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Peak Blood Lactate Levels and Peak Performance Markers in Two Groups of MS Patients Performing an Exhaustive Bicycle Ergometry

Dissertation

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1. Introduction

1.1 Multiple sclerosis

1.1.1 Epidemiology and natural history of MS

Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) characterized by inflammatory demyelination and loss of axons in the brain and in the spinal cord (Milo and Miller 2014). MS was first depicted by the Scottish physician Robert Carswell in 1838, who illustrated the pathological changes of the spinal cord in an anatomical atlas (Carswell 1838). Though the exact cause and pathomechanism for MS remain unknown until today, it is generally regarded as an autoimmune disease. Between 60% and 70% of the patients affected by MS are women (Goodin 2014). Northern Europe ranks among the world's regions with the highest percentage of MS, with a prevalence in Germany of 54–150/100.000 and an incidence of 1.9–4.6/100.000 per year (Milo and Kahana 2010). The first symptoms typically show up during early adulthood, the mean age of onset is approximately 30 years (Noseworthy et al. 2000; Milo and Miller 2014). Fifteen years after onset of the disease, 50% of the patients are no longer able to walk without assistance (Weinshenker et al. 1989). Consequently, MS constitutes an important cause for disability in young and middle-aged adults. After the onset of MS, the median time to death is about 30 years, which means overall life expectancy is reduced by 5–10 years (Brønnum-Hansen et al. 2004). However, the prognosis is variable: 10% of the patients lead a life largely unaffected by MS even 20 years after the onset (Weinshenker et al. 1989).

1.1.2 Diagnosis and causes of MS

The current diagnostic algorithm for MS combines clinical information on neurological symptoms with findings of magnetic resonance imaging (MRI), results of evoked electrophysiological potential testing and the analysis of cerebrospinal fluid (CSF) (Polman et al. 2011). MS is diagnosed if these sources confirm a disease activity reflecting focal demyelination which has affected the CNS in more than one place (dissemination over space) and on more than one occasion (dissemination over time) (Compston and Coles

2008). The exact roles of the genes and of the environment in the development of MS have yet to be determined, but there is evidence that environmental factors like viral exposure might serve as a trigger to start the disease in persons who carry a genetic risk for MS (Compston and Coles 2008; Goodin 2014).

1.1.3 Disease courses: RRMS, SPMS, PPMS, PRMS

In clinical practice and research, MS is classified into four, or lately three, disease courses (Lublin and Reingold 1996; Lublin et al. 2014). The most common disease course, present at the beginning in 85–90% of the patients, is the relapsing-remitting form (RRMS). It is usually diagnosed retrospectively and characterized by self-limited attacks of neurological symptoms, also called episodes or flares. The attacks occur at unpredictable intervals, last for several days or weeks and then often improve spontaneously. Between relapses, patients recover completely or to a large extent (see Fig. 1a). MRI of RRMS patients typically shows old and new lesions at the same time (Goodin 2014). In about 65% of the RRMS patients, at some point the neurologic function begins to deteriorate steadily and relapses become less frequent, the disease course thus turning into the secondary-progressive form (SPMS, see Fig. 1b). The mean age for the transition from RRMS to SPMS lies around 40 years, which means that on average around 10 years pass between the onset of RRMS and the transition to SPMS (Weinshenker et al. 1989; Confavreux and Vukusic 2006). Between 10% and 20% of MS patients experience, from the beginning, a steady decline in their neurological function without remitting (see Fig. 1c). This disease course is called primary-progressive MS (PPMS) (Noseworthy et al. 2000; Confavreux and Vukusic 2006). PPMS manifests itself later in life,

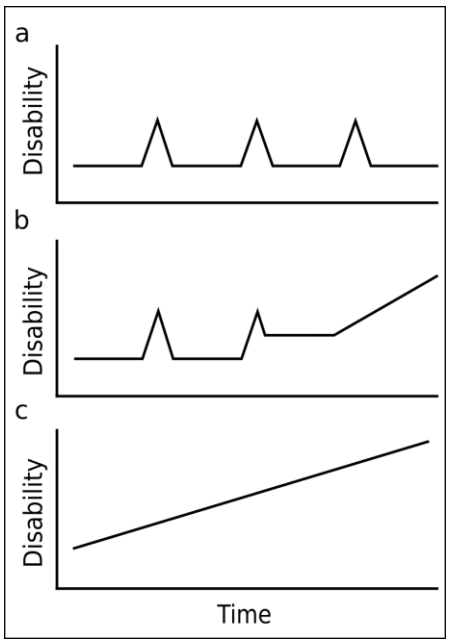

Fig. 1: Clinical courses of MS. a: RRMS, b: SPMS, c: PPMS. Redrawn after Hassan-Smith and Douglas (2011)

with a mean age of onset around 40 years. Noticeable, almost 50% of the patients affected by PPMS are men (Rice et al. 2013). It has been argued that PPMS and SPMS might actually represent the same clinical entity, but this issue remains highly controversial (Goodin 2014).

A fourth disease course has originally been described as progressive-relapsing MS (PRMS). Patients affected by this form were reported to show worsening neurological symptoms from the beginning, but to experience once in a while acute exacerbations of neurological dysfunction additional to their otherwise steadily progressing disease. In the latest revisions of the diagnostic criteria for MS, the authors recommended to eliminate the category "PRMS" and to classify subjects with the described disease course characteristics as "PPMS patients with disease activity" (Polman et al. 2011). It remains an unsolved question if the different disease courses are actually just clinically different expressions of the same underlying pathomechanism or if they rather reflect distinct entities with distinct pathomechanisms (Goodin 2014).

1.1.4 Symptoms

Symptoms of MS are diverse and mostly not specific for the disease. The neurologic examination of RRMS patients might yield signs of an involvement of the motor, sensory and autonomic system as well as the visual system (Compston and Coles 2008). Patients can experience muscle weakness, spasticity, sensory loss, loss of vision as well as impaired coordination and balance, reflecting disease activity in the cerebellum or cerebellar pathways. Autonomic symptoms include bladder dysfunction, erectile impotence and constipation. Two clinical signs relatively characteristic for MS are Lhermitte's sign (the sensation of electric shocks as a reaction to neck flexion) and the Uhthoff phenomenon, which describes the worsening of neurologic symptoms triggered by an increase of body temperature, e.g. during hot baths or exercise. Subjects diagnosed with MS frequently suffer from neuropsychological symptoms like fatigue and depression. Depending on the progression of the disease, some display cognitive impairments like attention deficits, problems with executive function and eventually dementia (Noseworthy et al. 2000; Compston and Coles 2008; Durstine et al. 2009). The symptoms of the primary- and secondary-progressive forms of MS differ

somewhat from the ones seen in the relapsing-remitting disease course: PPMS and SPMS mostly manifest as spinal syndrome, causing gradually worsening gait disorder and resulting in spastic paraparesis. Symptoms emanating from the cerebrum and cerebellum also occur in PPMS, but only in a smaller number of patients compared to RRMS (Rice et al. 2013; Wingerchuk and Carter 2014).

1.1.5 Pathology and MRI

The pathologic correlate of active RRMS are multifocal “plaques”, areas in the CNS characterized by infiltrates of inflammatory cells, demyelination and, eventually, axonal degeneration, axonal loss and gliosis. These plaques or lesions can be detected by MRI in their different stages. They cluster preferentially in the optic nerve, the cortex and the subcortical white matter, around the lateral ventricles and the corpus callosum, in the brain stem and the spinal cord (Compston and Coles 2008). After a relapse in RRMS, neuronal function might be partly or temporarily restored by redistribution of sodium channels or by remyelination, but ultimately, structural damage remains. If the disease enters the secondary-progressive stage (SPMS), diffuse axonal and neuronal degeneration sets in, while the MRI evidence for active inflammation lesions subsides. Accordingly, MRI of PPMS patients shows less lesions of active inflammation from the beginning (Goodin 2014). It remains an unsolved question if inflammation is the driving process of PPMS or if it should be regarded as a mainly neurodegenerative disorder, with inflammation being only a secondary response (Compston and Coles 2008).

1.1.6 Drug therapy and other therapeutic interventions

Acute episodes in RRMS are usually treated by short-time corticosteroid pulse therapy. If the symptoms do not abate, plasmapheresis might be considered as a second choice treatment (Compston and Coles 2008). In contrast to this, the so-called disease-modifying therapies (DMTs) are administered continuously and aim to reduce the frequency and severity of attacks (Wingerchuk and Carter 2014). Although DMTs often fulfill these aims and evidentially lower the number of new lesions detected by MRI, their effect on

the long-term clinical outcome of MS patients is still somewhat questionable (Carrithers 2014).

The three first generation DMTs, still widely used, are the immunomodulatory substances Interferon β-1b, Interferon β-1a and Glatiramer acetate, all of which are self-injectable drugs (subcutaneous or intramuscular).

Three different DMTs are administered via intravenous transfusion: Mitoxantrone is a general immunosuppressive drug; its use is limited to two or three years because it evokes cumulative dose-related cardiomyopathy. Natalizumab is a humanized monoclonal antibody. It was withdrawn temporarily from the market in 2006 because a small number of patients developed progressive multifocal leukoencephalopathy (PML) under therapy. In the meantime, it has been reintroduced but is given to MS patients mainly after failure of other DMTs and according to a risk mitigation strategy. Alemtuzumab, the third and last intravenously administered DMT, is another humanized monoclonal antibody. It has been associated with an increased risk for developing secondary autoimmune reactions like thyroid disease.

Three oral DMTs are currently available: Fingolimod, Teriflunomide and Dimethyl-Fumarate, which are all immunomodulatory agents. Dimethyl-Fumarate and Teriflunomide are first line treatments for mild or moderate RRMS, Fingolimod is a first line treatment for active or highly active RRMS (Deutsche Gesellschaft für Multiple Sklerose 2015).

Disease-modifying drugs are often given as a sequential monotherapy, meaning that one DMT drug is replaced by another if episodes “break through” under the current therapy. An alternative treatment mode is the induction and maintenance strategy, which is based on the concept of “hitting hard and early”, then preserving the remission state with a comparably safe and mild DMT (Wingerchuk and Carter 2014).

Importantly, until today, none of the DMTs has been proven to affect PPMS. The only disease-modifying substance approved for SPMS so far is Mitoxantrone, which has severe side effects and is limited to two years of use (Wingerchuk and Carter 2014). As Rice et al. stated in a review (2013, p. 1102), “nothing reverses, stops, or even appears significantly to slow progressive disability once established”.

Nonetheless, a number of drugs are available which do not influence the prognosis or progression of the disease itself, but mitigate symptoms like depression, bladder incontinence and spasticity. Examples for this are antidepressants, anticholinergic agents and muscle relaxants (Thompson et al. 2010). Symptomatic therapies play an important role in the treatment of all possible disease courses of MS, especially for the patients already affected by serious disability and impairments. Besides physicians who oversee medication-based treatment strategies and monitor the neurologic status, a number of other health care professionals are involved in the support of MS patients, including neuropsychologists, social workers, specialist nurse practitioners and physical therapists (Compston and Coles 2008).

1.2 Exercise in MS

In the past, MS patients were traditionally discouraged from physical exercise. This was, in part, a consequence of the observation of the Uhthoff phenomenon: In some patients, the neurologic symptoms were reported to worsen during exercise, triggered through an increase in the body temperature. Furthermore, many health care professionals were of the opinion that by exercising, MS patients would waste valuable energy which they were believed to need for their daily activities (Dalgas et al. 2008).

However, in the last two decades, this assessment has been proven to be misleading. The exacerbation of symptoms during exercise has been shown to be only temporary, with fatigue and neurological function of the subjects not differing from pre-exercise levels 24 hours post-exercise (Smith et al. 2006).

Exercise prescription is now increasingly regarded as an important therapeutic intervention to prevent MS patients from physical inactivity and the resulting loss in functional capacity (White and Dressendorfer 2004).

Aerobic exercise has been proven to be beneficial for MS patients by a number of randomized controlled trials (RCTs). Training increases cardiorespiratory fitness, walking ability, muscle strength, endurance and exercise tolerance in MS patients (White and Dressendorfer 2004; Rietberg et al. 2005; Heesen et al. 2006). It improves quality of life and emotional functioning and counteracts secondary diseases linked to physical inactivity, like cardiovascular diseases, diabetes or osteoporosis. The physical and psychological benefits of exercise in MS patients

seem to be similar to those apparent in healthy subjects (O'Donovan et al. 2010; Bouchard et al. 2012) and might even compensate deficits frequently seen in MS patients, like fatigue (Asano and Finlayson 2014), depression or cognitive impairment (Briken et al. 2014).

To guarantee the safety of patients and to account for disease- and patient-specific physical characteristics, it has been recommended to individualize exercise programs by prescribing each patient the appropriate training intensity (Dalgas et al. 2008; Durstine et al. 2009). For this purpose and to evaluate the effectiveness of training programs, performance diagnostics (or exercise tests) are undertaken with MS patients. Because the disease symptoms frequently affect patients' walking ability, ergometries for clinical exercise testing are preferably conducted on the bicycle, not on the treadmill (Durstine et al. 2009).

1.3 Exercise testing

1.3.1 Principles of incremental bicycle spiroergometry

Physical exercise provokes a dramatic increase in the metabolism of skeletal muscle fibers. This challenges the body's physiologic homeostasis on different levels: Muscle contraction and relaxation requires adenosine triphosphate (ATP), cell oxygenation needs to be sustained, acidosis due to excess lactate has to be buffered and controlled. To fulfill these demands, a number of functional systems in the body need to interact in a coordinated manner: Energy in the form of ATP has to be supplied via different metabolic pathways (see chapter 1.4); the ventilatory system has to ensure sufficient gas exchange in the lungs; the heart and the circulatory system have to provide adequate systemic and pulmonary blood flow (Systrom and Lewis 2015). The basic idea of a spiroergometric performance test is to measure the subject's individual response of these main functional systems to physical exercise (Kenney et al. 2012).

In a typical incremental spiroergometric exercise test, the subject cycles on a bicycle ergometer against an increasing workload (Rost 2001). This protocol is continued until the subject reaches volitional exhaustion.

During the test, the subject wears an air-tight breathing mask with an integrated turbine flow-meter that measures the respiratory ventilation ($\dot{V}E$). An

external analyzer determines the concentrations of oxygen (O_2) and carbon dioxide (CO_2) in the breathing air, from which the inhaled O_2 volume ($\dot{V}O_2$) and the exhaled CO_2 volume ($\dot{V}CO_2$) are calculated. The heart rate is registered by electrocardiographic tracking and the blood pressure is measured repeatedly during the test. As a marker for the metabolic response to exercise, lactate levels can be determined in capillary blood drawn from the earlobe at regular intervals.

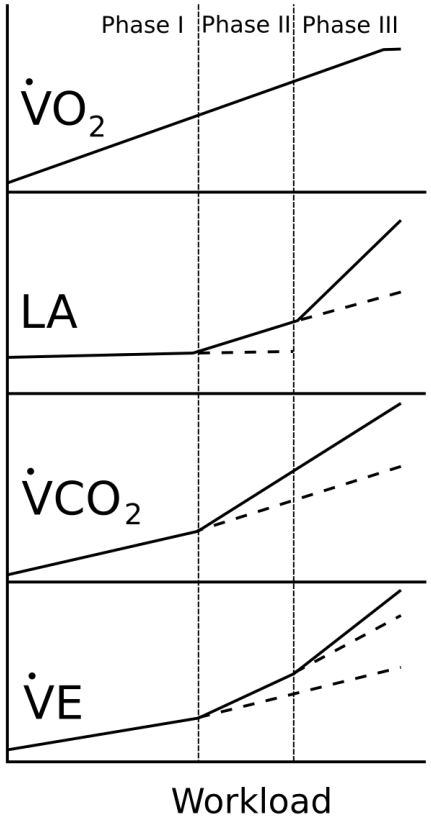

Fig. 2: Spiroergometric curves of ventilatory parameters ($\dot{V}E$ = ventilation, $\dot{V}O_2$ = inhaled O_2 volume, $\dot{V}CO_2$ = exhaled CO_2 volume) and blood lactate concentration (LA) during the three phases of energy supply. Redrawn after Pokan et al. (2004a)

Based on these physiological measurements, a number of other parameters can be calculated and plotted over time during the bicycle ergometry, like the respiratory quotient (RQ), which is calculated by dividing $\dot{V}CO_2$ by $\dot{V}O_2$ (Kroidl et al. 2010).

From the resulting curves of ventilatory, circulatory and metabolic parameters at a given workload (see Fig. 2), conclusions can be drawn about the subject's general fitness, aerobic and anaerobic capacity. Points on the spiroergometric curves that depict the transition from one energy supply phase to another (see chapter 1.4) are referred to as ventilatory or metabolic thresholds (Jones 1997; Cooper and Storer 2001; Wasserman et al. 2011).

Training changes the response of the main functional systems to exercise, which leads to altered curves and thresholds during spiroergometry (Poole and Gaesser 1985). Consequently, spiroergometric exercise tests are used not only to assess the physical fitness of subjects and to determine the right exercise intensity for training, but also as a longitudinal measure to evaluate the effectiveness of training interventions, in healthy subjects as well as in patients (Dickhuth et al. 2007). In chronically ill subjects, such as patients with

chronic obstructive pulmonary disease (COPD) or with coronary artery disease, spiroergometric tests are sometimes conducted to determine the grade of dysfunction of the pulmonary or the cardiovascular system (Gallagher 1994; Fletcher et al. 2001).

1.3.2 Key parameters and reference values for spiroergometric testing

Two markers frequently used to assess the cardiorespiratory fitness of subjects during bicycle spiroergometry are the maximal oxygen uptake ($\dot{V}O_{2max}$) and the maximal workload (W_{max} or W_{max}/kg) (Fletcher et al. 2001; Wasserman et al. 2011; Motl and Fernhall 2012). In healthy subjects and in patients, it is also common to measure the blood lactate response to exercise (see chapter 1.5) (Weltman 1995; Goodwin et al. 2007).

$\dot{V}O_{2max}$ is a direct measure for the aerobic capacity of subjects as it reflects "the maximal ability of a person to take in, transport, and use oxygen" (Systrom and Lewis 2015). Theoretically, $\dot{V}O_{2max}$ is characterized by a plateau in $\dot{V}O_2$ despite increasing exercise intensity in an incremental protocol, signaling that the upper limit of O_2 intake has been reached. Because spiroergometric curves of most subjects do not show the characteristic plateau, the term "$\dot{V}O_{2peak}$" has been introduced to describe the highest oxygen intake achieved. The cardiovascular system is regarded as the main limiting factor for $\dot{V}O_{2max}$, while the other functional systems (respiratory and metabolic) seem to play a lesser role (Kenney et al. 2012). The equations for $\dot{V}O_{2peak}$ reference values, according to Jones, are (60 – 0.55 x age in years) ml/min/kg for healthy male subjects and (48 – 0.37 x age in years) ml/min/kg for healthy female subjects (Jones 1997).

