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Altered signaling for mitochondrial and myofibrillar biogenesis in skeletal muscles of multiple sclerosis patients

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Abstract

Patients with MS (pwMS) experience muscle weakness and lowered muscle oxidative capacity. To explore the etiology for the development of such muscle phenotype we studied skeletal muscle AMP-activated protein kinase (phospho-AMPKα, governing mitochondrial biogenesis) and mammalian target of rapamycin (phospho-mTOR, governing myofibrillar biogenesis) phosphorylation in pwMS. After assessment of body composition, muscle strength, exercise tolerance and muscle fiber type, muscle phospho-AMPKα and phospho-mTOR was assessed in 14 pwMS and 10 healthy controls (part 1). Next, an endurance exercise bout was executed by 9 pwMS and 7 healthy subjects, with assessment of changes in muscle phospho-AMPKα and phospho-mTOR (part 2). Elevated basal muscle phospho-AMPKα and phospho-mTOR was present in MS ($p<0.01$) and independently related to MS. Correlations between muscle phospho-AMPKα or phospho-mTOR and whole-body fat mass, peak oxygen uptake and expanded disability status scale ($p<0.05$) were found. After endurance exercise muscle phospho-AMPKα and phospho-mTOR remained elevated in pwMS ($p<0.01$). Muscle signaling cascades for mitochondrial and myofibrillar biogenesis are altered in MS and related to impairment and disability level. These findings indicate a link between muscle signaling cascades and level of disability/impairment, and thus may open a new area for the development of novel therapies for peripheral muscle impairment in MS.

Keywords: multiple sclerosis, AMPK, mTOR, skeletal muscle biochemistry, exercise

Abbreviations

AMPK, AMP-activated protein kinase

BMI, body mass index

CSA, cross-sectional area

HR, heart rate

pwMS, patients with MS

MS, multiple sclerosis

mTOR, mammalian target of rapamycin

RER, respiratory gas exchange ratio

RPE, ratings of perceived exertion

VO2peak, peak oxygen uptake

Wmax, cycling power output

Introduction

Multiple sclerosis (MS) is associated with peripheral muscle alterations such as muscle weakness and lowered muscle oxidative capacity [1-3]. In accordance, a smaller type 1 and 2 skeletal muscle fiber diameter, lower succinate dehydrogenase activity, delayed phosphocreatine resynthesis after isometric exercise, blunted intramuscular metabolic responses during isometric exercise and complex-1 deficiency in skeletal muscle mitochondria is present in patients with MS (pwMS) [4-9]. These data collectively indicate significantly disturbed skeletal muscle cell biochemistry and composition in pwMS.

Although inactivity, which is associated with pwMS [10], could contribute to muscle weakness and lowered endurance exercise tolerance, it remains unknown whether the above-mentioned biochemical skeletal muscle cell and fiber abnormalities are also related to disturbed molecular signaling pathways.

Two 'master switches' (skeletal muscle kinases) are known to mediate muscle biochemistry and composition in humans: AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) [11,12]. AMPK is a serine-threonine (Ser/Thr) kinase which is activated during repetitive muscle contractions, hypoxemia, ischemia, oxidative stress, metabolic poisoning and nutrient deprivation [11]. Such AMPK activation in skeletal muscle cells enhances mitochondrial biogenesis through phosphorylation of PGC1α and hereby elevates skeletal muscle oxidative capacity [11]. mTOR is a Ser/Thr kinase which is activated by heavy-load muscular contractions, anabolic hormones (insulin-like growth factor-1, insulin) and high amino acid availability [12]. Activation of mTOR leads to myofibrillar biogenesis and hereby lean tissue mass gain [12].

Examining skeletal muscle AMPKα and mTOR phosphorylation in pwMS might provide important clues to the etiology of disturbed skeletal muscle biochemistry and composition, and would be promising to improve treatments for peripheral muscular impairment in MS. In

the present study, we examined basal skeletal muscle AMPKα and mTOR phosphorylation in pwMS and hypothesized that significant anomalies in AMPKα and/or mTOR phosphorylation are present in skeletal muscle cells of pwMS.

To counteract muscle wasting and lowered muscle oxidative capacity pwMS participate in exercise therapy interventions [13]. The demonstration of altered skeletal muscle AMPK and/or mTOR 'master switch' pathways in MS could enhance the level of evidence of peripheral muscle involvement and might have important consequences for treatment strategies. However, changes in skeletal muscle AMPKα and mTOR phosphorylation to acute exercise in pwMS are presently unknown. In healthy subjects, acute endurance exercise significantly alters muscle AMPKα and mTOR phosphorylation [14-21]. These changes are instrumental to mitochondrial and myofibrillar biogenesis. To optimize the selection of training modalities and to induce significant beneficial changes in muscle phenotype of pwMS, the biochemical responses of skeletal muscle cells to acute exercise should be studied. In the second part of the present study, we studied the effect of acute endurance exercise on skeletal muscle AMPKα and mTOR phosphorylation in pwMS. We hypothesized that disturbed changes in muscle AMPKα and/or mTOR phosphorylation are present in pwMS after exercise.

