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master in de biomedische wetenschappen: klinische moleculaire wetenschappen

Masterproef

Impact of exercise therapy on the development of impaired glucose tolerance and skeletal muscle contractile properties in MS and EAE

Promotor :
Prof. Bert OP 'T EIJNDE

Lauren Kosten

Masterproef voorgedragen tot het bekomen van de graad van master in de biomedische wetenschappen , afstudeerrichting klinische moleculaire wetenschappen

Maastricht University

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Finally, I want to thank everyone who supported me in any way, not only this year, but throughout the past 5 years.

List of abbreviations

APC	Antigen presenting cell
aPKC	atypical protein kinase C
APS	adapter protein with Pleckstrin homology and Src homology 2 domains
BBB	Blood-brain barrier
C3G	Crk SH3-binding guanine nucleotide-releasing factor (C3G)
CAP	c-Cbl associated protein
Cbl	Casitas B-lineage Lymphoma
CD4	cluster of differentiation 4
CFA	Complete Freund's adjuvant
CON	Control group
CrkII	Cdc2-Related Kinase II
CSA	Cross-sectional area
DEXA	dual energy X-ray absorptiometry
EAE	Experimental autoimmune encephalomyelitis
ECG	electrocardiogram
EDL	Extensor digitorum longus muscle
EX	Exercise group
GH	Growth hormone
GLUT4	Glucose transporter type 4
Grb2	Growth factor receptor-bound protein 2
GTP	Guanine triphosphate
HR	Heart rate
IgG/M	Immunoglobulin type G/M
IGT	Impaired glucose tolerance
IL	Interleukin
IR	Insulin resistance
IRS	Insulin receptor substrate
MAPK	mitogen-activated protein kinases
MBP	myelin basic protein
MetS	Metabolic syndrome
MHC	Major Histocompatibility Complex
MOG	myelin oligodendrocyte glycoprotein
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MS	Multiple Sclerosis
OGTT	Oral glucose tolerance test
PBS	Phosphate buffered saline
PI3K	Phosphoinositide 3-kinase
PIP2	Phosphatidylinositol- 4,5-bisphosphate
PIP3	phosphatidylinositol-3,4,5- trisphosphate
PKB/Akt	protein kinase B
PLP	proteolipid protein
PPMS	Primary progressive multiple sclerosis
PRMS	Progressive-relapsing multiple sclerosis
RRMS	Relapse-remitting multiple sclerosis
SED	Sedentary group
SH2	Src homology-2
SOCS	Suppressor of cytokine signaling
SOL	Soleus muscle
Sos	Son of sevenless
SPMS	Secondary progressive multiple sclerosis
TA	Tibialis anterior muscle
tAUC	Total area under the curve
TMEV	Theiler's murine encephalomyelitis virus
TNF	Tumor necrosis factor

Summary

Background and aims: Multiple sclerosis (MS) is an autoimmune disorder of the central nervous system leading to a wide range of symptoms, including muscle weakness and fatigue, that amongst others result in an inactive lifestyle. Inactivity can also lead to secondary health risks like osteoporosis and aspects of the metabolic syndrome, including diabetes mellitus which is preceded by impaired glucose tolerance (IGT) and insulin resistance (IR). In other pathologies, several studies demonstrated that physical exercise can be implemented in the treatment or prevention of IR in patients with metabolic syndrome or patients predisposed to develop type-2 diabetes. Physical exercise is also used for the improvement of MS symptoms due to inactivity. However, the effect of physical exercise on IR (and IGT) in MS and experimental autoimmune encephalomyelitis (EAE), an animal model of MS, is unknown.

This study is based on the hypothesis that exercise reduces symptoms of muscle weakness and IGT. Both are assumed to be caused by inactivity, counteracted by physical exercise improving muscle properties in MS patients. Muscle power is lost due to a muscle fiber type shift. Muscle atrophy has an effect on this fiber type distribution and is seen in both EAE and MS. Moreover, IR development as a consequence of inactivity is already suggested in MS patients. The study aims to investigate the exercise effect on IR development in EAE and MS during one part; and on characteristics and contractile properties of skeletal muscles in EAE and muscle power in MS during the other part.

Methods: (1) First a clinical study was conducted, in which the glucose profile of MS patients was compared to the glucose tolerance of matched healthy controls by means of a 2 hour lasting fasting oral glucose tolerance test. Next, MS patients participated in a 24 weeks combined physical training program whereupon muscle force (isometric dynamometry) and glucose tolerance were tested.

(2) Second, an animal study was performed, in which sedentary and exercised EAE rats (EAE SED and EAE EX respectively) were investigated on IGT by means of an oral glucose tolerance test, and on skeletal muscle properties, both functionally (isokinetic dynamometry) and morphologically. The results of these tests were compared with data of sedentary and exercised healthy rats (CON SED and CON EX respectively).

Results: (1) ***Patient study:*** The glucose profile differed between MS patients and healthy controls ($p= 0,006$). After 24 weeks total area under the glucose curve (tAUC) remained stable in the MS intervention group (EX), while it tended to worsen in the MS control group (CON) ($p= 0,07$). Total fat percentage of EX decreased significantly ($p= 0,02$), while CON remained stable ($p= 0,1$). Moreover, the lean BMC and lean mass to height² ratio of EX increased significantly. After 24 weeks of exercise, the isometric strength of the hamstrings of the most impaired leg of EX improved up to 50%, while the strength of the quadriceps improved 25%. Moreover, an intervention effect could be visualized between CON and EX ($p < 0,05$). The isometric muscle strength of the biceps in the most affected arm improved up to 31%, while the strength of the triceps improved 14%. An intervention effect could also be visualized in the most affected arm between CON and EX ($p < 0,05$). The 10 RM tests also confirmed effectiveness of the protocol.

(2) Rat study: EAE EX showed the first clinical symptoms 1 day later compared to EAE SED. The tAUC does not differ either between CON SED and EAE SED, and CON EX and EAE EX.

Muscle work declined 25,93% for CON EX, while CON SED remained stable. Moreover, an intervention effect between CON SED and CON EX could be detected, while muscle work in EAE CON and EX remained stable during repetitive contractions.

Conclusions: The data of the present study suggest that MS patients seem to have a higher risk of developing IGT, compared to matched healthy controls, which can probably be stabilized by physical exercise.

Muscle weakness, predominantly knee flexion weakness, can be significantly diminished by means of the applied training program in an MS population, with a positive impact on fatigue and general lifestyle.

There is a clear training effect on clinical symptoms in EAE rats, yet further research is required to confirm effects on contractile properties. The outcomes can help to develop exercise-based therapies to reduce fatigue and the risk of secondary health problems for MS patients.

Samenvatting

Achtergrond en doelen: Multiple sclerose (MS) is een auto-immuun aandoening van het centrale zenuwstelsel dat leidt tot diverse symptomen, waaronder spierzwakte en vermoeidheid, hetgeen onder andere resulteert in een inactieve levensstijl. Inactiviteit kan ook leiden tot secundaire gezondheidsrisico's zoals osteoporose en het metabool syndroom, waaronder diabetes mellitus wat vooraf gegaan wordt door een verstoorde glucosetolerantie (VGT) en insulineresistentie (IR). In andere pathologieën hebben verschillende studies aangetoond dat fysieke training geïmplementeerd kan worden in de behandeling of preventie van IR, bijvoorbeeld in patiënten met het metabool syndroom of patiënten met aanleg om type 2 diabetes te ontwikkelen. Fysieke training wordt ook gebruikt voor de verbetering van MS gerelateerde symptomen, die te wijten zijn aan inactiviteit. Echter, het effect van fysieke training op IR (en VGT) in MS en experimentele autoimmune encephalomyelitis (EAE), een MS diermodel, is onbekend. Deze studie is gebaseerd op de hypothese dat fysieke training symptomen van spierzwakte en VGT, beiden verondersteld veroorzaakt te worden door inactiviteit, kan reduceren. Bovendien zou fysieke training de spierkarakteristieken verbeteren in patiënten met MS. Verlies in spierkracht wordt veroorzaakt door een shift in spiervezeltype. Spieratrofie heeft een effect op deze siervezeltype verdeling en kan worden waargenomen in zowel EAE als MS. Bovendien werd in het verleden reeds een associatie gerapporteerd tussen de ontwikkeling van IR en inactiviteit bij MS patiënten. Het doel van deze studie is het nagaan van de invloed van fysieke activiteit op de ontwikkeling van IR in EAE en MS enerzijds; en op karakteristieken en contractiele eigenschapen van skeletspieren in EAE en spierkracht in MS anderzijds.

Methoden: (1) Eerst werd een klinische studie uitgevoerd waar het glucose profiel van MS patiënten werd vergeleken met de glucose tolerantie van een gelijkaardige groep gezonde controle personen, aan de hand van een orale glucose tolerantie test (OGTT). Vervolgens participeerden de MS patiënten in een 24 weken durend fysiek trainingsprogramma, waar op vast tijdstippen spierkracht (isometrische dynamometrie) en glucosetolerantie werden getest. **(2)** Vervolgens werd een dierstudie uitgevoerd, waarin sedentaire en getrainde EAE ratten (EAE SED en EAE EX) werden onderzocht op VGT door middel van een OGTT, en op skeletspierkarakteristieken, zowel functioneel (isokinetische dynamometrie) als morfologisch. De resultaten werden vergeleken met data van sedentaire en getrainde gezonde ratten (CON SED en CON EX).

Resultaten: (1) ***Patiëntenstudie:*** Het glucoseprofiel verschilde tussen MS patiënten en gezonde controles ($p= 0,006$). Na 24 weken bleef de totale oppervlakte onder de glucose curve (tAUC) stabiel in de MS interventiegroep (EX), terwijl deze neigde te verergeren in de MS controlegroep (CON) ($p= 0,07$). Het totale vetpercentage van de EX verminderde significant ($p= 0,02$), terwijl CON stabiel bleef ($p= 0,1$). Bovendien verhoogden de vetvrije massa en vetvrije massa/lengte ratio van EX ook significant. Na 24 weken van fysieke training verbeterde de isometrische kracht van de hamstrings in het zwakste been tot 50%, terwijl de kracht van de quadriceps 25% verbeterde. Bovendien kon een interventie effect gevisualiseerd worden tussen CON en EX ($p < 0,05$). Verder verbeterde de isometrische spierkracht van de biceps in de zwakste arm tot 31%, terwijl de kracht van de triceps 14% verbeterde. Een interventie effect kon ook waargenomen worden bij de zwakste arm tussen CON en EX ($p < 0,05$). De 10 RM testen bevestigden eveneens de effectiviteit van het protocol. **(2)** ***Rattenstudie:*** EAE EX toonde de eerste klinische symptomen 1 dag later dan EAE SED. De tAUC van CON SED, EAE SED, CON EX en EAE EX verschilden niet. Spierkracht daalde 25,93% voor CON EX, terwijl CON SED stabiel bleef. Verder kon een interventie effect worden waargenomen tussen CON SED en CON EX, terwijl de spierkacht stabiel bleef in EAE SED en EX, tijdens herhaalde contracties.

Conclusies: De data van deze studie suggereren dat MS patiënten een hoger risico hebben om VGT te ontwikkelen, in vergelijking met gezonde controles, hetgeen waarschijnlijk gestabiliseerd kan worden door fysieke training. Spierzwakte, voornamelijk knie flexie zwakte, kan door middel van het gehanteerde trainingsprotocol verminderd worden in een MS populatie, met een positieve impact op vermoeidheid en algemene levensstijl. Er is een duidelijk trainingseffect op klinische symptomen in EAE ratten, maar verder onderzoek is nodig om effecten op contractiele eigenschappen te bevestigen. Deze bevindingen kunnen helpen bij de ontwikkeling van therapieën om vermoeidheid en het risico op secundaire gezondheidsproblemen te reduceren bij MS patiënten.

Introduction

2.1 Multiple Sclerosis

Multiple sclerosis (MS) is an inflammatory autoimmune disorder of the central nervous system (CNS). This disease is, depending on the type of MS, often characterized by relapses alternating with periods of remission. Symptoms usually vary amongst patients, because the location of each plaque and severity of each attack can be different (1). MS has an incidence of approximately seven individuals per 100 000 every year. Its prevalence is around 120 per 100 000, and the risk of developing MS during a lifetime is one in 400. The majority of patients (80%) have relapsing/remitting MS (RRMS) from the onset, meaning that the disease has phases of relapses with full recovery and relapses with persistent deficit. After several years secondary progression can occur. This stage is called secondary progressive MS (SPMS). A minority of patients (20%) doesn't start with a relapsing/remitting phase, but suffers from the beginning from the progressive form. In this case it is called primary progressive MS (PPMS) (2). This primary progressive MS can later on develop into progressive-relapsing MS (PRMS), which is the fourth and rarest form of the disease. This form presents initially as PPMS but patients develop acute worsening of neurologic problems with incomplete recovery and ongoing deterioration (3). North America and Europe are regions where MS is common, but in other regions there are also cases of PPMS. The age at onset of PPMS and SPMS is about 40 years, which is 10 years older than in RRMS, which starts around the age of 30. Men tend to suffer two to three times more from RRMS than women, a tendency that is not seen in PPMS. In children a progressive disease course from onset is virtually non-existing (4).

2.1.1 Symptoms

Amongst typical symptoms like chronic pain, temperature sensitivity, spasticity and poor balance, muscle weakness and fatigue also occur very often. Fatigue is one of the most frequent and severe symptoms reported by MS patients (5). Disuse of muscles or general inactivity, that often occurs with MS patients, is in part responsible for weakness and fatigue (6), and can be improved by exercise (7). Fatigue is an overwhelming exhaustion or tiredness affecting daily life. It interferes with normal work, family and social situations; although there is no clear correlation between disability and fatigue in MS. Research with magnetic resonance imaging (MRI) and neurophysiological studies show that the frontal cortex and basal ganglia are involved in the occurrence of fatigue. This means that there probably is a dysfunction of the circuits between the thalamus, basal ganglia and frontal cortex. These circuits might be disturbed by the MS lesions itself or by the products of inflammation. The sense of fatigue in MS patients could also be due to a higher working load that is needed to perform mental and physical activity in the brain (5).

2.1.2 Pathological physiology & anatomy

In MS, oligodendrocytes (Figure 1), responsible for myelin synthesis, are a principal target of the immune system, causing primary demyelination of the axons and worsening of the impulse propagation or conduction. Normally signal conduction occurs saltatory (Figure 2), referring to the passive conduction of action potentials between nodes of Ranvier (voltage-gated sodium channels clustering) along myelinated axons (2).

The pathological spectrum starts with primary demyelination due to little oligodendrocyte damage, followed by extensive oligodendrocyte loss, finally followed by very extensive macrophage activation together with tissue damage. Not only myelin and oligodendrocytes are involved, but also axons and astrocytes (8).
Formation of inflammatory focal white matter lesions (demyelination) is most prominent in the pathology of RRMS. Within these active lesions, complete demyelination is seen together with acute axonal atrophy and injury, varying in severity. In progressive MS – PPMS and SPMS – these newly formed inflammatory demyelinating plaques are still present, but rare. Here the normal-appearing white matter (NAWM) is affected by a diffuse inflammation and microglia activation, diffuse axonal atrophy of the gray and white matter and secondary demyelination, which is myelin breakdown that follows neuronal damage (9).

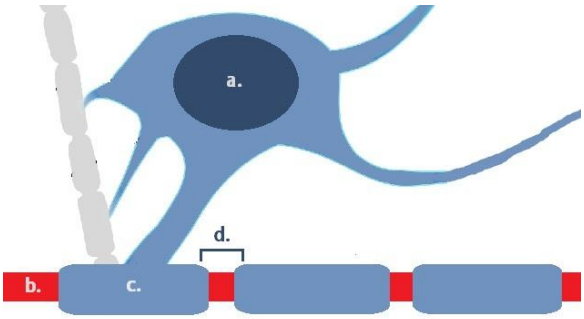


Figure 1: Oligodendrocyte. a) Nucleus of the oligodendrocyte, b) axon, c) myelin sheet, d) node of Ranvier

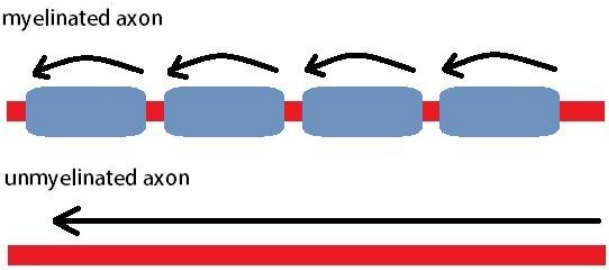


Figure 2. Unidirectional conduction of action potentials along the axon

2.1.3 Diagnosis

Individuals are classified based on diagnostic criteria in three categories: MS, not MS, or possible MS. Evidence from magnetic resonance imaging (MRI) is incorporated. Patients must have repeated attacks (at least two), suggesting the appearance of lesions separated in time and space, which means that more than one anatomical site is affected by different attacks. The second lesion however does not need to be clinically expressed (2; 3). Diagnosis by imaging is not a requisite in case of a history of relapsing disease affecting multiple sites of the central nervous system. Therefore it is most often used for patients with clinically isolated lesions or progressive disease at a single site. MRI scans show T2-weighted abnormalities in the white matter of more than 95% of patients with MS. These abnormalities however are not diagnostic because they occur more frequently than new clinical events and there is less cerebral involvement in patients with PPMS than in those who have comparable disability from secondary progression (2). Nine brain lesions, two spinal cord lesions or four to eight brain lesions and one spinal cord lesion are required to suggest the diagnosis of PPMS (3). Electrophoresis of the cerebrospinal fluid proteins can show oligoclonal IgG bands in more than 90% of MS patients, confirming the inflammatory underlying pathology. This is the only diagnostic value, so electrophoresis can be useful in excluding alternative explanations like progressive spinal cord syndromes and normally occurring imaging abnormalities in elderly patients (2).

2.1.4 Treatment

a. Pharmacological Therapy

There are different pharmacological therapies available to treat MS. Although MS can still not be cured, recent therapies reduce the number of relapses, reduce disability caused by relapses, manage neurological symptoms, prevent disability during the progressive phase and treat the established progression (2). First line treatment consists of treatment with immunomodulators based on interferons (IFN-β1b known as Betaferon® or Extavia® and IFN-β1a known as Avonex® and Rebif®), glatiramer acetate (Copaxone®) or corticosteroids (methylprednisolone known as Medrol®). These first line treatments tackle the inflammation in the CNS and the associated symptoms (8). Second line treatment is applied when first line treatment proves to be insufficient for the patient. This usually consists of prescribed use of natalizumab (Tysabri®) or mitoxantrone (9).