The W_{max} reached during incremental ergometry has traditionally been used to measure overall exercise capacity (Kroidl et al. 2010). W_{max} has been shown to depend not only on the subject's fitness, but also on weight and age. The reference value for healthy male subjects is 3.0 W/kg, with 10% discount for every decade after the age of 30. Healthy female subjects should achieve 2.5 W/kg, with 8% discount for every decade after the age of 30 (Rost 2001).

The heart rate is another key parameter recorded during spiroergometry. In incremental exercise tests, it increases in a linear fashion with workload and $\dot{V}O_2$. This reaction is first mediated by a reduction in vagal tone, then by an

increase of sympathetic stimulation on the heart and the vascular system (Fletcher et al. 2001). Shortly before volitional exhaustion is achieved, the heart rate reaches a plateau, similar to $\dot{V}O_{2max}$. Importantly, the maximal heart rate (HR_{max}) declines with age. This is taken into account by equations for HR_{max}, e.g., HR_{max} = 208 – (0.7 x age in years) (Kenney et al. 2012).

1.4 Phases of energy supply and recruitment of muscle fiber types

1.4.1 Three-phase-model of energy supply

The required energy for muscle contraction and relaxation has to be provided via different metabolic pathways in the form of ATP. Consequently, all routes of energy supply result in the formation of this molecule (Kenney et al. 2012). During incremental exercise, the organism preferentially uses the most economic process available at the respective exercise intensity to synthesize ATP (Dickhuth et al. 2007). Traditionally, energy supply during incremental exercise is divided into three consecutive phases: 1) aerobic, 2) aerobic-anaerobic and 3) anaerobic (Kindermann et al. 1979; Skinner and McLellan 1980; Pokan et al. 2004a). These phases and the thresholds marking the transitions between them are still highly relevant for exercise testing today, although it has been recognized in the meantime that there is no such thing as an exclusive aerobic or anaerobic phase. Rather, all forms of energy supply contribute to the body's energy needs during all forms of physical activity, with specific pathways merely dominating the different phases during incremental exercise (Kenney et al. 2012).

1.4.2 Aerobic phase

During low-intensity exercise, ATP synthesis is mainly realized through aerobic or oxidative pathways, meaning that oxygen (O_2) is required for the key chemical reaction. This reaction, the electron transport chain, takes place in specialized organelles of the muscle cells, the mitochondria (Kenney et al. 2012). At very low exercise intensities (below 50–60% of the maximal oxidative capacity of the muscle), fatty acids are the main energy substrate. They are converted into acetyl coenzyme a (CoA) via β-oxidation, then entering the citric acid cycle and, eventually, driving the electron transport

chain (Dickhuth et al. 2007). The β-oxidation of fatty acids yields more than 100 ATP molecules per substrate molecule, thus constituting the most economical way of energy supply. On very low intensity levels, β-oxidation can provide ATP for hours up to days (Kenney et al. 2012).

With rising exercise intensity, β-oxidation becomes too slow to meet the utilization rate of ATP in the muscles. Instead, acetyl CoA is now increasingly synthesized out of carbohydrates (glucose or glycogen, which is converted to glucose via glycogenolysis). Both flow into glycolysis, resulting in two molecules of pyruvate per molecule glucose. In the aerobic phase, pyruvate is then transformed into acetyl CoA, which, again, enters the citric acid cycle (de Marées 2002). Burning of carbohydrates yields only 36–39 molecules of ATP per substrate molecule. However, this it outweighed at higher exercise intensities by the quicker formation rate of 2.5 ATP per second (as opposed to only 1.5 ATP per second for fatty acids). Because glucose and glycogen stores in the body are limited, this form of energy supply can be maintained for about 90 minutes at the most, and only under the condition that the exercise intensity remains low enough for the rate of ATP production (Kenney et al. 2012).

On a neuromuscular level, mainly type I (slow-twitch oxidative) muscle fibers are recruited during low exercise intensities. These fibers are characterized by a high concentration of mitochondria and oxidative enzymes (Skinner and McLellan 1980). Type I fibers contract slowly, but are very resistant to fatigue, meaning they can sustain their power output over a long stretch of time. A high percentage of type I muscle fibers and a corresponding high aerobic capacity is typical for endurance athletes like distance runners, whose performance depends on energy storages lasting longer than a few seconds or minutes (Kenney et al. 2012).

1.4.3 Aerobic-anaerobic phase

With exercise intensity increasing further, the ATP production provided by burning carbohydrates via aerobic glycolysis, citric acid cycle and electron transport chain becomes too slow to satisfy the increasing energy demand of the muscle cells. From this point on, a growing percentage of ATP is synthesized through anaerobic glycolysis. This pathway is even less economic, yielding only two molecules of ATP per substrate molecule

(glucose). But again, its production rate (5 molecules of ATP per second) is even higher than the one reached by aerobic carbohydrate burning (Kenney et al. 2012). Anaerobic glycolysis takes place in the sarcoplasm of the muscle cell, outside of the mitochondria, and does not require O_2 for its reactions. The product of glycolysis, pyruvate, is generated too fast in this phase to be completely converted into acetyl CoA and fed into the citric acid cycle. Instead, growing amounts of pyruvate accumulate and are chemically reduced to lactic acid. This metabolite spontaneously dissociates into lactate and protons, which ultimately causes an increase of lactate and a decrease of pH in the blood. During spiroergometric performance tests, measuring of the blood lactate level is frequently used to estimate the contribution of anaerobic pathways to the total energy supply, and thereby, also the aerobic capacity (Dickhuth et al. 2007; Kroidl et al. 2010).

In this “intermediate” phase of combined aerobic and anaerobic energy supply, type IIa (fast-twitch oxidative-glycolytic) fibers are increasingly recruited (Skinner and McLellan 1980). Type IIa fibers have a high glycolytic capacity and a moderate oxidative capacity (a moderate concentration of mitochondria and oxidative enzymes), reflecting the ability to use both aerobic and anaerobic pathways for energy supply. They contract faster than type I fibers but are less resistant to fatigue (Kenney et al. 2012).

1.4.4 Anaerobic phase

If the exercise intensity exceeds a certain level, the energy demand is covered almost exclusively by anaerobic glycolysis. This leads to a quick accumulation of lactic acid, which dissociates into lactate and protons. Because the key enzyme of glycolysis, phosphofructokinase, is inhibited by acidosis, anaerobic burning of carbohydrates via this pathway is self-limited (Rost 2001). Exercise fuelled solely by anaerobic glycolysis can be sustained for about one minute (Kenney et al. 2012). In the blood, the protons are buffered by bicarbonate (HCO_3^-), resulting in the formation of water (H_20) and CO_2. The elevated CO_2 concentration in the blood stimulates the respiratory center in the brain stem, evoking an increase of pulmonary ventilation and CO_2 release in the lung. In spiroergometric tests, the corresponding sharp increase in the ventilation

curve ($\dot{V}E$) depicts the onset of the anaerobic phase and is therefore regarded as a ventilatory threshold (see Fig. 2) (Kroidl et al. 2010).

In this last phase of the performance test before exhaustion, type IIx (fast-twitch glycolytic) muscle fibers can be recruited (Skinner and McLellan 1980). Fibers of this type are characterized by a high glycolytic and a low oxidative capacity. They contract very fast, but their resistance to fatigue is minimal (Kenney et al. 2012).

1.5 Blood lactate kinetics during incremental exercise

1.5.1 Lactate accumulation in the blood

As mentioned above, lactate and protons are the dissociation products of lactic acid, a metabolite accumulating gradually with increasing ATP production via anaerobic glycolysis. Importantly, this reaction takes place in the sarcoplasm of the muscle cells, with the cell membrane as a barrier between the muscle fibers and the surrounding interstitial tissue that contains the blood vessels.

Only a small part of the lactate flux across this membrane occurs via diffusion of undissociated lactic acid. The major part of the lactate and proton flux is facilitated by an H^+-monocarboxylate cotransporter. A third, small proportion is mediated by an antiporter exchanging lactate molecules for HCO_3^- or Cl^- (Goodwin et al. 2007).

Crucially, the lactate concentration measured in the blood is always the result of a dynamic equilibrium between 1) lactate production and release from the muscles (and other tissues) to the blood and 2) lactate uptake and removal from the blood through different tissues (Brooks 1991). Thus, as Goodwin states in a review article (2007, p.562), “when a person exhibits an increase in blood lactate during exertion, it could represent (1) an increase in lactate production and release from muscle, (2) a decrease in lactate uptake and removal, or (3) a relatively greater increase in production and release in comparison to uptake and removal”. Skeletal muscles may be the major source for lactate, especially during exercise, but other body tissues including the intestine, the skin and the liver have also been identified as lactate producers (Weltman 1995).The most important tissues responsible for

eliminating lactate from the blood are the liver, the heart and inactive or moderately active oxidative muscle fibers (Stallknecht et al. 2007).
Lactate can be re-oxidized to pyruvate and utilized as a carbohydrate energy source in the citric acid cycle by these tissues. In the liver, pyruvate is used as a substrate for gluconeogenesis and for replenishment of the glycogen store. Therefore, lactate is today no longer regarded as a "dead end product" of anaerobic glycolysis, but rather as a central player in the distribution of carbohydrate energy. In the "cell-to-cell lactate shuttle" hypothesis, the blood stream in which lactate levels are measured during ergometry merely constitutes a route which connects the carbohydrate metabolism of tissues throughout the body (Brooks 2000).
Nonetheless, changes in the blood lactate level reflect the body's metabolic response to incremental exercise. Consequently, the blood lactate concentration still plays an important role as a parameter both in clinical exercise testing for patients and in performance testing for athletes (Goodwin et al. 2007; Kenney et al. 2012).

1.5.2 The curvilinear lactate profile and lactate threshold concepts

Under resting conditions, the blood lactate concentration is between 0.4 and 1.5 mM/l (Pokan et al. 2004b). This resting level underlines the fact that lactate

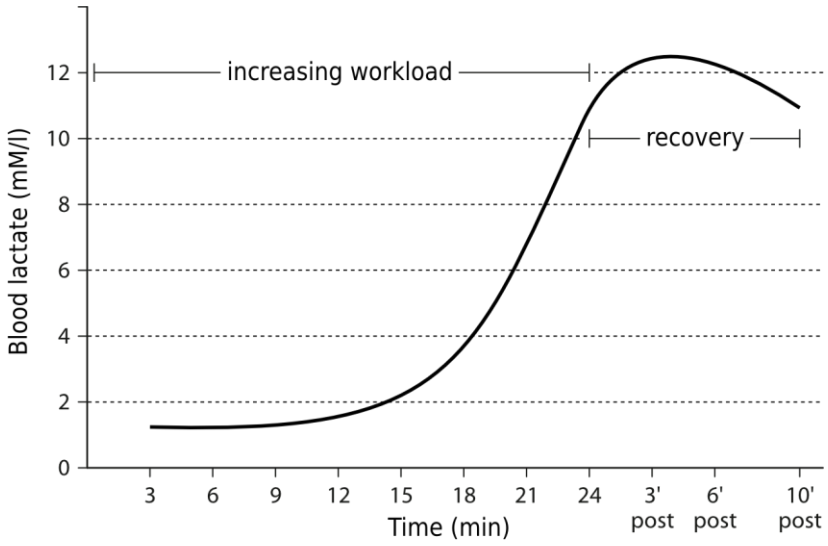

Fig. 3: Typical curvilinear lactate profile during an incremental, progressive exercise test, including recovery phase. Redrawn after Kindermann et al. (2007).

is produced and released to the blood constantly, not only during exercise. During progressive, incremental exercise, the lactate level rises slowly at first,

then more rapidly, in the fashion of a hyperbolic function (Rost 2001). In incremental exercise tests, the endpoint of the hyperbolic curve (the blood lactate level at the point of volitional exhaustion) usually lies between 8 and 10 mM/l (Goodwin et al. 2007). As it takes some time for the lactate produced in the muscles to reach the bloodstream, the actual peak of blood lactate is not reached at the break-off point, but between one and ten minutes later (see Fig. 3). For break-off concentrations lower than 12 mM/l, the blood lactate peak is usually reached with a delay between one and five minutes (de Marées 2002). The initial rise of the blood lactate concentration above resting levels was first described in 1964 by Wasserman and McIlroy as the "lactate threshold" (LT, see Fig. 4) or "anaerobic threshold", marking the transition from a solely aerobic energy supply phase to a second phase in which anaerobic mechanisms set in (Wasserman and McIlroy 1964).

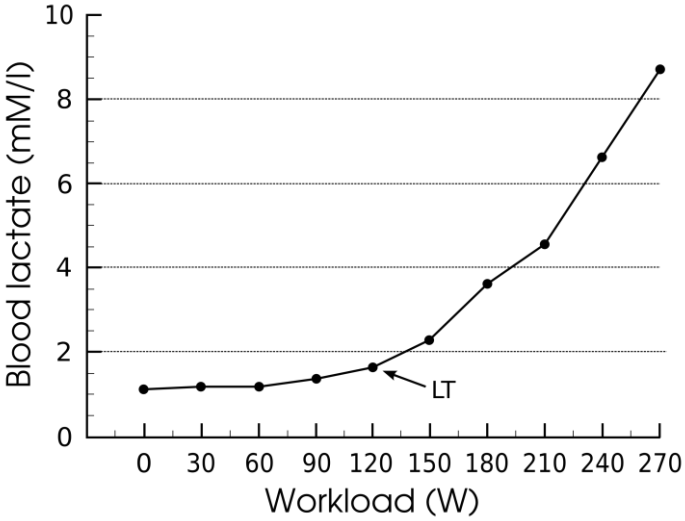

Fig. 4: Lactate response to a progressive, incremental exercise test, LT representing the "lactate threshold" LT_1. Redrawn after Goodwin et al. (2007).

In the three-phase-model described above, this second stage is called the aerobic-anaerobic phase. In 1976, Mader introduced a fixed lactate threshold at 4 mM/l, which should designate the endpoint of this phase (Mader et al. 1976; Westhoff et al. 2013). Because there has been considerable confusion about the terms "aerobic threshold" and "anaerobic threshold", it has been proposed to call the endpoint of the aerobic phase "lactate threshold 1" (LT_1) and the starting point of the anaerobic phase "lactate threshold 2" (LT_2) (Westhoff et al. 2013). In 1979, a fixed concentration of 2 mM/l was proposed for the LT_1, with the aerobic-anaerobic phase now theoretically confined between the fixed points of 2 and 4 mM/l (Kindermann et al. 1979).

However, it quickly became apparent that metabolic thresholds differ between individuals and are not represented adequately by a fixed lactate blood level. In the following years, the concept of an “individual anaerobic threshold” (again meaning the LT_2) was introduced by Keul, Stegmann and Kindermann (Keul et al. 1979; Stegmann and Kindermann 1981; Stegmann et al. 1981). In their physiologic concept of the transition, this threshold is the point where the highest possible rate of lactate elimination from the blood is reached and is still in a dynamic balance with the lactate production and release into the blood. If the exercise intensity and the lactate production increase further, the elimination rate cannot keep up with it any longer, resulting in the end of the dynamic equilibrium and a steeper increase in the curvilinear blood lactate profile. This balanced state of the blood lactate level just before LT_2 has later been termed the “maximal lactate steady state” (MLSS) (Heck et al. 1985). The increase in production of lactic acid at LT_1 and the accelerated increase after MLSS (at LT_2) provoke threshold-like transitions in the ventilation (see Fig. 2). These transitions can be detected in the ventilatory curves during spiroergometry and have been termed “ventilatory thresholds 1 and 2” (VT_1 and VT_2). They are causally connected with the metabolic thresholds LT_1 and LT_2 and correlate highly with these, but do not always occur at the exact same exercise intensity (Kroidl et al. 2010; Westhoff et al. 2013).

Although over the years more than 60 concepts of lactate thresholds have been presented, the determination of the MLSS, or rather of the exercise intensity at which a subject reaches the MLSS (or LT_2) during spiroergometry, has been the primary aim of lactate performance diagnostics (Westhoff et al. 2013). The workload or $\dot{V}O_2$ level at which LT_2 happens is a widely accepted parameter for the aerobic endurance performance and general cardiovascular fitness (Poole and Gaesser 1985; Faude et al. 2009). An improvement in the aerobic capacity elicits a shift to the right of the complete curvilinear lactate blood profile (including MLSS and LT_2), with lower blood lactate concentrations at the respective submaximal workloads (see Fig. 5) (Rost 2001).

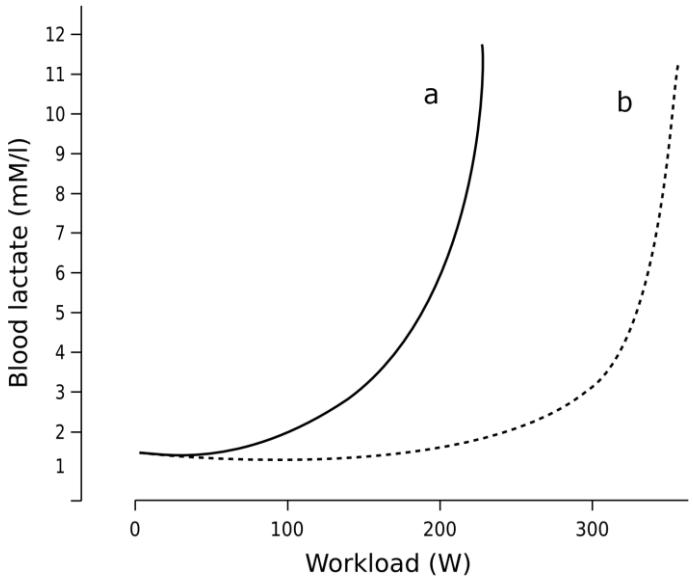

Fig. 5: Differences between an untrained runner (a) and a trained runner (b) in terms of the blood lactate response to exercise. Redrawn after Rost (2001).

Although the concept of a fixed LT_2 is theoretically outdated, the workload or $\dot{V}O_2$ at a blood lactate level of 4 mM/l is still frequently used as a measure for aerobic capacity in spiroergometric tests – at least in Germany, where traditionally metabolic thresholds are determined, while ventilatory threshold measurements are more common in the USA and in GB (Westhoff et al. 2013). In designated lactate performance tests, the workload is usually increased stepwise, with the individual steps usually lasting three minutes or longer (Dickhuth et al. 2007). This approach takes into account the time lag between lactate production in the muscle and the appearance in the blood and therefore warrants a correct allocation of measured blood lactate concentrations to the corresponding workloads (Westhoff et al. 2013).
In contrast, in spiroergometric tests like the ones that were analyzed in this work, protocols with a linear increase of workload are used. These so-called ramp protocols deliver more accurate values for ventilatory parameters like $\dot{V}O_2$ and are therefore suited better for the recording of “linear” spiroergometric curves (Kroidl et al. 2010).
The lactate profile obtained during such ramp protocols is not eligible for application of the current lactate threshold concepts, because the measured blood lactate values relate to workloads which lie already in the past by the time of measurement (Dickhuth et al. 2007). Nevertheless, maximal lactate concentrations are often determined in spiroergometric ramp protocols. They

are regarded as closely related to the RQ, but can provide additional information on the metabolic state of the subject (Kindermann et al. 2007). For the spiroergometric performance tests analyzed in this work, peak lactate concentrations were determined. The time lag was accounted for by taking multiple blood samples for lactate measurement during a five-minute period after break-off (see chapter 2.3).