Materials and methods

Study design

This study was part of a large study examining the impact of exercise training in MS (executed from March 2013 to June 2013). This was a combination of a cross-sectional study (part 1) and a prospective observational study (part 2) (Figure 1). Basal phosphorylation of skeletal muscle AMPKα (phospho-AMPKα) and mTOR (phospho-mTOR) was studied in Caucasian pwMS and healthy subjects in part 1, and the impact of acute endurance exercise on phospho-AMPKα and phospho-mTOR was examined in a subsample of subjects in part 2. Following expanded disability status scale (EDSS) determination [22] and screening of medication intake, body weight and height was measured from which body mass index (BMI) was calculated. Next a dual x-ray absorptiometry scan was executed to analyze body composition, a dynamometry test was executed in pwMS to determine which leg was the weakest, and a maximal cardiopulmonary exercise test was executed to asses peak oxygen uptake (VO_{2peak}). One week later subjects came to the laboratory in fed condition between 9-11AM for the collection of a muscle biopsy. A subgroup of participants then completed an endurance exercise bout, after which a second muscle biopsy of the same leg was collected. Subjects were advised to refrain from heavy exercise or exercise training for at least 3 days ahead of laboratory visit.

Participants

Potential study candidates with MS were approached by telephone from a list of pwMS that have contributed to previous studies and healthy participants were searched by local advertisements.

Part 1: 14 pwMS were compared with 10 healthy subjects. These subjects were primarily matched for age, gender and BMI.

Part 2: 9 pwMS were compared with 7 healthy subjects before and after an acute endurance exercise bout. These subjects were primarily matched for age, gender and BMI. Exercise volume and intensity was matched between groups. The sample of part 2 was smaller because from some subjects we were unable to collect a second muscle biopsy, too small muscle samples were collected or subjects refused to undergo a second muscle biopsy.

Patients with MS had been diagnosed for at least 12 months ahead of study. None of the participants were diagnosed with cardiovascular, renal or pulmonary disease. The research was carried out according to The Code of Medical Ethics of the World Medical Association (Declaration of Helsinki), informed consents were obtained from all subjects, and the author's institutional review board (Jessa Hospital, Hasselt) approved the study.

Sample size was primarily based on previous studies examining the impact of acute endurance exercise on phospho-AMPKα and phospho-mTOR [14-21]. In these studies 6-16 participants were studied and significant changes with sufficient statistical power in phospho-AMPKα and phospho-mTOR due to acute exercise were found.

Primary outcome measures

Skeletal muscle phospho-AMPKα and phospho-mTOR.

Secondary outcome measures

Skeletal muscle fiber type composition, body composition, peak oxygen uptake.

Measurements

Maximal cardiopulmonary exercise test

Peak oxygen uptake (VO_{2peak}) and cycling power output (W_{max}) were assessed during an incremental exercise test till exhaustion on an electronically braked cycle ergometer (cycling

frequency of 70rpm) using a 1-min work stage protocol [23]. VO_2 and respiratory gas exchange ratio (RER) measurements were performed continuously (Jaeger Oxycon, Erich Jaeger GmbH, Germany) to assess peak oxygen uptake (VO_{2peak}). Cardiac function was monitored using a 12-lead electrocardiogram with heart rate (HR) recorded continuously. Peak cycling power output (W_{peak}) was reported. All subjects were encouraged to exercise until volitional exhaustion and the test was ended when the subjects could not maintain a cycling frequency of >55rpm.

Body composition

Following body weight (mechanical column scale with beam, Seca, Birmingham, UK) and length assessment, segmental and whole-body fat mass and lean tissue mass were determined using whole-body dual x-ray absorptiometry (DXA; Lunar DPXL, Wisconsin, USA) [24]. Lean tissue mass of the biopsied leg was also measured.

Muscle strength

Maximal voluntary unilateral knee-extensor strength of both legs was evaluated on an isokinetic dynamometer in pwMS only (Biodex Medical Systems®, Shirley, New York, USA). After a 5-minute standardized warm-up on a cycle ergometer, strength tests were performed in a seated standardized position on a backward inclined (5°) chair [25]. Two maximal isometric knee-extensions (4 seconds) at knee angle of 45° were performed. The highest isometric extension torque (Nm) of the manually smoothed curves at this knee angle was selected as maximal isometric torque. The weakest leg was then selected as the leg from which a muscle biopsy was taken in pwMS. A maximal isometric knee extension torque of 107±46Nm was observed in the weakest leg, and of 125±39Nm was observed in the strongest leg (leading to a 26±38% difference in peak torque between legs in pwMS).

