $p<0.05$) of IL-10 concentrations after 8 weeks resistance. Because of the explorative nature of these studies and the existence of other (immunologic) mechanisms/parameters that could be responsible for affecting the EAE disease course further research is necessary in this domain (see future perspectives).

3.3. Limitations and future perspectives

3.3.1. Limitations of the current studies

The present Ph.D. thesis consists both animal experiments and studies with persons with MS. The authors have specifically chosen this approach in order to broaden the scope of this thesis. However, given the fact EAE is not equal to MS and vice versa, this approach, at some points, also narrows the scope of this work. The following paragraphs will discuss, either or not combined with the work of others, methodological adjustments for future research.

3.3.1.1. Animal research

(i) In his review Heesen et al (319) indicated that acute and chronic stress on EAE prior or after induction inhibited clinical EAE symptoms. Study 1 suggested that very intense swim exercise worsened overall hindquarter paralysis while intense treadmill running (study 2) slightly delayed the onset of hindquarter paralysis. Given the fact that the hypothalamic-pituitary-adrenal axis response, which is exercise mode dependent (191), can affect the EAE disease process (320) this possibly explains the difference between study 1 and 2. **(ii)** A familiarization period was conducted in both studies (1 and 2) to familiarize the rodents to their experimental exercise intensities. As a consequence and given the fact that different exercise intensities were applied (study 2), the aerobic capacity of the exercising animals compared to the sedentary rats differed between groups at induction. **(iii)** Furthermore, EAE was injected in both hind paws of the rodents. During treadmill running this might have caused inconvenience that possibly may have affected the running performance. **(iiii)** To calculate the minimum sample size literature based effect sizes are used for *a priori* power analysis. To the author's knowledge, however, the animal studies performed in this thesis are the first to investigate this matter. As a result, it was difficult to calculate statistical

power a priori. Post hoc power analysis based on the actual effect size of the current studies (253), however, indicated that statistical power reached (or was just below) the required threshold. The statistical power of hindquarter paralysis and muscle strength of study 1 and TNFα and IL-10 of study 2 reached the threshold of 0.80. Power of muscle strength data and muscle fiber determination of study 1 was 0.74 and 0.75, respectively. Hindquarter paralysis, muscle fiber morphology and IL-6 of study 2, however, was between 0.45-0.56. **(iiiii)** Given the fact that the impact of endurance training on muscle fiber characteristics in EAE may have been reduced by inactivity during the paralysis period (Study 1 and 2) this may have clouded exercise effects. **(iiiiii)** Finally, both animals studied the impact of exercise on muscle contractile properties was investigated in the glycolytic TA and oxidative EDL muscles because during swimming these muscles are important (321). As such, to compare between studies and because these muscles are active during (inclined) running (321) as well we also assessed muscle contractile properties in TA en EDL of the second animal study. However, treadmill running mainly affects gastrocnemius and soleus. Therefore, we may have missed possible training effects.

3.3.1.2. Human research

(i) In the human part of this thesis a priori statistical power analysis are lacking. However, post hoc power analysis were performed on the actual effect sizes of each study. In accordance to the animal part, many variables of these studies reached the threshold of 0.80. More particular, unilateral leg strength power analysis of study 3 reached a power of 0.86 (265). In addition, IL-10 and IL-6 of study 5 and all regression analyses of study 6 were also above 0.8. In study 3 post-hoc power analysis of the training results, using the actual instead of the literature-based effect size, reached a power of 70.3%. However, power analysis using a larger (+15%), literature-based, effect size would have reached a power of 0.83 (265). Very similar findings were found for study 4 because the main effect was just below 0.8 (0.72). Finally, IL-8, IL-1β, TNFα, IL-12p70 and IL-1ra were underpowered (power <0.5). **(ii)** Another limitation was investigator blinding, which was only the case for the research neurologist and all the investigators during baseline measurements. As such, it could be argued that the investigators were biased and may have influenced the results. Although this limitation is acknowledged, it is also apparent that only modest gains in muscle strength and no gains in functional mobility were reported, therefore an overestimation of the effects is rather unlikely. **(iii)** Furthermore, subjects of the resistance training study and WBV intervention were randomized based on EDSS, gender and age and not on baseline muscle strength because we assumed that similar disability levels would render similar lower limb muscle

strength. **(iiii)** Although the WBV study included a control group, we did not apply 'placebo' vibrations and an individualized training progression approach using optimal individual vibration parameters assessed for example by electromyography or calf muscle strength measurements **(iiii)** Finally, in study 3 (published) we concluded that unilateral strength training improved training efficiency. However, because we did not compared the unilateral and bilateral outcomes it is probably better to conclude that unilateral training also improves muscle strength of severely affected legs.