1.6 Peak blood lactate levels

1.6.1 Peak blood lactate levels in healthy subjects

The accumulation of a blood lactate concentration above 8 mM/l has been used as a criterion for true exhaustion in untrained subjects performing incremental ergometry protocols (Izquierdo et al. 2001; Langeskov-Christensen et al. 2015). Accordingly, the standard curvilinear blood lactate response to incremental exercise is depicted with a maximum between 8 and 12 mM/l (Rost 2001; de Marées 2002; Goodwin et al. 2007; Faude et al. 2009; Kroidl et al. 2010; Wasserman et al. 2011; Kenney et al. 2012). The lactate curve of trained subjects is shifted to the right when compared to untrained subjects (Kindermann et al. 2007), but this shift usually has only little or no influence on the lactate maximum (see Fig. 5) (Kumagai et al. 1982; Rost 2001). However, low peak lactate levels have been reported for specialized endurance athletes, such as marathon runners, whose metabolism is extremely well adapted to aerobic performance and lactate clearance (Föhrenbach et al. 1987).

In 180 male participants of the Baltimore Longitudinal Study of Aging, average blood lactate concentrations after multi-stage, maximal treadmill exercise were found to decrease consistently with age. In spite of this trend, even the subgroup of subjects aged 50–59 years still reached mean maximal lactate levels over 8 mM/l (or 72 mg/dl). Subjects aged 30–39 and 40–49 reached levels between 8.8 mM/l (80 mg/dl) and 9.4 mM/l (85 mg/dl), respectively. Subjects aged 20–29 reached levels above 10 mM/l (or 90 mg/dl) (Tzankoff and Norris 1979). In a group of 2038 physically active men performing a maximal cycle ergometer test, researchers from the Netherlands found peak plasma lactate values of 11.5 mM/l in the subgroup aged 40–44 (n=1057),

11.4 mM/l in the subgroup aged 45–49 (n=530), and 10.2 mM/l in the subgroup aged 50–54 (n=242) (Bovens et al. 1993). As plasma measurements have been reported to yield higher lactate concentrations than measurements in whole blood, these values have to be treated with caution (Goodwin et al. 2007). However, in a newer study, maximal whole blood lactate concentrations were determined in a group of untrained middle-aged and elderly men performing a multi-stage incremental cycling test. Maximal lactate levels were 9.7 mM/l for the middle-aged group (mean age 42 years, n=26) and 8.05 mM/l in the elderly group (mean age 65 years, n=21) (Izquierdo et al. 2001).
Data concerning peak lactate levels of healthy women are sparse and more contradictory: In an Australian study, a peak plasma lactate level of 10.2 mM/l was found in untrained women (mean age 31.4 years, n=22) five minutes post-exercise after an incremental bicycle ergometry (Sargent and Scroop 2007). In the Dutch study mentioned before, peak plasma lactate values of 8.5 mM/l were measured in physically active women in the subgroups aged 40–44 (n=426) and 45–49 (n=213). In the subgroup aged 50–54 (n=140), the peak plasma lactate was 8.2 mM/l (Bovens et al. 1993). In contrast, a research group from France measured a peak blood lactate level of only 6.1 mM/l in a group of sedentary, healthy women (mean age 47 years, n=20) five minutes post-exercise after an incremental bicycle ergometry (Ba et al. 2009). It has to be considered, though, that the blood samples for the measurements in this last study were taken from venous blood. Lactate levels in venous samples can be biased towards lower values if the tissue draining into the vein is a net consumer of lactate at the time of measurement (Goodwin et al. 2007). Importantly, there seems to be a sex difference, with women frequently displaying lower lactate levels than men at all stages of incremental exercise tests (Sargent and Scroop 2007). However, to our knowledge, peak blood lactate levels below 6 mM/l have not been described for healthy, untrained women performing an incremental bicycle ergometry.

1.6.2 Peak blood lactate levels in patients with chronic diseases

The peak blood lactate response of patients has been shown to differ from that seen in healthy, untrained individuals in a number of chronic conditions.

In a French study with 236 males referred for the diagnosis of coronary artery disease (CAD), a lower blood lactate level at maximal exercise has been identified as a significant predictor for CAD: Patients which were afterwards diagnosed with CAD reached a mean peak blood lactate level of only 7.68 mM/l (determined in haemolyzed capillary whole blood samples), while the non-diseased subjects in the study reached a mean value of 10.56 mM/l (Barthelemy et al. 1996).

In patients suffering from angina pectoris, subjects who reach the lowest absolute maximal workload (measured in W, as opposed to W/kg) reach the lowest peak blood lactate values and vice versa (Karlsson et al. 1984; Pokan et al. 2004a). This relation between absolute maximal workload and peak lactate levels (see Fig. 6) is, to our knowledge, not usual in healthy subjects.

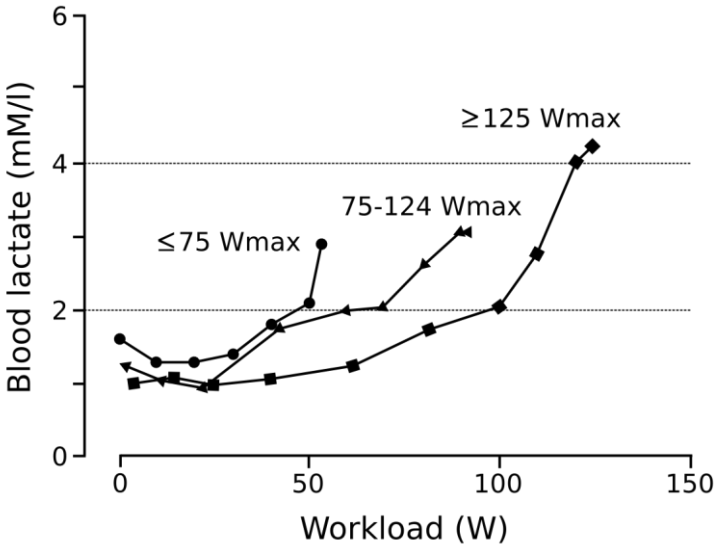

Fig. 6: Relation between blood lactate and maximal workload (W_{max}) in patients with angina pectoris. Redrawn after Karlsson et al. (1984).

Patients suffering from neoplastic diseases have recently been reported to exhibit a curvilinear blood lactate profile with a reduced maximal lactate level (Banzer et al. 2012). Similarly, patients with cystic fibrosis (CF) have been repeatedly shown to display lower blood lactate levels at both submaximal and maximal exercise intensities than healthy individuals (Fezer 2004). In one study, patients with CF accumulated a blood lactate of 5.4 mM/l at peak exercise, compared to a concentration of 8.3 mM/l in the control group (McLoughlin et al. 1997).

To our knowledge, it has never been explored if the peak blood lactate response to exercise is altered in MS patients. The blood lactate levels of MS patients have, until now, been determined in only one study conducted by our own working group, in which the peak concentrations were not analyzed (Schulz et al. 2004).

As the importance of exercise interventions is growing, performance tests can be expected to become more common procedures for MS patients. Because measurement of the blood lactate response is a method frequently used for exercise testing in Germany, it is important to clarify if this method is also applicable for different groups of MS patients. If subjects suffering from MS show a reduced peak lactate response, this would raise the question if the threshold concepts derived from lactate performance tests with healthy subjects can be transferred directly to these patients.

In addition, peak lactate levels and the resulting lactic acidosis have traditionally been associated with muscular fatigue limiting exercise capacity (Wasserman et al. 2011), and MS patients have been shown to suffer from fatigue – central and/or peripheral – even before the onset of the typical neurologic symptoms (Davis and Walsh 2010). In a group of patients with mild MS performing voluntary exercise, Kent-Braun et al. found less lactic acidosis than in healthy controls (Kent-Braun et al. 1994), suggesting a failure of muscle activation. For MS patients, especially those with a progressive disease course, one would therefore expect a low maximal workload coupled with a low peak blood lactate, similar as in patients with angina pectoris (Karlsson et al. 1984).

Consequently, the objective of the present study is to examine the peak blood lactate response to exercise in two different groups of MS patients and to understand its relation with W_{max}. This work is mainly descriptive and exploratory; aiming to characterize the two groups and to find out if the MS patients share the altered blood lactate response with patients suffering from different chronic conditions.

In addition to W_{max}, other key spiroergometric peak parameters ($\dot{V}O_{2peak}$ and HR_{max}) were used to analyze the interrelation of peak lactate with patients' performance in the ergometry. Furthermore, we correlated peak blood lactate and maximal workload with patients' grade of disability, walking ability and

psychological parameters to identify possible factors that might influence patients' performance and blood lactate accumulation in exhaustive exercise protocols. In the last part of this work, the peak lactate values, the other peak performance markers, the walking ability and the psychological measures were compared between the two groups of MS patients.

2. Patients and Methods

2.1 Study design

The present work is a cross-sectional study. Its primary aim is to characterize the peak blood lactate response and its relation with the maximal workload (W_{max} or W_{max}/kg) in two different groups of MS patients performing an incremental, progressive bicycle ergometry. The two patient groups that underwent the exercise test differ in two basic characteristics: Firstly, their disease course (see chapter 1.1.3) and secondly, their grade of disability according to the expanded disability status scale (EDSS).

The first group included 41 MS patients with a secondary- or primary progressive disease course and moderate to severe disability (EDSS score 4–6). This group is termed “group P” (p for progressive) below. The second group included 25 MS patients with a relapsing-remitting disease course and mild to moderate disability (EDSS score 0–3.5). It is termed “group R” (r for relapsing-remitting) below.

In addition to W_{max} and W_{max}/kg, we measured $\dot{V}O_{2peak}$/kg and HR_{max} and investigated the interrelations of our peak performance markers in both groups. In group P, in which the MS patients were more severely disabled, the peak respiratory quotient (RQ_{peak}) was also determined to assess the degree of exhaustion at break-off.

We conducted, in both groups, an explorative analysis for potential correlations of the peak blood lactate level and W_{max} with other parameters: Patients’ grade of disability (EDSS score), performance in the six-minute-walking test (6MWT) and the psychological parameters depression and fatigue.

Finally, we drew a comparison between group P and group R in the peak performance markers reached during the bicycle ergometry, the walking ability and the psychological measures depression and fatigue.

2.2 Patient recruitment, inclusion and exclusion criteria

2.2.1 Group P

The data for group P analyzed in this work was obtained from a baseline performance test for an exercise intervention pilot study which is already published (Briken et al. 2014). The trial was approved by the ethics committee

of the chamber of physicians, Hamburg, Germany (registration number PV3689). The participants signed a written consent form before taking part in the study.

Patients were recruited between August 2011 and January 2012 in the MS outpatient clinic at the University Hospital Hamburg-Eppendorf (UKE), by advertisement on the homepage of the German Multiple Sclerosis Society (DMSG) and by leaflets in neurologic practices. In addition, patients in an UKE database were contacted which had agreed to be informed about new studies. To be included in group P, patients had to meet diagnostic criteria for clinically definite MS according to the McDonald criteria (Polman et al. 2011) with a secondary-progressive disease course (Lublin and Reingold 1996) and moderate to severe disability (EDSS 4–6). To promote the recruitment, later patients with a primary-progressive disease course were included as well if they fulfilled the EDSS criterion. All neurologic, psychological and functional tests for both groups were conducted by specially trained staff.

Patients were excluded if they had medical contraindications for exercise, which were assessed by self-report using the revised Physical Activity Readiness Questionnaire (rPAR-Q) (Thomas et al. 1992). Patients were also excluded if they had started a new therapy with disease-modifying drugs (DMTs) within the last 6 months, undergone steroid therapy within the last 4 weeks or if they had documented relapses within the last 12 months. Further exclusion criteria were an abnormal liver or kidney function, infection with hepatitis B or C virus (or severe chronic diseases of the liver) and diagnosis of immunodeficiency. Patients were excluded if they were taking psychotropic medication or if they suffered from any neurological disorders other than MS. 50 potential subjects were examined for the study in the MS outpatient clinic at the UKE. Out of the 50, 47 were included in the original study (Briken et al. 2014). Out of the 47 patients, three did not perform a complete baseline test; their data was excluded for this work. Another three patients had motoric problems which were so severe that they could not keep up a constant number of revolutions per minute (rpm) on the bicycle ergometer. As the spiroergometric data of these patients cannot be regarded as reliable, they were also excluded from the data analysis for this work. Consequently, group P consists of a total of 41 patients.

2.2.2 Group R

The patients included in group R were recruited for a successive trial that followed the pilot study with group P and also comprised an exercise intervention, specifically an aerobic endurance training. The data analyzed for group R was obtained from the baseline performance test of that second trial. The trial was approved by the ethics committee of the chamber of physicians, Hamburg, Germany (registration number PV4356). Like in group P, the participants signed a written consent form before taking part in the study. Patients for group R were recruited over a database in the MS outpatient clinic at the UKE and by advertisement on the homepage of the DMSG. Later on, patients were also addressed directly during consultations in the MS outpatient clinic. Recruitment started in December 2013 and is still going on at present. Until the end of October 2014, 28 patients were included in the original study. Out of those, 25 had completed a baseline performance test and were therefore included for this work.

To be included in group R, patients had to meet criteria for clinically definite MS according to the McDonald criteria (Polman et al. 2011) with a relapsing-remitting disease course (Lublin and Reingold 1996) and mild to moderate disability (EDSS 0–3.5). MS disease duration had to be less than ten years and patients were required to be in remission at the time of the trial. Furthermore, patients had to be either stable under immunomodulatory therapy for more than three months or without plans for a new therapy in the next year. Patients had to be able to come to the UKE for training sessions two or three times a week.

For group R, patients were excluded if they had cognitive deficits or psychiatric comorbidities that prevented them from understanding the concept of the study. Patients were also excluded if they were taking psychotropic medication and if they had any medical contraindications for aerobic endurance training, like severe heart diseases. Further exclusion criteria were a highly active disease course and an unstable disease course under the current immunomodulatory medication. Because magnetoencephalography (MEG) and MRI were part of the study, patients were excluded if they had any implants or body modifications, like piercings or tattoos, which could interfere with these imaging techniques.

2.3 Bicycle ergometry

A bicycle ergometer (Ergofit® 3000) was used in combination with a portable spiroergometer (Metalyzer3b® by Cortex Biophysik Gmbh, Leipzig, Germany). Patients were informed about the procedure beforehand. Before cycling, a 12-lead resting electrocardiogram was recorded and assessed by an experienced physician. The bicycle ergometry was started only if the electrocardiogram was without pathological findings. The staff conducting the ergometries was trained by an experienced sports scientist. Blood lactate was measured using an EKF diagnostics analyzer (Biosen C-Line®). Ventilatory parameters were determined by a turbine flow-meter integrated in a breathing mask and by a connected analyzer measuring the concentrations of O_2 and CO_2 in the breathing air. The subjects' blood pressure was measured repeatedly during the test. If the systolic blood pressure exceeded 210 mmHg or the diastolic blood pressure exceeded 110 mmHg, the bicycle ergometry was stopped.

All participants underwent a ramp protocol. Importantly, the incline of the protocol differed between the groups: Group P patients originally started cycling at 25 W with an incline of 12.5 W/min until volitional exhaustion was achieved. Because some patients (n=19) were unable to perform this WHO standard ergometry protocol, an alternative protocol was used in these cases, starting at 8 W with an increase of 8 W/min (Briken et al. 2014). For the patients in group R, an easier protocol was used from the beginning: They started cycling at 10 W, with the workload remaining constantly at 10 W for the first five minutes, followed by an incline of 10 W/min. After exhaustion, all patients in both groups went on cycling for five minutes on a low intensity level.

The different ramp protocols are the result of an optimizing process: In the first study with group P, it became obvious that the patient collective was heterogeneous regarding the ability to perform the standard WHO ergometry. Some patients managed the incline without problems, while others were not able to maintain a constant frequency of rotation during this protocol. For these patients, the easier ramp protocol was used. In the second study with group R, an easier protocol was used from the beginning, including a warm-up phase of five minutes in which patients could get used to the cycling movement. In both groups, patients were instructed to try to keep up a constant pedaling rate, if possible above 70 rpm.

2.4 Spiroergometric outcome measures

The main outcome measures for this work were the peak blood lactate concentration and the maximal workload (W_{max} and W_{max}/kg). Lactate levels were determined in capillary whole blood. The blood samples for the lactate measurement were obtained from the patients' earlobes in the first, third and fifth minute of the recovery phase. The highest value out of the three samples was regarded as peak blood lactate.

Workload, $\dot{V}O_2$ and heart rate were recorded continuously during the ergometry. As most patients did not reach a true $\dot{V}O_{2max}$ (defined by leveling off of $\dot{V}O_2$ (< 2 ml/min/kg) despite increased workload), the term $\dot{V}O_{2peak}$ is used here instead. $\dot{V}O_{2peak}$ was determined as the mean of the three successive highest $\dot{V}O_2$ registrations achieved.

In the ergometries with group P, RQ_{peak} was determined as the highest ratio of $\dot{V}CO_2$ produced and $\dot{V}O_2$ absorbed ($\dot{V}CO_2/\dot{V}O_2$). $RQ_{peak} \geq 1.1$ was regarded as certain exhaustion, $RQ_{peak} \geq 1.0$ and < 1.1 as incipient exhaustion (Bouchard et al. 2012).

We determined how many subjects in group P and group R reached the peak blood lactate concentrations that have been reported in the literature for healthy untrained individuals (Heck et al. 1985; Wasserman et al. 2011). $\dot{V}O_{2peak}$/kg, W_{max} and W_{max}/kg of both groups were directly compared to adjusted reference values (Rost and Hollmann 1982; Jones 1997).

2.5 Disability (EDSS)

The grade of disability in both groups was determined by the patients' score on the expanded disability status scale (EDSS, see Fig. 7). This instrument was introduced by Kurtzke in 1983 and is now widely used to classify the clinical status of MS patients and to describe their disease progression (Kurtzke 1983; White and Dressendorfer 2004). The EDSS is a disease-specific standardized assessment scale that evaluates different functional systems of the CNS which are typically affected by MS (e.g. cerebral, pyramidal, sensory, visual, cerebellar, bladder and bowel) from grade 0 (normal) to 6 (severe disability). From the evaluation of the functional systems, the final score is derived.