Skeletal muscle fiber cross sectional area and AMPKα and mTOR phosphorylation

Muscle biopsies were obtained from the middle part of the m. quadriceps femoris vastus lateralis (Bergström needle technique). Muscle biopsies of pwMS were obtained from the weakest leg, as determined by dynamometry test. In healthy controls muscle biopsies were obtained from a random leg. The collected muscle tissue was freed from connective tissue and immediately embedded in Tissue-Tek, frozen in isopentane cooled with liquid nitrogen and stored at -80°C until analysis.

Immunohistochemistry. Serial transverse sections (9µm) from muscle samples were cut at -20°C and stained by means of ATPase histochemistry, after preincubation at pH 4.4, 4.6 and 10.3. The serial sections were visualized and analyzed using a Leica DM2000 microscope and a Leica Hi-resolution Color DFC camera (Leica, Stockholm, Sweden) combined with image-analysis software (Leica Qwin, Leica, Stockholm, Sweden). On average 170±10 fibers were calculated and included in the cross-sectional area (CSA) and fiber type analyses.

AMPK and mTOR phosphorylation. Skeletal muscle AMPKα phosphorylation (phospho-AMPKα Thr172) and mTOR phosphorylation (phospho-mTOR Ser2448) were examined by commercially available ELISA kits (Cell Signaling Technology Inc.) according to manufacturer's protocol and as executed in previous studies [26,27]. Phospho-AMPKα and phospho-mTOR was measured spectrophotometrically at an absorbance of 450 nm.

Endurance exercise bout

In part 2, participants completed a single endurance exercise bout on an electronically braked cycle ergometer. All pwMS had to complete 3 6-min exercise bouts at 70% of W_{peak}, interspersed by a 6-min recovery. In order to match relative exercise intensity and exercise

volume, healthy subjects were instructed to exercise at 70% of W_{peak} in bouts of 6 min, but for a shorter total exercise duration (third exercise bout was shorter or absent) to elicit an equal caloric expenditure. As a result, exercise intensity and caloric expenditure was hereby matched between groups. HR was monitored continuously by ambulatory monitor (Polar, Kempele, Finland) (from which caloric expenditure was calculated) and ratings of perceived exertion (RPE, an a 20-point scale) were requested after each exercise bout. During and after exercise only water was consumed. A second skeletal muscle biopsy was collected at 34±8 minutes after exercise.

Statistical analysis

SPSS v. 22.0 was used for statistical analyses. Data were expressed as means±SD. According to Shapiro-Wilk tests data were not normally distributed. To compare parameters between pwMS and healthy controls Mann-Whitney U-tests were used in part 1. Univariate relations between phospho-AMPKα or phospho-mTOR and subject characteristics (age, body weight and height, BMI, VO_{2peak}, whole-body fat mass, whole-body and leg lean tissue mass, EDSS, isometric leg extension peak torque, muscle fibre CSA) were examined by Spearman correlations. Next, a multivariate linear regression model was created in which relations between phospho-AMPKα or phospho-mTOR and subject characteristics (age, gender, body weight and height, BMI, presence of MS) were examined. To assess the impact of acute exercise on muscle phospho-AMPKα and phospho-mTOR Wilcoxon Signed Ranks tests were applied in part 2. Relations between parameters were examined by Spearman correlations. Statistical significance was set at $p<0.05$ (2-tailed). Observed statistical power was calculated by use of GPower, v. 3.1.2.

Results

Part 1: comparison between pwMS and healthy subjects

Subject characteristics

Fourteen pwMS (disease duration: 9.6±6.5 years, n=3 with SPMS, n=9 with RRMS, n=2 with PPMS) and 10 healthy participants were included in part 1 (Table 1). Between groups subject characteristics were comparable ($p>0.05$), except for type 2a muscle fiber CSA and VO_{2peak} ($p<0.05$).

AMPKα and mTOR phosphorylation

Muscle phospho-AMPKα (1.57±0.42 mg/ml in pwMS vs. 1.14±0.24 in healthy subjects, $p<0.01$, observed statistical power α=0.82) and phospho-mTOR (0.64±0.15 mg/ml in pwMS vs. 0.29±0.02 in healthy subjects, $p<0.001$, observed statistical power α=0.99) was significantly higher in pwMS as opposed to healthy subjects (Figure 2).

Regression analysis

A trend for an independent relation (model r=0.62 and p=0.16) between muscle phospho-AMPKα and MS was found (p=0.064). Muscle phospho-mTOR (model r=0.89 and $p<0.001$) was independently related to MS ($p<0.001$).