Most pharmacological treatments have side effects, some of which might affect the outcomes in this study. The synthetic corticosteroid Medrol® is known to cause IR (10). In general, corticosteroids inhibit glucose utilization and are characterized by switching of skeletal muscle energy metabolism from glucose to lipids. Elevated plasma glucose levels, resulting in diabetes, occur due to enhanced gluconeogenesis together with inhibition of insulin-directed glucose disposal in skeletal muscle. A study by Pellacani *et al.* showed that acute administration of Medrol® to healthy subjects had effects on the glucose tolerance 2 hours after the dose

intake. The effects showed to be fully reversible 24 hours after administration of the glucocorticoid (11). Zarcovic *et al.* confirmed this effect on insulin, where glucocorticoid - induced IR develops quickly and does not change during further glucocorticoid treatment (12). A rat study by Johansen *et al.* showed that the combination of growth hormone (GH) and Medrol® decreased insulin action on circulating glucose levels, causing IR (13).

When it comes to fatigue, IFN-β1a has been reported with higher incidence of fatigue symptoms in MS patients. The same applies to glatiramer acetate (14). The second line therapy with natalizumab also has the common side effect of fatigue (15). To exclude the different side effects of MS medication, the use of an animal model is appropriate.

b. Rehabilitation therapy

Besides the use of medication, physical rehabilitation is an important part of overall treatment of MS. Since there is no cure and the disease does have an important impact on daily activities, including balance and coordination impairment and spasticity, patients need to adjust daily routines to their increasing disability. As mentioned above, muscle weakness and fatigue are frequent symptoms. This is partly attributable to disuse or inactivity (6), which can be improved by physical training (7). Exercise therapies however are not evident for MS patients because they need more rest than healthy persons. These therapies also have more benefit for ambulatory patients, since it can improve fitness and functioning in MS patients in a mild stage. More severely disabled MS patients benefit from therapy focusing on mobilization and coordination rather than strength and endurance training programs, focusing the exercise therapy on maintenance of function instead of fitness (18). In ambulatory patients, moderate exercise is well tolerated, but it is suggested that intensive exercise might have better effects, in analogy with healthy persons. It is however feared that intensive exercise in MS patients might be harmful, evoking relapses etc. Nevertheless, the value of physical therapy during the acute relapse phase and in patients who have disability due to incomplete recovery after a relapse has been confirmed by clinical trials showing improvements in the patient's condition between relapses. This shows the benefit of intensive exercise therapy throughout the disease, besides the management of symptoms like spasticity and fatigue (16). Nevertheless, to further investigate the effects of high intensity training, the use of an animal model is required.

Exercise therapy comprises different components. Endurance training has its effects on the cardiorespiratory system, improving capacities like muscle energy metabolism (both aerobic and anaerobic). Resistance training on the other hand promotes muscle hypertrophy and muscle capillary density, improving the contractile properties of skeletal muscles. Until now, limited research was performed to evaluate the influence of a combined training program, which is therefore the applied training program in this study. Physical rehabilitation can possibly also play an important role in the treatment or even prevention of secondary health risks like IR, because MS patients have a decreased activity level that leads to these secondary problems related to inactivity (6).

2.2 Glucose uptake

2.2.1 Non-MS Population

Normally, mechanisms are, postprandial, triggered to regulate blood glucose levels. Insulin plays a key role in this regulation. Insulin is a hormone which has an important effect on glucose homeostasis. It is released from β cells in the pancreas in response to stimulation by eating a meal. It then promotes the storage of the ingested glucose as glycogen and triglycerides in the liver. Not all glucose will be taken up by the liver, making the glucose levels in circulation rise. At this point, glucose transporters in other tissues will be activated by insulin to minimize changes in blood glucose concentration (Figure 3). GLUT4 is one of these glucose transporters and will be discussed later on (17).

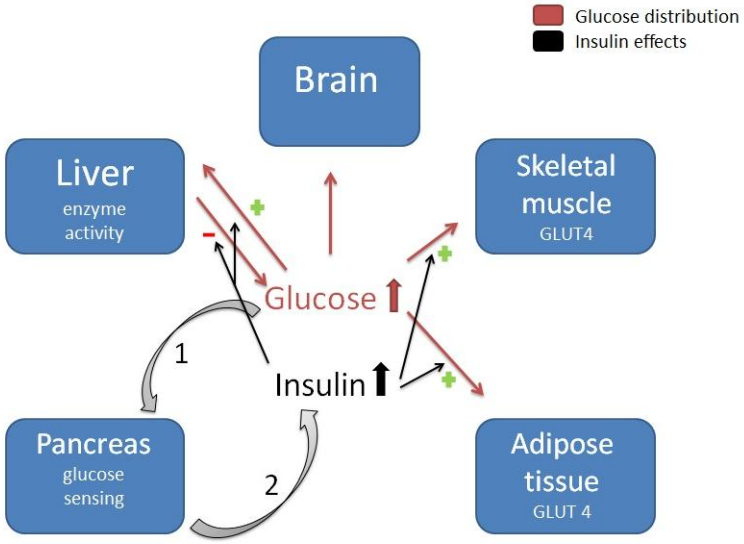


Figure 3: Insulin promotes postprandial glucose uptake.

The insulin receptor is widely expressed in mammalian cells, but levels are variable between cell types. The insulin receptor binds insulin, transmitting a signal to the cytosol via intrinsic tyrosine-specific protein kinase activity. What follows is the phosphorylation of intracellular substrates, the insulin receptor substrates (IRSs). These IRSs in turn activate signaling proteins. Phosphoinositide 3-kinase (PI3K) is recruited to the membrane where it activates a pathway involving protein kinase B (PKB) (17; 18). Two signals seem to be most important in insulin action. These signals are transmitted by PI3K and the guanine nucleotide exchange factor Grb2/Sos. PI3K generates phosphatidylinositol(3,4,5)tris-phosphate (PIP3) at the cytosolic face of membranes and Grb2/Sos activates Ras, a small G-protein. Both signals are involved in switching the tyrosine phosphorylation cascade to serine/threonine signaling cascades, which involve PKB and mitogen-activated protein kinases (MAPKs). PI3-kinases are divided in classes: Class Ia PI3K is activated by IRSs, while class Ib PI3K is activated by heterotrimeric G-proteins (18).

Muscle and fat tissue have their own glucose-transport system (the GLUT4 isoform) that can quickly be up regulated (Figure 4) (19). GLUT4 is almost completely excluded from the plasma membrane without stimulation (exercise or a meal). This in contrast with other GLUT isoforms that constitutively reside in the cell surface membrane (20). When insulin is released after a meal or when a person exercises, GLUT4 shifts from this location inside the cell to the plasma membrane (Figure 5) (21; 19). During exercise the metabolism of skeletal muscle increases tremendously, increasing the need for higher energy uptake. More glucose needs to be shifted into the muscle cells, making the GLUT4 up regulation a crucial system.

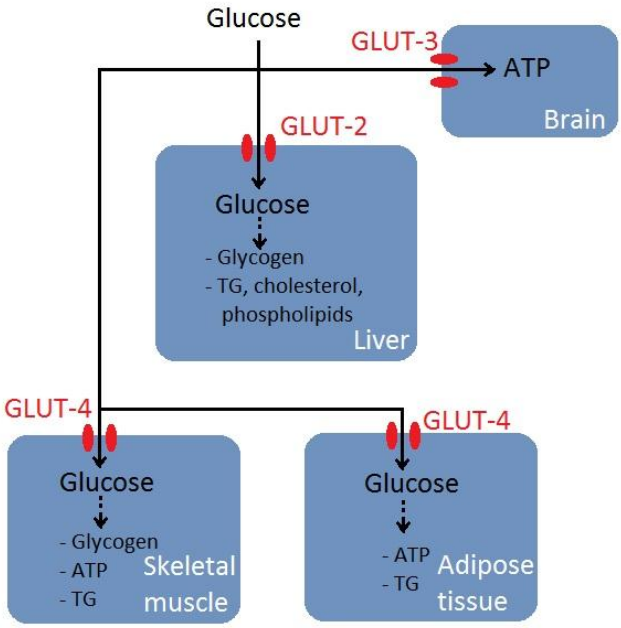


Figure 4: Glucose transport into different tissues through glucose transporters (GLUT).

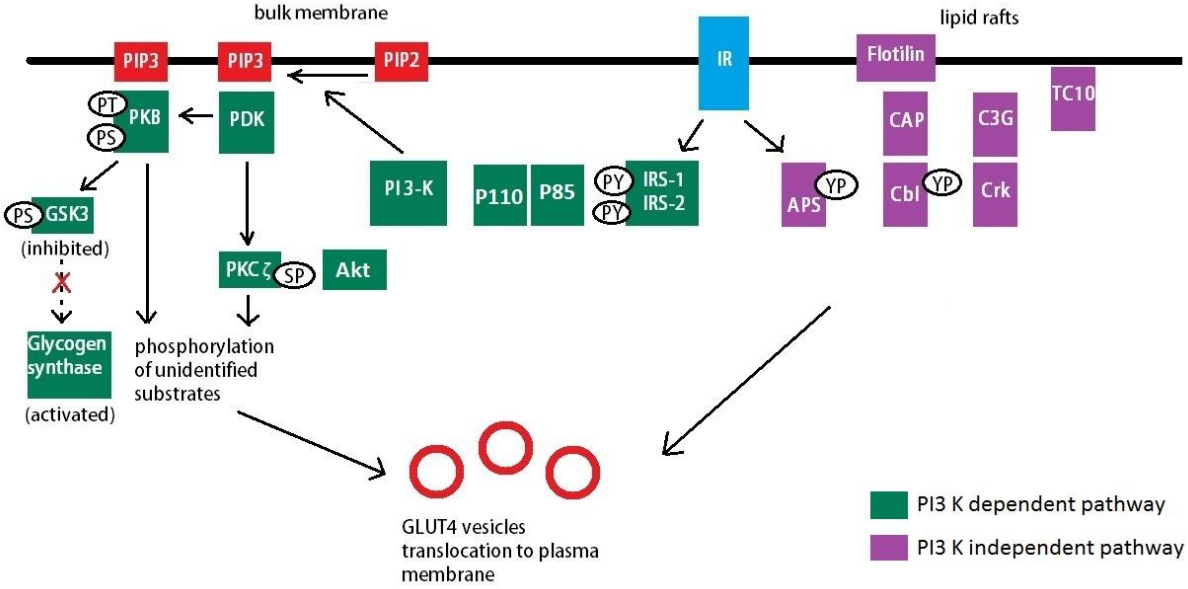


Figure 5: Insulin signaling to GLUT4 translocation to the plasma membrane. PI3 K dependent and independent pathway.

Molecularly, GLUT4 translocation occurs through the binding of insulin (which is released in response to exercise or a meal) to its receptor. The receptor conformation changes and thereby stimulates intrinsic tyrosine kinase activity. The insulin receptor then targets IRS proteins. P110 is activated by interaction of IRS-1/-2 (phosphorylated by tyrosine) with the SH2 domain of p85. Phosphatidylinositol- 4,5-bisphosphate (PIP2) is a substrate of PI3K and becomes phosphorylated, resulting in PIP3. Because of this, there is a rise in PIP3 at the plasma membrane, attracting downstream signaling molecules. Downstream of IRS/PI3K, Akt and aPKC-λ/ζ, are activated, which are serine/threonine protein kinases further phosphorylating substrates leading to the translocation of GLUT4.

Besides this PI3K pathway, there is a PI3K independent pathway leading to GLUT4 translocation (as demonstrated in adipose tissue (21)). APS and CAP are recruited to the insulin receptor, engaging Cbl. Cbl in

turn becomes tyrosine phosphorylated, and binds the complex of CrkII and Crk SH3-binding guanine nucleotide-releasing factor (C3G). C3G is a proposed exchange factor for the small GTP-binding protein TC10. The termination of the insulin signaling pathway has mechanisms that are potentially important for the development of IR. Insulin receptors are down regulated by insulin by means of endocytosis and degradation. The IRS proteins are inactivated by the serine/threonine kinases. The activity of both the insulin receptor and IRS proteins is also reduced by a third mechanism, namely activation of suppressor of cytokine signaling (SOCS) proteins (19).
This resulting translocation of GLUT4 to the plasma membrane in response to physical exercise causes glucose to be taken up in the muscles more easily to address the higher energy requirement. This effect causes a better glucose tolerance.

These processes of insulin signaling, resulting in glucose uptake, can fail and cause conditions such as IGT, IR and diabetes mellitus. IR is a reduced biological effect for any given concentration of insulin. It can be caused both by a deficiency of insulin production or a shortage of insulin receptors (with sufficient insulin production). The overall glucose tolerance of a person is defined by three factors: the biological action of insulin, the amount of circulating insulin and the impact of glucose on its own insulin-independent disposal. About 90% of insulin-mediated glucose disposed in peripheral tissues is taken up in skeletal muscle, making this tissue central to the insulin-resistant state (18).
After glucose intake, insulin is released and promotes the storage of glucose as glycogen and triglycerides in the liver. The glucose that is not metabolized by the liver contributes to the rise in blood glucose levels (17). Glucose is then transported from the peripheral circulation into the tissues through, above mentioned, glucose transporters (GLUT), which have different isoforms, depending on the tissue. Therefore insulin sensitivity occurs mainly because of reduced insulin-mediated glycogen synthesis and defects in glucose transport (18). When the glucose uptake into muscle and fat cells fails, it is defined as IGT and IR, leading to (the onset of) diabetes mellitus type 2.
The pathogenesis of diabetes mellitus type 2 usually starts with a combination of genetic predisposition and lifestyle factors, leading to IR. IR then results in hyperplasia of the β cells in the pancreas to compensate. In this stage there still is normoglycemia. The next stage is early β cell failure, resulting in IGT, followed by more severe late β cell failure, ending in the stage of diabetes mellitus. In rare cases the pathogenesis starts with primary β cell failure immediately resulting in diabetes (22) (Figure 6).

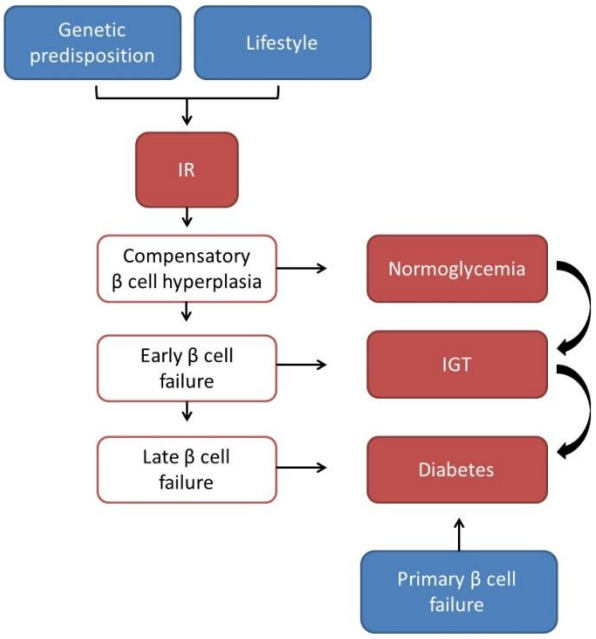


Figure 6: Pathogenesis of type 2 diabetes mellitus (adapted from (22)).

Treatment of IR primarily consists of physical exercise. Several studies have already established that physical training has potential as a treatment or even prevention measure for IR (IGT) in patients with the metabolic syndrome (23) and people predisposed to develop type 2 diabetes (24). Exercise is proposed as treatment because of some immediate effects on glucose metabolism, which primarily occur through the level of GLUT4 trafficking, more specifically, a shift of GLUT4 from the cytosol to the plasma membrane of muscle and fat cells. The additive translocation of GLUT4 to the plasma membrane has been demonstrated to occur in response to muscle contractions (insulin independent glucose uptake) (20) rather than through up regulation of insulin signaling at the inulin receptors or at the level of IRS and PI3K (19). In the past, it has been shown that physical exercise on a regular basis enhances PI3K activation by insulin in skeletal muscle, improving glucose uptake in healthy human subjects (25; 26). In healthy Sprague-Dawley rats increased levels of PI3K and GLUT4 mRNA are shown in response to endurance training (27). This exercise treatment has to be repeated however, since this positive effect on insulin sensitivity and glucose uptake disappears with inactivity. Exercise therapy proves to result in a persistent effect on glucose tolerance in IR individuals (23).
On a side note, the clinical relevance of these findings becomes clear with a study of Goodyear *et al.*, showing that obesity (IR subjects) causes a decrease in tyrosine phosphorylation of the insulin receptor, phosphorylation of IRS-1, content of the p85 subunit of PI3K, and PI3K activity associated with IRS-1 in the skeletal muscle (28).

2.2.2 MS Population

In the literature case reports indicate a higher incidence of diabetes mellitus in persons with MS compared to people without MS (29). Warren *et al.* demonstrated that significantly more MS patients, compared to healthy controls, had diabetes (30). Insulin dependent diabetes was reported by Wertman *et al.* to occur 94.5 times more in MS patients than in a healthy population (31). Because MS patients are more often inactive due to the disabling symptoms, the risk at developing IR increases. However, it remains unclear whether this increased risk to develop IR (and other secondary health risks) is a consequence of the disease per se, or a result of the reduced physical activity. Effects from medication should also be kept in mind, which can influence glucose tolerance as mentioned above. Moreover, this study will investigate whether or not MS patients can reduce this intolerance by moderate physical exercise, as already proven in other populations, suffering from IR. This effect occurs trough the glucose independent pathways that are triggered by exercise, as described above.

Above mentioned muscle characteristics playing a role in the loss of muscle power and possibly IR also improve due to exercise therapy (32). Some muscle characteristics of interest for insulin sensitivity that can be improved by exercise are glucose transport capacity, GLUT4 expression and insulin-stimulated glycogen storage (33). The effect on the development of IGT, in humans with MS and EAE rats, needs further investigation.

2.3 Skeletal muscles

2.3.1 Non-MS Population

As generally accepted, physical training improves muscle strength in healthy populations (32). This improvement depends on exercise-induced changes in muscle energy metabolism and physiology, which is fiber type dependent. Two main types of muscle fibers have been identified. Type I fibers have a higher capillary density and oxidative potential, compared to type II fibers. The energy cost is greater in type II in comparison with type I skeletal muscles (34). Not all muscles have the same fiber type distribution. In general, muscles that maintain posture (e.g. leg muscles) contain more type I oxidative fibers, while muscles for short force outbursts comprise more type II fibers (35).

2.3.2 MS Population

In an MS population physical training has the same global effect as in a healthy population, but the response to exercise might differ since these patients often suffer from muscle weakness and fatigue caused by inactivity. Muscle fatigue is highly attributable to muscle fiber type and fiber atrophy, since energy metabolism and physiology in individual skeletal muscles largely depends on the structure and type of the muscle fibers (35;

36). Loss of muscle strength in MS patients is explicable by a shift in muscle fiber type, which can be attributed to MS patients' tendency to have less type I fibers (6). This fiber type distribution would indicate that MS patients have a lower capacity to supply energy through aerobic-oxidative means because of this shift in fiber type. In other words, their energy supply occurs mainly by anaerobic mechanisms by conversion of fiber characteristics to more glycolytic properties (6).