The scale extends from the lowest score of 0.0 (normal neurological exam) over 1.0 (no disability, minimal signs in 1 functional system) and then in steps of 0.5 to

the highest score of 10.0 (death due to MS). As described in a systematic review by Meyer-Moock et al., the lower scale values of the EDSS measure impairments based on the neurological examination, while the upper range of the scale (EDSS >6) rather measures handicaps of patients with MS. In particular, the determination of EDSS 4–6 is heavily dependent on aspects of walking ability (Meyer-Moock et al. 2014). Importantly, not every MS patient has to go through the whole scale: Some stay stationary at an intermediate EDSS score, and some move up slowly through the scale but never reach the upper range. In a study conducted by neurologists in the US, 20 years after the onset of the disease, only 25% of patients with RRMS had reached an EDSS score of 3.0. Patients with SPMS reached an EDSS score of 6.0 after a median time of ten years post-diagnosis, while patients with PPMS reached this score after a median of three years (Pittock et al. 2004).

Kurtzke Expanded Disability Status Scale (EDSS)

- ❑ 0.0 - Normal neurological exam (all grade 0 in all Functional System (FS) scores*).
- ❑ 1.0 - No disability, minimal signs in one FS* (i.e., grade 1).
- ❑ 1.5 - No disability, minimal signs in more than one FS* (more than 1 FS grade 1).
- ❑ 2.0 - Minimal disability in one FS (one FS grade 2, others 0 or 1).
- ❑ 2.5 - Minimal disability in two FS (two FS grade 2, others 0 or 1).
- ❑ 3.0 - Moderate disability in one FS (one FS grade 3, others 0 or 1) or mild disability in three or four FS (three or four FS grade 2, others 0 or 1) though fully ambulatory.
- ❑ 3.5 - Fully ambulatory but with moderate disability in one FS (one grade 3) and one or two FS grade 2; or two FS grade 3 (others 0 or 1) or five grade 2 (others 0 or 1).
- ❑ 4.0 - Fully ambulatory without aid, self-sufficient, up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1), or combination of lesser grades exceeding limits of previous steps; able to walk without aid or rest some 500 meters.
- ❑ 4.5 - Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance; characterized by relatively severe disability usually consisting of one FS grade 4 (others or 1) or combinations of lesser grades exceeding limits of previous steps; able to walk without aid or rest some 300 meters.
- ❑ 5.0 - Ambulatory without aid or rest for about 200 meters; disability severe enough to impair full daily activities (e.g., to work a full day without special provisions); (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combinations of lesser grades usually exceeding specifications for step 4.0).
- ❑ 5.5 - Ambulatory without aid for about 100 meters; disability severe enough to preclude full daily activities; (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combination of lesser grades usually exceeding those for step 4.0).
- ❑ 6.0 - Intermittent or unilateral constant assistance (cane, crutch, brace) required to walk about 100 meters with or without resting; (Usual FS equivalents are combinations with more than two FS grade 3+).

❑ 6.5 - Constant bilateral assistance (canes, crutches, braces) required to walk about 20 meters without resting; (Usual FS equivalents are combinations with more than two FS grade 3+).

❑ 7.0 - Unable to walk beyond approximately 5 meters even with aid, essentially restricted to wheelchair; wheels self in standard wheelchair and transfers alone; up and about in wheelchair some 12 hours a day; (Usual FS equivalents are combinations with more than one FS grade 4+; very rarely pyramidal grade 5 alone).

❑ 7.5 - Unable to take more than a few steps; restricted to wheelchair; may need aid in transfer; wheels self but cannot carry on in standard wheelchair a full day; May require motorized wheelchair; (Usual FS equivalents are combinations with more than one FS grade 4+).

❑ 8.0 - Essentially restricted to bed or chair or perambulated in wheelchair, but may be out of bed itself much of the day; retains many self-care functions; generally has effective use of arms; (Usual FS equivalents are combinations, generally grade 4+ in several systems).

❑ 8.5 - Essentially restricted to bed much of day; has some effective use of arm(s); retains some self-care functions; (Usual FS equivalents are combinations, generally 4+ in several systems).

❑ 9.0 - Helpless bed patient; can communicate and eat; (Usual FS equivalents are combinations, mostly grade 4+).

❑ 9.5 - Totally helpless bed patient; unable to communicate effectively or eat/swallow; (Usual FS equivalents are combinations, almost all grade 4+).

❑ 10.0 - Death due to MS.

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*Excludes cerebral function grade 1.

Note 1: EDSS steps 1.0 to 4.5 refer to patients who are fully ambulatory and the precise step number is defined by the Functional System score(s). EDSS steps 5.0 to 9.5 are defined by the impairment to ambulation and usual equivalents in Functional Systems scores are provided.

Note 2: EDSS should not change by 1.0 step unless there is a change in the same direction of at least one step in at least one FS.

Sources: Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 1983 Nov;33(11):1444-52.

Haber A, LaRocca NG. eds. Minimal Record of Disability for multiple sclerosis. New York: National Multiple Sclerosis Society; 1985.

Fig. 7: Kurtzke Expanded Disability Status Scale.
Source: US National Multiple Sclerosis Society (2015)

2.6 Walking ability (6MWT)

Patients' walking ability in both groups was assessed by using the total distance in meters achieved in the 6MWT. In this functional performance test, the patients are instructed to walk as far as possible in six minutes on an even underground. The 6MWT is a measure originally used to determine walking capacity in patients suffering from cardiac or pulmonary diseases. In MS, it has been shown to be a feasible, reproducible and reliable measure which is strongly related to patients' disability and subjective assessment of ambulation and physical fatigue (Goldman et al. 2008).

2.7 Psychological parameters: Depression and Fatigue

In group P, depression was assessed using the self-reported version of the 30-item "Inventory of Depressive Symptoms" (IDS-SR_{30}) (Trivedi et al. 2004). In group R, a shorter, 16-item version of the inventory was used, the self-reported "Quick Inventory of Depressive Symptoms" (QIDS-SR_{16}). Both inventories are designed to measure the symptom severity of depression by assessing the criterion symptom domains for a major depressive episode as defined in the 4th edition of the American Psychiatry Association Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). To warrant comparability between the groups, the official translation table was used to convert IDS-SR_{30} total scores of group P into QIDS-SR_{16} total scores, which range from 0 to 27 (University of Pittsburgh Epidemiology Data Center 2015).

Fatigue was measured in both groups by the fatigue subscale of the Hamburg Quality of Life Questionnaire in Multiple Sclerosis (HAQUAMS) (Gold et al. 2001). The HAQUAMS is a questionnaire designed to measure the disease-specific quality of life in MS patients. The revised version used for this work (HAQUAMS 10.0) includes a total of 44 items (Schäffler et al. 2013). 28 out of those 44 items form six subscales concerning 1) the mobility of the lower extremities, 2) mobility of the upper extremities, 3) cognition, 4) fatigue, 5) mood and 6) communication. The fatigue subscale which is used in this work consists of four items which are rated on a Likert scale from 1 (strongly disagree) to 5 (strongly agree). The total score for the fatigue subscale is generated by calculating the mean score of the four items.

2.8 Statistics

In both groups, correlations and partial correlations were calculated using the Pearson coefficient. As the EDSS score and the HAQUAMS fatigue subscale were regarded as ordinal-scaled rather than interval-scaled, Spearman's rho was used for correlations with these parameters.

Because of the exploratory character of the work, no Bonferroni correction was conducted for the correlations. In this approach, a possible cumulation of type I errors is accepted rather than the possibility to miss an existing correlation due to a conservative calculation.

The significance of differences between group P and group R in interval-scaled clinical characteristics like age and height was determined by t-tests, while Chi^2-tests were used for nominal-scaled clinical characteristics like gender and drug therapy. T-tests were also used to determine the significance of inter-group differences in the main spiroergometric outcome measures (mean peak blood lactate level, mean W_{max}/kg, mean $\dot{V}O_{2peak}$/kg and mean HR_{max}), in the total distance achieved in the 6MWT, the mean score in the QIDS-SR_{16} and the mean score on the HAQUAMS fatigue subscale.

On an intra-group level, t-tests were performed to investigate the significance of differences in the mean peak blood lactate level and mean W_{max}/kg between patients of different gender, medication, disease course (only applicable for group P) and ramp protocol (only applicable for group P).

In group P, patients were divided into three subgroups depending on their absolute maximal workload (W_{max}). The subgroups were chosen according to the literature for patients with angina pectoris (lowest group ≤ 74 W; intermediate group 75-124 W; highest group ≥ 125 W) (see Fig. 6) (Karlsson et al. 1984; Pokan et al. 2004a). Analysis of Variance (ANOVA) was conducted to determine if the mean peak lactate differs significantly between these subgroups in group P.

For group R, the analysis was modified because the W_{max} limits did not apply to this patient collective (only one subject reached a W_{max} of ≤ 74 W). Instead, the patients were divided in a subgroup with W_{max} < 125 W and a subgroup with W_{max} ≥ 125 W. Instead of ANOVA, a t-test was then conducted to determine if the mean peak lactate differs significantly between these two subgroups of group R.

For the correlation between EDSS score and peak blood lactate, we conducted an exploratory analysis in which all patients in group P and group R were pooled

and treated as if belonging to one group. This was done because the section of the EDSS in each group was rather small (0–3.5 in group R and 4–6 in group P). By merging the groups, the range of the score was expanded (0–6 in the pooled group). With the expanded EDSS range, a correlational analysis between EDSS and peak blood lactate is possible without yielding false zero correlations due to the limitation of variance in the confined groups.

An alpha level of .05 (two-sided) was used for all statistical analyses.

3. Results

3.1 Descriptive statistics

3.1.1 Group P

For this group, the data of 41 patients with SPMS and PPMS were analyzed (23 females and 18 males). EDSS scores ranged from 4.0 to 6.0, with a median of 4.5 (see Table 1 for clinical characteristics of the group).

Subjects reached a mean peak blood lactate concentration of 4.1 mM/l (see Fig. 8 and 10). Only 3 patients (all male) were able to accumulate a peak blood lactate concentration of 8 mM/l and above; no female patient reached the concentration of 8 mM/l. Out of the 23 female patients, only 2 were able to accumulate a peak blood lactate concentration of 6 mM/l and above. In total, 17 out of the 41 patients (41.5%) reached a lactate level of 4 mM/l.

Patients in group P achieved a mean W_{max} of 102 W and a mean W_{max}/kg of 1.5 W/kg (see Fig. 12 and 14). Mean $\dot{V}O_{2peak}$ was 1450 ml/min, mean $\dot{V}O_{2peak}$/kg was 21 ml/min/kg (see Table 2 for means and standard deviations). Patients reached between 60% and 72% of the W_{max}, W_{max}/kg and the $\dot{V}O_{2peak}$ they should have reached according to the reference values for healthy, untrained individuals (see Table 3a for comparison to reference values).

Patients' mean HR_{max} was 138 bpm in group P (80% of the reference value for HR_{max}, see chapter 1.3.2). The mean RQ_{peak} was 1.08 ± 0.10 (range 0.92–1.33, median 1.07). Out of 41 patients, 30 reached an $RQ_{peak} \geq 1.0$. Out of those 30 patients, 17 patients reached a $RQ_{peak} \geq 1.1$ and 13 patients reached a $RQ_{peak} \geq 1.0$ and < 1.1.

Patients in group P reached a mean total distance of 315 ± 106 m in the 6MWT (range 69–542, median 316). The mean score in the QIDS-SR_{16} was 6.7 ± 3.6 (range 0–16, median 7) and the mean score on the HAQUAMS fatigue subscale was 2.6 ± 0.9 (range 1–4.5, median 2.5).

Table 1: Clinical characteristics

	Group P	Group R	p
Subjects	41	25	
Age (years)	50 ± 8	40 ± 11	<.001
Sex (f/m)	23/18	16/9	.526
Disease duration (years)	16 ± 8	8 ± 7	<.001
EDSS score (range, median)	4–6, 4.5	0–3.5, 2	
MS type (SPMS/PPMS/RRMS)	31/10/0	0/0/25	
Protocol (1/2/3)	19/22/0	0/0/25	
DMT (y/n)	12/29	14/11	.031
Weight (kg)	68.2 ± 11.3	72.3 ± 21.1	.308
Height (m)	1.71 ± 0.07	1.72 ± 0.11	.679
BMI (kg/m^2)	23.1 ± 3.3	24.1 ± 5.5	.393

Note: EDSS: expanded disability status scale; SPMS: secondary-progressive multiple sclerosis; PPMS: primary-progressive multiple sclerosis; RRMS: relapsing-remitting multiple sclerosis; Protocol 1: Start at 8 W, incremental increase of 8 W/min; Protocol 2: Start at 25 W, incremental increase of 12.5 W/min; Protocol 3: Start at 10 W, constant workload for five minutes, then incremental increase of 10 W/min; DMT: Patient takes disease-modifying drug therapy; BMI: body mass index. Data given as total number or mean (M) ± standard deviation (SD), for EDSS: range and median.

3.1.2 Group R

For this group, the data of 25 patients with RRMS were analyzed (16 females and 9 males). EDSS scores ranged from 0.0 to 3.5, with a median of 2.0 (see Table 1 for clinical characteristics of group R and the significance of differences to group P).

Subjects reached a mean peak blood lactate concentration of 6.5 mM/l (see Fig. 9 and 11). 5 patients (4 male, 1 female) out of the 25 were able to accumulate a peak blood lactate concentration of 8 mM/l and above; 7 out of the 16 females reached a peak blood lactate concentration of 6 mM/l and above. In total, 22 out of the 25 patients in group R (88%) reached a lactate level of 4 mM/l or above.

Patients achieved a mean W_{max} of 146 W in group R and a mean W_{max}/kg of 2.1 W/kg (see Fig. 13 and 15). Mean $\dot{V}O_{2peak}$ was 1904 ml/min, mean $\dot{V}O_{2peak}$/kg 27 ml/min/kg (see Table 2 for means and standard deviations). Patients reached between 72% and 92% of the W_{max}, W_{max}/kg and the $\dot{V}O_{2peak}$ they should have reached according to the reference values for healthy, untrained individuals (see Table 3b for comparison to reference values). Patients' mean HR_{max} was 166 bpm (92% of the reference value for HR_{max}, see chapter 1.3.2). RQ_{peak} was not determined in group R.

Patients in this group reached a mean total distance of 460 ± 94 m in the 6MWT (range 300–700, median 440). The mean score in the QIDS-SR_{16} was 6.5 ± 3.5 (range 0–14, median 6) and the mean score on the HAQUAMS fatigue subscale was 2.6 ± 1.1 (range 1–4.5, median 3).

3.1.3 Clinical characteristics of the pooled group

If group P and group R are treated as one group, this pooled group contains 66 patients (39 females and 27 males). EDSS scores range from 0.0 to 6.0, with a median of 4.0. Patients' mean age is 47 ± 10 years, mean disease duration 13 ± 9 years. The mean height is 1.72 ± 0.09 m, mean weight 69.7 ± 15.7 kg, mean BMI 23.5 ± 4.2 kg/m^2.

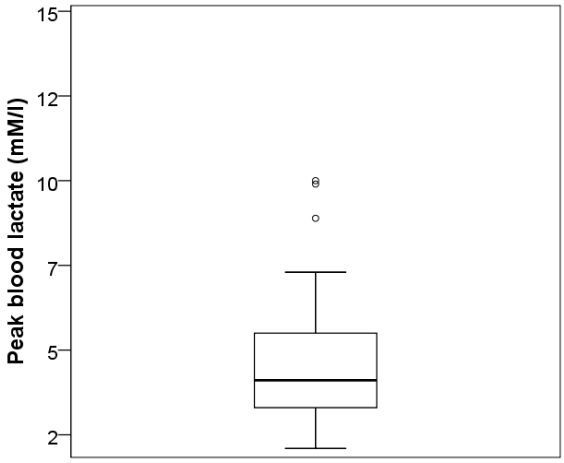

Fig. 8:
Peak blood lactate levels in group P.

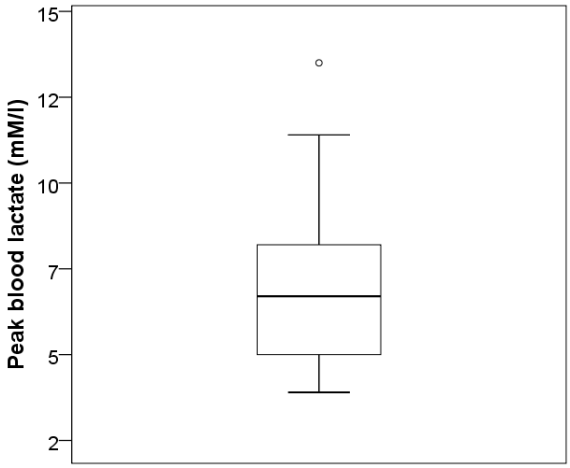

Fig. 9:
Peak blood lactate levels in group R.

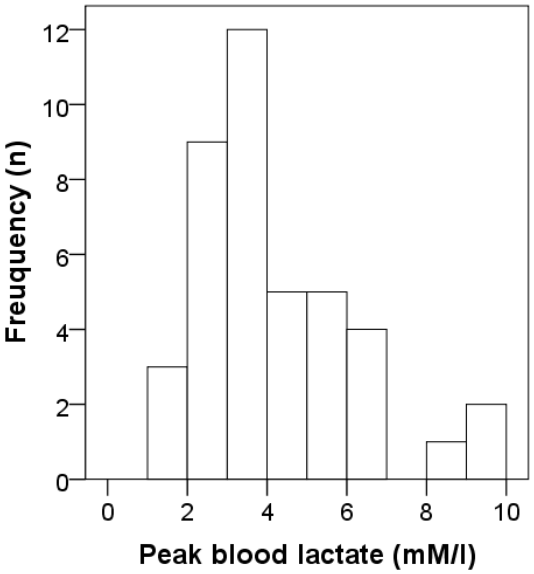

Fig. 10:
Frequency distribution of peak blood lactate levels in group P.

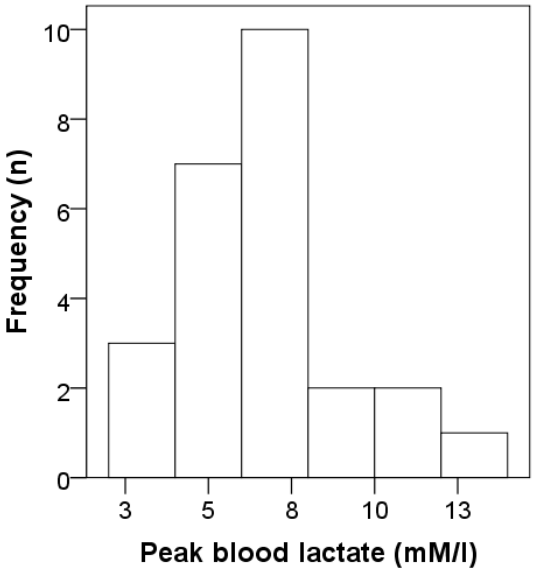

Fig. 11:
Frequency distribution of peak blood lactate levels in group R.