Correlations

Significant relations ($p<0.05$) were found between muscle phospho-AMPKα and whole-body fat mass (r=0.54), VO_{2peak} (r=-0.47), EDSS (r=0.60), and between muscle phospho-mTOR and VO_{2peak} (r=-0.58), whole-body fat mass (r=0.55), and EDSS (r=0.74). Muscle phospho-AMPKα correlated significantly ($p<0.05$) with muscle phospho-mTOR (r=0.67).

Part 2: impact of acute endurance exercise

Subject characteristics

Nine pwMS (disease duration: 9.4±5.8 years, n=2 with SPMS, n=6 with RRMS, n=1 with PPMS) and 7 healthy participants were included in part 2 (Table 2). Between groups body height, VO_{2peak} and fat mass was different ($p<0.05$).

Exercise bout characteristics

Although cycling power output was significantly higher in healthy controls (167±58 W) when compared to pwMS (96±41W, $p<0.01$), the observed exercise intensity (%HR_{peak} 89±10% vs. 90±5%, respectively), ratings of perceived exertion (12.7±3.8 vs. 12.9±1.7), and caloric expenditure (123±48 kcal vs. 117±48 kcal, respectively) were comparable between groups ($p>0.10$).

AMPKα and mTOR phosphorylation

Basal muscle phospho-AMPKα was significantly ($p<0.05$) different between groups (1.46±0.24 mg/ml vs. 1.09±0.26 mg/ml in pwMS vs. healthy subjects, respectively, observed statistical power α=0.76), as well as muscle phospho-mTOR (0.63±0.14 mg/ml vs. 0.29±0.02 mg/ml in pwMS vs. healthy subjects, respectively, observed statistical power α=0.98) (Figure 3). After endurance exercise muscle phospho-AMPKα was significantly ($p<0.01$) different between groups (1.66±0.51 mg/ml vs. 1.12±0.23 mg/ml in pwMS vs. healthy subjects, respectively, observed statistical power α=0.69) as well as muscle phospho-mTOR (0.71±0.19 mg/ml vs. 0.28±0.02 mg/ml in pwMS vs. healthy subjects, respectively, observed statistical power α=0.96). Within pwMS and healthy subjects muscle phospho-AMPKα and phospho-mTOR did not change significantly ($p>0.05$). Relative change in muscle phospho-AMPKα (+19±53% vs. +4±13% in pwMS vs. healthy subjects, respectively) and muscle phospho-

mTOR (+14±20% vs. -1±10% in pwMS vs. healthy subjects, respectively) after endurance exercise was comparable between groups ($p>0.10$).

Correlations

In total group, changes in muscle phospho-AMPKα and muscle phospho-mTOR after endurance exercise correlated significantly ($r=0.62$, $p<0.05$). Relative change in muscle phospho-AMPKα correlated significantly with exercise HR ($r=0.73$, $p<0.01$) and exercise $\%HR_{peak}$ ($r=0.77$, $p<0.01$).

Discussion

In the present study basal skeletal muscle AMPKα phosphorylation (phospho-AMPKα) and mTOR phosphorylation (phospho-mTOR) was significantly elevated in ambulatory patients with multiple sclerosis (pwMS). Such elevation in phospho-AMPKα and phospho-mTOR was related to greater whole-body fat mass, higher expanded disability status scale (EDSS), and lower peak oxygen uptake (VO_{2peak}). Muscle phospho-AMPKα and phospho-mTOR remained elevated after endurance exercise in pwMS.

After proper matching of age, gender and body composition between groups muscle phospho-AMPKα and phospho-mTOR were significantly elevated in pwMS (part 1). Moreover, elevated skeletal muscle phospho-AMPKα ($p=0.064$) and phospho-mTOR ($p<0.001$) was independently related to MS. According to the observed statistical power and p-values, especially basal muscle phospho-mTOR seemed to deviate in pwMS. The etiology of an elevated basal skeletal muscle phospho-AMPKα and phospho-mTOR in MS remains speculative. Even in healthy individuals, causes for basal alterations in muscle phospho-AMPKα and phospho-mTOR are highly speculative. Due to the complexity of the pathophysiology of MS we can only speculate how this disease may cause alterations in muscle phospho-AMPKα and phospho-mTOR. This limitation in current knowledge should be kept in mind throughout this discussion.