In the literature researchers have been exploring contractile property changes, both morphologically and functionally, in skeletal muscles of MS patients. In a study from Garner *et al.*, vastus lateralis muscle biopsies from MS patients' weakest legs and from healthy controls were studied to assess the mechanisms and characteristics of contraction. When measuring the peak force by activation with standard activating solution (with Ca^{2+}), type I fibers from the MS patients were 13% stronger than fibers from healthy controls. It was argued that this difference in muscle force resulted from a significantly smaller cross-sectional area (CSA) in MS biopsies and a reduced specific force (force per fiber CSA). As to type IIa fibers from MS patients, 11% less peak force was produced compared to healthy controls. The muscle force deficit in type IIa fibers was completely attributed to their smaller fiber CSA. Shortening velocity, i.e. contraction was approximately fourfold faster in type IIa fibers compared to type I fibers. The relative number of fibers that contained type I, I/IIa, IIa/IIx or IIx Major Histocompatibility Complex (MHC) was similar between groups. Exclusive expression of the type IIa isoform was relatively less seen in MS biopsies (37). De Haan *et al.* demonstrated that patients with MS were able to voluntarily generate only about three-quarter of the maximal force-generating capacity of their muscles. Comparisons were made with control subjects. Their muscle force and speed characteristics did however not differ significantly from the control results. The muscles of male MS patients showed a greater fatigability during repeated exercise compared to male control subjects (36).

MS patients tend to be cautious about physical activity because of the risk of exacerbations or relapses. However, as mentioned before, this inactivity brings some negative health consequences, like loss of muscle strength and muscle fiber atrophy. To diminish this weakness, the problem of inactivity should be met, which is why physical training ought to be investigated in MS patients. Petajan *et al.* already demonstrated increased isometric muscle strength in exercising MS patients (38). Moreover, another study also showed that isometric muscle strength increased after physical training in MS patients (39). The typical weakness that is unilaterally experienced in MS patient's legs might be an obstacle for actively participating in training programs, but patients should keep in mind that unilateral training, even in severely affected legs, can potentially improve muscle strength (39).

2.3.3 Skeletal muscles and IR

Physical training induces muscle hypertrophy, an effect that plays an important role in glucose uptake capacity. Some important muscle characteristics for insulin sensitivity that can be improved by exercise are glucose transport capacity, GLUT4 expression and insulin-stimulated glycogen storage in the skeletal muscles (33; 32). Generally, during hypertrophy the muscle mass increases, leading to an elevated amount of muscle tissue witch is able to store more blood glucose. This change in tissue mass can be measured in patients by dual energy X-ray absorptiometry (DEXA) and confirmed by a muscle force measurement. Due to the invasive techniques on MS patients, to investigate muscle properties of individual fibers, this can be examined into depth in an animal model first.

2.4 Animal MS models

Due to the arguments mentioned above, the use of an animal model seems appropriate:

- To exclude the side effects of some MS medication on IR and some symptoms
- To investigate the effect of an intense training program on the disease course, the symptoms and the development of IR. More specific, the use of an intense training program has not yet been proven to be safe in MS patients, due to the fear to develop a relapse or to worsen the symptoms.
- To investigate the tissue properties and composition of the muscles after the intense training program.

2.4.1 Experimental autoimmune encephalitis

One of the most used existing animal models for MS is EAE. The pathological lesions occurring in this model are primary demyelination, similar to the events in human MS (40). EAE can be induced in different species such as rodents and primates, making this the most commonly used animal model for MS (41). EAE is induced by inoculation with several different proteins present in the CNS (42). Concretely this is most often done by means of immunization with a mixture in complete Freund's adjuvant (CFA) containing a homogenate of mouse spinal cord, myelin basic protein (MBP, a protein from the CNS), proteolipid protein (PLP) or peptides from the major encephalitogenic regions of MBP, PLP, or myelin oligodendrocyte glycoprotein (MOG). MOG makes up less than 0.5% of all myelin proteins and is situated on the surface of myelin sheaths. Classic EAE shows the development of tail and hind leg paralysis, which progresses to the forelimbs. Next to paralysis, weight loss occurs. This progression of symptoms reflects the fact that inflammation preferentially starts in the spinal cord, usually after a prodromal interval of 10–20 days. Scoring of the disease progression is therefore based on motor deficits resulting from spinal cord lesions. The disease course depends on the peptide injected and the animals used (Figure 7).

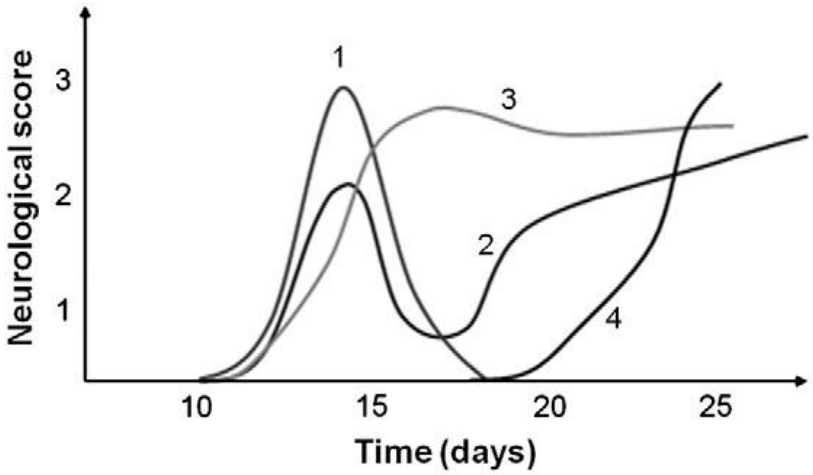


Figure 7: EAE disease course for different animal models (adapted from (43)). 1: MBP in Lewis rats, 2: MOG in dark agouti rats, 3: PLP in SJL/J mice, 4: rhMOG in marmoset. The time curves and neurological scores are not exact but rather an approximation. The neurological scores on a scale from 1 to 10 are as follows; 1: limp tail, 2: limp tail and weakness of hind limbs, 3: limp tail and complete paralysis of hind legs, 4: limp tail, complete hind leg and partial front leg paralysis, 5: complete hind and complete front leg paralysis.

The pathogenesis of classical EAE models mainly involves MHC class-II-restricted CD4+ T lymphocytes. Peripheral myelin-specific CD4+ T cells that have escaped immune tolerance are activated. These myelin-reactive CD4+ T cells are primed during the induction phase of actively induced EAE, and expand in the peripheral lymphoid organs. During the effector phase activated myelin specific T cells migrate to the CNS and cross the blood–brain barrier (BBB) (40; 44). Antigen-presenting cells (APCs) need to present myelin peptides for the CNS-infiltrating CD4+ T cells to reach full reactivation. When these CD4+ T cells are fully reactivated, a cascade of events is initiated. Chemokines that recruit macrophages to the site of inflammation are secreted.

These macrophages in turn secrete pro-inflammatory cytokines, such as TNF-α and IL-1, which are important for perpetuating inflammation, but also contribute to CNS tissue damage. Besides macrophages, microglia are also activated in EAE (44).

2.4.2 Acute vs. chronic EAE

EAE induction in most rat strains causes an acute disease without relapses. Demyelinating lesions in the CNS are sparse. MS however is a chronic disease, characterized by relapses and demyelination (45).
Immunization of Lewis rats with a mixture of MBP with Freund's adjuvant induces acute EAE, with clear clinical symptoms. In guinea pigs however, the same antigen induces chronic EAE (46). To make a chronic EAE rat model, immunization with guinea pig spinal cord homogenate can be used, but the outcome depends on the rat strain. In a chronic model the focus is on demyelinating plaques, rather than distinct symptoms. Lewis rats need pharmacological manipulation to develop severe relapsing EAE after immunization with a spinal cord homogenate. This is not the case with Dark Agouti rats (45). In this study acute EAE Lewis rats will be used because the peak of the disease is easy to detect and visualize, and the focus is not on the CNS lesions, but on the management of clinical symptoms, which would not be clear in a chronic model.

2.4.3 Theiler's Murine Encephalomyelitis Virus

Besides the most commonly studied animal for MS, namely EAE, another model has shown to have great potential. This model is based on a neurotropic viral infection. One of these neurotropic viral infection models for MS is Theiler's murine encephalomyelitis virus (TMEV) infection (41). The inflammatory demyelinating disease caused by TMEV can only be induced in mice. Since the use of rats will be needed for this study, an elaboration on this animal model is obsolete.

Aims of the study

The first aim of the present study was to evaluate whether or not MS patients are more glucose intolerant compared to healthy controls. Hereafter, this study investigated the effect of moderate and intense physical training on the development of IGT and IR, the precursor of diabetes mellitus, in MS patients (Aim 1.1) and EAE rats (Aim 1.2), respectively. It was expected that the area under the glucose curve would decrease after physical exercise, meaning an improvement of the IGT and IR, as already proven in other populations. The second goal of this study was to investigate the contractile properties of skeletal muscles after exercise therapy. More specific, this study investigated the changes in muscle strength in MS patients, after a moderate training program (Aim 2.1) and on characteristics and contractile properties of skeletal muscles and muscle power in EAE rats, after an intense training program (Aim 2.2).
The expected outcomes were an increase of muscle strength for both rats and patients, which means that they are stronger, and possibly a change in muscle fiber type due to exercise (in rats). The cross sectional area of the rat's muscles was also expected to increase after exercise.

The main hypothesis of this study implied that exercise can reduce symptoms of muscle weakness and IGT - assumed to be caused by inactivity - by improving muscle properties in MS patients.

Materials & methods

3.1 MS patient study

3.1.1 Participants

45 healthy controls and 47 MS patients participated in this study with written informed consent. After comparison of the glucose profile of both groups, 42 MS patients participated in a physical training or sedentary program during 24 weeks, while maintaining normal living habits (Figure 8). Patients were excluded from the study when they were diagnosed with diabetes mellitus type 2 prior to the study, in case of pregnancy or other conditions that can prove to be a contraindication against physical exercise, in case of psychological problems, or in case of serious illness other than MS, like cancer and aids. MS patients that had more than 2 serious relapses in the past two years prior to the study and patients participating in other clinical studies concerning physical activity were also excluded. Patients that were included were diagnosed with MS, at least 18 years of age, had an EDSS score between 0 and 6, and were available during 6 months for tests and/or training sessions.

3.1.2 Study design

First healthy controls and MS patients underwent a 2 hour fasting oral glucose tolerance test (OGTT), to compare their glucose profiles. Next, MS patients proceeded with the study by performing baseline tests to investigate muscle force, by means of dynamometry, and glucose tolerance by means of an OGTT. Body composition was measured by means of the DEXA scan. After the initial testing period, the patients enrolled in the training sessions were asked to participate in the training program 2 to 3 times a week. The same tests were performed again after 6, 12 and 24 weeks, to evaluate the influence of physical exercise (or sedentary lifestyle) on the different tests. This study was approved by the Hasselt University Ethics Committee according to the Helsinki declaration.

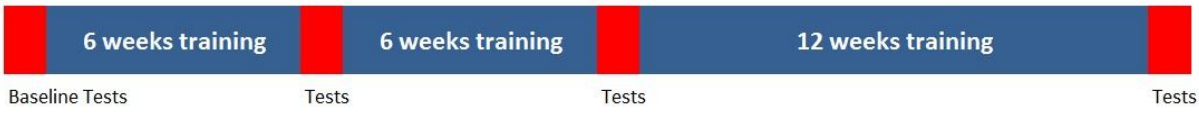


Figure 8: Course of the patient study. All patients undergo baseline tests before starting the training. After 6, 12 and 24 weeks they are tested again on glucose tolerance, muscle strength and body composition.

The training program consisted of endurance training on the treadmill and exercise bike; resistance training of lower body with leg curl, leg extension and leg press exercises and upper body strength training with arm curl, chest press and vertical traction exercises (Technogym equipment, Cesena (FC), Italy). The repetitions and thus the level of difficulty for each exercise increased during the study (Figure 9).

	Power	*Endurance*
Baseline	1x10	6 min
after 6 weeks	2x15	2x7 min
after 12 weeks	2x20	2x10 min
In week 24	4x15	3x10 min
	Repetition sets per exercise	Minutes on treadmill and bike

Figure 9: Training schedule for the patient study, short version

Training session durations gradually increased from approximately one hour to 2,5 hours. The complete training schedule can be consulted in appendix table 1.

3.1.3 Oral glucose tolerance test (OGTT)

Glucose tolerance was measured by means of a fasting, 2-hour lasting OGTT. At the start of the test venous (BD Vacutainer SST II Advance Plus Blood Collection Tubes, BD, Plymouth, UK) and capillary blood samples (Analox instruments Ltd, London, UK) were drawn from the patient before they were asked to drink a glucose solution (1g of glucose per kg body weight in 200 ml water). From then on venous blood samples were taken after 1 and 2 hours from an elbow vein and capillary blood samples were taken from the earlobe at intervals of 20 minutes. After 30 min, the venous blood samples were centrifuged for 10 minutes at 2500 rpm and the remaining serum was stored at -80°C for insulin measurement later on by means of the ADVIA Centaur Insulin assay (Siemens healthcare diagnostics Inc.). The capillary blood samples were immediately tested to determine the whole blood glucose concentration with the Analox GM7 Micro-stat (Analox instruments Ltd, London, UK). With these values, a glucose curve can be composed. Total area under the glucose curve (tAUC), a measure of glucose sensitivity, was calculated using the trapezoidal rule applied to the glucose curves during the OGTT. The use of medication was registered during the complete course of the study and deviating tests, immediately after the use of Medrol and/or other corticosteroids, were excluded.

3.1.4 Body composition

The body composition of the subjects was determined by means of dual energy X-ray absorptiometry (DEXA) of the whole body (Delphi W S/N 70331, Hologic, Vilvoorde, Belgium). The positioning of the subject was standardized. The software (APEX version 3.0.1, Hologic, Bedford, MA, USA) calculated the fat mass and lean mass (g) in the body, as well as the ratio per region: arms, trunk, legs, head, android region and gynoid region.

3.1.5 Skeletal muscles

The maximum voluntary muscle force of the knee and elbow flexor and extensor muscles was measured by isokinetic dynamometry for maximum force-endurance measurements and isometric dynamometry for maximum force measurements. Left and right were measured separately because of the bilateral difference in MS patients.

The dynamometer (Biodex, Enraf-Nonius, Shirley, New York) consists of an electrometer and a chair with an electromagnetic arm that measures the force generated by the contracting muscles. The patient was stabilized in a sitting posture with the back of the chair at an angle of 85°, while the axis of the knee or elbow was on the same line with the transversal axis of the dynamometer. The subject was stabilized with belts and was told to hold on to handles on the sides. The protocol was standardized by using the same settings of the chair for each measurement and by having the same researcher executing each test.

The maximum isometric muscle force (Nm) of the knee extensor muscles and flexor muscles was measured by making the subject flex and extend at a maximum during 4 seconds, with the knee making an angle of 45° and 90°. The subject performed these flexions and extensions in duplo with a rest period of 30 seconds between each contraction. The highest peak torque of the extension and flexion movements done in duplo was selected as the maximum isometric muscle force.

For the measurement of the flexor and extensor muscles of the elbow, an elbow angle of 60° and 105° was used. Contractions were also done in duplo and with the same time intervals as with the isometric knee contractions.

The maximum dynamic (isokinetic) force (Joule per repeated contraction) was measured by making the subject bend and extend their knee 30 times (speed: 180° per second) at an angle of 70° (between 90° and 160°). To know the fatigue of the muscles from these measurements, the total workload of the muscle from the first 3 and last 3 contractions were compared and the difference was expressed in percentages.

3.1.6 10 RM Test

The 10 RM test is used to measure the ten repetition maximum. In weight training, this is the maximum weight a person can lift in 10 repetitions for a given exercise. This test is performed to see if the participants' capacity to lift weights increases throughout the training protocol, meaning that the protocol is effective enough to increase muscle strength.

3.2 Laboratory EAE rat study

3.2.1 Animals

100 female Lewis rats (6-7 weeks old, 100-124 grams, Harlan CPB, Zeist, The Netherlands) were housed in the animal breeding center on a 12:12 light/dark cycle and 22°C temperature. They were fed rat pellets (Carfil RN-01-K12, Harlan) ad libitum. The animal Ethics Committee of Hasselt University approved the study protocol in accordance with the Helsinki declaration.

3.2.2 Study design

The complete laboratory animal study comprised 100 female Lewis rats, twenty rats were assigned to the pilot study to optimize the protocol, while all other rats were divided in a phase I (n= 40) and a phase II (n= 40, data not included) group of the actual study (Figure 10). Initially, all rats had one week of habituation (day -21 till day -15) to the environment, followed by two weeks of habituation to the training protocol (day -14 till day -1). In the first three habituation training sessions the rats ran at a speed of 4m/min and inclination of 0°. In the second habituation week, treadmill inclination was set at 25° and running speed was progressively increased day by day from 12m/min to 18m/min (Figure 11). All test groups consisted of healthy (n= 10) and EAE (n= 10) sedentary rats (CON SED and EAE SED respectively) and healthy (n=10) and EAE (n=10) rats that underwent physical training on the treadmill (IITC model 800 expandable treadmill, IITC Life Science, Woodland Hills, CA) at a speed of 18m/min and inclination of 25° during 10 days (CON EX and EAE EX respectively). At day 0, after EAE induction, the actual training program started.. Treadmill running mainly affects gastrocnemius and soleus (SOL) muscles, while the inclination activated tibialis anterior (TA) and extensor digitorum longus (EDL) muscles, indicating the importance of setting an inclination. Sedentary control rats underwent the same manipulations by being placed on the stationary treadmill, experiencing the same stress level. The complete training schedule can be consulted in appendix table 2. In phase I, the EAE rats went through the complete disease course (18 days, Figure 12) and underwent the tests at day 17, a week later compared to the rats in the phase II group (data not included), who were tested at day 11, 2 days after their last treadmill training (on day 9) . A day by day description of the animal study is attached in table 3 (see appendix). The tests performed at day 10 or day 17 were an OGTT, dynamometry of the rat's hind leg and a morphologic study of the skeletal muscles in the hind leg. TA and EDL were dissected because they show a distribution of type I and type II fibers that possibly changed due to exercise; and SOL because it mostly consists of type I fibers and this muscle has not been used in this type of research yet (Figure 13).

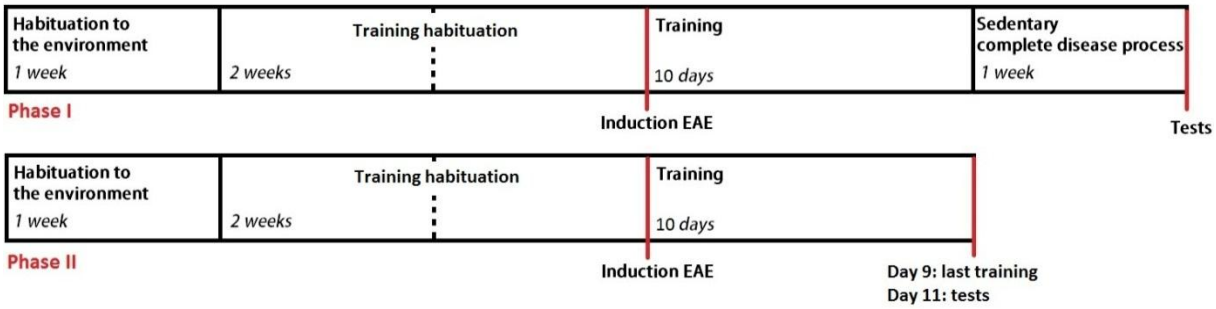


Figure 10: Course of the rat study. All rats start with habituation upon arrival followed by a period of adjusting to the treadmill.

	week 1	*week 2*		*10 days*
Training habituation	4m/min	12-18m/min	**Training**	18m/min
	0°	25°		25°
	15-30 min	45-60 min		60 min

Figure 11: Training schedule. In the weeks to habituate to the training, speed went from 4m/min to 18 m/min. The initial habituation training session had a duration of 15 minutes, going up to 1 hour after 2 weeks. The inclination was set to 25° in the second week.