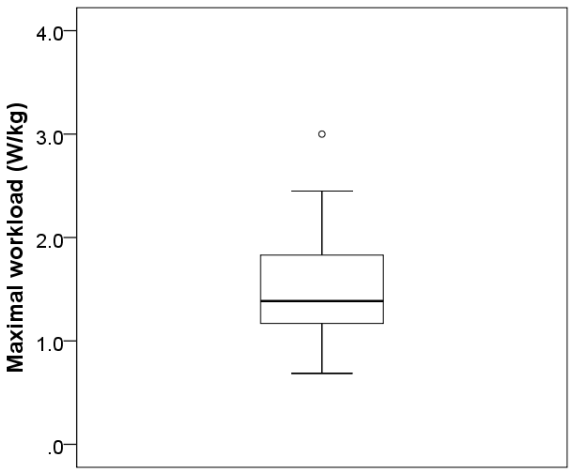

Fig. 12:

Maximal workload in group P.

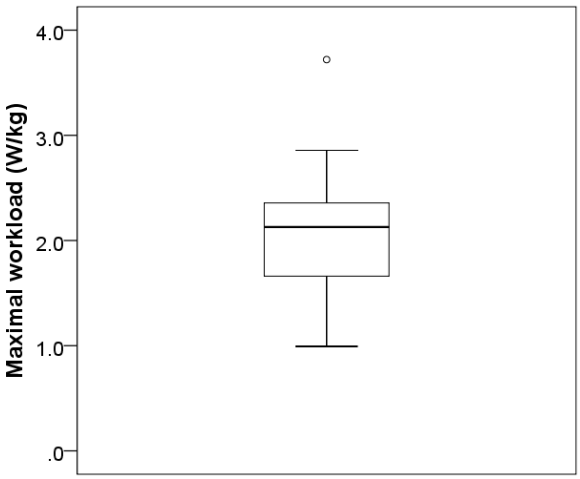

Fig. 13:

Maximal workload in group R.

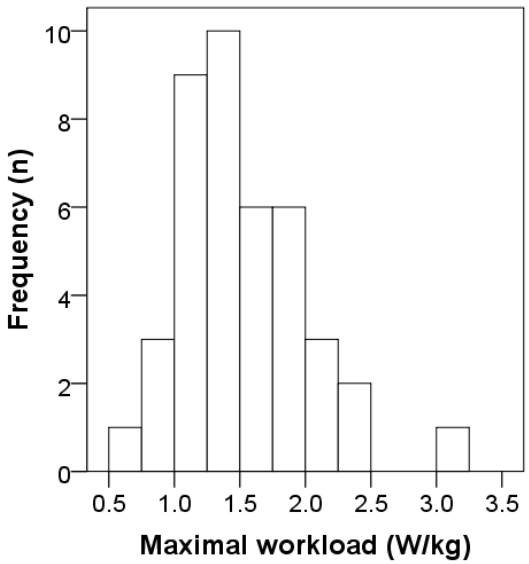

Fig. 14:

Frequency distribution of maximal workload in group P.

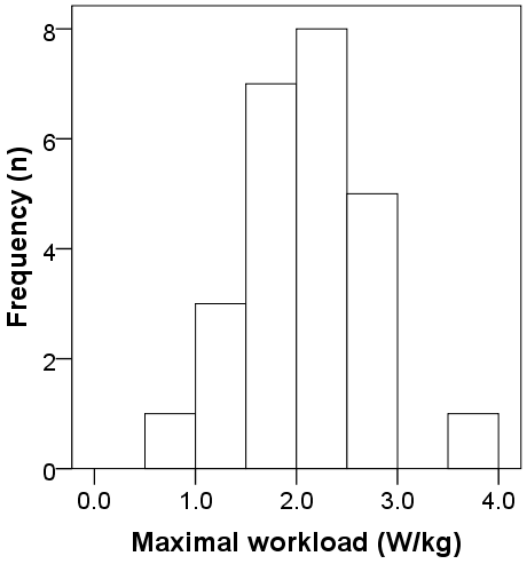

Fig. 15:

Frequency distribution of maximal workload in group R.

Table 2. Peak performance values for group P and group R.

		M ± SD	Range	Median
Peak blood lactate (mM/l)	Group P	4.1 ± 2	1.6–9.5	3.6
	Group R	6.5 ± 2.4	3.4–13.0	6.2
W_{max}/kg (W/kg)	Group P	1.5 ± 0.5	0.7–3.0	1.4
	Group R	2.1 ± 0.6	1.0–3.7	2.1
W_{max} (W)	Group P	102 ± 32	48–191	98
	Group R	146 ± 44	68–263	140
$\dot{V}O_{2peak}$ (ml/min)	Group P	1450 ± 404	702–2652	1397
	Group R	1904 ± 600	1110–3480	1750
$\dot{V}O_{2peak}$/kg (ml/min/kg)	Group P	21 ± 6	11–34	20
	Group R	27 ± 7	14–49	27
HR_{max} (bpm)	Group P	138 ± 21	101–183	139
	Group R	166 ± 22	132–210	165

Note: W_{max}: maximal workload; $\dot{V}O_{2peak}$: peak oxygen consumption; HR_{max}: maximal heart rate. Data given as mean (M) ± standard deviation (SD), range, median.

Table 3a. Comparison of performance markers in group P with reference values for healthy, untrained individuals.

	Female subjects				Male subjects			
	M ± SD	Ref.	% of Ref.	p	M ± SD	Ref.	% of Ref.	p
$\dot{V}O_{2peak}$/kg (ml/min/kg)	20 ± 5	30	67 %	<.001	23 ± 6	32	72 %	<.001
W_{max} (W)	91 ± 23	128	71 %	<.001	115 ± 36	193	60 %	<.001
W_{max}/kg (W/kg)	1.4 ± 0.4	2.1	67 %	<.001	1.6 ± 0.6	2.4	67 %	<.001

Note: Performance markers $\dot{V}O_{2peak}$/kg: peak oxygen consumption, W_{max}: maximal workload, and W_{max}/kg reached by male and female subjects. Data given as mean (M) ± standard deviation (SD); reference values (Ref.) for (M); and the percentage of the reference value that was reached.
Reference values for $\dot{V}O_{2peak}$/kg (age-adjusted) and for W_{max} (height-, weight- and age-adjusted) according to calculation formula by Jones (1997), for W_{max}/kg (age-adjusted) according to calculation formula by Rost and Hollmann (1982).

Table 3b. Comparison of performance markers in group R with reference values for healthy, untrained individuals.

	Female subjects				Male subjects			
	M ± SD	Ref.	% of Ref.	p	M ± SD	Ref.	% of Ref.	p
$\dot{V}O_{2peak}$/kg (ml/min/kg)	25 ± 4	33	76 %	<.001	29 ± 10	38	76 %	.027
W_{max} (W)	126 ± 29	137	92 %	.166	182 ± 43	254	72 %	.001
W_{max}/kg (W/kg)	2.0 ± 0.5	2.3	87 %	.044	2.2 ± 0.8	2.7	81 %	.083

Note: Performance markers $\dot{V}O_{2peak}$/kg: peak oxygen consumption, W_{max}: maximal workload, and W_{max}/kg reached by male and female subjects. Data given as mean (M) ± standard deviation (SD); reference values (Ref.) for (M); and the percentage of the reference value that was reached.
Reference values for $\dot{V}O_{2peak}$/kg (age-adjusted) and for W_{max} (height-, weight- and age-adjusted) according to calculation formula by Jones (1997), for W_{max}/kg (age-adjusted) according to calculation formula by Rost and Hollmann (1982).

3.2 Correlations among the performance indicators

3.2.1 Group P

Our main outcome measures, the peak blood lactate level and W_{max}/kg, were significantly correlated in group P (see Table 4). W_{max}/kg and peak blood lactate were also significantly correlated with the other peak performance markers $\dot{V}O_{2peak}$/kg and HR_{max}.

After controlling for W_{max}/kg, the correlation of peak blood lactate with $\dot{V}O_{2peak}$/kg became insignificant ($r = .16$; $p = .336$), as well as the correlation of peak blood lactate with HR_{max} ($r = .24$; $p = .145$). The correlation between W_{max}/kg and $\dot{V}O_{2peak}$/kg remained highly significant when controlled for peak blood lactate ($r = .76$; $p < .001$) whereas the correlation between W_{max}/kg and HR_{max} did not ($r = .26$; $p = .111$).

3.2.2 Group R

In group R, the peak blood lactate level and W_{max}/kg were also significantly correlated with each other and with $\dot{V}O_{2peak}$/kg and HR_{max} (see Table 4).

After controlling for W_{max}/kg, the correlation of peak blood lactate with $\dot{V}O_{2peak}$/kg became insignificant ($r = .29$; $p = .170$), while the correlation of peak blood lactate with HR_{max} stayed significant in this group ($r = .53$; $p = .007$). The correlation between W_{max}/kg and $\dot{V}O_{2peak}$/kg remained highly significant when controlled for peak blood lactate ($r = .85$; $p < .001$) whereas the correlation between W_{max}/kg and HR_{max} did not ($r = .30$; $p = .152$).

Table 4. Correlation of peak performance markers with clinical, psychological and sociodemographic variables.

	Group P		Group R	
	W_{max}/kg	Peak lactate	W_{max}/kg	Peak lactate
W_{max}/kg	1	.57***	1	.73***
Peak lactate	.57***	1	.73***	1
$\dot{V}O_{2peak}$/kg	.84***	.55***	.93***	.80***
HR_{max}	.43**	.42**	.70***	.77***
EDSS score	-.45**	-.04	-.64**	-.76***
6MWT	.48**	-.02	.24	.30
Depression	-.10	-.15	-.14	-.28
Fatigue	-.09	-.16	-.15	-.18
Disease duration	.10	-.19	-.49*	-.41*
Age	-.13	-.08	-.46*	-.53**

*Note: W_{max}: maximal workload, $\dot{V}O_{2peak}$/kg: peak oxygen consumption, HR_{max}: maximal heart rate, EDSS: expanded disability status scale, 6MWT: total distance in the six minute walking test. *** indicating p values <.001, ** indicating p values <.01, * indicating p values <.05.*

3.3 Correlation with disability and walking ability

3.3.1 Group P

EDSS scores showed a significant inverse correlation with W_{max}/kg, but not with peak blood lactate (see Fig. 16 and 17). Similarly, the total distance achieved in the 6MWT was significantly related to W_{max}/kg, while there was no significant relationship with peak blood lactate (see Table 4).

3.3.2 Group R

In this group, EDSS scores showed a significant inverse correlation with W_{max}/kg and an even stronger inverse correlation with peak blood lactate levels (see Fig. 18 and 19). The total distance achieved in the 6MWT was neither significantly related to W_{max}/kg nor to peak blood lactate (see Table 4).

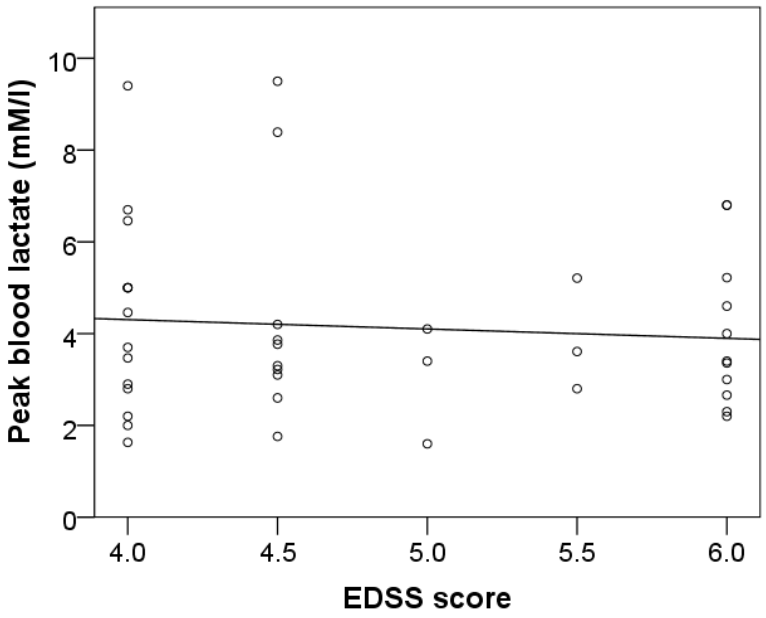

Fig. 16:
Relation between peak blood lactate levels and EDSS score in group P (r^2 linear = .007)

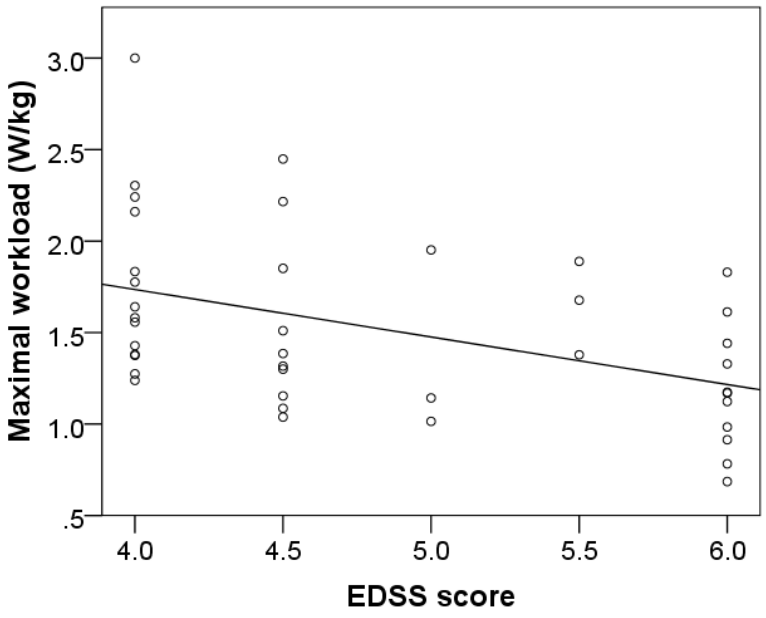

Fig. 17:
Relation between maximal workload and EDSS score in group P (r^2 linear = .194)

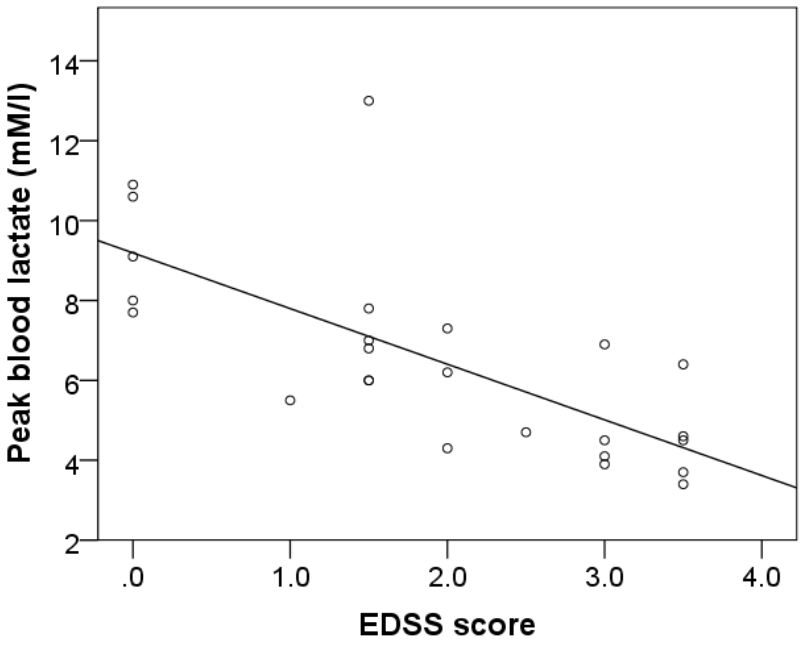

Fig. 18:
Relation between peak blood lactate levels and EDSS score in group R (r^2 linear = .514)

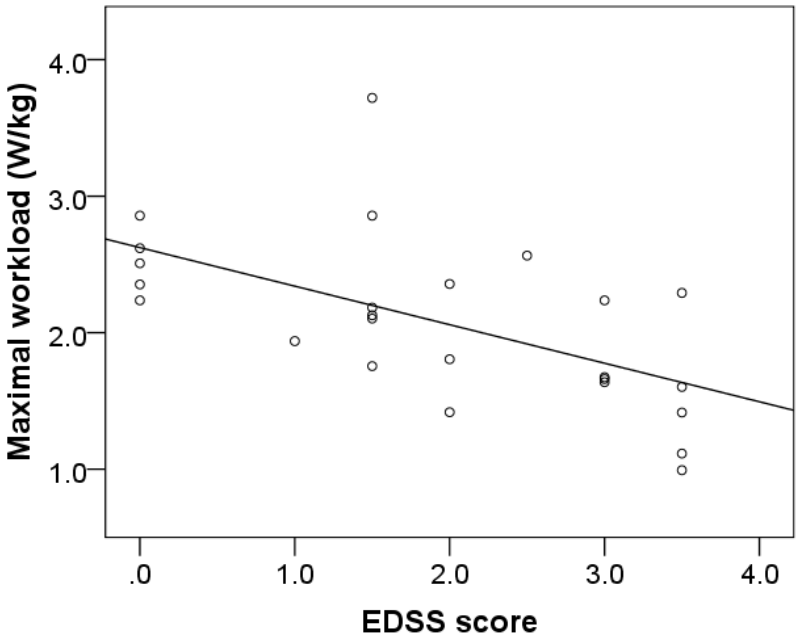

Fig. 19:
Relation between maximal workload and EDSS score in group R (r^2 linear = .342)

3.3.3. Correlation with EDSS in the pooled group

If group P and group R were treated as one big group, for this pooled group the EDSS score was correlated highly significant with peak blood lactate levels ($r = .54$; $p < .001$) and with W_{max}/kg ($r = .62$; $p < .001$) (see Fig. 20 and 21).