Muscle phospho-AMPKα is up regulated during repetitive muscle contractions, hypoxemia, ischemia, oxidative stress, metabolic poisoning or nutrient deprivation [11]. Muscle phospho-mTOR is up regulated when muscle cells are exposed to greater amino acid concentrations and anabolic hormones, but also after heavy-load muscular contractions [12]. In ambulatory pwMS it could be hypothesized that, at least in part, basal muscle phospho-AMPKα was increased because of changes in blood or muscle cytokine content [28] and/or vitamin D deficiency [29]. Elevation in muscle cell interleukin-18 content and the induction of acute

systemic inflammation leads to elevation in muscle phospho-AMPKα [30,31]. Moreover, supplementation of vitamin D3 lowers muscle phospho-AMPKα phosphorylation [32]. Because MS is associated with systemic inflammation and vitamin D deficiency, it could be proposed that such clinical status leads to elevations in basal muscle phospho-AMPKα. However, this hypothesis remains to be verified and it should be examined further how MS can alter basal muscle phospho-AMPKα. To explain an elevated basal muscle phospho-mTOR in MS is much more difficult. Vitamin D supplementation up regulates muscle phospho-mTOR [33] and inflammation suppresses muscle phospho-mTOR [34]. In MS a lowered muscle phospho-mTOR would thus be anticipated. It remains to be examined why the opposite result was found in our study. We advocate further studies to discover the contributing factors (physical activity, blood hormones, inflammatory markers, amino acids, genetics, immunological changes, blood vitamin D level) to an elevated basal skeletal muscle phospho-AMPKα and phospho-mTOR in MS. Such studies will very likely contribute to novel and improved treatment of peripheral muscle impairment in MS.

Even though MS was associated with an elevated basal muscle phospho-AMPKα and phospho-mTOR, exercise tolerance and cross sectional area of muscle fiber type 2a was paradoxically decreased. In fact, an elevated basal muscle phospho-AMPKα and phospho-mTOR correlated with a lower exercise tolerance (VO_{2peak}) and greater level of disability (based on EDSS). Although correlations do not indicate causality, these data may indicate that when MS progresses skeletal muscle phospho-AMPKα and phospho-mTOR increases accordingly. Such increase in basal skeletal muscle phospho-AMPKα and phospho-mTOR during clinical MS progression and worsening in disease symptoms could be due to unknown parallel physiological changes. It could be hypothesized that inappropriate downstream signaling after phosphorylation of muscle AMPKα and mTOR occurs in MS. Insufficient activation of muscle peroxisome proliferator-activated receptor-γ coactivator (PGC1α) could

occur after AMPKα phosphorylation, and suppressed protein synthesis and activation could occur after mTOR phosphorylation, in pwMS. Due to this inappropriate downstream signaling, mitochondrial and myofibrillar biogenesis is not stimulated sufficiently. In turn, skeletal muscle AMPKα and mTOR compensate by elevated phosphorylation. Future studies should focus on downstream molecular signaling after AMPKα and mTOR phosphorylation in muscle cells of pwMS to develop new treatments for prevention of muscle wasting and reduction in muscle oxidative capacity.

In part 2 of the present study no significant changes in muscle phospho-AMPKα and phospho-mTOR were found after endurance exercise in both groups. Muscle phospho-AMPKα and phospho-mTOR remained thus elevated in pwMS. However, it remained unknown whether the downstream signaling changes due to AMPKα and mTOR phosphorylation were equally affected by exercise in pwMS. Even though long-term exercise training is a successful intervention for increasing oxidative capacity and muscle strength in pwMS [13], it remains thus uncertain whether the magnitude of improvement would be the same in healthy subjects when following the same exercise intervention.

However, a greater relative exercise intensity (based on heart rate) correlated with greater increases in muscle phospho-AMPKα. These data are in line with previous observations in healthy subjects and illustrate that endurance exercises of greater intensities are more likely to stimulate mitochondrial biogenesis and thus also improve oxidative capacity or endurance capacity [35].

It may be questioned whether the observed reductions in VO_{2peak} and/or type 2a muscle fiber type diameter in pwMS are mainly due to inactivity or altered skeletal muscle phospho-AMPKα and phospho-mTOR. Unfortunately, physical activity was not measured by objective methods in the present study. It thus follows that there only can be speculated whether the observed reduction in type 2a muscle fiber diameter and endurance capacity in pwMS is due

to physical inactivity or alterations in muscle signaling. It is very likely that the examined pwMS were physically less active when compared with their healthy counterparts [36]. However, when pwMS were exposed to endurance exercise of the same intensity and volume (in part 2), as in healthy subjects, anomalous skeletal muscle phospho-AMPKα or phospho-mTOR remained present. It thus follows that when physical activity level is acutely restored in pwMS, skeletal muscle signaling for mitochondrial and ribosomal biogenesis remains anomalous. These findings seem to indicate that altered skeletal muscle signaling cascades for mitochondrial and ribosomal biogenesis in pwMS can, at least in part, be instrumental to lowered muscle strength and/or endurance capacity, regardless of physical activity. However, future studies should examine the relations between skeletal muscle phospho-AMPKα or phospho-mTOR and long-term physical activity in pwMS.