3.3.2. Future perspectives

In an attempt to elucidate the effects of exercise therapy on muscle contractile properties, functional capacity and cytokine balance, other questions arose that could be the basis for future research. They, in part, are based on new insights presented in this Ph.D. project either or not combined with the work of others. They will be described in the following paragraphs.

3.3.2.1. Aerobic exercise

(i) Given the fact that intense running exercise slightly delayed the onset of the hindquarter paralysis symptoms in EAE, it seems worthwhile investigate the impact of high intensity exercise training aerobic exercise capacity in MS. In this regard, Collet et al. already suggested that higher-intensity exercise is more effective with respect to the performance of a 2 minutes walking test. This may also support our findings that clinical alterations might be intensity related. However, these authors also reported a higher dropout rate in this training group (218). **(ii)** Furthermore, under the conditions as applied in study 2 chronic systemic cytokine serum levels were not altered but other (immunologic) mechanisms/parameters, however, could be responsible for affecting the acute EAE disease course. Because in other animal models cytokine responses were locally up regulated such as in the muscle (210) or in different parts of the brain (216,322) without affecting serum cytokine concentrations (322). Therefore, it may be interesting to investigate the impact of endurance and/or resistance training on acute cytokine responses not only in the serum but also in the muscle and CNS of EAE rodents. **(iii)** Castellano and coworkers (288) also explored the blood concentrations of brain derived neurotrophic factor (BDNF) in persons with MS. They suggested that BDNF was up regulated (288). In addition, another study also reported that BDNF levels in the CSF and active MS lesions were higher in MS patients (323). Sarchielli et al (324) also documented higher concentrations of BDNF (CSF and serum) during a relapse and the following remission phase compared to a stable disease phase and they assumed that lower BDNF levels might be responsible for

disease progression and axonal damage (324). Based on the above mentioned facts, the impact of exercise therapy on these potential MS modifying mechanisms should be investigated in the future. **(iiii)** Given the fact that acute EAE mimics only the inflammation process, the effect of high intense treadmill running on the hindquarter paralysis should also be investigated in a chronic, EAE model

3.3.2.2. Resistance training

(i) Compared to the strength gains (~+10%, Study 3) we reported a higher-intensity resistance training program might further enhance muscle strength. In this regard, Dalgas et al. already investigated the effect of an intense resistance training program of 12-weeks and reported knee extensor strength improvements of ~+15% (130). This additional strength gain (~+5%) after an intense resistance training protocol may be responsible for the alterations of the functional capacity that was also reported by these authors. However, to affect functional capacity and according to the general training principle of specificity (102), it also seems useful to evaluate walking capacity after a combined exercise protocol (resistance training and endurance training) which includes treadmill exercises. **(ii)** Furthermore, the results of the resistance training study (Study 3) indicate that exercise effects were already reached after 10 weeks of training. Hereafter training effects remained stable. Clearly, longer-term exercise studies including gradually increased training intensities are necessary to investigate if higher training effects can be expected. **(iii)** In our MS training studies we did not investigated muscle fiber morphology. To date, Dalgas et al. are the only investigators that explored the effect of a standardized resistance training program on muscle fiber size and distribution (132). These authors reported increased type II fibers of the vastus lateralis muscle while type I muscle fibers and the distribution were not changed (132). However, these histological muscle fiber changes should be investigated in a larger sample size. Furthermore, to date the impact of exercise therapy on biochemical changes such as the metabolism of the muscle are still unknown **(iiii)** In Study 6 different muscle strength measures were correlated with walking capacity and the outcome suggested that isokinetic muscle endurance strength and knee flexor strength, respectively, are highly related, especially in moderately impaired persons with MS. Based on these facts, it seems worthwhile to investigate the impact of a resistance training program, that consists of strength endurance and knee flexor exercises, on the walking capacity of persons with MS. **(iiiii)** Finally, the long-term (>6months) effects are not clear. Here different set-ups ranging from standardized resistance training programs in a fitness center with or without personnel guidance to standardized home based maintenance strength training might be interesting to investigate.

3.4. Conclusions

3.4.1. EAE

- EAE reduces the cross sectional area of type IIb muscle fiber of the foot extensors. This may explain the absence of the isokinetic peak work during a series of successive muscle contractions.
- The impact of exercise on EAE disease is probably intensity depended. Exercise intensities near the anaerobic threshold probably delays the onset of hindquarter paralysis while exercise intensities that exceed the anaerobic threshold worsen the disease course.
- Under the condition of the present study exercise does not alter basal cytokine serum levels in EAE.