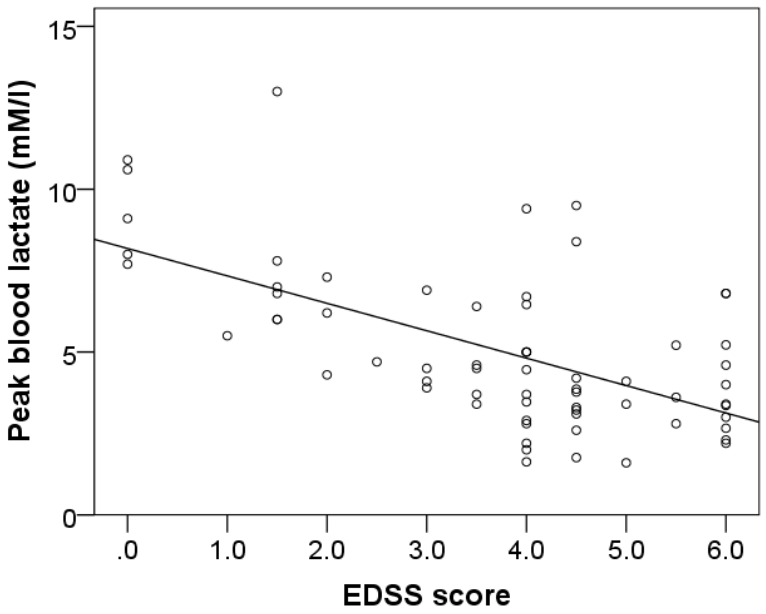

Fig. 20:
Relation between peak blood lactate levels and EDSS score in the pooled group
(r^2 linear = .362)

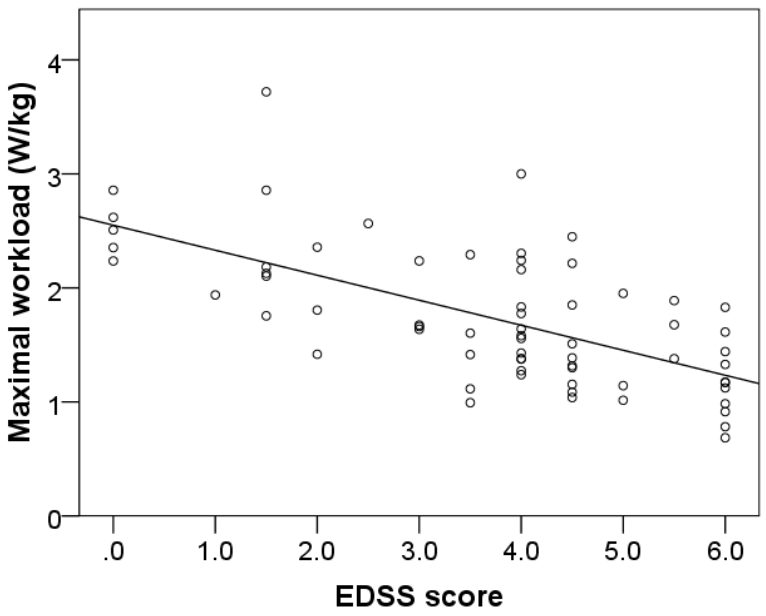

Fig. 21:
Relation between maximal workload and EDSS score in the pooled group
(r^2 linear = .410)

3.4 Correlation with psychological measures

3.4.1 Group P

Neither W_{max}/kg nor peak blood lactate correlated significantly with depressive symptoms (according to the total score of QIDS-SR_{16}) or with fatigue (according to the score in the HAQUAMS fatigue subscale) (see Table 4). EDSS scores showed a significant relationship with both depressive symptoms ($r = .32$; $p = .039$) and fatigue ($r = .39$; $p = .013$). Depressive symptoms and fatigue were highly correlated with each other ($r = .55$; $p < .001$).

3.4.2 Group R

Neither W_{max}/kg nor peak blood lactate levels correlated significantly with depressive symptoms or with fatigue (see Table 4). In this group, EDSS scores did not show a significant correlation with depressive symptoms ($r = .34$; $p = .101$) or fatigue ($r = .39$; $p = .013$). Depressive symptoms and fatigue were not significantly correlated ($r = .36$; $p = .081$).

3.5 Correlation with disease duration and age

3.5.1 Group P

Neither peak blood lactate nor W_{max}/kg correlated significantly with disease duration or age (see Table 4). Patients' age showed a significant inverse correlation with HR_{max} ($r = -.40$; $p = .010$).

3.5.2 Group R

In this group, both peak blood lactate and W_{max}/kg showed a significant inverse correlation with disease duration and age (see Table 4). Patients' age also correlated significantly and inversely with HR_{max} ($r = -.61$; $p = .001$).

3.6 Comparison of patient subgroups

3.6.1 Group P

Patients with SPMS and patients with PPMS did not differ significantly regarding their mean W_{max}/kg or mean peak lactate (see Table 5a). Similarly, no significant difference was found between patients who received DMT and patients who did not.

The different ramp protocols discriminated weakly regarding W_{max}/kg, but not regarding peak blood lactate levels. Female subjects reached a lower mean W_{max}/kg and a lower mean peak lactate than male subjects, but the differences were not statistically significant.

Table 5a. Comparison of mean peak blood lactate and mean maximal workload in different subgroups of patients in group P.

		Peak blood lactate (mM/l)		t-Test		Maximal workload (W/kg)		t-Test	
Subgroups	n	M	SD	t (39)	p	M	SD	t (39)	p
SPMS	31	4.0	± 1.8	.71	.483	1.5	± 0.4	.27	.789
PPMS	10	4.5	± 2.4			1.5	± 0.7		
No DMT	29	4.0	± 1.9	.73	.471	1.5	± 0.5	.94	.351
DMT	12	4.5	± 2.2			1.6	± 0.4		
Protocol 1	19	3.5	± 1.4	1.94	.060	1.3	± 0.3	2.28	.028
Protocol 2	22	4.7	± 2.3			1.7	± 0.6		
Male	18	4.5	± 2.6	1.13	.265	1.6	± 0.6	1.56	.127
Female	23	3.8	± 1.3			1.4	± 0.4		

Note: *Data given as mean (M) ± standard deviation (SD) reached by the different subgroups. SPMS: secondary-progressive multiple sclerosis, PPMS: primary-progressive multiple sclerosis. DMT: Patients received disease-modifying drug therapy. Protocol 1: Start at 8 W, incremental increase of 8 W/min. Protocol 2: Start at 25 W, incremental increase of 12.5 W/min.*

In group P, ANOVA was conducted to determine if the mean peak lactate of MS patients differs significantly between the three performance subgroups described in the literature for heart patients (Karlsson et al. 1984; Pokan et al. 2004a). The 7 patients in the lowest performance group reached a mean peak blood lactate of 2.8 ± 0.8 mM/l. The 27 patients in the intermediate group reached a mean peak blood lactate of 4 ± 1.6 mM/l. The 7 patients in the highest group reached a mean peak blood lactate of 6 ± 2.9 mM/l. The group effect was significant ($F(2, 38) = 5.78$, $p = .006$) (see Fig. 22).

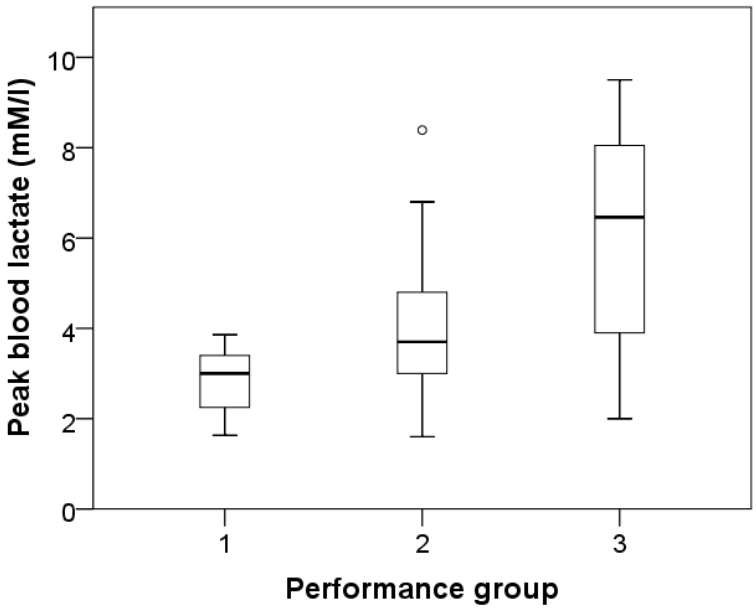

Fig. 22: Peak blood lactate in the three performance subgroups of group P (grouped by W_{max}).
Group 1: ≤ 74 W, Group 2: 75-124 W, Group 3: ≥ 125 W.

3.6.2 Group R

Patients who received DMT reached no significantly different peak blood lactate levels or W_{max}/kg compared with patients who did not receive DMT (see Table 5b). Female subjects reached a lower mean W_{max}/kg and a lower mean peak lactate than male subjects, but the difference was not significant.

Table 5b. Comparison of mean peak blood lactate and mean maximal workload in different subgroups of patients in group R.

		Peak blood lactate (mM/l)		t-Test		Maximal workload (W/kg)		t-Test	
Subgroups	n	M	SD	t (23)	p	M	SD	t (23)	p
No DMT	11	6.5	± 3.2	.04	.971	2.1	± 0.8	.04	.969
DMT	14	6.5	± 1.8			2.1	± 0.4		
Male	9	7.7	± 3.0	1.86	.076	2.2	± 0.8	.64	.531
Female	16	5.9	± 1.9			2.0	± 0.5		

Note: *Data given as mean (M) ± standard deviation (SD) reached by the different subgroups. DMT: Patients received disease-modifying drug therapy.*

In group R, a t-test was conducted to determine if the mean peak lactate of MS patients differs significantly between the performance group with W_{max} <125 W and the performance group with W_{max} ≥ 125 W. The 7 patients in the lower performance group reached a mean peak blood lactate of 4.6 ± 1.1 mM/l. The 18 patients in the higher group reached a mean peak blood lactate of 7.3 ± 2.4 mM/l. The group effect was significant (t (23) = 2.79, p =.010) (see Fig. 23).

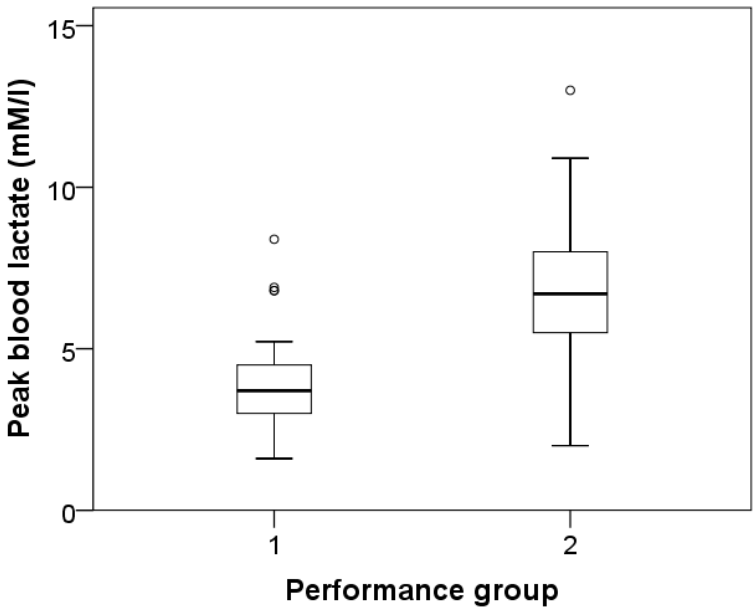

Fig. 23: Peak blood lactate in the two performance subgroups of group R (grouped by W_{max}). Group 1: < 125 W, Group 2: ≥ 125 W.

3.7 Comparison of peak performance markers between group P and group R

The patients in group R reached a higher peak blood lactate, a higher W_{max}/kg, a higher $\dot{V}O_{2peak}$/kg and a higher HR_{max} compared with the patients in group P. All of these differences were highly significant (see Table 6).

Table 6. Inter-group comparison of the peak performance markers.

		n	M ± SD	t(64)	p
Peak blood lactate (mM/l)	Group P	44	4.1 ± 2	4.35	< .001
	Group R	25	6.5 ± 2.4		
W_{max}/kg (W/kg)	Group P	44	1.5 ± 0.5	4.16	< .001
	Group R	25	2.1 ± 0.6		
$\dot{V}O_{2peak}$/kg (ml/min/kg)	Group P	44	21 ± 6	3.42	.001
	Group R	25	27 ± 7		
HR_{max} (bpm)	Group P	44	138 ± 21	5.14	< .001
	Group R	25	166 ± 22		

Note: W_{max}: maximal workload; $\dot{V}O_{2peak}$: peak oxygen consumption; HR_{max}: maximal heart rate. Data given as mean (M) ± standard deviation (SD) reached by the groups.

3.8 Comparison of walking ability and psychological measures between group P and group R

The total distance achieved in the 6MWT achieved by patients in group P (315 ± 106 m) was significantly lower than in group R (460 ± 94 m) (t (64) = 5.62, p <.001).

There was no significant difference between the groups in the mean score of the QIDS-SR_{16} (t (64) = .22, p = .824) and in the mean score of the HAQUAMS fatigue subscale (t (64) = .06, p = .954).

4. Discussion

4.1 Characterization of the groups according to peak performance markers

In the incremental bicycle spiroergometry conducted for this work, neither the patients in group R (relapsing-remitting MS, EDSS 0–3.5) nor the patients in group P (progressive MS, EDSS 4–6) were able to reach the peak performance values predicted for healthy, untrained subjects (see Tables 3a and 3b). This confirms a decrease in aerobic and functional capacity which has previously been described as linked to disease severity in MS (White and Dressendorfer 2004; Heesen et al. 2006; Dalgas et al. 2008; Heine et al. 2015; Langeskov-Christensen et al. 2015).

For example, in a study with 92 MS patients (EDSS 1.0–5.5, mixed disease courses), Finnish researchers found a maximal workload of 154 W for male subjects and 113 W for female subjects (Romberg et al. 2004). These W_{max} values are higher than the ones reached by group P in the present work (115 W for males, 91 W for females), but lower than the ones reached by group R (182 W for males, 126 W for females). Similarly, in a study with 26 MS patients (EDSS 1.0–6.5, mixed disease courses), a Swiss group found a $\dot{V}O_{2max}$/kg of about 23 ml/min/kg (Mostert and Kesselring 2002). Again, this performance marker lies between the $\dot{V}O_{2peak}$/kg values determined for the two groups in this work (21 ml/min/kg for group P and 27 ml/min/kg for group R).

Considering the comparisons mentioned above, one could reason that we examined a similar patient sample as the described studies, but went a step further by discriminating between the progressive and the relapsing-remitting disease courses and dividing the group according to the EDSS score. Our descriptive results characterizing the spiroergometric performance of group P and group R (see Table 2) might be of help in the future for planning exercise tests and exercise studies with similar subgroups of MS patients, as they allow estimating the peak performance more accurately than the existing literature.

4.2 Comparison of the peak performance in the two groups

We could show that subjects in group P reached lower spiroergometric peak performance values than subjects in group R (see Table 6). This difference was

highly significant for all the performance markers we tested (peak blood lactate, W_{max}/kg, $\dot{V}O_{2peak}$/kg, HR_{max}).

Importantly, the difference between the groups is probably rather under-estimated in this work: As described in section 2.2.1, three patients in group P had to be excluded because of motoric problems. As these patients were on the upper end of the EDSS section of group P (4–6), their peak performance values presumably would have been in the lower ranges of the group's results, thus further underlining the difference to group R.

The difference in the peak performance values cannot be explained by the mean age difference of ten years, because the discrepancy between group P and group R also was evident in the comparison with the age-adjusted reference values (see Tables 3a and 3b).

Besides age, the only clinical characteristic that differed significantly between the groups (see Table 1) was the mean disease duration, which was 8 years in group R and 16 years in group P. As the disease duration was required to be less than 10 years in the inclusion criteria for group R (see chapter 2.2.2), this is not surprising. Moreover, the longer disease duration in group P has to be regarded as a "side-effect" of the grouping according to disease courses: Group P contains a majority of patients with SPMS, which is usually diagnosed about ten years after the onset of RRMS.

According to Goodin (2014, p. 234) it has been suggested "that the emergence of the progressive phase of MS (either SPMS or PPMS) is just an effect of chronological age and that the different disability milestones are reached on a predefined schedule not influenced by relapses (i.e., that PPMS is just RRMS in which the relapsing phase has been skipped)"(Confavreux and Vukusic 2006). With this controversial hypothesis in mind, the age difference between our groups could also be explained by the grouping according to disease courses – meaning that the subjects with progressive MS examined in this work are just further along in the "schedule" of the disease and therefore chronologically older.

Regardless of this, the comparison between the groups shows that the performance of MS patients in spiroergometric tests is strongly associated with the patients' disease course and EDSS score. These two clinical characteristics should therefore be taken in consideration in the planning phase of exercise studies with MS patients.

The same applies for the ergometry protocols: The present work shows that for different subgroups of MS patients, different ramp protocols have to be chosen. Within the rather small EDSS section of group P (4–6), some patients were able to perform the standard WHO ergometry protocol, while for others an easier protocol had to be chosen. Even then, some patients were not able to keep the rate of revolutions on the bicycle ergometer over 70 per minute. This is due to motoric coordination problems frequently encountered by MS patients and might have influenced the spiroergometric peak performance values in group P. The protocol that was subsequently designed for the second study with group R was feasible for all patients in this group, but the small incline resulted in a rather long ergometry for the "healthier" subjects, which also might have had an impact on the peak performance values.

4.3 The EDSS score and spiroergometric performance

The percentages of the reference peak performance values that were reached by group P and group R (see Tables 3a and 3b) are especially interesting if they are regarded with the EDSS score in mind. In group R, which includes patients with an EDSS score of 0.0 up to 3.5, the percentages range from 92% of the reference value (best result) down to 72% of the reference value (worst result). The EDSS scores of group P directly fall into line with those of group R, beginning at 4.0 and ranging up to 6.0. Intriguingly, the percentages of the spiroergometric reference values reached by group P show just the same trend: The highest percentage in this group is 72% of the reference value, which is exactly the lower limit of group R, and percentages then stretch down to the lowest value of 60%.

Furthermore, it is noticeable that the "smooth transition" between healthy subjects and the non- or minimally-disabled subjects in group R (with an EDSS score of 0.0, meaning a normal neurological exam) is also represented in the percentages of reference values reached in this group: Two out of six results do not differ significantly from the reference values, meaning that the spiroergometric performance in the upper range in group R borders on the performance of healthy, untrained subjects.

These observations indicate that the EDSS score allows an estimation of the spiroergometric performance (or rather, the decreased performance in

comparison to healthy, untrained subjects) of MS patients, at least in the EDSS range that was examined in this work (0–6).

4.4 Walking ability, depression and fatigue in the two groups

Patients in group R achieved a greater total distance in the 6MWT than patients in group P (460 m vs. 315 m). This result is not surprising because EDSS scores ≥ 4.0 are influenced directly by the walking distance (see Fig. 7).

Goldman et al. already reported an interrelation between the 6MWT and the EDSS score (2008). In their study, patients with an EDSS score of 0.0–2.5 reached a mean total distance of 603 m, patients with an EDSS score of 3.0–4.0 reached 507 m, and patients with an EDSS score of 4.5–6.5 reached 389 m. Compared with these values, the distances reached by our subjects in both groups seem to be rather low. However, the study by Goldman et al. included patients with mixed disease courses (RRMS, PPMS and SPMS). This explains the lower distance reached by the subjects in our group P: After all, it consisted exclusively of patients with progressive MS, which often suffer from more severe gait disorder than patients with the relapsing-remitting disease course. It remains unclear why the patients in our group R did not achieve the distance of 507 m that was reached by patients with an EDSS of 3–4 in the Goldman study: Our group R had a lower EDSS score of 0–3.5, and it consisted solely of patients with the relapsing-remitting MS disease course. Both facts should have rendered the group more able for the 6MWT.