Because of anomalous muscle signaling cascades for mitochondrial and ribosomal biogenesis in pwMS, it could be hypothesized that a different selection of training modalities during exercise intervention should be preferred in clinical practice. To maximize muscle phospho-AMPKα and phospho-mTOR (or downstream signaling cascades), prolonged participation (at least 8 weeks), greater exercise volumes and frequencies (at least 3 days/week) and higher endurance and strength training intensities are preferable. Despite the application of a sufficient exercise volume, frequency and program duration in most rehabilitation programs for pwMS, most often low-to-moderate exercise intensities are selected [37,38] in the belief that greater exercise intensities are intolerable and/or could provoke exacerbations. However, VO_{2peak} and whole-body lean tissue mass increases with significantly greater magnitude when following a high-intensity interval endurance training program vs. a continuous moderate-intense endurance training program in pwMS (Wens et al., submitted observations), even though this new type of training was feasible and safe (no orthopedic injuries, greater drop-out or exacerbations). Another study found non-significant greater improvements in walking

capacity, but unfortunately greater drop-out rates and adverse events in the high-intensity trained group [39]. It thus should be studied further how to safely implement high-intensity endurance exercise training in pwMS to prevent premature drop-out but maximize the clinical benefits. To stimulate muscle phospho-mTOR and hence lean tissue mass gain or muscle strength, greater strength training intensities and/or volumes may be preferred [40], although more studies are warranted to verify these findings. Moreover, when voluntary muscle contractions are difficult for pwMS, muscle electro stimulation programs may be initiated: this type of high-intensity muscle strength training in very effective to improve muscle strength, muscle mass and endurance capacity in pwMS [41-43]. As to whether (high-intensity) endurance or strength training is capable of improving muscle phospho-AMPKα and phospho-mTOR (downstream signaling cascades) in pwMS remains however to be studied.

In the present study a muscle biopsy was obtained from the weakest leg in pwMS, and from a random leg in healthy participants. In healthy subjects, peak isometric or isokinetic leg extension torques are not very different between dominant and non-dominant leg (up to ~5%), at least in subjects not involved in specific sports disciplines and without leg injuries [44,45]. However, the lack of a great difference in quadriceps muscle strength between dominant and non-dominant leg in healthy persons does not guarantee equal muscle phospho-AMPKα or phospho-mTOR between different legs. In pwMS, differences in quadriceps muscle strength between dominant and non-dominant leg were significant: a 26±38% difference between legs was noticed in our study. It remains thus to be studied whether muscle phospho-AMPKα or phospho-mTOR varies according to leg dominance in MS patients.

In this study certain limitations were present. The study sample size was low (with small subgroups of RRMS, SPMS and PPMS patients), although the observed statistical power for detection of differences in muscle phospho-AMPKα and phospho-mTOR between groups was

sufficient. A larger group of MS patients should be examined to be able to understand the impact of different MS subtypes and/or pharmacotherapy on muscle phospho-AMPKα and phospho-mTOR. In addition, EDSS was relatively low: pwMS with higher EDSS remain to be studied. Drop-out rate from the first to the second part of the study was high. In this study, it was not examined whether neurological deficits were present in the biopsied leg of pwMS. Insufficient quantity of muscle tissue was collected to study downstream signaling cascades after phosphorylation of AMPKα and mTOR.

In conclusion, basal and post-exercise skeletal muscle AMPKα and mTOR phosphorylation is elevated in MS, and this elevation is related to lowered exercise tolerance and greater level of disability. These data provide insights in etiology of peripheral muscle impairment in MS and could open an area for the development and study of novel therapies for peripheral muscle impairment.

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Figure legends

Figure 1

Thirty pwMS and 15 healthy controls were invited to participate in the present study. From those subjects, 14 pwMS and 10 healthy controls fully participated in part 1 of the study, and 9 pwMS and 7 healthy controls fully participated in part 2.

Figure 2

Muscle phospho-AMPKα and phospho-mTOR was significantly higher in pwMS as opposed to healthy subjects.

Figure 3

Basal muscle phospho-AMPKα and phosph-mTOR was significantly different between groups before exercise. After endurance exercise muscle phospho-AMPKα and phospho-mTOR remained different between groups.