3.4.2. MS

- Standardized ACSM based resistance training increased maximal muscle strength in persons with MS, while additional electro-stimulation did not further enhance muscle strength. Whole Body Vibration does not affect maximal muscle strength.
- Unilateral resistance training increased muscle strength even in severely impaired legs.
- Despite the fact that muscle strength was improved, functional capacity was not affected by resistance training either or not combined with additional electro-stimulation or WBV.
- In PwMS with moderate ambulatory dysfunction knee-extensor endurance strength, as well as isometric knee flexor strength are important predictors for walking capacity.
- Resistance training either or not combined with additional electro-stimulation and WBV did not affect chronic cytokine levels in MS.

Chapter IV:

Nederlandstalige samenvatting

4.1. Achtergrond

Multiple Sclerose (MS) is een progressieve auto-immune aandoening van het centrale zenuwstelsel (CZS). Deze chronische ziekte begint meestal bij jongvolwassenen met een gemiddelde leeftijd tussen 20 en 40 jaar. Wereldwijd zijn er meer dan 2,5 miljoen mensen gediagnosticeerd met deze ziekte. Ondanks intensief onderzoek is de onderliggende oorzaak van deze ziekte nog steeds niet gekend. Gebaseerd op de meest gangbare hypothese wordt MS veroorzaakt door een ontregeling in het immuunsysteem. In het immuunsysteem van personen met MS keren bepaalde immuuncellen, de zogenaamde autoreactieve T-cellen, zich tegen lichaamseigen myeline en veroorzaken een chronische ontstekingsreactie gekenmerkt door een cytokine gemedieerde inflammatoire cascade reactie. Deze ontstekingsreactie is de oorzaak van het beschadigen van het myeline waardoor de neurologische symptomen ontstaan omdat op de plaats van de ontsteking littekenweefsel wordt gevormd. De functie van dit myeline is het omgeven van de zenuwuitlopers met een isolerende laag zodat de prikkelgeleiding optimaal en snel verloopt. Verder worden ook de myeline producerende cellen (oligodendrocyten) in het CZS aangetast. Tot op heden is MS helaas moeilijk te diagnosticeren. Vaak neemt het een hele periode in beslag vooraleer de correcte diagnose kan gesteld worden. Hiervoor worden de weefsellaesies in de hersenen en ruggenmerg in kaart gebracht (MRI) en wordt de progressie van het littekenweefsels in deze weefsels opgevolgd. Verder zijn er nog andere klinische en paraklinische tekenen die bijdragen tot het stellen van de diagnose, zoals het onderzoeken van het ruggenmergvocht op de aanwezigheid van zogenaamde 'oligoclonale banden'. In tegenstelling tot andere aandoeningen, zijn er geen specifieke parameters in het bloed die bruikbaar kunnen zijn voor diagnosestelling. Wel zijn er bloedparameters die gebruikt worden om de graad van inflammatoire opflakkeringen (cytokines) in te schatten en zo de behandeling hiervan (zie later) efficiënter uit te voeren.

MS komt voor in verschillende vormen maar het meest frequent voorkomende type is de relapsing-remitting (RR) vorm die gekenmerkt wordt door ziekteopflakkeringen die de klinische symptomen doen toenemen. Na enige tijd verdwijnen deze, en het functioneren van de persoon wordt hersteld tot ongeveer op het niveau van voor de ziekte opstoot. Vaak gaat na verloop van tijd dit RR-type over in een secundair progressieve vorm (SP). Deze vorm is gekenmerkt doordat de opflakkeringen verdwijnen maar dat de ziekte en klinische symptomen progressief blijven toenemen. Hiernaast is er nog een primair progressieve MS vorm (PP). Deze is analoog aan de SP vorm, enkel zijn er nooit ziekte opstoten merkbaar, maar treedt er vanaf het ontstaan van de ziekte een geleidelijke toename van de ziektesymptomen op. Aangezien de weefsellaesies op overal in het CZS kunnen ontstaan, leidt MS tot een brede waaier van klinische symptomen. De meest voorkomende symptomen zijn

visuele en sensorische problemen, cognitieve stoornissen maar vooral ook motorische problemen zoals spierzwakte. Deze symptomen, die rechtstreeks het gevolg zijn van een verminderde prikkelgeleiding over de zenuwuitlopers van het CZS, worden vaak de primaire MS symptomen genoemd. In vele gevallen leiden ze ook tot secundaire consequenties. Deze kunnen best samengevat worden onder de noemer "inactiviteitgebonden problemen/gevolgen" zoals een verminderde aerobe capaciteit en gedaalde spierkracht.