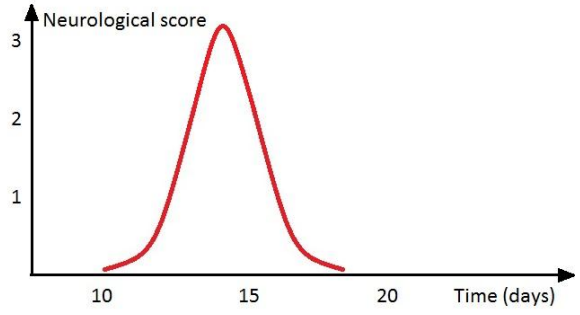


Figure 12: EAE disease course in MBP induced Lewis rats

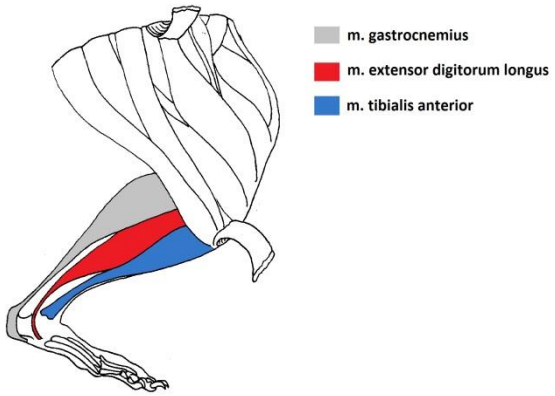


Figure 13: Location of the used muscles in the right hind leg of a rat, lateral view. SOL is situated underneath m. gastrocnemius

3.2.3 EAE induction & clinical scoring

Acute EAE was induced by a single percutaneous injection (100 µl/foot) in both footpads that for immunization of 4 rats consists of 192 µl purified MBP (25 mg/ml) together with 200 µl mycobacterium tuberculosis (20 mg/ml, H37Ra, Difco, Detroit, MI), 256 µl PBS and 960 µl complete Freunds adjuvant (CFA, Difco). EAE animals are scored daily on clinical symptoms. Scores range from 0,5 to 5 points (0,5 = partial paralysis tail, 1 = complete paralysis tail, 2= ataxia, 2,5 = partial hind limb paralysis, 3 = complete hind limb paralysis, 4= paralysis to the diaphragm, 5 = death by EAE)

3.2.5 Oral Glucose tolerance test

The IGT was tested by means of a fasting 2-hour OGTT. After a fasting period of 16 hours the test started with the collection of an arterial blood sample (Multivette 600 serum, Sarstedt, Germany) under isoflurane gas anesthesia (IsoFlo, Abbott Laboratories Ltd., Berkshire, UK) together with a capillary blood sample in a capillary tube (Analox instruments Ltd, London, UK) from the tip of the tail. Then they were administered a glucose solution (1g/kg body weight) with a concentration of 1M by means of a probe. Every 20 minutes a new capillary blood sample was collected from the tail. Arterial blood samples were collected every hour. The arterial blood samples were centrifuged after 20 minutes (4800 rpm, 6 minutes) and the remaining serum was stored at -80°C for insulin measurement later on by means of two-site sandwich ELISA (ADVIA Centaur Insulin assay, Siemens healthcare diagnostics Inc., Tarrytown, NY, USA). The capillary blood samples were immediately tested to determine the whole blood glucose concentration by means of an Analox GM7 Micro-stat (Analox instruments Ltd, London, UK). The composed glucose curve was used to calculate the tAUC by means of the trapezoidal rule, an expression of glucose sensitivity.

3.2.6 Skeletal muscles: dynamometry

To test the muscle force, electrical percutaneous needle stimulation of the common peroneal nerve was used on the extensor muscles of the left hind limb, causing contraction. The rat was anesthetized with an IP injection of pentobarbital sodium (Nembutal, 1ml/kg body weight, Ceva Sante Animale, Brussel, Belgium) and the skin and muscles were dissected to expose the common peroneal nerve. The electrode was attached to the nerve and the rat was fixated with the knee and ankle joint in the dynamometer. The fixated hind leg of the rat was in a 35° angle with the dynamometer and the movement of the hind leg during stimulation had a speed of 100° per second. Stimulation of the nerve (1mA) during 250 ms (3s rest intervals) caused 120 isokinetic dorsiflexions of the foot, according to the used protocol. Muscle fatigue was calculated in percentages, the peak torque during the first 30 subsequent contractions was adjusted as 100%.

3.2.7 Skeletal muscles: morphology

To study morphologic characteristics of the skeletal muscles, the rat was anesthetized with an IP injection of Nembutal (Ceva Sante Animale) and connective tissues and blood were removed. The first muscle to be removed was TA at the front of the rat's hind leg. Second, the EDL was taken out. At the back of the limb, m. gastrocnemius was dissected to reach SOL, which was the last muscle to be dissected. Part of each muscle was snap-freezed with liquid nitrogen for future biochemical analyses (not covered in this thesis) and stored at -80°C. The muscle tissue that was taken for immunohistochemistry was embedded in Tissue-Tek® (Miles Laboratories), frozen in 2-methylbutane (Sigma-Aldrich, St. Louis, MO, USA) (in liquid nitrogen) and also stored at -80°C.

The embedded biopsies were stained with triple-coloring to determine the fiber type distribution. First, 8 µm thick cross sections were made at -20°C and air-dried during 30 minutes. Then the sections were washed during 5 minutes in 0,5% Triton-X100 in phosphate buffered saline (PBS). Finally they were rinsed in PBS for another 5 minutes. The triple-coloring consisted of a first incubation during 45 minutes with a mix in PBS of 2 mouse monoclonal antibodies against myosin heavy chain I (1:50; A4.840 supernatant, Developmental Studies Hybridoma Bank, Iowa, USA), IIa (1:50; N2.261 supernatant, Developmental Studies Hybridoma Bank, Iowa, USA) and 1 rabbit polyclonal laminin antibody (1:100; L-9393, Sigma, Zwijndrecht, The Netherlands). After the incubation, the sections were washed 3 times during 5 minutes with PBS. The second incubation also took 45 minutes and comprised a mixture of secondary antibodies (1:500 goat-anti-mouse IgM AlexaFluor 555, 1:200 goat-anti-mouse IgG1 AlexaFluor 488 and 1:130 goat-anti-rabbit IgG AlexaFluor 350; Molecular probes, Invitrogen, Breda, Nederland) in PBS to visualize the muscle fiber type. The sections were washed again 3 times during 5 minutes in PBS before they were mounted with Dako fluorescent mounting medium (Dako, North America, California, USA) and dried overnight. Visualization of the staining was done with a Nikon E800 fluorescence microscope (Nikon, Badhoevedorp, The Netherlands) and analyzed with NIS elements software (Nikon, Badhoevedorp, The Netherlands).

3.3 Statistical analyses

Normal distribution was checked with the Kolmogorov-Smirnoff test. Baseline differences between groups were analyzed using one-way ANOVA. Training effects were analyzed by means of a 2x4 ([Control, Exercise] x [Baseline, 6 weeks, 12 weeks, 24 weeks]) mixed model repeated measurements ANOVA. Between groups data were tested with two-sample equal variance student t-tests and within groups data with paired t-tests.

Results

4.1 MS Clinical study

4.1.1 Biometrical data and drop out

Forty-five healthy controls and 47 MS patients (EDSS = 3,3 ± 1,35) participated in the first part of the study. Both groups were matched on age (HC: 46,52 ± 12,35; MS: 48.95 ± 9.91), gender (male/female ratio HC: 0,69 ± 0,47; MS: 0,60 ± 0,49) and BMI (HC: 23,39 ± 5,93; MS: 24.65 ± 4.59). Afterwards, 42 MS patients (EDSS = 3,31 ± 1,37) proceeded to the second part of the study. After randomization the sedentary control group (CON, n= 16) and the exercise intervention group (EX, n= 26) were assembled, as represented in table 1. However, two patients were forced to leave the second part of the study early. The first left the study after 4 weeks, due to a severe relapse, followed by hospitalization. The second terminated the program after 8 weeks, due to mental problems. Moreover, after completing the first 12 weeks of the protocol 7 patients decided to end their training program.

Table 1: Biometrical data from the MS patients (n= 42).

		Age	*Height (m)*	*Weight (kg)*	*BMI*	*EDSS*	*Gender*
Exercise	Mean	48,08	1,7	71,87	24,9	3,28	female n = 16
	SD	10,19	0,08	15,64	4,69	1,33	male n = 10
Control	Mean	47,94	1,67	70,77	24,23	3,26	female n = 9
	SD	11,41	0,26	17,04	5,49	1,37	male n = 7

4.1.2 Oral glucose tolerance test

a. MS patients versus healthy controls

The whole blood glucose concentration of the healthy controls and the MS patients are represented in figure 14-A. From the start the glucose concentrations were significantly different between healthy controls and MS patients to the detriment of the MS patients. Two hours after the glucose load glucose concentrations of both groups were similar. Moreover, tAUC differed significantly between both groups (p= 0,006) (Figure 14-B).

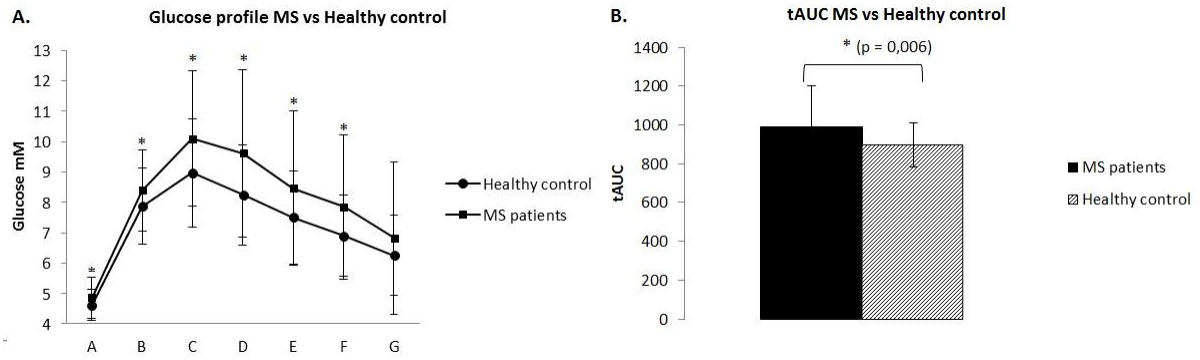


Figure 14: Mean values ± SD are plotted. A) tAUC in MS patients and healthy controls. Samples were collected every 20 minutes (A-G).: p ≤ 0,05. B) Results of the OGTT in MS patients and healthy controls.*

b. MS training versus MS sedentary control

At baseline there were no differences between CON and EX. Following 24 weeks of combined training tAUC (Figure 15) and the glucose profile (Figure 16) remained stable in EX, compared to baseline, while tAUC tended to worsen in CON (p= 0,07).

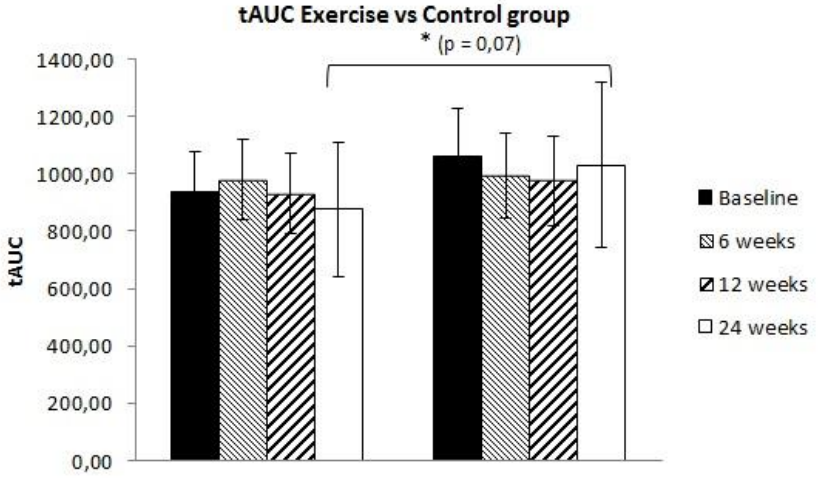


*Figure 15: tAUC from the training intervention study. Mean values ± SD are plotted. Left: EX; Right: CON. *: p= 0,07*

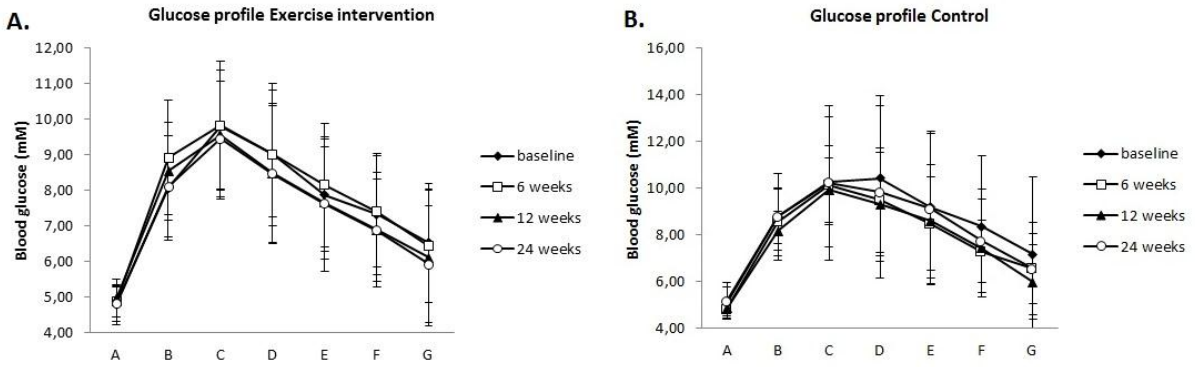


Figure 16: Mean values ± SD are plotted. A) Glucose profile EX. B) Glucose profile CON. Curves are shown at baseline, 6 weeks, 12 weeks and 24 weeks.

c. Correlations

The tAUC data and biometrical data from the MS patients were analyzed on correlations. Pearson correlation coefficients were calculated showing values between -1 and 1, values closer to 1 showing a stronger correlation. The tAUC from MS patient's glucose profile is significantly correlated to age and to EDSS score (Table 2).

Table 2: Correlations of baseline tAUC with biometric data from all MS patients

	Age	*BMI*	*EDSS*	*Gender*
Pearson Correlation	0,448	-0,072	0,344	0,03
p - value	* 0,003	0,65	* 0,032	0,846

4.1.3 Body composition

The results of the DEXA scan are represented in table 3. After 24 weeks and compared to baseline, the total fat % of EX decreased significantly (p= 0,02), while CON remained stable (p= 0,4). Moreover, the lean BMC and lean mass to height² ratio also increased significantly in EX, while these parameters decreased in CON after 24 weeks. The lean mass between EX and CON also differs significantly at this point (p= 0,01). Other parameters remained stable throughout the study or even worsened in CON.

Table 3: Change in body composition of CON and EX at the different test moments within (compared to baseline) and between groups.

		Δ Change compared to baseline (%)									
		fat mass	lean BMC	total mass	total body fat %	fat mass/height²	android/gynoid ratio	% fat trunk/%fat legs	trunk/limb fat mass ratio	lean mass/height²	
6 weeks	Exercise	-1,85 ± 3,91	-3,92 ± 18,61	-0,74 ± 2,53	-1,13 ± 3,04	-1,66 ± 3,77	419,83 ± 1967,88	-2,04 ± 4,21	0,17 ± 5,64	0,07 ± 2,74	Mean ± SD
		* 0,02	0,17	0,09	* 0,05	* 0,03	0,16	* 0,02	0,45	0,45	p vs baseline
	Control	0,56 ± 3,27	-0,83 ± 2,55	-0,58 ± 1,73	1,12 ± 3,28	0,67 ± 3,02	1,88 ± 3,51	0,58 ± 4,08	2,85 ± 5,39	-0,61 ± 2,76	
		0,30	0,16	0,16	0,15	0,25	0,06	0,33	0,06	0,25	p vs baseline
		* 0,05	0,30	0,43	* 0,03	* 0,05	0,25	0,05	0,11	0,26	P T vs C
12 weeks	Exercise	-1,44 ± 5,45	0,67 ± 3,38	-0,08 ± 3,25	-1,4 ± 4,08	-1,31 ± 5,32	1,37 ± 6,64	-1,65 ± 4,82	-0,94 ± 6,29	0,6 ± 3,08	
		0,11	0,18	0,46	0,06	0,13	0,17	0,06	0,24	0,18	p vs baseline
	Control	-0,82 ± 5,13	-0,88 ± 1,93	-1,15 ± 2,01	0,25 ± 3,92	-0,75 ± 4,88	1,82 ± 4,67	-3,29 ± 6,03	-2,61 ± 6,30	-0,66 ± 1,92	
		0,30	0,07	* 0,03	0,41	0,30	0,10	* 0,04	0,09	0,13	p vs baseline
		0,37	0,07	0,15	0,13	0,38	0,42	0,19	0,23	0,10	P T vs C
24 weeks	Exercise	-2,32 ± 7,36	1,44 ± 3,25	-0,01 ± 3,67	-2,42 ± 4,74	-2,22 ± 7,07	1,1 ± 7,88	-1,03 ± 5,17	-1,91 ± 7,68	1,21 ± 2,95	
		0,09	* 0,03	0,49	* 0,02	0,09	0,27	0,19	0,14	* 0,04	p vs baseline
	Control	-0,5 ± 8,32	-1,31 ± 2,55	-0,99 ± 2,99	0,31 ± 6,51	-0,53 ± 7,92	2,01 ± 6,63	0,07 ± 8,73	-0,98 ± 8,27	-1,29 ± 2,49	
		0,43	0,07	0,16	0,44	0,42	0,18	0,49	0,36	0,07	p vs baseline
		0,27	* 0,01	0,23	0,10	0,28	0,38	0,33	0,38	* 0,01	P T vs C

4.1.4 Skeletal muscle dynamometry

a. Knee isometric force

The isometric 90° knee extension and flexion strength of EX improved in both legs after 24 weeks of training and compared to baseline. After 24 weeks and compared to baseline, hamstrings strength of the most impaired leg improved up to 50,95% (p= 0,00027) while quadriceps strength improved 25,80% (p= 0,009). Moreover, the muscle strength of CON remained stable (Figure 17A-D). An intervention effect can be visualized between CON and EX after 12 and 24 weeks of exercise ($0,05 \leq p \geq 0,002$). Comparable results were seen for the isometric 45° knee extension and flexion strength.

b. Elbow isometric force

The isometric 105° elbow extension and flexion strength of the EX improved in the most affected elbow after 6, 12 and 24 weeks of training compared to baseline. The biceps strength in the most affected arm improved up to 31,35% after 24 weeks of exercise (p= 0,09), while the triceps strength improved 14,1% (p= 0,008). The muscle strength of CON remained stable (Figure 18). Moreover, an intervention effect can be visualized in the most affected arms between CON and EX after 6 and 12 weeks of exercise (6 weeks: Most affected elbow extension 105° p= 0,05; 12 weeks: Most affected elbow flexion 105° p= 0,03). There is also a training effect visible between CON and EX for the least affected elbow flexion 105° (p= 0,02). For the isometric 60° elbow extension and flexion strength improved significantly after 6 weeks for EX in both arms (Most affected arm extension: p= 0,0006; flexion p= 0,02; Least affected elbow extension: p= 0,0005; flexion: p= 0,03). However, a training intervention effect could not be visualized in an elbow angle of 60°.

c. Knee isokinetic force

Data not included in this paper, the results need further analysis.