In contrast, the 6MWT result of our group R (460 m) fits in with the results of another study in which the performance of patients with RRMS in the 6MWT was examined (Bosnak-Guclu et al. 2012). In this work, the patient group with an EDSS score of 0–2 reached a mean total distance of 582 m (n=23), while the group with an EDSS score of 2.5–4.5 reached 446 m (n=20).

Neither the depressive symptoms according to the QIDS-SR_{16} nor the fatigue according to the HAQUAMS fatigue subscale differed significantly between group P and group R.

In the case of the HAQUAMS fatigue subscale, the number of items might have been too small to detect a difference between the groups. A longer questionnaire specifically designed to investigate fatigue, like the full-length modified fatigue impact scale (MFIS) with 21 items, would have been more suited, but

unfortunately the HAQUAMS fatigue subscale was the only test for fatigue that was conducted with both groups.

The mean total score in the QIDS-SR_{16} in both groups would be classified as just fulfilling the criteria for "mild depression". This result is surprising as patients with progressive MS and a more severe disability according to the EDSS could have been expected to show more depressive symptoms. One possible explanation is that the uncertainty of new episodes in the relapsing-remitting disease course triggers the feeling of "learned helplessness", and therefore depressive symptoms, while patients in the progressive phase of the disease know better what to expect, and in this way gather some kind of "control". This might prevent them from becoming more depressed in the progressive disease phase, although they are usually impaired more severely in their daily life by MS symptoms than patients with the relapsing-remitting disease course.

4.5 A reduced peak blood lactate response to exercise in MS patients

Although the patients underwent the protocol until volitional exhaustion, the vast majority of subjects both in group P and in group R was not able to accumulate a peak blood lactate level of 8 mM/l, which is typically reached by healthy individuals in exhaustive exercise tests (Izquierdo et al. 2001; Wasserman et al. 2011; Langeskov-Christensen et al. 2015).

In group P, the mean peak blood concentration was 4.1 mM/l (see Fig. 8). Out of the 41 subjects, only 3 male patients reached a level of 8 mM/l, and only 2 female patients reached a concentration of 6 mM/l and above, which is the lowest described peak lactate level we found in the literature for healthy untrained women. Only 17 out of the 41 patients in group P (41.5%) reached a concentration of 4 mM/l, which is still routinely used in exercise tests as a fixed lactate threshold (see chapter 1.5.2) (Heck et al. 1985; Westhoff et al. 2013).

In group R, the mean peak blood lactate concentration was 6.5 mM/l (see Fig. 9). This is significantly higher than in group P (see Table 6), but still lower than in healthy individuals. Only 5 out of the 25 patients in this group reached a level of 8 mM/l (4 males and 1 female). Out of the 16 females, 7 reached a peak lactate concentration of 6 mM/l and above. Different than in group P, the majority of the patients (88%) did reach the fixed lactate threshold of 4 mM/l.

Our results clearly show a reduced peak blood lactate response to exhaustive exercise in both groups. This finding is, to our knowledge, novel for MS patients. On the one hand, this outcome is remarkable because lower fitness levels in healthy individuals are traditionally associated with an earlier increase of blood lactate (reflecting a shift of the lactate curve to the left), but usually not with a reduced peak lactate level (see Fig. 5). Peak lactate levels as low as 4 mM/l, as they were found in group P, have to our knowledge not been described for healthy subjects. As explained more detailed in section 1.6.1, healthy persons tend to reach peak blood lactate levels above 8 mM/l or, in the case of females, at least 6mM/l in exhaustive exercise tests, even if they are untrained and the absolute maximal workload achieved at the point of exhaustion is low.

Low peak lactate levels have been reported for endurance athletes, whose metabolism is accustomed to aerobic performance and lactate clearance (Föhrenbach et al. 1987). This is in contrast to the poor aerobic capacity of our subjects, which is reflected in their low $\dot{V}O_{2peak}$/kg.

On the other hand, our findings are consistent with the low peak lactate levels described in the literature for patients suffering from other chronic medical conditions, such as heart diseases (CAD and angina pectoris), neoplastic diseases and cystic fibrosis (see section 1.6.2).

The mean peak blood lactate levels we found in group P and group R (4.1 mM/l and 6.5 mM/l, respectively) are comparable to the ones described in a doctoral thesis about tumor patients suffering from fatigue (Voigt 2008): In an exhaustive treadmill test, the subjects reached a maximal blood lactate concentration of 5.1 ± 2 mM/l. Similar as in group P, 39% of the tumor patients in that trial had to break off the exercise test because of exhaustion before they had reached the fixed lactate threshold of 4 mM/l.

A critical question is, therefore, if the peak blood lactate levels of our subjects are low due to secondary deconditioning shared with patients suffering from other chronic conditions, or if the reason of this phenomenon is MS-specific. This topic will be discussed in detail below.

Although its traditional role has been challenged (Gladden 2004), the accumulation of lactate and the low muscle pH caused by lactic acidosis is still seen as a major limiter and an important cause for muscular fatigue in exhaustive exercise (Kenney et al. 2012). However, it is unlikely that a peak blood lactate

level as low as 4.1 mM/l or even 6.5 mM/l reflects a muscle acidosis severe enough to prevent patients from continuing with the protocol. In healthy subjects, an only slightly lower blood lactate level of 3.7 mM/l has been identified as the average value for maximal lactate steady state (Goodwin et al. 2007), which, after all, is defined as a blood lactate level that can be maintained over time without further lactate accumulation (Pringle and Jones 2002).

If lactic acidosis was not the limiting factor for the subjects examined in this work, this raises the question what confines lactate accumulation, causes the onset of exercise-limiting fatigue and eventually limits exercise capacity in MS patients.

4.6 The question of cardiorespiratory exhaustion in group P

The mean HR_{max} of 138 bpm in group P (80% of the age-adjusted reference value) indicates that the patients did not achieve full cardiologic exhaustion during the bicycle ergometry. This problem has already been described in other exercise studies with MS patients (Rasova et al. 2005). However, the mean RQ_{peak} of 1.08 and the fact that 30 out of 41 subjects in group P reached RQ_{peak} values of ≥ 1.0 suggest that the majority of patients was at least approaching respiratory exhaustion. 17 out of the 30 patients reached an RQ_{peak} ≥ 1.1, which is regarded as definite exhaustion.

It is noticeable that the mean RQ_{peak} is comparatively high considering the low mean peak blood lactate – both values should theoretically be coupled, as it is lactic acidosis which provokes an increase of $\dot{V}CO_2$ and therefore causes the RQ to rise to levels ≥ 1.0. This discrepancy suggests that the complex process linking muscle lactate to blood lactate, blood pH and CO_2 production might be altered in MS patients.

4.7 Exercise limitation and fatigue

Regardless of the cardiorespiratory results, one can argue that the stopping point in the incremental spiroergometry constitutes some kind of exhaustion or fatigue. Irrespective of the underlying mechanism, patients reached volitional exhaustion, which is proposed as a suitable endpoint in recommendings for exercise tests with MS patients (Durstine et al. 2009). As it has been stated by Kenney et al. (2012, p. 133), “unless they are highly motivated, most individuals terminate exercise before their muscles are physiologically exhausted”.

In the language of exercise physiology, the expression “fatigue” is used to describe “decrements in muscular performance with continued effort” which are “accompanied by general sensations of tiredness” (Kenney et al. 2012, p. 128). For the following part of the discussion, it is important to clarify that this fatigue concept is not identical with the fatigue measured by the HAQUAMS fatigue subscale or the MFIS. The concepts overlap regarding the perceived tiredness, but differ insofar as fatigue in exhaustive exercise tests is a natural phenomenon also experienced by healthy individuals and athletes. In contrast to this, a constant sense of tiredness in the daily life is by no means healthy, but rather a serious symptom that is common in a number of chronic diseases. People suffering from this symptom without a causally connected disease are ascribed an independent medical category, the chronic fatigue syndrome.

In MS, fatigue in the daily life is a symptom experienced by many patients, sometimes before the onset of the more typical neurologic signs. It is imaginable that this perceived fatigue influences patients’ peak performance in exercise tests and in this way de facto overlaps with the “healthy” fatigue in the sense of exercise physiology. However, it has been demonstrated in a US study that while the muscular fatigue of MS patients is correlated with their disability grade, it is not related to the perceived every-day fatigue measured by a 28-item questionnaire (Sharma et al. 1995).

According to Davis et al. (2010, p. 164), fatigue in the sense of exercise physiology “may have origins that span from the cerebral cortex to muscle cross-bridging.” To structure the possible causes, fatigue is traditionally divided into peripheral fatigue (i.e., the cause lies within the muscle) and central fatigue (i.e., the cause lies within the central nervous system). Notwithstanding this classification, fatigue is a complex phenomenon, normally occurring at more than one place and being driven by more than one cause.

As possible sources for peripheral fatigue, Kenney et al. list the following: 1) a decreased rate of energy delivery, 2) accumulation of by-products like lactate and H^+, and 3) failure of the muscle fibers’ contractile mechanisms (2012, p. 128). Considering the low peak blood lactate levels of our subjects, the second point can probably be ruled out as a cause of their fatigue. In the following section, the existing literature on the muscle energy metabolism and the muscle fiber characteristics of MS patients will be examined. The crucial role of central

fatigue, defined as alterations in neural control of muscle contraction, will be discussed subsequently.

4.8 Muscle energy metabolism and muscle fiber characteristics in MS patients

Having in mind the concept of a fixed lactate threshold at 4 mM/l, one could hypothesize that because they are not used to exercise, the subjects in group P stopped cycling shortly after anaerobic energy supply mechanisms became dominant (shortly after the onset of the third phase in the three-phase energy supply model described in section 1.4.1). This would imply that the peak lactate levels of the subjects in group P are actually intermediate points of the blood lactate curve seen in untrained, healthy subjects, i.e. that our subjects just stopped cycling at an earlier point, which results in "truncated" lactate curves, with the upper part of the curve missing.

However, this explanation of a "missing anaerobic phase" is not supported by the literature. On the contrary: The muscles of MS patients have been demonstrated to possess more anaerobic characteristics in comparison to healthy individuals. In a study by Kent-Braun et al. (1997), muscle biopsies of 9 MS patients (EDSS 2–6) were analyzed and compared to biopsies of healthy controls. MS patients showed a lower activity of succinic dehydrogenase (SDH), a key enzyme of the electron transport chain, essential for aerobic energy supply. As stated in the paper, "the reduction in oxidative enzyme activity of our MS group approached that observed in complete spinal transection. This reduction is remarkable, considering that this group of MS patients was largely ambulatory" (1997, p. 2003). The Kent-Braun group also found less type I muscle fibers in the muscle biopsies of the MS patients (66% of the total muscle fibers vs. 76% in the healthy controls). The decreased oxidative enzyme activity was especially pronounced in type I fibers, indicating that even in the "left-over" slow-twitch oxidative muscle fibers, the aerobic capacity is restricted. All muscle fibers showed signs of atrophy (26% less cross-sectional area in MS patients vs. controls), but it was especially pronounced in type II fibers.

Transferred to our subjects, these findings do not only provide the pathophysiologic basis for the poor aerobic capacity, reflected in the low $\dot{V}O_{2peak}$ in group P and group R. Furthermore, they make it very unlikely that the patients in group P skipped the last, anaerobic phase in the three-phase-model of energy

supply: If MS patients show a “greater tendency for muscle to supply energy via anaerobic means”, as inferred by Kent-Braun et al. (1997, p. 2003), it is much more probable that the subjects in group P had already made the transition to a mainly anaerobic energy supply when they stopped cycling – although the majority did not reach the lactate threshold value of 4 mM/l where the average healthy subject makes this transition.

Most likely, our subjects did undergo all three phases of muscular energy supply during their spiroergometry, though the thresholds seem to occur at lactate levels considerably lower than in healthy subjects.

If this is true, it poses the question if the lactate performance tests used routinely for healthy individuals can simply be transferred to MS patients, especially those with higher grades of disability and progressive disease courses. The clarification of this issue is of special importance as exercise interventions and performance tests with MS patients are becoming more and more common, and lactate performance tests are widely used in Germany.

In group R, the application of the 4 mM/l lactate threshold during the spiroergometry would have been possible, as the majority of the patients was able to reach this blood lactate concentration before exhaustion. The most obvious reason is the lower grade of disability, with the subjects in group R representing something like an “intermediate stage” between healthy subjects and group P. However, even in this group the peak blood lactate levels are lower than normal. In conjunction with the literature regarding alterations in the muscle energy metabolism and fiber type composition, there is enough evidence to also question the applicability of routine lactate performance tests for MS patients with RRMS and a mild to moderate degree of disability.

4.9 A vicious circle of muscle fiber conversion and atrophy in MS patients

There is a special significance in the fact that in the muscle biopsies of MS patients, Kent-Braun et al. (1997) found 1) a smaller percentage of type I fibers and 2) a greater atrophy of type II fibers than in healthy controls. These results have been confirmed by a newer study which showed that type IIa fibers of MS patients experience twice the atrophy as type I fibers (Garner and Widrick 2003).

It has been known for a long time that a decreased utilization of skeletal muscle can lead to a conversion of muscle fibers in the slow (type I) to fast (type II)

direction (Scott et al. 2001). It is also known that physical inactivity is a frequent behavior of MS patients (Motl 2008).

If physical inactivity leads to a conversion of type I muscle fibers to type II, and if type II muscle fibers are more prone to atrophy, this signifies that physical inactive behavior could maneuver MS patients into a vicious circle, which further aggravates the loss of muscular mass and the weakness already caused by demyelination and axonal loss in the CNS.

From a practical point of view, this underlines the importance of therapeutic exercise interventions for MS patients. Physical activity could maybe break the vicious circle by stopping the conversion of type I fibers into type II fibers, thus mitigating the atrophy. According to Scott et al. (2001, p. 1814), endurance training can even evoke some “fast-twitch” characteristics in type I fibers. By this means, endurance training might not only increase the aerobic capacity of MS patients, but maybe even compensate a part of the functional deficit experienced due to the loss of type II fibers.

4.10 The role of the CNS in exercise limitation

The MS-specific muscle characteristics discussed so far concern potential peripheral causes for fatigue. However, MS is a disease of the CNS, with impaired motor function being one of the best-known symptoms. Consequently, an altered central motor activation is the most likely candidate for the primary cause of muscular fatigue and exercise limitation in MS.

Voluntary muscle contraction requires an electrophysiological signal from the upper motoneuron in the central motor cortex. This signal has to be delivered by the axon, through the subcortical white matter and down the spine to the cell body of the lower motoneuron, which is located in the anterior horn of the spinal cord. The cell body of the lower motoneuron is still part of the CNS, while its axon, leaving the spine in the spinal nerve and delivering the signal to the muscle via the neuromuscular junction, already belongs to the peripheral nervous system (PNS). At very low exercise intensities, the muscle force is generated mainly by the gradual recruitment of motor units (defined as one alpha motoneuron and the muscle fibers innervated by it). To reach moderate or maximal voluntary contraction, the firing frequency of the motor units is increased (Milner-Brown et al. 1973). Because patients with MS show inflammatory demyelination and loss

of axons in the brain and in the spinal cord (Milo and Miller 2014), it can be hypothesized that the transmission of the signal from the motor cortex to the muscle is disturbed in these patients, resulting in a loss of neural muscle activation. Indeed, a prolonged central motor electrical conduction time (van der Kamp et al. 1991) and reduced electromyography (EMG) frequencies (Ng et al. 2004) have been demonstrated in MS patients. These findings suggest a disease-specific lack of muscle activation, limiting patients' performance independent of cardiorespiratory reasons. As the cause for the lack of muscle activation (despite continued effort) lies within the CNS, this phenomenon would be categorized as central fatigue.

In their work, Kent-Braun et al. (1997, p. 2003) discussed an incomplete motor unit activation and chronically reduced maximum discharge rates as possible causes for the aberrant peripheral muscle characteristics they found in MS patients. Taken together, there is abundant evidence for a reduced central motor activation of the muscles in MS, possibly leading to secondary muscular atrophy and anaerobic characteristics in the muscles – e.g., a low percentage of type I fibers and a reduction in oxidative enzyme activity.

MS might also affect the limitation of exercise capacity in patients through other mechanisms than the classic motor pathway: In his central governor model, Noakes stresses the role of the CNS in limiting physical exercise, defining fatigue as a "mechanism by which the CNS ensures that homeostasis is maintained" (Noakes 2011). The brain accomplishes this "by regulating the number of motor units recruited in the exercising muscles in a feed-forward manner on a moment-to-moment basis" (p. 26). As MS lesions affect several parts of the CNS, the central regulation mechanism of homeostasis during exercise could well be impaired in our subjects. This means that MS may not only interfere with the transmission of the activation signal from the upper motoneuron to the muscle, it might also alter the formation and the strength of the signal itself in the motor cortex.

In addition to these motor mechanisms, the processing of sensory information is frequently disturbed in MS patients. If peripheral information about muscle activation and the leg movement during bicycle ergometry is not transmitted properly in the CNS, this could influence patients' perception and evaluation of exhaustive exercise. Furthermore, neuropsychological symptoms of MS might

play a role in the complex process of exercise limitation, although in this work no link could be found between the peak performance markers and depressive symptoms or perceived fatigue.

In 2011, Motl et al. presented experimental support for the hypothesis that neurological disability and physical inactivity are independently associated with the aerobic exercise capacity in MS patients. This information helps to settle the question if the peak performance markers of our patients are only low because of secondary deconditioning which they share with patients suffering from other chronic diseases.

The answer is no: There is an MS-specific component (reflected in the neurological disability, i.e., the EDSS score) which reduces the exercise capacity of patients. The primary cause probably lies within the demyelination and axonal loss in the supraspinal and spinal CNS, i.e., the disturbed transmission of the central motor activation signal to the muscle. Furthermore, the perceptual mechanisms in the afferent pathways might be altered in MS patients, as well as the formation of the signal itself. The pathological correlates of the low aerobic capacity that were found in the muscles of MS patients might be secondary symptoms of this altered central motor activation and altered feedback.

However, in addition to the MS-specific component, the physical inactivity of the patients has to be considered. This second component is prevalent not only in MS, but also in a number of other chronic diseases (although it might be triggered by different reasons, e.g. the limitation of coronary blood flow in CAD and the limitation of pulmonary ventilation in CF). Physical inactivity also results in a weaker stimulation of the muscles, in secondary deconditioning and in a reduced exercise capacity.