Aangezien er tot op heden geen curatieve therapie bestaat voor MS, spitsen de huidige behandelingen zich vooral toe op het onderdrukken van het immuunsysteem (inflammatoire cytokines) en de bijhorende functionele symptomen. Deze medicamenteuze behandeling heeft tot doel de frequentie en de ernst van de opflakkeringen te verminderen. Naast deze farmacologische behandeling zijn er ook verschillende vormen van revalidatietherapieën voor de behandeling van MS. Gelet op de huidige ongeneeslijkheid van de ziekte, richten deze zich vooral op het zo goed mogelijk behouden van de functionele capaciteit/mobiliteit van MS patiënten. Deze therapieën bestaan hoofdzakelijk uit kinesitherapie en ergotherapie welke vooral als doel hebben om het participatieniveau van de patiënt te handhaven of te verbeteren. Daarenboven zijn er ook gespecialiseerde verpleegkundigen die de patiënten kunnen wegwijs maken in hun dagdagelijkse verzorging en hygiëne. Verder zijn er ook verschillende vormen van oefentherapieën onderzocht die vaak een onderdeel zijn van de kinesitherapeutische behandelingen. Deze kunnen ook aanzien worden als een volledig apart onderdeel in de behandeling van MS omdat deze vormen zich vooral toeleggen op het behandelen van de inactiviteitgebonden secundaire MS gevolgen. Verder wordt er ook gesuggereerd dat oefentherapie misschien het potentieel heeft om het inflammatoire proces van MS positief te beïnvloeden.

4.2. Inhoud doctoraatsproefschrift

Het laatste decennium is er op het gebied van onderzoek met betrekking tot de fysieke revalidatie van personen met MS duidelijk een inhaalbeweging bezig. Vaak concentreren de wetenschappelijke revalidatiestudies zich vooral op het verbeteren van het gereduceerde fysiologische profiel van de MS patiënt met behulp van diverse vormen van oefentherapie. Oefentherapie kan opgesplitst worden in twee grote oefenmodaliteiten, namelijk uithoudings- en krachttraining. Uiteraard is er al onderzoek naar de effecten van deze twee vormen van oefentherapie bij personen met MS gebeurd maar wel voornamelijk met betrekking tot de invloed van uithoudingstraining op het inspanningsvermogen. Algemeen wordt aangenomen dat deze trainingsvorm veilig is en tot de verbetering van het aerobe uithoudingsniveau van de MS patiënt leidt. Voor

uithoudingstraining wordt het advies geformuleerd om aan een frequentie van 2 tot 3 maal per week met een lage tot matige intensiteit te trainen. Helaas, is tot op heden niet geweten of personen met MS ook hogere trainingsintensiteiten verdragen. Indien zo, zou dit de efficiëntie van uithoudingstraining nog aanzienlijk kunnen verbeteren. Bijgevolg kan men momenteel niet aangeven wat de optimale trainingsintensiteit is voor deze patiëntenpopulatie. Verder is er ook, maar in zeer beperkte mate, onderzocht wat de impact is van uithoudingstraining op spierkracht. Uit enkele van deze studies blijkt dat spierkracht kan verbeteren, terwijl andere auteurs geen veranderingen rapporteerden. De effecten van uithoudingstraining op de samenstelling en distributie van spiervezels zijn evenwel nog nooit bestudeerd. Verder zijn er ook indicaties dat uithoudingstraining de inflammatoire cytokine balans in een positieve richting kan doen kantelen. Helaas zijn deze indicaties exploratief waardoor er geen definitieve besluiten kunnen worden geformuleerd.

Bij de start van dit doctoraatsproject en in tegenstelling tot uithoudingstraining, kon op het gebied van krachttraining nog geen wetenschappelijk gefundeerde conclusie geformuleerd worden. De hoofdreden hiervan was dat de methodologische kwaliteit van de uitgevoerde studies beperkt was waardoor we de getrokken conclusies best nuanceren. Meerbepaald werden er zeer diverse en korte trainingsprogramma's en uiteenlopende evaluatietechnieken gebruikt, die vaak niet gestandaardiseerd waren. Ook was er nood aan het opnemen van een controlegroep om zo de trainingsprogressies beter te kunnen vergelijken en een objectiever oordeel te kunnen vellen. Uit andere wetenschappelijke studies weten we dat spieren de mogelijkheid bezitten om cytokines te produceren en dus het potentieel bezitten om ontstekingsreacties te kunnen beïnvloeden. Helaas is de invloed van krachttraining op de perifere cytokinebalans enkel door één studie summier onderzocht. De voorbij jaren stellen we evenwel een verbetering vast op het gebied van de methodologische kwaliteit van de krachttrainingstudies waardoor men de uitgevoerde studies beter kan vergelijken.