Table 1 Subject characteristics in part 1

	MS patients	healthy subjects	p-value
general characteristics			
n	14	10	
age (y)	48 ± 9	48 ± 8	0.93
males (n of total group)	10	6	0.56
body height (cm)	168 ± 6	173 ± 7	0.18
body weight (kg)	74 ± 13	70 ± 10	0.38
body mass index (kg/m²)	26.1 ± 4.1	23.4 ± 3.4	0.14
disease characteristics			
EDSS	2.8 ± 1.2	-	
type of MS (n)*			
SPMS	3	-	
RRMS	9	-	
PPMS	2	-	
body composition			
Whole-body adipose tissue mass (kg)	26.3 ± 7.9	21.4 ± 9.4	0.13
Whole-body lean tissue mass (kg)	46.6 ± 8.6	48.1 ± 8.1	0.61
Biopsied leg lean tissue mass (kg)	7.6 ± 1.5	8.6 ± 1.9	0.17
muscle characteristics			
type 1 CSA	4,235 ± 977	4,843 ± 1236	0.20
type 2a CSA	3,911 ± 1475	5,171 ± 1705	0.046
type 2x CSA	3,167 ± 1404	3,404 ± 926	0.31
type 1 %	44.6 ± 5.8	50.0 ± 14.2	0.29
type 2a %	33.6 ± 10.7	34.1 ± 12.4	0.75
type 2x %	22.9 ± 8.9	18.7 ± 11.6	0.51
VO_{2peak} (ml/min)	1,910 ± 627	3,143 ± 1019	0.006
RER_{peak}	1.17 ± 0.09	1.12 ± 0.04	0.172

Data are expressed as means±SD. Abbreviations: EDSS, Expanded Disability Status Scale; SPMS, secondary progressive multiple sclerosis; RRMS, relapsing remitting multiple sclerosis; PPMS, primary progressive multiple sclerosis; CSA, cross-sectional area; VO_{2peak}, peak oxygen uptake; RER_{peak}, peak gas exchange ratio.

Table 2 Subject characteristics in part 2

	MS patients	healthy subjects	p-value
general characteristics			
n	9	7	
age (y)	48 ± 9	47 ± 10	0.76
males (n of total group)	7	3	0.15
body height (cm)	166 ± 5	176 ± 6	0.012
body weight (kg)	70 ± 14	69 ± 11	0.76
body mass index (kg/m²)	23.4 ± 4.7	22.1 ± 2.5	0.17
disease characteristics			
EDSS	2.6 ± 1.0	-	
type of MS (n)*			
SPMS	2	-	
RRMS	6	-	
PPMS	1	-	
body composition			
adipose tissue mass (kg)	24.5 ± 8.9	16.8 ± 5.9	0.018
lean tissue mass (kg)	44.0 ± 7.5	50.2 ± 8.4	0.11
VO_{2peak} (ml/min)	1,954 ± 648	3,143 ± 1019	0.016
RER_{peak}	1.22 ± 0.10	1.19 ± 0.17	0.172

Data are expressed as means±SD. Abbreviations: EDSS, Expanded Disability Status Scale; SPMS, secondary progressive multiple sclerosis; RRMS, relapsing remitting multiple sclerosis; PPMS, primary progressive multiple sclerosis; CSA, cross-sectional area; VO_{2peak}, peak oxygen uptake.

Figure 1 Study flowchart

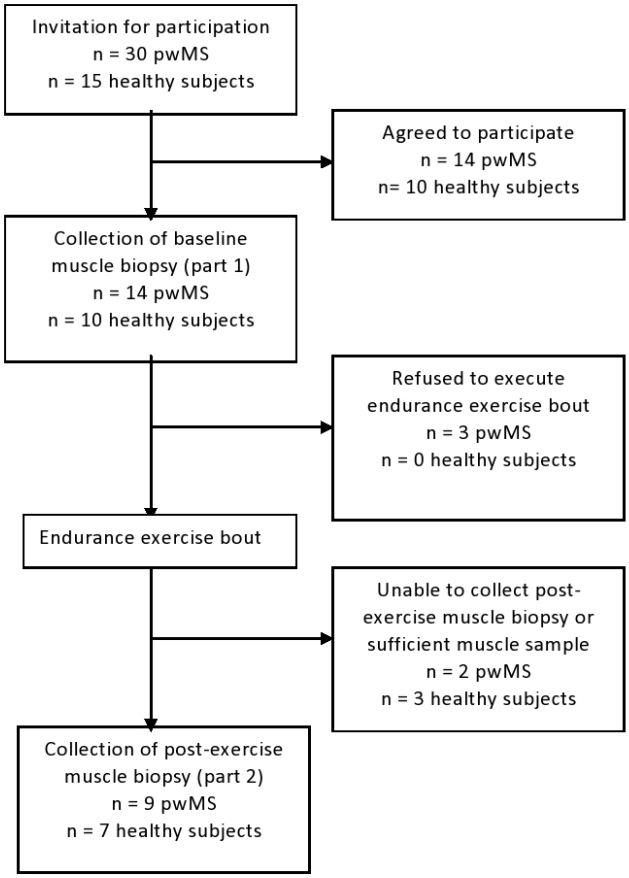


Figure 2 Basal muscle AMPKα and mTOR phosphorylation in pwMS and healthy subjects