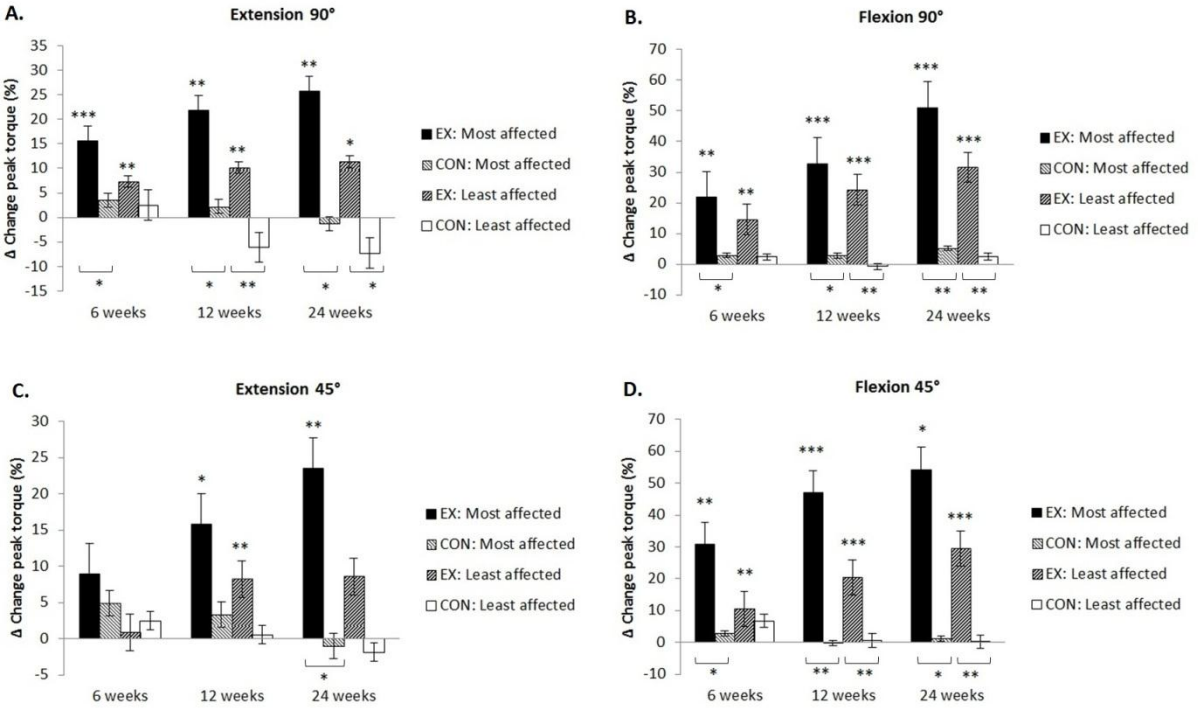


*Figure 17: Change in isometric peak torque in the knee compared to baseline after 6 weeks, after 12 weeks and after 24 weeks. Mean values ± SE are plotted. Different angles are tested. A)Extension 90°, B) Flexion 90°, C) Extension 45°, D) Flexion 45°. *: p ≤ 0,05; **: p ≤ 0,01; ***: p ≤ 0,001.*

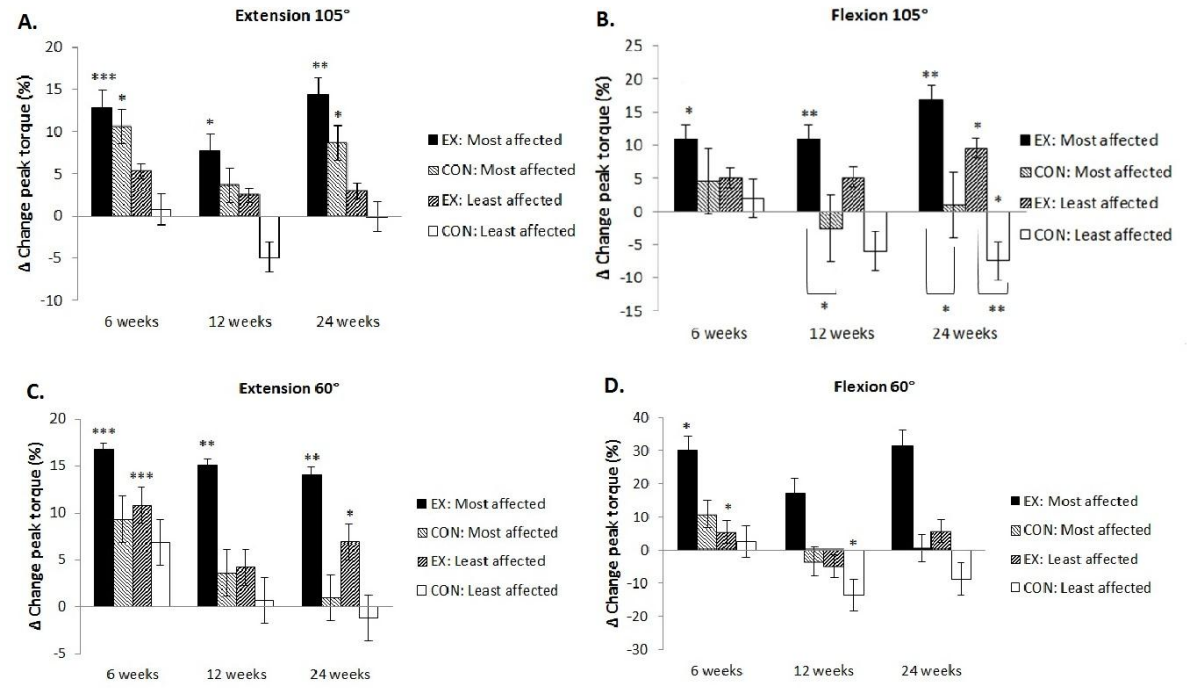


*Figure 18: Change in isometric peak torque in the elbow compared to baseline after 6 weeks, after 12 weeks and after 24 weeks. Mean values ± SE are plotted. Different angles are tested. A)Extension 105°, B) Flexion 105°, C) Extension 60°, D) Flexion 60°. *: p ≤ 0,05; **: p ≤ 0,01; ***: p ≤ 0,001.*

4.1.5 10 RM test

The 10 RM subjects reached significantly improved after 12 and 24 weeks and compared to baseline, for every exercise ($3{,}41\ 10^{-7} \leq p \geq 2{,}006\ 10^{-12}$). Figure 19 depicts the change in 10 RM for every exercise.

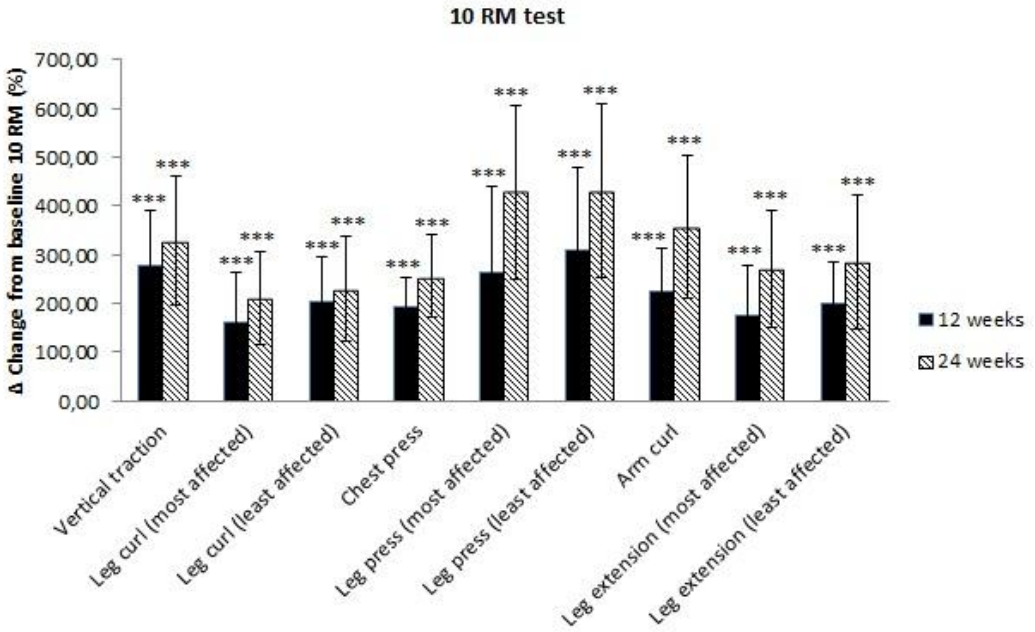


*Figure 19: Change in 10 RM compared to baseline. Mean values ± SD are plotted. ***: p ≤ 0,001*

4.2 Laboratory EAE rat study

4.2.1 Drop out

One EAE EX animal died early during phase I of the study course.

4.2.2 Body weight and food intake

As illustrated in figure 20, the body weight of EAE rats decreased throughout the disease course, more specific after day 1, immediately after the EAE induction. However, during the disease course and especially during the prevalence of the clinical symptoms, the weight of EAE EX decreased less, compared to EAE SED. The body weight of the healthy rats increased steadily during the complete course of the study. The corresponding food intake is represented in figure 21. Before day 0 the food intake was comparable between the different groups. Immediately after EAE induction the food intake decreased dramatically during 1 to 2 days. Afterwards, the food intake of the EAE groups normalized. Starting at day 10, after introduction of the clinical symptoms, the food intake of the EAE rats decreased again. The food intake of the healthy rats remained stable during the complete course of the study.

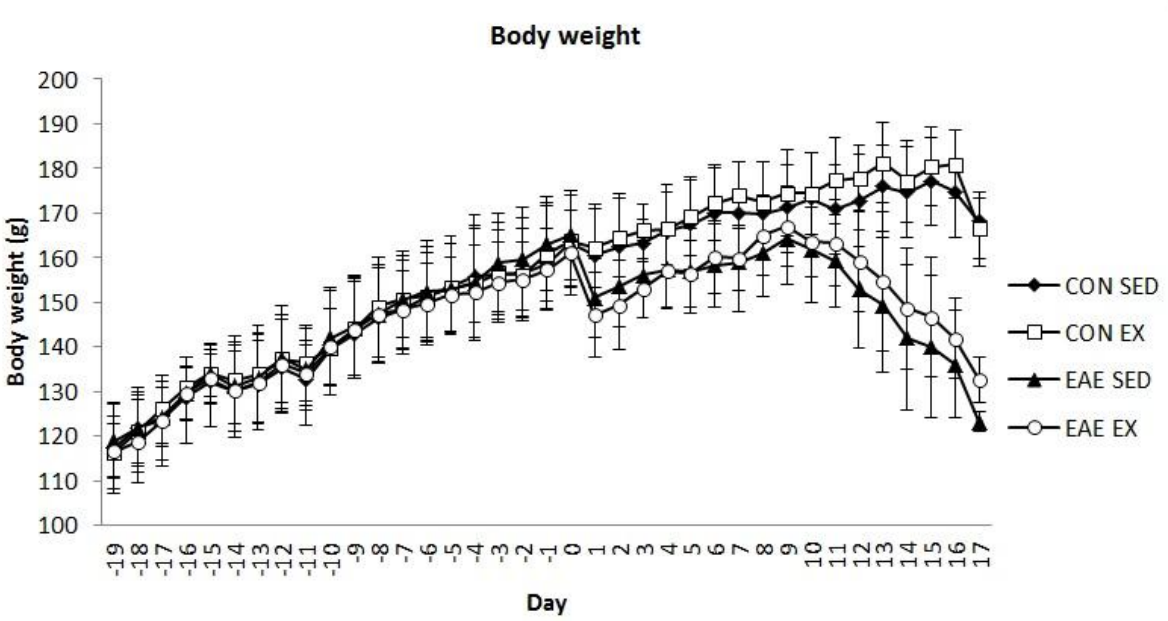


Figure 20: Body weight of the rats throughout the study. Mean values ± SD are plotted.

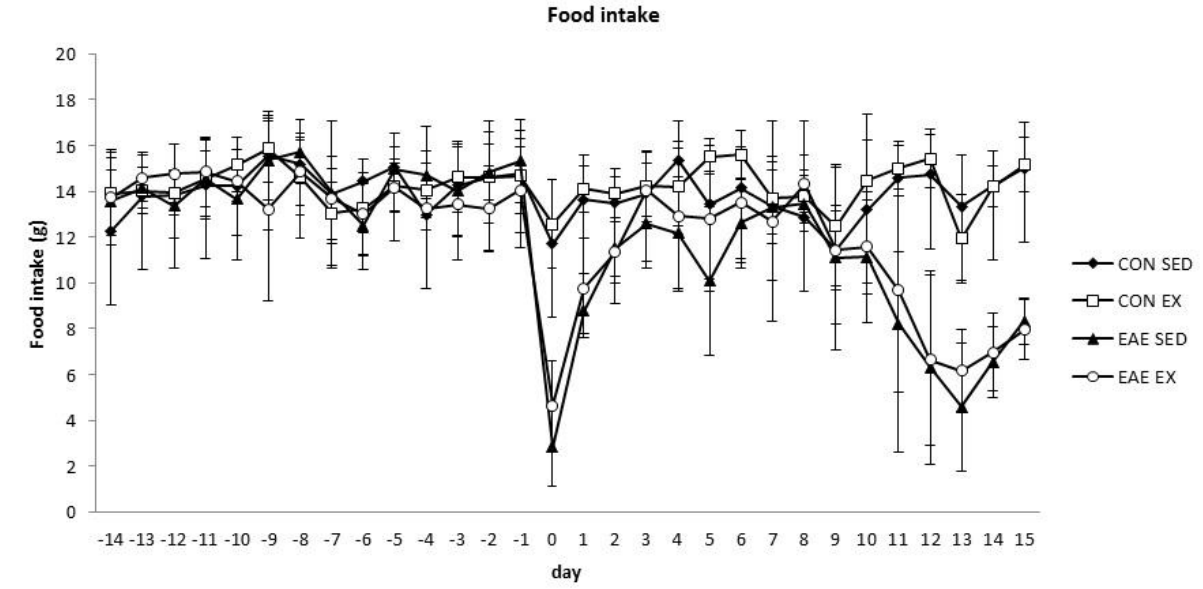


Figure 21: Food intake of the rats throughout the study. Mean values ± SD are plotted.

4.2.3 Clinical score

The clinical score of the EAE rats is represented in figure 22. During the first 10 days of the disease course no clinical symptoms were visible. During phase I the first symptoms appeared at day 11 for EAE SED, while EAE EX showed the first clinical symptoms 1 day later. The maximum score these rats reached is a mean score of 2,33 for EAE SED and 2,06 for EAE EX on day 14.

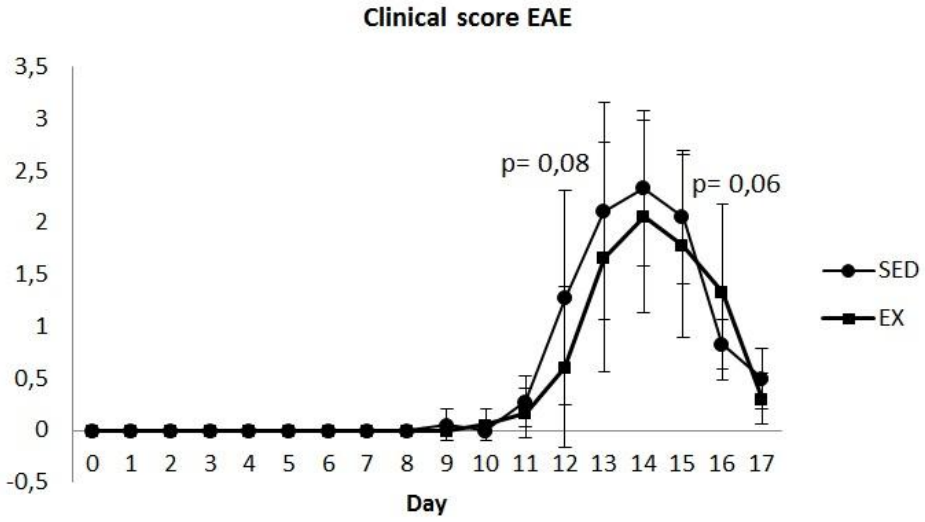


Figure 22: Clinical score EAE rats. Mean values ± SD are plotted. Scores range from 0,5 to 5 points (0,5 = partial paralysis tail, 1 = complete paralysis tail, 2= ataxia, 2,5 = partial hind limb paralysis, 3 = complete hind limb paralysis, 4= paralysis to the diaphragm, 5 = death by EAE)

4.2.4 Oral glucose tolerance test

The whole blood glucose concentration of the rats in phase I is represented in figure 23. At the start, the glucose concentrations were not significantly different between the four groups. After 20 and 40 minutes, there was a difference in blood glucose between CON EX and EAE EX (p= 0,05 and 0,04 respectively). Throughout the rest of the test, no differences were detected. Furthermore, the tAUC did not differ either between all groups.