Naast deze twee klassieke vormen van oefentherapie, zijn er ook een aantal andere trainingvormen die het potentieel hebben om spierkracht te doen toenemen. Dit zijn trainingsvormen die spierkracht kunnen verbeteren door het geven van bijvoorbeeld een additionele elektrostimulatieprikkel tijdens een gestandaardiseerd krachttrainingsprogramma of pees/spiervibraties via een trilplaat. Uiteraard passen deze trainingsvormen perfect binnen de context van deze thesis. Tot op heden werden ze slechts zeer summier onderzocht bij personen met MS waardoor dezelfde opmerkingen kunnen geformuleerd worden als bij krachttraining. Er is dus nood aan studie(s) met een goede methodologische kwaliteit, met de inclusie van een controle groep en met het bestuderen van langere termijn training.

Samenvattend kunnen we stellen dat er een aantal specifieke

tekortkomingen zijn om wetenschappelijk gefundeerde conclusies te maken omtrent de impact van oefentherapie op de contractiele eigenschappen van een spier of spiergroep bij personen met MS. Dit vormt de eerste doelstelling van deze thesis. Vanuit een klinisch oogpunt is het tevens belangrijk om na te gaan of spierkrachtverbeteringen zich ook vertalen in betere functionele prestaties zoals de evenwichts- als wandelcapaciteit. Dit is het tweede onderzoeksobjectief van deze thesis. Ten slotte zijn er aanwijzingen dat oefentherapie positieve effecten zouden kunnen hebben op het MS gerelateerde ontstekingsproces. Daarom gaan we in deze doctoraatsthesis, ten derde, na hoe, met behulp van verschillende vormen en intensiteiten van oefentherapie, pro- en anti-inflammatoire cytokine balans kan worden beïnvloed. Deze verschillende methodes hebben als doel om de oefentherapie voor personen met MS verder te optimaliseren.

4.2.1. Dierexperimentele studies

De eerste twee studies van dit proefschrift hadden als doel om de optimale intensiteit van de uithoudingstraining en de invloed op de contractiele spiereigenschappen te bepalen. Zoals hierboven beschreven, is het nog niet gekend of intensieve uithoudingstraining door personen met MS verdragen wordt. Daarom, en ook omdat spiervezeldeterminatie een invasieve techniek omvat, werd er geopteerd om deze studies uit te voeren bij een MS proefdiermodel, namelijk acute Experimentele Auto-immune Encefalomyelitis (EAE). Het inflammatoire proces van dit proefdiermodel vertoont veel gelijkenissen met het ontstekingsproces van MS en leidt ook tot klinisch waarneembare verlammingsverschijnselen, zoals paralyse van de achterpoten van de knaagdieren. De ziekte wordt door middel van een injectie in de beide voetzolen in de dieren geïnduceerd. Het geinjecteerde product bevat een olieachtige substantie waaraan mycobacteriën en MBP (myelin based proteine) werden toegevoegd. In de eerste studie werden de ratten (EAE en gezonde controle dieren) onderworpen aan zwemtraining waarvan de intensiteit werd verhoogd door een bijkomend gewicht (5.5% van het lichaamsgewicht) aan de staart te bevestigen. Na een gewenningsperiode van 7 dagen werd EAE geïnduceerd en zwommen de ratten een uur per dag gedurende 10 opeenvolgende dagen. Aangezien na deze periode de verlammingssymptomen gradueel optreden, werd de training stopgezet. Tijdens deze fase bereiken de klinische verlammingsverschijnselen een piek om vervolgens weer volledig te verdwijnen (ongeveer 17 dagen na ziekte inductie). Vanaf het begin van de studie werden lichaamsgewicht en de voedselopname dagelijks geregistreerd. Vanaf de EAE inductie werd dagelijks de klinische neurologische verlammingsverschijnselen van het achterkwartier aan de hand van een schaal gescoord. Op de laatste dag van het experiment (dag 17 post EAE inductie) werd de isokinetische (50°/s) uithoudingskracht (115

concentrische contracties) van de dorsiflexoren van de rechter enkel gemeten en werden van de linker achterpoot spierbiopten van m.tibialis anterior (TA) en m. extensor digitorum longus (EDL) genomen.