In the case of MS, this means the peripheral implications of physical inactivity overlap with the peripheral implications of an MS-specific reduced muscular activation, and both processes are hard to distinguish from each other. The anaerobic characteristics found in muscle biopsies of MS patients are probably a mixed result of both causes, and the same can be assumed for the low overall exercise capacity of our subjects.

4.11 How central and peripheral factors could cause low peak lactate levels

Kenney et al. mention three mechanisms of lactate clearance (and re-utilization of lactate as a metabolite) from the working muscle cell (2012, p. 128):
1) direct oxidation of the lactate by mitochondria in the same muscle cell;
2) transportation out of the muscle fiber and uptake by cells with a high density of mitochondria, like other type I (oxidative) muscle fibers, cardiac muscle, and liver cells; 3) reconversion into glucose in the liver and transportation back to the working muscle.

As MS patients show distinct anaerobic muscle characteristics and have a higher percentage of type II (non-oxidative) muscle fibers, it can be concluded that their lactate clearance via pathways 1) and 2) will probably be reduced. Regarded isolated, this should lead to higher lactate concentrations in the muscle cells of MS patients in comparison to healthy individuals.

The results of a study conducted by Näveri et al. with patients suffering from congestive heart failure are interesting in this context: After exhaustive exercise, these patients actually showed higher lactate concentrations in active muscle cells than healthy controls, while at the same time displaying lower blood lactate concentrations (Näveri et al. 1997). One reason that was proposed to explain this discrepancy is a reduced peripheral blood flow, resulting in a slower lactate clearance from the muscle cells.

Theoretically, a similar mechanism could apply for our subjects, meaning that the low peak blood lactate levels might not reflect corresponding low lactate levels in the muscle cells. In the anterior tibial muscle of MS patients, Sharma et al. (1995) indeed found a greater decrease in intracellular pH (reflecting greater lactic acidosis) compared to healthy controls. However, in this work the muscle was stimulated directly by electrical excitation of the peroneal nerve. In another work, where the muscle was contracted voluntarily by the patients, the pH was unchanged (Kent-Braun et al. 1994).

Kent-Braun et al. explained this through lack of neural muscle activation. As our subjects also performed voluntary exercise, their low peak blood lactate levels could reflect a corresponding low intracellular muscle lactate.

Possibly, a reduced lactate clearance through the pathways 1) and 2) leads to a faster accumulation of the metabolite in the cell at the onset of exercise, but

further accumulation of blood lactate is then restricted by the lack of central motor activation (or disturbed transmission of the activation signal).

Ultimately, MS patients with higher grades of disability might not be able to recruit the muscle mass necessary to produce blood lactate concentrations of 6 or 8 mM/l. Moreover, the muscle mass ready for recruitment might be reduced due to the ongoing atrophy of muscle fibers in MS.

Viewed from this perspective, the low peak blood lactate levels could actually be used as an indicator: If they really reflect the MS-specific lack of neuromuscular activation and the muscular atrophy, they could serve as a criterion to discriminate between poor exercise performance due to physical inactivity, which can also be found in healthy subjects (low performance but normal peak blood lactate levels), and poor exercise performance due to MS-specific disability (low performance and low peak blood lactate concentration).

In group R (EDSS 0–3.5), we determined peak blood lactate levels that were still lower than normal, but considerably higher than the peak blood lactate levels of group P (EDSS 4–6). It can be hypothesized that a group of MS patients even less disabled than group R, e.g. with an EDSS score of 0–2, would have reached higher peak blood lactate levels bordering on the levels described for healthy, untrained individuals. This is supported by the highly significant correlation that was found in the pooled group between the EDSS score of the patients and the peak blood lactate they accumulated (see Fig. 20).

4.12 A model for the curvilinear blood lactate profiles in group P and group R

We were able to demonstrate that both in group P and in group R, the peak blood lactate values were linked to the maximal workload reached by the patients, similar as in patients suffering from angina pectoris (Karlsson et al. 1984; Pokan et al. 2004a): The subjects who reached the lowest maximal workloads at volitional exhaustion also tended to reach the lowest peak blood lactate levels and vice versa (see Fig. 22 and 23). This interrelation is, to our knowledge, a new finding for MS patients.

In healthy individuals, submaximal workload (as opposed to maximal workload) is typically linked to blood lactate levels: If a healthy individual breaks off an exercise test at a low workload before reaching exhaustion, the blood lactate level at this point will also be low. However, if a healthy subject achieves volitional

exhaustion in a bicycle ergometry, this subject usually reaches a peak blood lactate level above 8 mM/l (or 6 mM/l, for women), even if it is untrained and the individual maximal workload is low. This is underlined by the fact that a peak blood lactate concentration of 8 mM/l or above has actually been used as a criterion for true exhaustion in untrained subjects performing incremental ergometry protocols.

A modification of Fig. 5 illustrates the difference between the typical left-shift of the lactate curve that reflects a poor training status in healthy individuals and the reduced peak blood lactate levels, coupled to reduced maximal workload, which we found in the two groups of MS patients (see Fig. 24). Importantly, the course of the lactate curves is not based on real measurements, but on the assumption that the curves of MS patients show a similar hyperbolic profile as the curves of healthy subjects. The only data points that were determined experimentally are the peaks of the lactate curves in group P and group R.

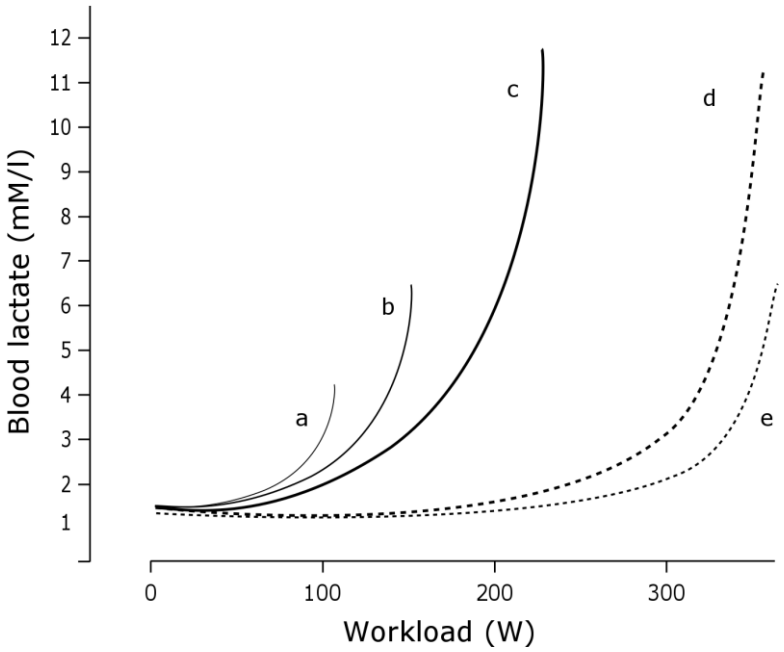

Fig. 24: Schematic curvilinear blood lactate profiles of a) group P (PPMS/SPMS, EDSS 4–6); b) group R (RRMS, EDSS 0–3.5); c) untrained healthy individual, d) trained healthy individual and e) endurance-trained marathon runner. Redrawn and modified after Rost (2001).

Fig. 24 also points out the difference between the blood lactate curves of MS patients and the blood lactate curves of endurance-trained subjects such as marathon runners, who exhibit low peak blood lactate levels as well, but reach their lactate peak at a much higher workload.

However, there is one similarity between the MS patients and the marathon runners: In both cases, the performance seems to be limited by factors other than the accumulation of lactate and the resulting lactic acidosis. In the case of the MS patients, the limiting factor was probably a lack of neural muscle activation. In the case of marathon runners, according to Kenney et al. (2012, p. 132), "the exercise-limiting fatigue is likely caused by inadequate energy supply, not excess lactic acid".

4.13 Interrelations of performance markers and possible influencing factors

Both in group P and group R, the peak performance markers were significantly correlated with each other (see Table 4). The highest correlations were found between W_{max}/kg and $\dot{V}O_{2peak}$/kg. This close interrelation has already been described for patients with RRMS and minimal disability by Motl et al. (2012). In their work, the W_{max} reached by the patients in a maximal, incremental bicycle ergometry was demonstrated to be an accurate predictor for $\dot{V}O_{2peak}$.

To identify possible factors influencing the exercise capacity in MS patients, we conducted an explorative analysis for correlations between the two main peak performance markers (W_{max}/kg and peak blood lactate) and patients' EDSS scores, walking ability (6MWT), depression and fatigue.

In group P, the 6MWT results correlated with W_{max}/kg, reflecting a relation between patients' mobility and exercise capacity. This makes sense insofar as the disability range in this group (EDSS 4–6) is defined to a large extent by the walking ability. Besides this relationship, no significant correlation was identified between the main peak performance markers and the total distance in the 6MWT.

Neither in group P nor in group R, significant interrelations were found between the peak performance markers and depressive symptoms or fatigue. This is consistent with the results of Rasova et al. (2005), who failed to find significant correlations between spiroergometric parameters and fatigue or depression in a study with 112 MS patients (mixed disease courses, mean EDSS 3.07). However, the Rasova group identified a significant correlation between depressive symptoms and fatigue. In the present work, this correlation was also significant in group P, but not in group R (see section 3.4).

Similarly, the EDSS score was significantly correlated to depression and fatigue in group P, but not in group R. As the total scores for depression and fatigue did

not differ significantly between group P and group R, one interpretation for these results is that in the progressive disease course, these symptoms occur less random, but more aligned with each other and with the advancing disability.
EDSS scores showed a significant inverse correlation with W_{max}/kg in both groups, which underlines the above-mentioned suitability of this scale to roughly estimate the performance of MS patients in exercise tests.
Interestingly, the correlation between EDSS scores and the peak blood lactate level was significant only in group R, not in group P (see Fig. 16 and 18). This might be a consequence of the small section of EDSS scores (4–6) in group P: It includes only five possible data points for EDSS scores (4.0 / 4.5 / 5.0 / 5.5 / 6.0), while the section in group R (0–3.5) includes seven data points (0.0 / 1.0 /1.5 / 2.0 / 2.5 / 3.0 / 3.5). Probably, the EDSS section in group P was just too small to detect the correlation: When the EDSS section was expanded by merging both groups into a bigger pooled group, the relation between EDSS scores and the peak blood lactate level became highly significant (see Fig. 20). It is remarkable that the best-fit line in this scatterplot actually starts at a concentration of 8 mM/l for the EDSS score of 0.0, meaning that peak blood lactate levels are the same as in healthy individuals in the non-disabled MS patients and then get worse with every 0.5 step on the EDSS score.

4.14 Conclusion

In this work, two different groups of MS patients were characterized by measuring the peak performance markers in an exhaustive bicycle spiroergometry.
Patients with a progressive disease course and a higher grade of disability according to the EDSS score were demonstrated to achieve significantly lower peak performance markers. A comparison of the peak performance data with reference values, as well as a highly significant correlation between EDSS scores and maximal workload, suggests that the EDSS score allows an estimation of the performance of MS patients in exercise tests.
A central finding of the present work is that the peak blood lactate response of MS patients is reduced in comparison to healthy, untrained individuals. This was the case in both groups, but more pronounced in patients with a progressive disease course and a higher EDSS score.

The peak blood lactate response in both groups was linked to the maximal workload. This finding is, to our knowledge, new for MS patients, though it has been described for patients with other chronic medical conditions. After reviewing the existing literature on peripheral and central causes for exercise-limiting fatigue in MS, it can be hypothesized that MS patients undergo all three phases of energy supply during spiroergometry, but pass the thresholds between these phases at lower blood lactate levels than healthy individuals. This raises the question if the lactate performance tests used routinely for healthy individuals can be transferred to MS patients, especially those with higher grades of disability and progressive disease courses.

The limitations in exercising and a reduced peak blood lactate response can be theoretically explained by a combination of two interdependent causes.

The first is an MS-specific central component, specifically a disturbed transmission of the activation signal from the motor cortex to the muscle. Moreover, the formation of this signal in the motor cortex might be altered, as well as afferent feedback mechanisms, limiting the adequate central processing of muscular sensations and thus perturbing motor adaptation. This leads to an inadequate neuromuscular activation and results in a secondary deconditioning of the muscles, eventually not only limiting maximal voluntary muscle contraction, but also preventing MS patients from recruiting enough muscle mass to produce normal peak lactate levels.

The second factor limiting the exercise capacity of MS patients is probably physical inactivity, which is a common behavior in MS as well as in other chronic diseases and in parts of the healthy population. Because physical inactivity creates a “non-pathologic” lack of neuromuscular activation and thus also results in secondary deconditioning, its long-term effects on the muscles are hard to distinguish from the pathological changes due to MS.

4.15 Limitations and prospect

The present work has several limitations. To begin with, there were no matched groups of healthy controls for the examined groups of MS patients. Instead, adjusted reference values from the literature had to be used.

Different spiroergometric protocols were used not only for the different groups, but also within group P. This is the result of an optimization process over time

and might even have some value as a feasibility result (see section 4.2), but it complicates the comparability of peak performance markers between the groups and within group P.

Instead of measuring the peak blood lactate levels after exhaustion, it would have been better to obtain complete curvilinear blood lactate profiles of all patients. The curves would have allowed drawing more detailed conclusions about the comparability of blood lactate dynamics between healthy subjects and MS patients. For example, a selection of lactate threshold concepts, including the individual anaerobic threshold, could have been applied to the lactate curves of the two groups and tested for feasibility. However, this would have required a stepwise increase of workload. In the exercise tests analyzed for this work, spiroergometric ramp protocols with a linear increase were used.

To clarify the complex process of exercise limitation, MS patients could have been interrogated after reaching volitional exhaustion about their exact reasons for stopping the ergometry (e.g., shortage of breath, pain in the legs, a feeling of muscular fatigue, spasticity etc.).

In future trials investigating the limitation of exercise and the blood lactate dynamics of MS patients, the patients' perceived exhaustion should be measured throughout the spiroergometry and at the point of exhaustion. Especially in the case of group P, it would have been important to know if the patients felt physically exhausted when they stopped cycling. The rated perceived exertion (RPE) scale has been used to this end in other exercise trials with MS patients, but the results have been contradictory so far: Morrison et al. found similar RPE levels in MS patients as in controls at submaximal and maximal exercise levels (Morrison et al. 2008), while Hansen et al. found significantly higher RPEs in MS patients compared to controls, explaining the difference through greater difficulties in muscle coordination patterns (Hansen et al. 2013). This topic seems to be unresolved and worth investigating.

5. Summary

In the present work, two groups of MS patients were characterized by analyzing their peak performance in an exhaustive, incremental bicycle spiroergometry. The groups differed regarding the disease course (primary- or secondary-progressive MS in group P vs. relapsing-remitting MS in group R) and the grade of disability according to the expanded disability status scale (EDSS; 4–6 in group P vs. 0–3.5 in group R). The main objective was to determine and compare the peak blood lactate response to exercise and the maximal workload and to investigate their interrelation.

In group P (n=41), subjects reached a mean peak blood lactate level of 4.1 mM/l and a mean maximal workload of 1.5 W/kg. In group R (n=25), the mean peak blood lactate level was 6.5 mM/l and the mean maximal workload was 2.1 W/kg. Spiroergometric peak performance in both groups was significantly lower than predicted for healthy, untrained individuals. Group R reached significantly higher values in all performance markers than group P, and peak blood lactate was correlated with maximal workload in both groups.

The peak blood lactate response was lower than normal in both groups, but the effect was more pronounced in group P: Only 5 out of 41 subjects in this group reached the blood lactate level expected at volitional exhaustion (≥ 8 mM/l for men and ≥ 6 mM/l for women). Less than half of the subjects reached the level of 4 mM/l, which is still routinely used in exercise tests as a fixed marker for aerobic capacity. A reduced peak blood lactate response to exercise has previously been described in other chronic diseases but is, to our knowledge, a novel finding for MS patients.

As exercise interventions and exercise tests are becoming more common in MS, these results call into doubt if the lactate performance tests and lactate thresholds used for healthy individuals can simply be transferred to MS patients.

Our results also raise the question which processes limit the exercise capability of the subjects in this study. After reviewing the literature, it seems probable that central manifestations of the disease cause a pathologic lack of neuromuscular activation and feedback. This aberrant motor processing, combined with the resulting atrophy and changes in the muscles' energy metabolism and fiber composition, presumably limits the exercise capacity and the muscular lactate production in MS patients. A habitual lack of physical activity, which is frequently seen not only in MS, might contribute to the limitation of exercise capability by causing a "physiologic" lack of neuromuscular activation, resulting in further muscular deconditioning.

6. List of Abbreviations

6MWT	six minute walking test
ANOVA	analysis of variance
ATP	adenosine triphosphate
BMI	body mass index
CAD	coronary artery disease
CF	cystic fibrosis
Cl^-	chloride
CNS	central nervous system
CO_2	carbon dioxide
CoA	coenzyme A
COPD	chronic obstructive pulmonary disease
CSF	cerebrospinal fluid
DMSG	German Multiple Sclerosis Society
DMT	disease-modifying therapy
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders (4^{th} edition)
EDSS	Expanded Disability Status Scale
EMG	electromyography
H^+	proton
H_20	water
HAQUAMS	Hamburg Quality of Life Questionnaire in Multiple Sclerosis
HCO_3^-	bicarbonate
HR_{max}	maximal heart rate
$IDS\text{-}SR_{30}$	self-reported 30-item Inventory of Depressive Symptoms
LT	lactate threshold
M	mean
MEG	magnetoencephalography
MFIS	modified fatigue impact scale
MLSS	maximal lactate steady state
MRI	magnetic resonance imaging
MS	multiple sclerosis
O_2	oxygen

PML	progressive multifocal leukoencephalopathy
PNS	peripheral nervous system
PPMS	primary-progressive MS
PRMS	progressive-relapsing MS
QIDS-SR_{16}	self-reported 16-item Quick Inventory of Depressive Symptoms
RPE	rated perceived exertion
RRMS	relapsing-remitting MS
RCT	randomized controlled trial
rPAR-Q	revised physical activity readiness questionnaire
RQ	respiratory quotient
RQ_{peak}	peak respiratory quotient
SD	standard deviation
SDH	succinic dehydrogenase
SPMS	secondary-progressive MS
UKE	University Hospital Hamburg-Eppendorf
$\dot{V}E$	ventilation
$\dot{V}CO_2$	exhaled CO_2 volume
$\dot{V}O_2$	inhaled O_2 volume
$\dot{V}O_{2max}$	maximal oxygen uptake
$\dot{V}O_{2peak}$	peak oxygen uptake
VT	ventilatory threshold
WHO	World Health Organization
W_{max}	maximal workload

7. Table Legend (6 Tables)

8. Figure Legend (24 Figures)

9. List of References

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11. Curriculum Vitae (entfällt in der elektronischen Version)

12. Eidesstattliche Versicherung

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Meta Josephina Maier

Hamburg, Mai 2015