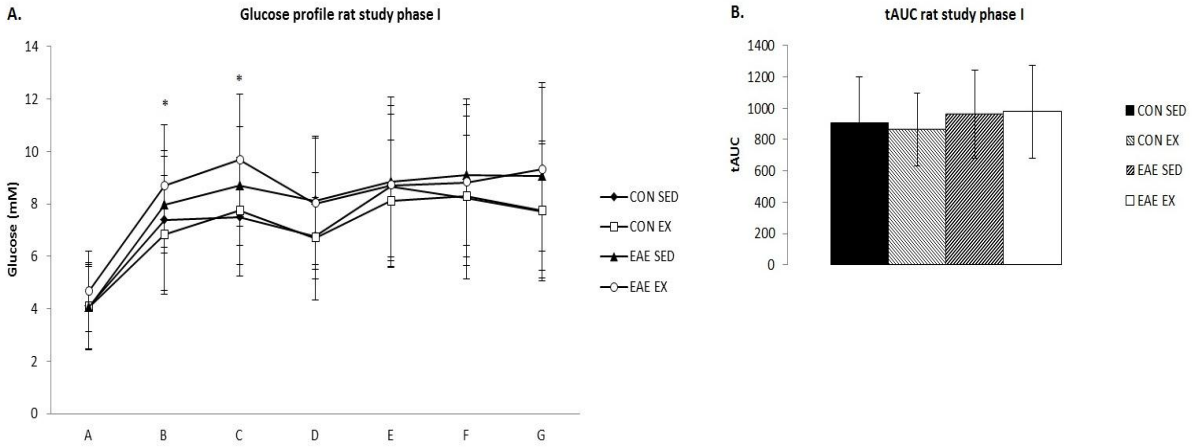


*Figure 23: Glucose curve and tAUC. Mean values ± SD are plotted. A) Results of the OGTT in phase I of the rat study. Samples were collected every 20 minutes (A-G). *: p ≤ 0,05. B) tAUC in rat study phase I.*

4.2.5 Skeletal muscles: dynamometry preliminary data

Figure 24 depicts the muscle power for 130 subsequent contractions by stimulation of the common peroneal nerve. The results of the measurements show a peak muscle work that was reached during the initial 15 contractions. This peak is absent in EAE rats. In order to calculate a decline in work, the first 15 contractions are left out (Figure 25-28).

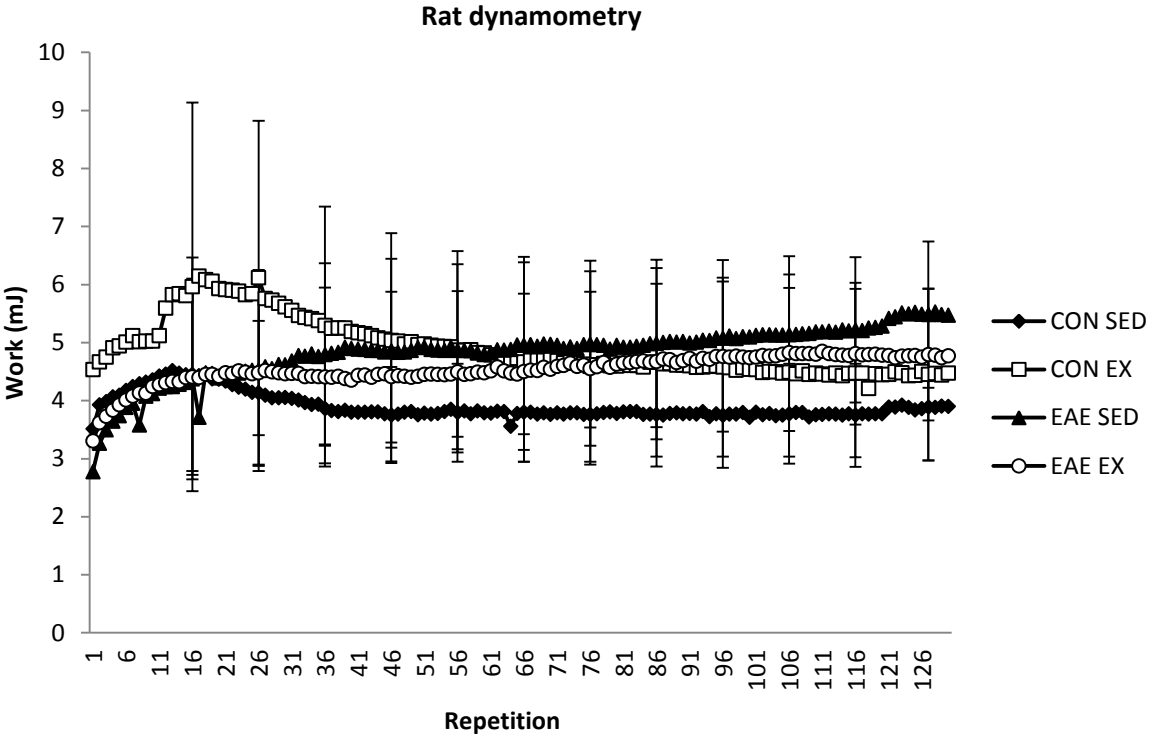


Figure 24: Rat dynamometry. Mean values ± SD are plotted.

Muscle work differed significantly between CON SED and CON EX during the complete protocol (0,01 ≤ p ≥ 0,048), indicating a higher energy expenditure and muscle force after 10 days of treadmill running. Muscle work remained stable (decline 12,87%; p= 0,2) for CON SED between the first and 110th contraction, while for CON EX the work declined 25,93% (p= 0,05) (Figure 25).

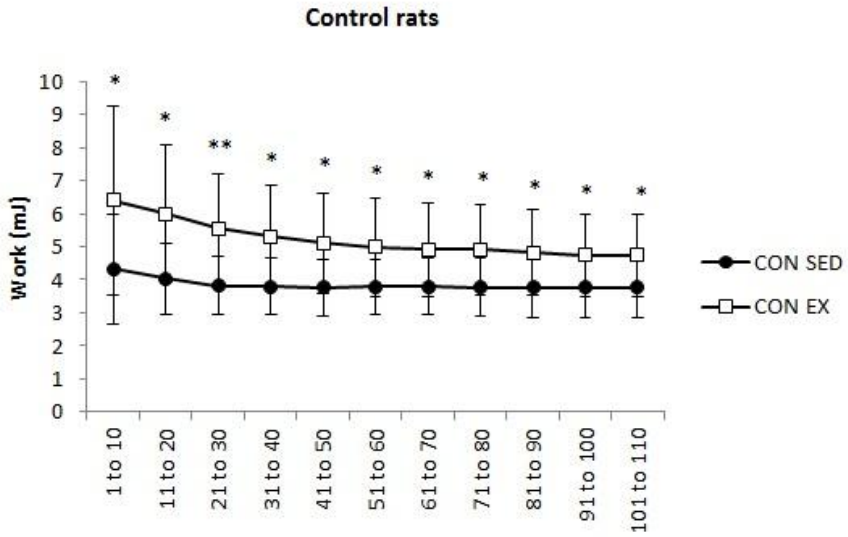


Figure 25: Muscle work control rats. Mean values ± SD are plotted.: p ≤ 0,05 **: p ≤ 0,01*

In EAE animals however, no training effect was detected. Muscle work remained stable in EAE EX, while an increase of 20,52% (p= 0,03) is seen in EAE SED muscle work (Figure 26).

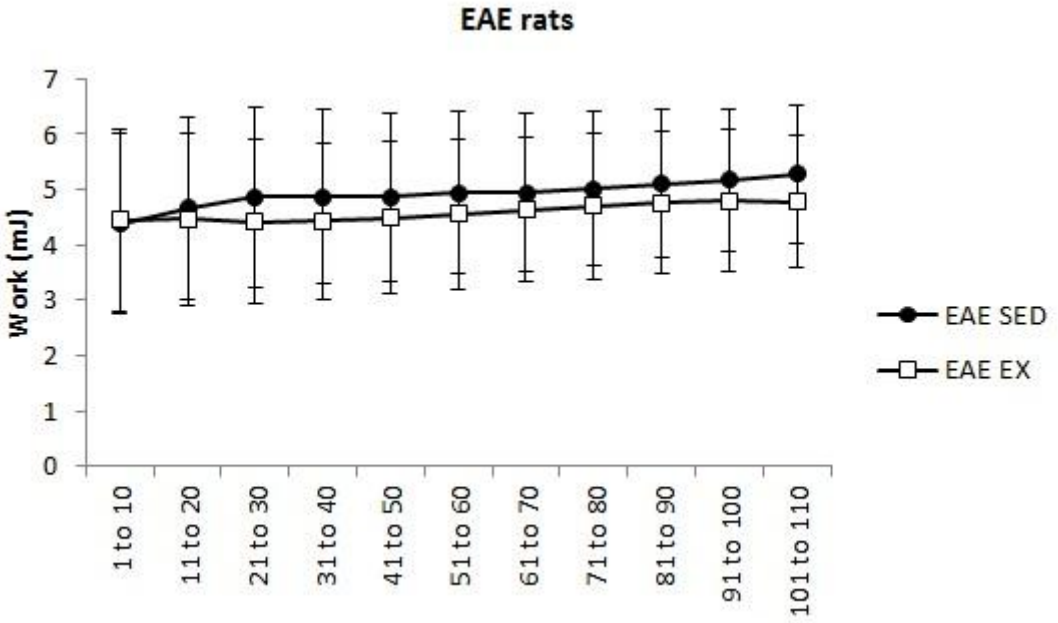


Figure 26 Muscle work EAE rats. Mean values ± SD are plotted.

Between CON EX and EAE EX a difference in muscle work is detected during the first 20 contractions (p= 0,05), showing a stronger start in the CON EX group. After this strong start the muscle work in CON EX declines 25,93 % (p= 0,05) (Figure 27).

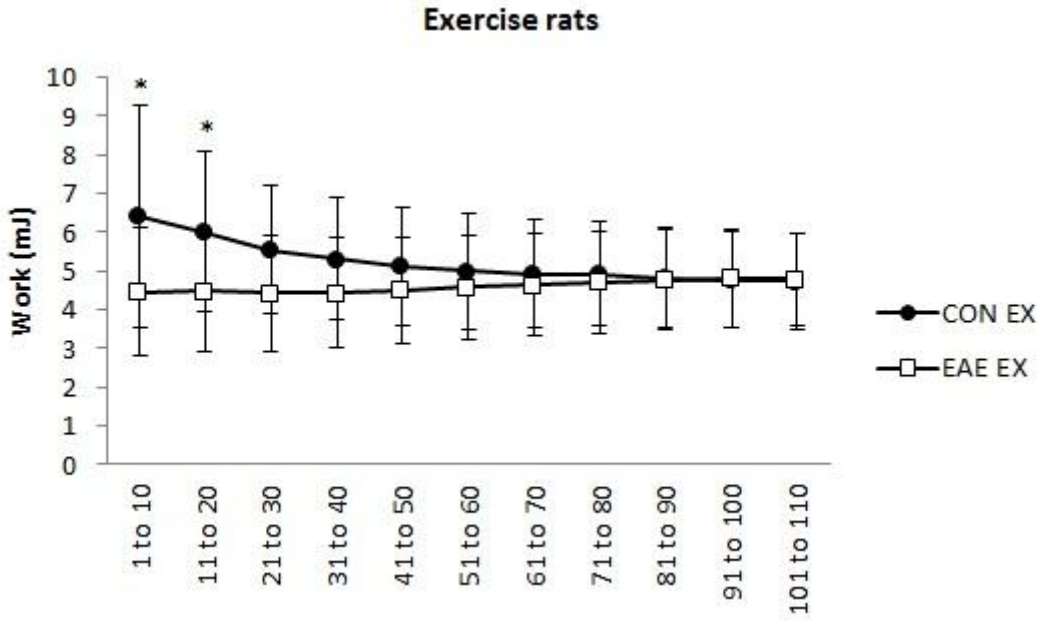


Figure 27 Muscle work exercise rats. Mean values ± SD are plotted.: p ≤ 0,05*

CON SED and EAE SED differed in muscle work from contraction 31 to 110 (0,007 ≤ p ≥ 0,05), muscle work being higher in the EAE rats (Figure 28).

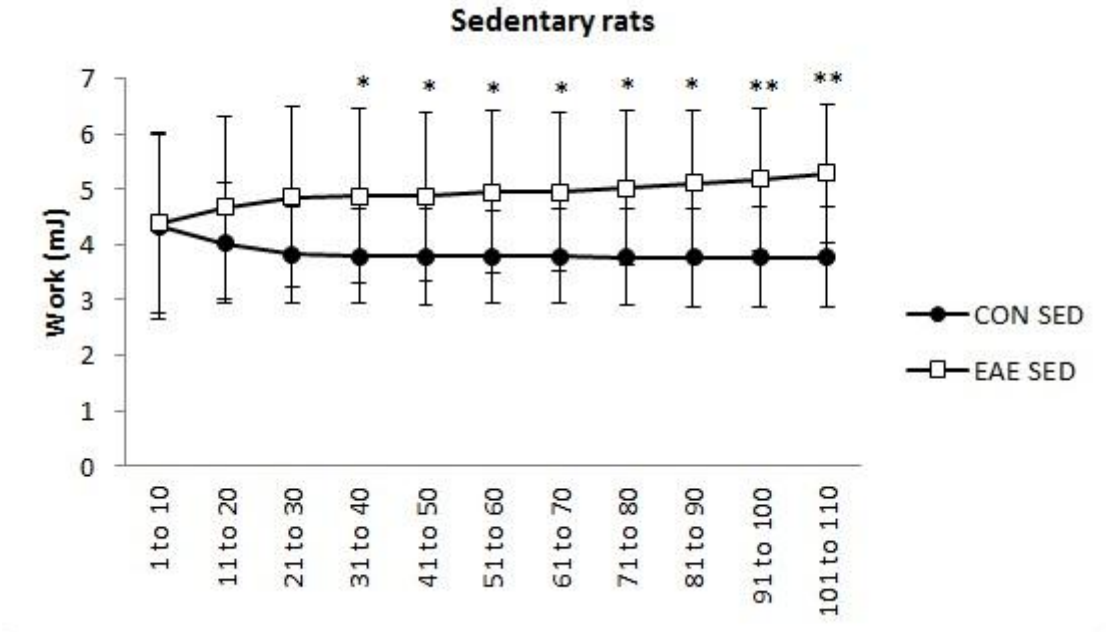


Figure 28: Muscle work sedentary rats. Mean values ± SD are plotted.: p ≤ 0,05 **: p ≤ 0,01*

4.2.6 Skeletal muscles: morphology

All muscle stainings show that the connective tissue (laminin) between fiber types is stained blue through the DAPI channel, type IIa fibers are colored green through the FITC channel and type I fibers are red through the TRITC channel.

Tibialis anterior

An example of the result of the fluorescent staining of TA is shown in figure 29.

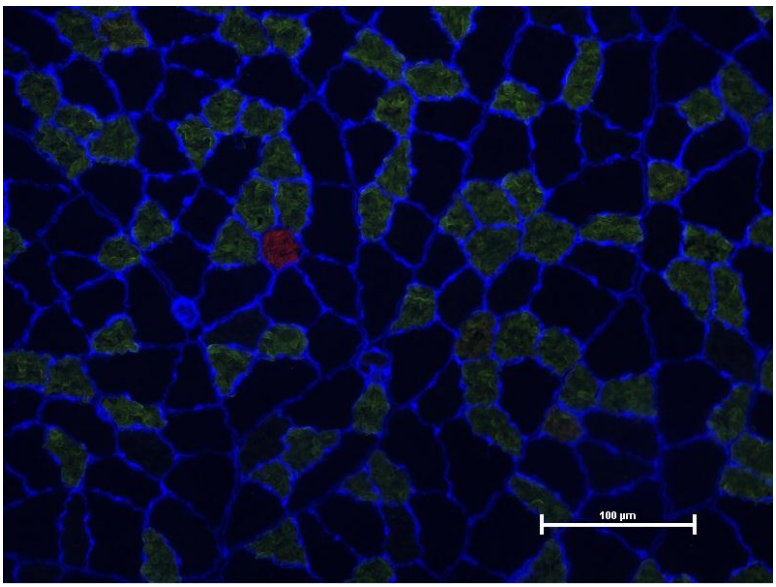


Figure 29: An example of TA from a sedentary control rat: Blue = laminin, green = fiber type IIa, red = fiber type I

The channels viewed separately are shown in figure 30.

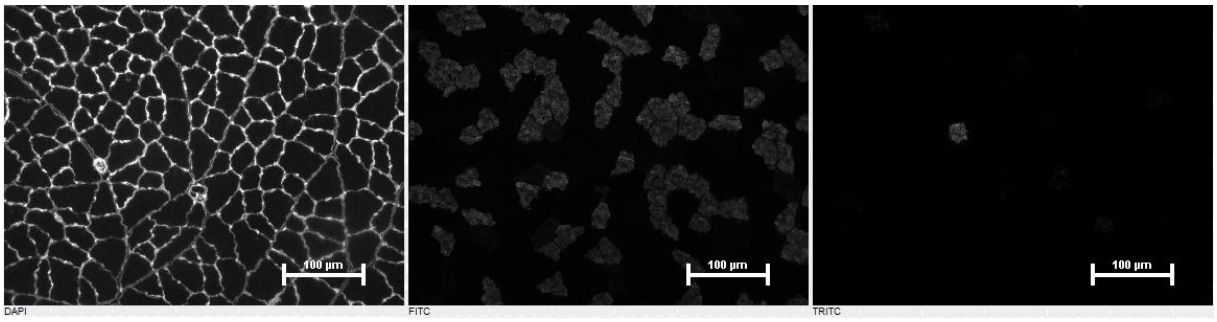


Figure 30: TA: Dapi (laminin), FITC (type IIa) and TRITC (Type I) channel

Fiber type distribution and cross sectional area were not fully analyzed for TA.

Extensor digitorum longus

An example of the fluorescent staining of EDL muscle is shown in figure 31.

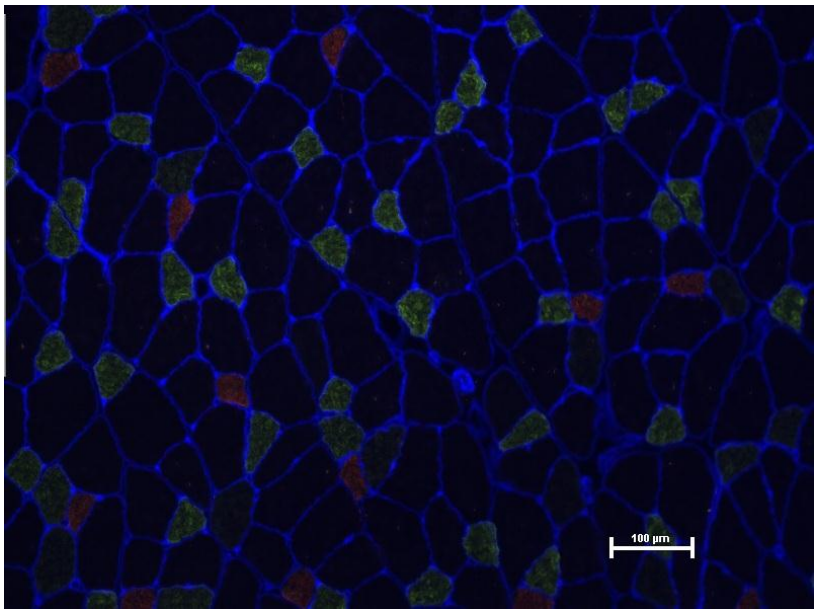


Figure 31: EDL from a sedentary control rat: Blue = laminin, green = fiber type IIa, red = fiber type I

The DAPI, FITC and TRITC channel are shown in figure 32.