Omdat in de eerste proefdierstudie de trainingsintensiteit moeilijk te controleren was, werden in de tweede studie de ratten (EAE en gezonde controledieren) getraind op een loopband waarbij na een 14-daagse gewenningsperiode en EAE inductie aan drie verschillende snelheden en inclinaties van het looptapijt werden gelopen. In een eerste trainingsgroep werd aan een lichte intensiteit gelopen (4m/min) zonder inclinatie. De tweede groep liep aan een matige snelheid (11m/min) en een inclinatie van 15°. De laatste trainingsgroep liep aan een hoge intensiteit, namelijk 18m/min met een helling van 25°. Naast deze trainingsgroepen werden er ook nog dieren in een sedentaire groep onderverdeelt. Ook in deze studie werden dagelijks het lichaamsgewicht en voedselopname geregistreerd en bij de EAE groep werden, na inductie, de klinische neurologische verlammingsverschijnselen ook genoteerd. Net zoals bij de eerste studie werden op de laatste experimentele dag (dag 17 post EAE inductie) de dieren onderworpen aan een isokinetische (50°/s) uithoudingskracht (115 concentrische contracties) van de rechter dorsiflexoren van de enkel en werden spierbiopten van TA en EDL van de linker achterpoot genomen. In deze tweede studie werden ook basale serum cytokine concentraties gemeten om het perifere inflammatoire proces in de verschillende subgroepen te exploreren. Dit is belangrijk aangezien bij MS ook ontstekingsreacties betrokken zijn en er zelfs verondersteld wordt dat oefentherapie anti-inflammatoire processen induceert die positieve invloed zouden kunnen hebben om de MS gerelateerde ontstekingsprocessen.

Op basis van de drie vooropgestelde hoofddoelen van deze thesis worden hieronder resultaten kort besproken volgens deze volgorde: *(i)* spier contractiele eigenschappen, *(ii)* functionele voordelen en de *(iii)* perifere cytokine response.

In beide studies werden zeer vergelijkbare contractiele eigenschappen gevonden. *(i)* Alle EAE subgroepen (zwemmers en lopers aan verschillende intensiteiten) toonden analoge isokinetische spieruithoudingskrachtprestaties. Bij deze dieren ontbrak namelijk, en in tegenstelling tot de gezonde controle dieren, de piekkracht tijdens de eerste 30 contracties uit een reeks van 115 isokinetische spiercontracties waardoor er geen daling in hun uithoudingskracht was. Deze resultaten werden bevestigd door de analyse van de spiervezels, namelijk de oppervlakte van de type IIb vezels (verantwoordelijk voor explosieve piekkracht tijdens de eerste spiercontracties) van de TA (studie 1 & 2) en EDL (studie 2) was significant afgenomen. Enkel bij de matige en intense lopers (studie 2) vonden we een kleine toename in de dwarsdoorsnede van type IIa van de TA. Helaas vonden we geen trainingseffecten op niveau van spieruithoudingskracht en spiervezelsamenstelling in de EAE subgroepen. *(ii)* Uit de loopband studie (studie 2) bleek ook dat er bij de intense

trainingsgroep (wellicht rond de anaerobe drempel) een vertraging optrad in het ontstaan van de verlammingssymptomen. In tegenstelling, zeer intense training (studie 1, duidelijk boven anaerobe drempel) een nadelig effect op het verloop van de achterpootverlamming van de proefdieren. Hieruit blijkt dat de training intensiteit belangrijk is om positieve effecten op de klinische symptomen te induceren. *(iii)* De basale cytokine profielen (inflammatoire parameter) veranderde niet door de looptraining. Dus waarschijnlijk kunnen deze niet verantwoordelijk worden geacht voor de vertraging in het ontstaan van de verlammingsverschijnselen. In beide dierexperimentele studies zijn er wel een paar opmerkingen die in acht moeten genomen worden bij het interpreteren van de resultaten. Tot onze verbazing vonden we geen trainingseffecten op spiervezeltypering ongeacht de trainingsmodaliteit en intensiteit. Dit zou mogelijks te verklaren kunnen zijn door het feit dat onze proefdieren voor de spierbiopsies 6 dagen inactief waren, wat mogelijk de trainingseffecten kan maskeren. Tot slot geven onze resultaten aan dat uithoudingstraining het cytokine profiel van het serum niet veranderd. Uiteraard zijn er nog andere potentiële 'neuroprotectieve' mechanismes bekent uit de literatuur die wij niet onderzochten. Deze kunnen misschien ook verantwoordelijk zijn voor de positieve impact op de klinisch neurologische symptomen.