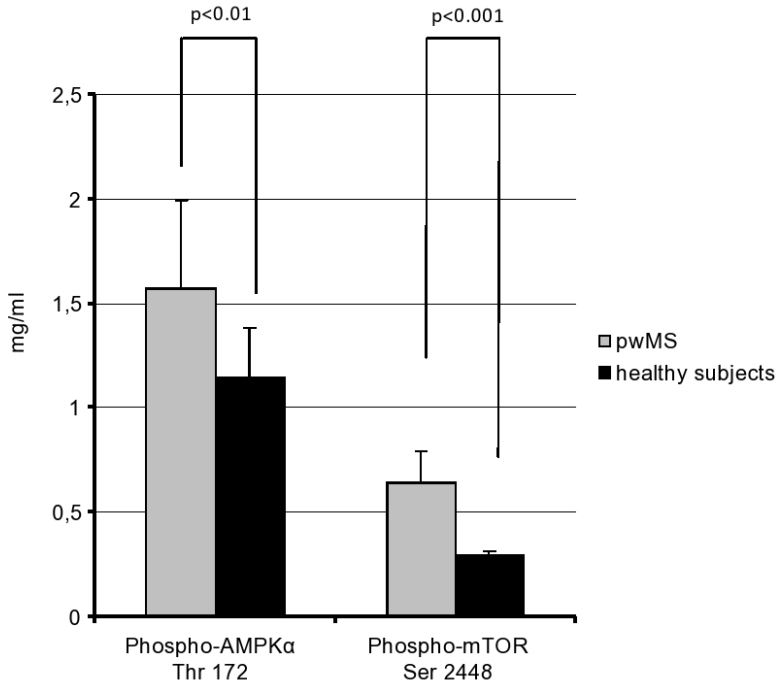
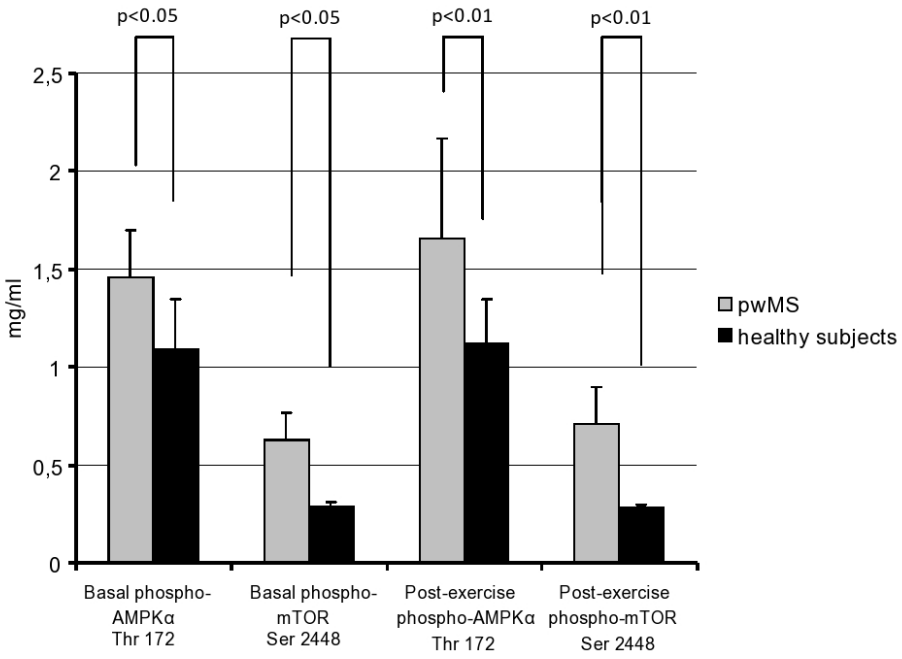


Figure 3 Impact of endurance exercise on muscle AMPKα and mTOR phosphorylation in pwMS and healthy subjects



Background: The etiology for the commonly observed peripheral muscle impairment in patients with MS (pwMS) remains uncertain. We studied skeletal muscle AMP-activated protein kinase (phospho-AMPKα, governing mitochondrial biogenesis) and mammalian target of rapamycin (phospho-mTOR, governing myofibrillar biogenesis) phosphorylation. Elevated basal muscle phospho-AMPKα and phospho-mTOR was present in MS and correlations with disability level was found. After endurance exercise muscle phospho-AMPKα and phospho-mTOR remained elevated in pwMS. Muscle signaling cascades for mitochondrial and myofibrillar biogenesis are altered in MS and related to disability level. Translational significance: A link between muscle signaling cascades and level of disability/impairment is present. This may open a new area for the development of novel therapies for peripheral muscle impairment in MS.