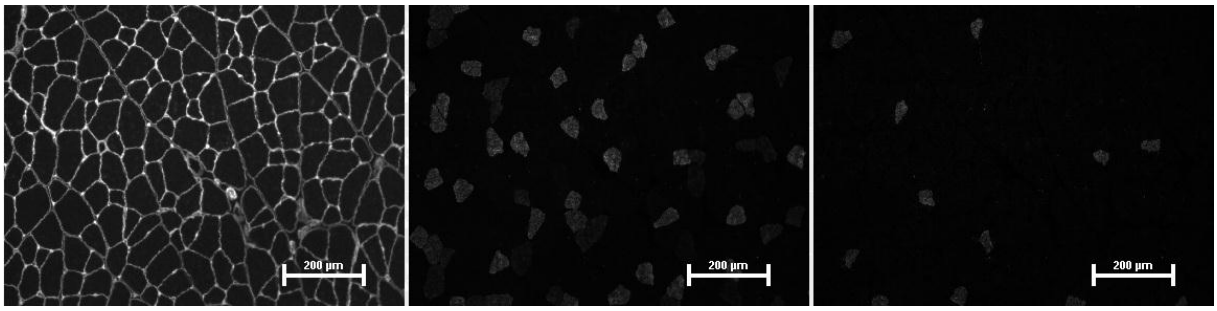


Figure 32: EDL: Dapi (laminin), FITC (type IIa) and TRITC (Type I) channel

The distribution and CSA of fiber type I, IIa and IIb need further analysis are not included in this paper.

Soleus

SOL had a staining result as shown in figure 33. This muscle is known to comprise for the majority of type I fibers (red color).

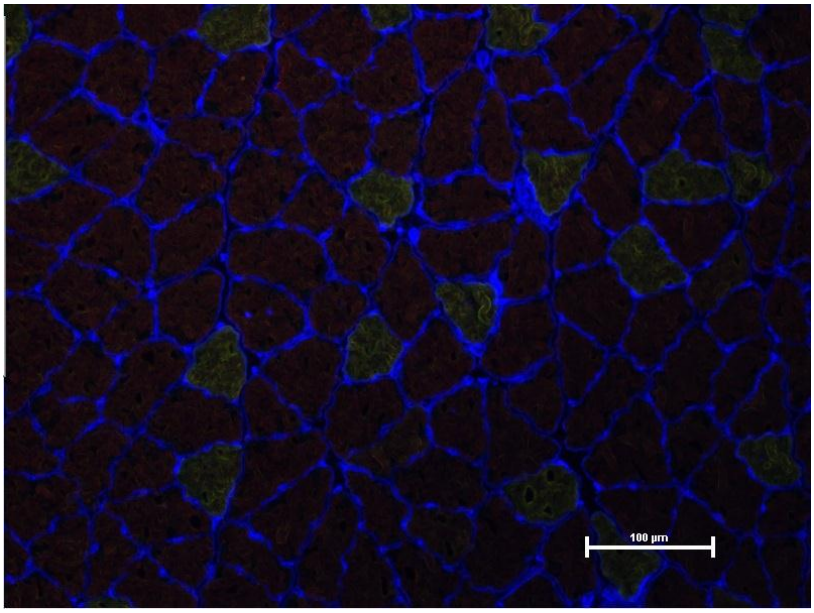


Figure 33: SOL from a sedentary control rat: Blue = laminin, green = fiber type IIa, red = fiber type I

All three channels split up are shown in figure 34.

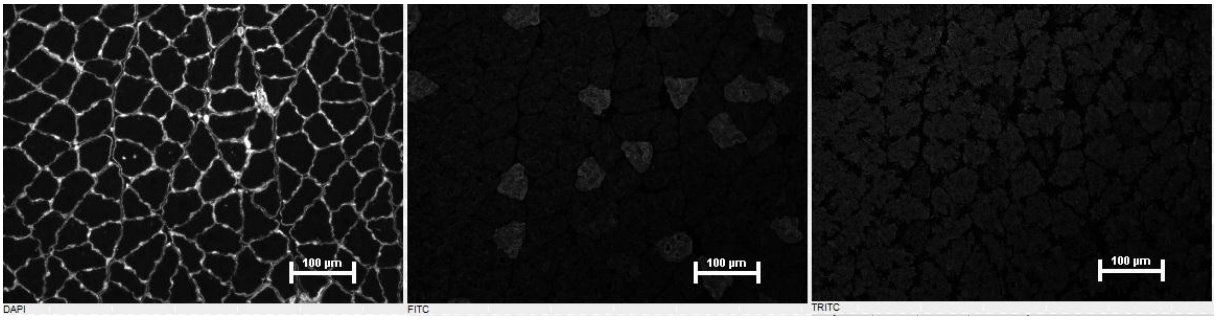


Figure 34: SOL: Dapi (laminin), FITC (type IIa) and TRITC (Type I) channel

Fiber type distribution and CSA need further analysis and are therefore not included in this paper.

Discussion & Conclusions

Muscle weakness frequently occurs in MS and is associated with fatigue and increased disability. As a consequence people with MS are physically inactive, a state that is linked to an increase in the majority of prevalent chronic diseases, including IGT, IR and diabetes. The data of the present study suggest that MS patients have IGT compared to matched healthy controls. Furthermore, exercise training might have a beneficial impact on glucose tolerance of MS patients, since it has been proven in other populations that the process of IGT can be influenced. The applied combined training program seems to improve predominantly knee flexion strength, counteracting the muscle fatigue. The rat study shows a clear positive training effect on clinical symptoms, however further research is needed to confirm effects on contractile properties. The different parameters will be discussed further. It should be noted that investigator blinding was not applied, which might have caused bias and influenced the results.

5.1 MS

5.1.1 Glucose tolerance

The 2 hour lasting fasting OGTT is the standard procedure in measuring blood glucose levels. The Analox GM7 microstat analyzer (Analox instruments Ltd.) has a compared accuracy of: y (Analox) = 0.9836x - 0.1335 mmol/L, r = 0.09991, n = 147 in method comparison vs Hexokinase (47).

Baseline tests showed that MS patients have a significantly higher tAUC and therefore are less glucose tolerant compared to healthy controls. This finding supports the reports from Warren *et al.* (30) and Wertman *et al.* (31) attempting to link MS with a higher risk of diabetes mellitus. Furthermore it provides a confirmation of the importance of this research goal. However, side effects of MS medication use should be kept in mind and were taken into account (see further).

Within the MS patient group, tAUC of the EX subgroup remained stable after 24 weeks of combined exercise training, while tAUC of CON tended to worsen after 24 weeks. These results indicate that regular exercise prevents aggravation of IGT. These results corroborate with reports in the literature on the positive effects of physical exercise on IR (23; 48; 49). However, serum insulin concentrations need to be determined in the future to provide further insight in insulin sensitivity.

Several studies already suggested the influence of body composition (fat and lean mass) on an altered glucose and insulin sensitivity (50; 51). More specific, central obesity is known to predispose individuals for IR, the metabolic syndrome and even diabetes type 2. Abdominal fat tissue produces TNF-α, which suppresses leptin and adiponectin production and increasing levels of free fatty acids, hereby indirectly reducing the insulin sensitivity of liver and muscle. Also due to abdominal fat tissue, cortisol levels rise locally, increasing blood glucose concentrations by stimulation of gluconeogenesis and decreasing GLUT4-mediated glucose uptake in skeletal muscle and adipose tissue (17). DEXA analyses of the subjects' body composition confirm a decrease in fat percentage after 24 weeks of combined exercise. However, no relevant changes on the glucose profile or tAUC were detected. In addition, serum insulin concentrations need to be determined in the future. Researchers assume a decrease of serum insulin concentration after 24 weeks of exercise resulting in an improved glucose tolerance.

An increase in lean body mass in the EX was also detected, suggesting that muscle atrophy due to inactivity might have a role in the development of impaired glucose tolerance (36; 52; 53), since skeletal muscle is important for blood glucose regulation. An increase in muscle mass possibly supports improvements in muscle power. Despite the changes of fat percentage and lean body mass, general weight loss is not detected, suggesting that the results of the OGTTs were not influenced by weight loss, which is also linked at an improvement of glucose tolerance in patients with IR and diabetes type 2 (50; 51). So, body mass, but also age, is known to influence the glucose profile, as already demonstrated in other populations (51; 52). Statistical correlations between age and OGTT confirmed these findings for MS patients. Also a correlation between OGTT and EDSS was detected, indicating the influence of MS on the presence of IGT of MS patients.

Besides body composition, side effects of pharmacological treatments can also affect the outcomes of this study. More specific, it has been mentioned that the synthetic corticosteroid Medrol is known to cause IR (10) by inhibition of glucose utilization. However, the use of medication is registered during the complete course of the study and deviating tests, immediately after the use of Medrol, were excluded.

In conclusion, MS patients have a higher risk of developing IGT, which can be stabilized by physical exercise. A future perspective in a training study would be to take muscle biopsies. Biopsies could contribute to a better understanding of the results concerning glucose tolerance and gain new insights.

5.1.2 Muscle strength

In exercise therapy, protocols specifically for MS patients have not been developed yet. Since MS patients and older people have similar activity levels, this initial protocol was based on the guidelines for elderly (54; 55). Adherence to the program was high and the level of difficulty was monitored by Borg scoring, indicating that the protocol was appropriate for this population. Effectiveness of the training protocol was measured and confirmed by means of 10 RM tests, a measure of strength gain on the training equipment. Assessment of changes in muscle strength was performed by means of the Biodex dynamometer, which is accepted as the golden standard for this purpose, including MS populations (56). Isometric muscle strength increased approximately 50% in quadriceps muscles and 25% in hamstrings which is higher compared to previous studies from Broekmans *et al.*, showing an increase of 10% in knee extensor muscles and an increase of 7-9 % in knee flexor muscles (39). These differences might be due to the use of a combined exercise program, while Broekmans *et al.* only used resistance training. Resistance training studies in other populations show similar results, such as Suetta *et al.* showing a 30% increase in isokinetic muscle strength after 12 weeks of resistance training in an elderly population (57). However, comparison between isokinetic and isometic strength is difficult. Dalgas *et al.* measured a 15,7% increase in knee extensor strength after 12 weeks of progressive resistance training in MS patients (58). In a population of stroke patients, Teixeira-Salmela *et al.* measured a 42.3% muscle strength improvement in the impaired leg muscles, including an 18% to 46% strength improvement in knee extensors (59). Within the same population, Weiss *et al.* also used a progressive resistance training intervention to increase isokinetic knee extensor strength in the impaired leg by 67% and in the non-impaired leg by 42% (60). These values are more comparable with our findings.

Since MS patients are mostly affected at one side, more specific at one leg, the used training protocol was optimized by applying unilateral strength training for the legs, while the arms were trained bilateral due to equal impairment of the arms. The unilateral muscle strength measurements in this study increased tremendously, showing that unilateral training has the potency to improve muscle strength even in the most impaired leg of the patient. However, strength measurement of the bilateral trained arms was not that significant. Maybe the test was too difficult to perform, due to the peculiar movement of the elbow, resulting in divergent test results. Or maybe the impairment of both arms is not as equal as assumed earlier, suggesting that unilateral strength training for the arms would gain better improvements. Further research is definitely required.

Isokinetic force measurements were not analyzed in this work. The purpose of the test was clarified in a standardized manner to each subject, yet many patients failed to execute the test sufficiently. Comprehension of the instructions may have been too difficult in this population. Further analysis is required in the future.

The clinical impact of combined exercise therapy is demonstrated by the benefits improved muscle strength has on walking capacity and balance, features that are diminished in MS patients. MS patients indicate a general improvement in well-being as well. It can be concluded that muscle weakness can be significantly diminished in an MS population, with a positive impact on fatigue and general lifestyle.

5.2 EAE

Acute EAE is a good model for several inflammatory aspects of MS, yet not all aspects of the disease are covered. Extrapolation of findings in this model to MS needs to be done with caution. The disease course of the EAE animals was recorded by assessment of clinical scores, body weight and food intake. The onset of clinical symptoms appeared to be delayed in the training group, which was paralleled by the body weight and food intake. This finding is supported by other authors, also reporting a delayed onset of the disease (57).

5.2.1 Glucose tolerance

No significant differences between the different groups were detected in the glucose profile and tAUC during phase I of the study. If physical exercise did have an effect on glucose tolerance, this effect was probably diminished by the sedentary week, after ending the training period and during the prevalence of the clinical symptoms. This hypothesis could potentially be confirmed in phase II of the study, further analysis is needed. A future perspective in an animal study would be to measure stress hormones, as Contarteze *et al.* have done previously (61). In the setup of this study, an increase in these hormones could have made the training too intense to temper EAE symptoms, so this effect needs to be excluded. Additionally, cortisol decreases GLUT4-mediated glucose uptake and stimulates gluconeogenesis, which influences the glucose profile (17).

5.2.2 Muscle strength and fiber type distribution

Isokinetic dynamometry is a highly standardized method for muscle strength measurements (56). However, comparison with the literature is difficult. Rossi *et al.* executed similar research, but they used voluntary and uncontrolled training (35). The study from Le Page *et al.* does not describe a protocol (57). It is very likely that the training intensity, used in this study, was higher compared to previous research. The applied treadmill running program of this study mainly affects m. gastrocnemius and SOL, while the inclination activated TA and EDL muscles. EAE injection was done in both footpads of the hind limbs, which may have interfered with the rat's performance on the treadmill. However, this was only an issue during the first 2 training days. During phase I isokinetic muscle fatigue measurements in healthy rats a peak was reached during the initial 30 contractions of the 120 contraction series, followed by a decline in muscle work, which improves after exercise. In EAE a peak in the measured muscle work was absent, and muscle work remained stable. This suggests that fast twitch type II fibers are more damaged in EAE rats, since this fiber type is predominantly responsible for peak strength (35). The absence of a peak in EAE rats is in contrast with the results from de Haan *et al.* (36), but the difference could be explained by the different muscles that were tested (TA & EDL vs. gastrocnemius muscle), since a study by Broekmans *et al.* showed similar results (39). Also, de Haan *et al.* performed isometric tests, while this study uses an isokinetic protocol. Nevertheless, some training effects on muscle force might have been missed, since muscle force measurement was not done on SOL.

Furthermore, the EAE rats showed a surprising result. The EAE SED rats were stronger compared to EAE EX. This is probably attributable to overtraining. However, these results are comparable with the findings of Broekmans *et al.*, where an intense swimming program was used as intervention. The intensity of the swimming can be comparable with the intensity of our treadmill training. Besides the possibility of overtraining, an additional loss of muscle mass (body mass), occurred in the sedentary week after ending the training sessions, can also be a possible explanation for these interesting results. More specific, the sedentary week (and the prevalence of the clinical symptoms) may have diminished the training effects in EAE. Based on this hypothesis, and to possibly exclude the diminishing effects of the sedentary week, rats in phase II were tested 48 hours after the last treadmill training. However, phase II of this study was not completed while ending this paper so these data were not included.

This study hypothesized that muscle fatigue is in part attributable to a switch in fiber type and could be improved by exercise. To confirm this hypothesis morphologically, muscle fiber composition and distribution were studied. However, analysis of the CSA and fiber type distribution is not completed yet and needs to be finished in the future. Expectations are that EAE rats show a lower CSA of type II muscle fibers, confirming the lower peak torque due to fewer fast twitch fibers. A similar study from de Haan *et al.* showed a lower peak

torque in EAE animals and significantly smaller CSA compared to control rats (36). These changes in contractile properties are likely due to the inactivity caused by paralysis of the hind limbs.

In conclusion, there is a clear training effect on clinical symptoms in EAE rats, yet further research is required to confirm effects on contractile properties. Investigating muscle contractile properties in MS patients is limited. So researching this aspect into depth in an animal model first may provide a better understanding of the physiological consequences of exercise therapy in MS patients. Afterwards, for better extrapolation between animal models and the patient study, biopsies from MS patients are needed in attempting a comparison of CSA and fiber type distribution between MS patients and the rat study. Doing the experiments with both MS patients and EAE rats gives a broader perspective of the subject. However, extrapolation needs to be done with caution: EAE is not equal to MS.

Exercise therapy proves to be an important implementation in the treatment of MS, because it has positive effects on some clinical symptoms. However, exercise therapies are not optimized yet, which is why research investigating this topic can provide the needed information for further rehabilitation protocols.

Bibliography

1. **Calabresi, P.** Multiple sclerosis and demyelinating conditions of the central nervous system. [book auth.] Ausiello D. Goldman L. *Cecile medicine.* 23. Philadelphia : Saunders Elsevier, 2007, p. 436.

2. *Multiple sclerosis.* **Compston , A. and Coles , A.** 359, 2002, Lancet, pp. 1221-31.

3. *Multiple sclerosis overview.* **Stüve, Olaf and Oksenberg, Jorge.** [ed.] Roberta Pagon, et al., et al. s.l. : University of Washington, Seattle, 2006, GeneReviews.

4. *Primary-progressive multiple sclerosis.* **Miller, D. and Leary, S.** 2007, Lancet Neurol, Vol. 6, pp. 903-912.

5. *Physiopathology of fatigue in Multiple Sclerosis.* **Leocani, L., Colombo, B. and Comi, G.** 29, 2008, Neurol Sci, pp. S241-S243.

6. *Strength, skeletal muscle composition, and enzyme activity in multiple sclerosis.* **Kent-Braun, A.** 6, 1997, J. Appl. Physiol., Vol. 83, pp. 1998-2004.

7. *Physical activity behaviour in individuals with SPMS.* **Motl, R.** 9, 2007, Int J MS Care, pp. 139-42.

8. *Will the newer oral MS agents be welcomed by managed care organizations?* **Lipsy, R.** 8 Supplement, 2010, Am J Manag Care, Vol. 16, pp. S227-S233.

9. *Switching Multiple Sclerosis Patients with Breakthrough Disease to Second-Line Therapy.* **Castillo-Trivino, T., et al., et al.** 2, 2011, Plos One, Vol. 6, pp. 1-5.

10. *Pharmacodynamic/Pharmacogenomic Modeling of Insulin Resistance Genes in Rat Muscle After Methylprednisolone Treatment: Exploring Regulatory Signaling Cascades.* **Yao, Zhenling, Hoffman, Eric and Ghimbovs, Svetlana.** 2, 2008, Gene Regulation and Systems Biology, pp. 141-161.

11. *Acute methylprednisolone administration induces a transient alteration of glucose tolerance and pyruvate dehydrogenase in humans.* **Pellacani, A., et al., et al.** 1999, European Journal of Clinical Investigation, Vol. 29, pp. 861–867.

12. *Glucocorticoid effect on insulin sensitivity: a time frame.* **Zarkovic, M., et al., et al.** 3, 2008, J Endocrinol Invest., Vol. 31, pp. 238-42.

13. *The role of growth hormone and glucocorticoid in glucose handling in vivo.* **Johansen, T., et al., et al.** 1999, Journal of Endocrinology, Vol. 162, pp. 87–93.

14. **Smith, B., et al., et al.** *Drug Class Review: Disease-modifying Drugs for Multiple Sclerosis: Final Update 1 Report.* Portland (OR): Oregon Health & Science University. 2010.

15. *Toxicities of immunosuppressive treatment of autoimmune neurologic diseases.* **Lallana, E. and Fadul, C.** 3, 2011, Curr Neuropharmacol., Vol. 9, pp. 468-477.