4.2.2. Studies bij personen met MS

In het humane luik (studies 3, t.e.m. 6) van dit proefschrift werd het effect van verschillende krachttrainingmodaliteiten (Studies 3 en 4) op spier contractiele eigenschappen (studies 3 en 4), functionele verbeteringen (studies 3 en 4), de onderlinge relatie van deze twee laatste factoren (studie 6) en impact op het chronische cytokine profiel (studie 5) bestudeerd. Zoals hierboven beschreven, was er vooral nood aan krachttrainingstudies (zowel klassieke vormen als andere potentiële krachttrainingvormen zoals additionele elektrostimulatie en trilplaattraining) met een gestandaardiseerde methodologie en met een langere trainingsduur. Daarom werd er in deze studies gebruik gemaakt van gestandaardiseerde oefenprogramma's (ACSM-gebaseerd) met objectieve en valide meetprincipes (isokinetische dynamometrie) en met een geïncludeerde controle groep. Hiernaast en gebaseerd op het feit dat personen met MS vaak lijden aan (kracht)asymmetrie van de onderste ledematen, werd er geopteerd om de krachttraining (studie 3) unilateraal uit te voeren teneinde de trainingsprikkel te optimaliseren voor elk been apart. In deze context werden er door de subjecten ook unilaterale oefeningen tijdens de trilplaattraining (studie 4) uitgevoerd. Verder werden een aantal functionele taken zoals wandelafstand, evenwicht en klinische symptomatologie (EDSS)

in beide studies geëvalueerd (studies 3 en 4). De trainingsduur van dit experiment omvatte telkens twee trainingsperiodes van 10 weken waarna de hierboven beschreven metingen telkens opnieuw herhaald werden. Naast deze klinische metingen werd er op de drie meetmomenten (baseline, na 10 en 20 weken training) ook telkens een bloedstaal afgenomen om de basale pro- en anti-inflammatoire cytokineconcentraties te bepalen (Studie 5).

Naar analogie van de bespreking van de dierexperimentele resultaten van deze thesis worden hieronder de resultaten kort besproken volgens de volgorde van de drie vooropgestelde hoofddoelen: *(i)* spier contractiele eigenschappen, *(ii)* functionele voordelen en de *(iii)* perifere cytokine response.

(i) De spierkracht nam toe na zowel de klassieke krachttraining als na de klachttraining met additionele elektrostimulatie. Er was evenwel geen verschil tussen de krachttoenames in deze groepen. Verder werd, via unilaterale training, ook het trainingspotentieel aangetoond van de zwakkere (afunctionele) benen, wat zeker een vernieuwende klinische bevinding is. In tegenstelling tot de krachttraining bleef de spierkracht onveranderd na trilplaat training (studie 4). *(ii)* Helaas vertaalde de gevonden krachtwinsten (studie 5) zich niet in een verbetering van de wandelcapaciteit. Enkel de lenigheid verbeterde na krachttraining maar dit zou vooral het gevolg kunnen zijn van het stretchen van de beenspieren die aan het einde van elke trainingssessie werden uitgevoerd als cooling down. *(iii)* Tot slot lijkt het er op dat de basale cytokine balans niet werd beïnvloed door krachttraining (klassieke noch de alternatieve krachttraining vormen; studie 5). Ook hier moeten we enkele opmerkingen in acht nemen met betrekking tot het interpreteren van onze resultaten. Om de statische kracht in toekomstig onderzoek te verhogen, is het opportuun om meer subjecten per groep op te nemen. Verder waren de testleiders en subjecten niet geblindeerd voor de ondergane therapievorm. Hoewel het moeilijk is om de subjecten te blinderen voor hun opgelegde therapie vorm is het toch aan te bevelen.

Aangezien andere krachttrainingstudies na maximale krachtwinst wel regelmatig verbeteringen rapporteerde op functioneel niveau, hebben we met onze baseline data van de verschillende kniestrekkers krachttypes (isometrisch, isokinetisch en krachtuithouding) en de isometrische kniestrekkers versus isometrische knie buigers, de relatie met de wandelcapaciteit bestudeerd (Studie 6). Door deze relatie te onderzoeken kunnen volgende krachttrainingstudies verder worden geoptimaliseerd. Verder werd er ook de hypothese gemaakt dat zowel spierkracht als wandelcapaciteit afhankelijk zijn van de functionele mogelijkheden van de persoon met MS. Dit werd gebaseerd op de EDSS score van de mensen (milde MS subgroep: EDSS ≤ 4.0 en matige MS subgroep: EDSS > 4.0 en ≤ 6.5). Deze veronderstelling werd door de resultaten bevestigd. Eveneens toonden we aan dat in de matige MS subgroep niet zozeer maximale

krachtverbetering van de kniestrekkers vereist is om de wandelcapaciteit te doen toenemen maar dat de uithoudingskracht van deze spieren een hogere relatie vertoonde en dus meer invloed op de wandelcapaciteit heeft. In de matige subgroep bleek dat ook een belangrijke rol toegeschreven moet worden aan de maximale knie buiger kracht om de wandelcapaciteit te laten toenemen.

Volledigheidshalve moet er ook aangegeven worden dat de studies uitgevoerd in het kader van dit doctoraatsproject beperkingen bevatten. *(i)* Zo kan men opmerken dat er geen a priori statische power analyses gemaakt werden. Maar om dit correct te berekenen moet men zich baseren op, in de literatuur beschikbare effectgrootte. De dierenstudies die we uitvoerden zijn deze de eerste studies, die enerzijds de invloed van heel intense zwemtraining als anderzijds de impact van laag tot hoog intense looptrainingen bij EAE ratten onderzocht. Hierdoor was het zeer moeilijk tot onmogelijk om een zo correct mogelijke inschatting te maken van de groepsaantallen. Een statistische power analyse na de dierexperimentele studies, die gebruikt maakte van de bekomen effectgrootte, blijkt dat er een aantal metingen de kritische grens van 0.8 halen, maar een aantal metingen (net) onder deze grens vallen. Voor het humane gedeelte kunnen we hetzelfde besluiten met het verschil dat op basis van een literatuur gebaseerde effectgrootte de power over de kritische grens zou zijn. *(ii)* Verder hebben we de strategische keuze gemaakt om een veelheid van aspecten te onderzoeken / exploreren om tot ruime conclusies te kunnen komen. Dit heeft dan wel tot gevolg dat de diepgang van ons onderzoek naar de onderliggende mechanismen mogelijks te beperkt was. Zo kunnen een aantal mechanismen zoals de lokale cytokine productie verder worden uitgediept. Zelfs andere mechanismes, die buiten de invalshoek van dit proefschrift vallen, kunnen verantwoordelijk zijn voor mogelijke neuro-protectieve effecten na oefentherapie(vb. BDNF). *(iii)* Om ethische redenen hebben we geen spiervezelkenmerken kunnen opnemen in het humane luik van dit project. In te toekomst zou dit wel moeten gebeuren aangezien dit in belangrijke mate kan bijdragen tot het verder optimaliseren van oefentherapie.

Gebaseerd op de resultaten van dit proefschrift kunnen we de volgende algemene conclusies / suggesties formuleren.

EAE:

- EAE verkleint de dwarsdoorsnede van de type IIb vezels van de voetextensoren. Dit kan een verklaring zijn voor de afwezigheid van de piekkracht tijdens de eerste 30 van de 115 concentrische isokinetisch spiercontracties.
- De invloed van oefentherapie op het EAE ziekte verloop lijkt afhankelijk van de intensiteit. Trainen rond de anaerobe drempel lijkt het ontstaan van de klinische symptomen te vertragen terwijl hogere intensiteiten deze lijken te verergeren.
- Uithoudingstraining verandert de basale cytokine concentraties van het serum bij EAE ratten niet.

MS:

- Gestandaardiseerde krachttraining verbetert de maximale spierkracht in personen met MS, terwijl additionele electro-stimulatie niet leidt tot een extra krachtstoename. Trilplaattraining had geen impact op de spierkracht.
- Unilaterale training verbeterde de spierkracht zelfs in zwakkere benen.
- Ondanks het feit dat de spierkracht verbetert bleef de functionele capaciteit van de personen met MS onveranderd door zowel krachttraining, al dan niet gecombineerd met additionele electro-stimulatie als trilplaattraining.
- In personen met matige MS beperkingen zijn de uithoudingskracht van de kniestrekkers als de maximale isometrisch kracht van de knie buigers belangrijke voorspellers van de wandelcapaciteit.
- Bij personen met MS beïnvloeden krachttraining, al dan niet gecombineerd met additionele electro-stimulatie, als trilplaattraining de chronische cytokine balans van het serum niet.

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Curriculum Vitae

Tom Broekmans werd geboren op 23 mei 1982 in Heusden-Zolder.

Hij voltooide zijn TSO studies in de afdeling Biotechnische Wetenschappen aan het OLV Instituut te Sint Truiden in 2000.

Aansluitend volgde hij de eerste kandidatuur in Kinesitherapie en Revalidatiewetenschappen aan de KUL (Katholieke Universiteit Leuven).

Het volgend academiejaar zette hij deze studies verder aan de Provinciale Hogeschool Limburg. Hij studeerde af met onderscheiding en behaalde zijn licentiaatsdiploma in 2005.

Hij begon zijn loopbaan als zelfstandig kinesitherapeut. In het kader van een Bijzonder Onderzoeks Fonds (BOF) beurs van de Universiteit Hasselt, ving hij in oktober 2006 aan met zijn doctoraat.

Tijdens zijn doctoraatsopleiding volgde hij enkele cursussen waaronder :

- Statistiek, 2 delig," Regression models" en "Analysis of Variance"
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- Onderwijsprofessionalisering
- Academisch Engels

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