16. *Rehabilitation in multiple sclerosis.* **Cabrera-Gómez, José A.** 2007, Multiple sclerosis for the practicing neurologist, Vol. 5, pp. 75-83.

17. **Porterfield, P. Susan and White, A. Bruce.** *Endocrine physiology.* 3rd. Philadelphia : Mosby Elsevier, 2007. ISBN-13: 978-0-323-03666-5.

18. **Kumar, S. and O'Rahilly, S., [ed.].** *Insulin Resistance: Insulin action and its disturbances in disease.* s.l. : Wiley, 2006.

19. *Turning Signals On and Off: GLUT4 Traffic in the Insulin-Signaling Highway.* **Thong, F., Dugani, C. and Klip, A.** 2005, Physiology, Vol. 20, pp. 271–284.

20. *Analysis of GLUT4 Distribution in Whole Skeletal Muscle Fibers: Identification of Distinct Storage Compartments That Are Recruited by Insulin and Muscle Contractions.* **Ploug, T., et al., et al.** 6, 1998, The Journal of Cell Biology, Vol. 142, pp. 1429–1446.

21. *Regulated transport of the glucose transporter GLUT4.* **Bryant, N., Govers, R. and James, D.** 2002, Molecular cell biology, Vol. 3, pp. 267-277.

22. **Kumar, V., et al., et al.** *Robbins basic pathology.* 8th. Philadelphia : Saunders Elsevier, 2007. pp. 778-781.

23. *Exercise training-induced improvements in insulin action.* **Hawley, J. and Lessard, S.** 192, 2008, Acta Physiol., pp. 127-135.

24. *Exercise as a therapeutic intervention for the prevention and treatment of insulin resistance.* **Hawley, J.** 20, 2004, Diabetes Metab Res Rev, pp. 383-393.

25. *Regular exercise enhances insulin activation of IRS-1-associated PI3-kinase in human skeletal muscle.* **Kirwan, J., et al., et al.** 2000, J. Appl. Physiol., Vol. 88, pp. 797–803.

26. *Effect of short-term exercise training on insulin-stimulated PI 3-kinase activity in human skeletal muscle.* **Houmard, J., et al., et al.** 1999, Am J Physiol Endocrinol Metab, Vol. 277, pp. E1055-E1060.

27. *Effect of Long-Term Exercise on Gene Expression of Insulin Signaling Pathway Intermediates of Insulin Signaling Pathway Intermediates.* **Kim, Y., et al., et al.** 1999, Biochemical and Biophysical Research Communications, Vol. 254, pp. 720–727.

28. *Insulin Receptor phosphorylation, Insulin Receptor Substrate-I phosphorylation, and phosphatidylinositol 3-kinase activity are decreased in intact skeletal muscle strips from obse subjects.* **Goodyear, L., et al., et al.** 1995, J. Clin. Invest, Vol. 95, pp. 2195-2204.

29. *Significant decreased insulin secretion in a diabetic patiënt with clinically probable multiple sclerosis.* **Katsuki, A.** 37, 1998, Internal Medicine, pp. 865-869.

30. *Multiple sclerosis and diabetes mellitus: further evidence of a relationship.* **Warren, S. and Warren, K.** 4, 1982, Can J Neurol Sci., Vol. 9, pp. 415-9.

31. *An association between multiple sclerosis and type I diabetes mellitus.* **Wertman, E.** 1, 1992, J Neurol., Vol. 239, pp. 43-5.

32. *Exercise attenuates the clinical, synaptic and dendritic abnormalities of experimental autoimmune encephalomyelitis.* **Rossi, S.** 36, 2009, Neurobiology of Disease, pp. 51-59.

33. *Exercise Induces Rapid Increases in GLUT4 Expression, Glucose Transport Capacity, and Insulin-stimulated Glycogen Storage in Muscle.* **Ren, J., et al., et al.** 20, 1994, The Journal of Bioloogical Chemistry, Vol. 269, pp. 14396-14401.

34. *adaptations of skeletal muscle to prolonged intense endurance training.* **Hawley, J.** 29, 2002, Clinical and Experimental Pharmacology and Physiology, pp. 218-222.

35. **Stevens, A. and Lowe, J.** *Histologie van de mens.* s.l. : Bohn Stafleu van Loghum, 1997. ISBN 90-313-2435-3.

36. *contractile properties and fatigue of quadriceps muscles in multiple sclerosis.* **de Haan, A., et al., et al.** 2000, Muscle & Nerve, Vol. 23, pp. 1534 - 1541.

37. *Cross-bridge mechanisms of muscle weakness in multiple sclerosis.* **Garner, D. and Widrick, J.** 2003, Muscle Nerve, Vol. 27, pp. 456–464.

38. *Impact of aerobic training on fitness and quality of life in multiple sclerosis.* **Petajan, J., et al., et al.** 4, 1996, Ann Neurol. , Vol. 39, pp. 432-441.

39. *Effects of long-term resistance training and simultaneous electro-stimulation on muscle strength and functional mobility in multiple sclerosis.* **Broekmans, T., et al., et al.** 4, 2011, Multiple sclerosis journal, Vol. 17, pp. 468-477.

40. *Multiple sclerosis: current etiological concepts.* **Seil, F.** 116, 1972, Calif. Med., pp. 25-33.

41. *Neuropathogenesis of Theiler's Murine Encephalomyelitis Virus Infection, An Animal Model for Multiple Sclerosis.* **Tsunoda, I. and Fujinami, R.** 2010, J Neuroimmune Pharmacol, Vol. 5, pp. 355–369.

42. *Studies in the Modulation of Experimental Autoimmune Encephalomyelitis.* **Libbey, J., Tsunoda, I. and Fujinami, R.** 2010, J Neuroimmune Pharmacol, Vol. 5, pp. 168–175.

43. *The experimental autoimmune encephalomyelitis model for proteomic biomarker studies: From rat to human.* **Rosenling, T., et al., et al.** 2011, Clinica Chimica Acta, Vol. 412, pp. 812–822.

44. *Modeling multiple sclerosis in laboratory animals.* **Schreiner, B., Heppner, F. and Becher, B.** 2009, Semin Immunopathol, Vol. 31, pp. 479-495.

45. *Characterization of acute versus chronic relapsing autoimmune encephalomyelitis in DA rats.* **Tanuma, N., Shin, T. and Matsumoto, Y.** 2000, Journal of Neuroimmunology, Vol. 108, pp. 171–180.

46. *Induction and Clinical Scoring of Chronic-Relapsing Experimental Autoimmune Encephalomyelitis.* **Beeton, C., Garcia, A. and Chandy, K.** 2007, Journal of visualized experiments, Vol. 5, p. 224.

47. Analysers: GM7. *Analox instruments.* [Online] 2011. [Cited: 05 21, 2012.] http://www.analox.com/analysers/gm7#specification.

48. *Physical activity: the forgotten tool for type 2 diabetes management.* **Colberg, S.** 70, 2012, Front Endocrinol, Vol. 3.

49. *Physical exercise as treatment of type 2 diabetes mellitus.* **König, D. and Berg, A.** 6, 2012, Internist, Vol. 53, pp. 678-687.

50. *Relative muscle mass is inversely associated with insulin resistance and prediabetes. Findings from the third National Health and Nutrition Examination Survey.* **Srikanthan, P. and Karlamangla, A.** 9, 2011, J Clin Endocrinol Metab., Vol. 96, pp. 2898-2903.

51. *Reversal of muscle insulin resistance by weight reduction in young, lean, insulin-resistant offspring of parents with type 2 diabetes.* **Petersen, K., et al., et al.** 21, 2012, Proc Natl Acad Sci U S A, Vol. 109, pp. 8236-8240.

52. *Influence of muscle wasting on oral glucose tolerance testing.* **Moxley, R., et al., et al.** 6, 1983, Clin Sci , Vol. 64, pp. 601-609.

53. *First dorsal interosseous muscle atrophy. Clinical sign of impaired glucose metabolism.* **Bienenstock, H., Davidson, S. and Forschner, T.** 8, 1971, N Y State J Med, Vol. 71, pp. 851-854.

54. **Balady, G., et al., et al.** *ACSM's guidelines for exercise testing and prescription.* 2000.

55. **Durstine, J. and Moore, G.** *ACSM's Exercise management for persons witch chronic diseases and disabilities.* second. s.l. : American college of sports medicine, 2003. ISBN 0-7360-3872-8.

56. *Muscle strength and fatigue during isokinetic exercise in individuals with multiple sclerosis.* **Lambert, C., Archer, R. and Evans, W.** 10, 2001, Med Sci Sports Exerc., Vol. 33, pp. 1613-1619.

57. *Resistance training induces qualitative changes in muscle morphology, muscle architecture, and muscle function in elderly postoperative patients.* **Suetta, C., et al., et al.** 1, 2008, J Appl Physiol., Vol. 105, pp. 180-186.

58. *Resistance training improves muscle strength and functional capacity in multiple sclerosis.* **Dalgas, U., et al., et al.** 2009, Neurology, Vol. 73, pp. 1478-1484.

59. *Muscle strengthening and physical conditioning to reduce impairment and disability in chronic stroke survivors.* **Teixeira-Salmela, L., et al., et al.** 10, 1999, Arch Phys Med Rehabil, Vol. 80, pp. 1211-1218.

60. *High intensity strength training improves strength and functional performance after stroke.* **Weiss, A., et al., et al.** 4, 2000, Am J Phys Med Rehabil, Vol. 79, pp. 369-376.

61. *Stress biomarkers in rats submitted to swimming and treadmill running exercises.* **Contarteze, R., et al., et al.** 151, 2008, Comp Biochem. Physiol A Mol. Integr. Physiol., pp. 415-422.

62. *The Immunopathology of Multiple Sclerosis: An Overview.* **Lassmann, H., Brück, W. and Lucchinetti, C.** 17, 2007, Brain Pathol, pp. 210-218.

63. *Biology of Oligodendrocyte and Myelin.* **Baumann, N. and Pham-Dinh, D.** 2, April 2001, Physiological reviews, Vol. 81, pp. 871-927.

64. *Exercise and rehabilitation for individuals with multiple sclerosis.* **Brown, T and Kraft, G.** 2, 2005, Phys Med Rehabil Clin N Am, Vol. 16, pp. 513-555.

65. *Relationship of body fat with insulin resistance and cardiometabolic risk factors among normal glucose-tolerant subjects.* **Gokulakrishnan, K., et al., et al.** 3, 2011, J Postgrad Med., Vol. 57, pp. 184-188.

66. *Association between insulin resistance and lean mass loss and fat mass gain in older men without diabetes mellitus.* **Lee, C., et al., et al.** 7, 2011, Vol. 59, pp. 1217-1224.

67. *An apparatus to measure in vivo biomechanical behavior of dorsi- and plantarflexors of mouse ankle.* **Ashton-Miller, J., et al., et al.** 72, 1992, J.Appl.Physiol., pp. 1205-1211.

68. *Effect of physical exercise on adoptive experimental autoimmune encephalomyelitis in rats.* **Le Page, C., et al., et al.** 73, 1996, Eur.J.Appl.Physiol Occup.Physiol., pp. 130-135.

Appendix

Table 1: Training protocol patient study

Week	Session	Resis	Endur	Week	Session	Resis	Endur
1	1	1x10	6 min	**13**	31	2x20	2x10 min
	2	1x10	6 min		32	2x20	2x10 min
2	3	1x15	8 min	**14**	33	3x12	3x7 min
	4	1x15	8 min		34	3x12	3x7 min
	5	1x15	8 min		35	3x12	3x7 min
3	6	1x15	8 min	**15**	36	3x12	3x7 min
	7	2x10	2x5 min		37	3x12	3x7 min
4	8	2x10	2x5 min	**16**	38	3x12	3x7 min
	9	2x10	2x5 min		39	3x15	3x8 min
	10	2x10	2x5 min		40	3x15	3x8 min
5	11	2x12	2x6 min	**17**	41	3x15	3x8 min
	12	2x12	2x6 min		42	3x15	3x8 min
6	13	2x12	2x6 min	**18**	43	3x15	3x8 min
	14	2x12	2x6 min		44	3x18	3x9 min
	15	2x15	2x7 min		45	3x18	3x9 min
7	16	2x15	2x7 min	**19**	46	3x18	3x9 min
	17	2x15	2x7 min		47	3x18	3x9 min
8	18	2x15	2x7 min	**20**	48	3x18	3x9 min
	19	2x18	2x8 min		49	4x10	3x10 min
	20	2x18	2x8 min		50	4x10	3x10 min
9	21	2x18	2x8 min	**21**	51	4x10	3x10 min
	22	2x18	2x8 min		52	4x10	3x10 min
10	23	2x20	2x9 min	**22**	53	4x10	3x10 min
	24	2x20	2x9 min		54	4x12	3x10 min
	25	2x20	2x9 min		55	4x12	3x10 min
11	26	2x20	2x9 min	**23**	56	4x12	3x10 min
	27	2x20	2x9 min		57	4x12	3x10 min
12	28	2x20	2x10 min	**24**	58	4x15	3x10 min
	29	2x20	2x10 min		59	4x15	3x10 min
	30	**10RM**			60	**10RM**	

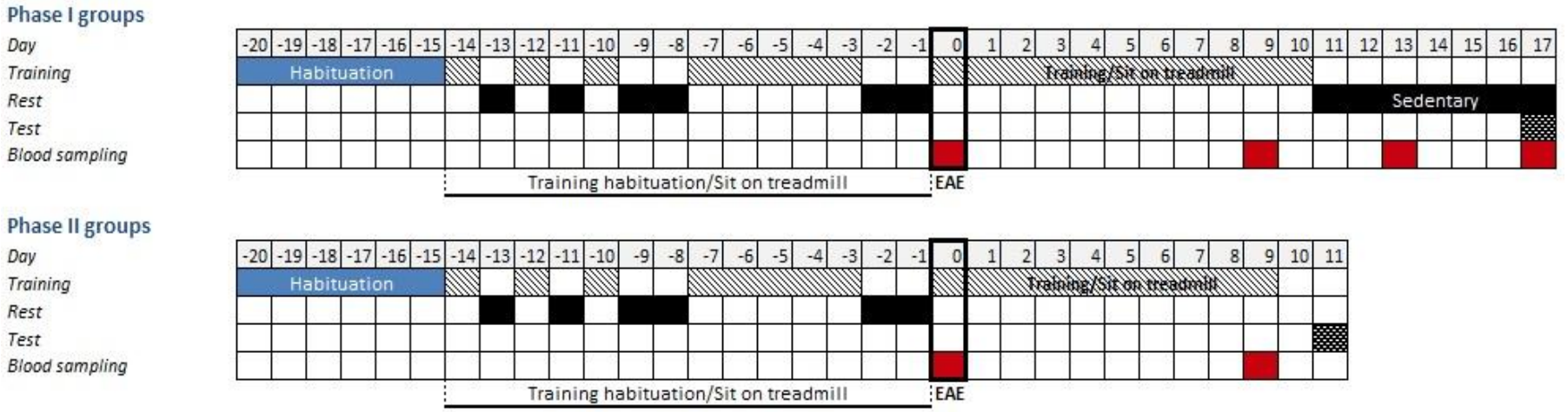
Table 1 shows the protocol for resistance and strength training of the patients participating in the study. For each training session, the number of repetitions is mentioned for the strength training and the number of minutes for the endurance training. After 12 and 24 weeks a 10RM test is taken.

Table 2: Training protocol rat study

Day	Group A	Group B	Speed	Ang	Duration	Group C	Group D	Speed	Ang	Duration
-20	AH	AH				AH	AH			
-19	H	H				H	H			
-18	H	H				H	H			
-17	H	H				H	H			
-16	H	H				H	H			
-15	H	H				H	H			
-14	ST	TH	4 m/min	0°	15	ST	TH	4 m/min	0°	15
-13	S	S				S	S			
-12	ST	TH	4 m/min	0°	20	ST	TH	4 m/min	0°	20
-11	S	S				S	S			
-10	ST	TH	4 m/min	0°	30	ST	TH	4	0°	30
-9	S	S				S	S			
-8	S	S				S	S			
-7	ST	TH	12 m/min	25°	45	ST	TH	12	25°	45
-6	ST	TH	13 m/min	25°	50	ST	TH	13	25°	50
-5	ST	TH	14 m/min	25°	55	ST	TH	14	25°	55
-4	ST	TH	16 m/min	25°	60	ST	TH	16	25°	60
-3	ST	TH	18 m/min	25°	60	ST	TH	18	25°	60
-2	S	S				S	S			
-1	S	S				S	S			
0	ST	T	18m/min	25°	60	ST	T	18	25°	60
1	ST	T	18 m/min	25°	60	ST	T	18	25°	60
2	ST	T	18 m/min	25°	60	ST	T	18	25°	60
3	ST	T	18 m/min	25°	60	ST	T	18	25°	60
4	ST	T	18 m/min	25°	60	ST	T	18	25°	60
5	ST	T	18 m/min	25°	60	ST	T	18	25°	60
6	ST	T	18 m/min	25°	60	ST	T	18	25°	60
7	ST	T	18 m/min	25°	60	ST	T	18	25°	60
8	ST	T	18 m/min	25°	60	ST	T	18	25°	60
9	ST (phase II stop)	T (phase II stop)	18 m/min	25°	60	ST (phase II stop)	T (phase II stop)	18	25°	60
10	ST	T	18 m/min	25°	60	ST	T	18	25°	60
11	S/Test phase II	S/Test phase II				S/Test phase II	S/Test phase II			
12	S	S				S	S			
13	S	S				S	S			
14	S	S				S	S			
15	S	S				S	S			
16	S	S				S	S			
17	S	S				S	S			
18	Test phase I	Test phase I				Test phase I	Test phase I			

Table 2 shows the trainings protocol of the rat study. Group A: sedentary control. Group B: training control. Group C: sedentary EAE. Group D: training EAE. AH = arrival and habituation. H = habituation. ST = sit on treadmill. TH = Training habituation. T= training. S= sedentary. = EAE induction.
The rats in the phase I group undergo the test immediately after the last training. The phase II rats have an extra sedentary week before the tests.

Table 3: Study design of the laboratory animal study.



	Group	Training	EAE induction
Phase I	A. Sedentary control	Sits on treadmill	No induction
	B. Training control	Trains on treadmill	No induction
	C. Sedentary EAE	Sits on treadmill	Induction on day 0
	D. Training EAE	Trains on treadmill	Induction on day 0
Phase II	A. Sedentary control	Sits on treadmill	No induction
	B. Training control	Trains on treadmill	No induction
	C. Sedentary EAE	Sits on treadmill	Induction on day 0
	D. Training EAE	Trains on treadmill	Induction on day 0

Table 1 shows the study design for the animal study. All animals are divided in a phase I and phase II group. Both groups have a further subdivision in 4 test groups: A. Sedentary control, B. Training control, C. Sedentary EAE and D. Training EAE. The last two subgroups will be induced with EAE on day 0. Group B and D are the test groups that will follow a training programme on the treadmill, while group A and C remain sedentary.

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Richting: **master in de biomedische wetenschappen-klinische moleculaire wetenschappen**
Jaar: **2012**

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Datum: **11/06/2012**