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FACULTEIT GENEESKUNDE EN LEVENSWETENSCHAPPEN
*master in de revalidatiewetenschappen en de
kinesithérapie*

Masterproef

The effect of acute exercise on the skeletal muscle energy metabolism in
Multiple Sclerosis patients

Promotor :
Prof. dr. Dominique HANSEN

Copromotor :
Prof. dr. Bert OP 'T EIJNDE

Melissa Moors , Niels Vansina

*Proefschrift ingediend tot het behalen van de graad van master in de
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ACKNOWLEDGEMENTS

The master thesis presented here, is the result of two years of hard work. We conducted the literature search last year and this year we focused on the research study itself.

First, we would like to thank our promotor Prof. Dr. Dominique Hansen for his guidance and knowledge. We would also like to thank him for his assistance during the statistical analyses, for reading our numerous revisions and giving us remarks.

Second, our gratitude goes out to Prof. Dr. Bert Op 't Eijnde for reading our thesis and Ir. Inez Wens, who made it possible for us to take part in her MS-HIIT doctorate study.

We would also like to thank all of the subjects, that took part in our study, for their time and contribution.

Also, thanks to our fellow students who endured this process with us.

Last but not least our gratitude goes out to our parents for supporting us and their love through these years.

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Oude Baan 69 - 3210 Linden, 25 June 2014

N. V.

RESEARCH CONTEXT

This duothesis belongs to the research domain cardiorespiratory rehabilitation, since the goal of this research was to investigate the influence of acute exercise on skeletal muscle metabolism in persons with Multiple Sclerosis (MS).

Research on this topic is important because MS is a common neurological diseases. For years it was believed that physical exercise in MS would be detrimental, but recent data suggests that participation in physical exercise is beneficial. Most of the MS patients have an inactive lifestyle which often results in a decrease of aerobic capacity and functional muscle strength.¹ Therefore it is important to examine the impact of acute exercise (endurance and/or resistance exercise) on adenosine monophosphate-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) in the energy metabolism of the skeletal muscle. Another important matter to investigate is the possible influence of exercise intensity, frequency and duration.

The study was the first part of a doctorate study of Inez Wens which consisted of two phases. This project is called 'MS-HIIT' and was financed by a doctorate fellow. Research was performed at the research facility REVAL of the University of Hasselt and Biomed at Diepenbeek. In phase one, the influence of an acute exercise bout on both AMPK and mTOR phosphorylation patterns was investigated.

Together with our promoter PhD Dominique Hansen and co-promoter PhD Bert Op 't Eijnde we tried to find an answer to the following question: "What is the effect of acute exercise on energy metabolism in MS patients?".

The research protocol was given to us by our promoter PhD Dominique Hansen. The intervention (muscle biopsies and acute endurance/resistance exercise) started in April 2013 and ended in June 2013. Muscle analyses were conducted in November 2013.

An overview of the task division (writing and data-analysis) is present in Appendix 1. We conducted the statistical analysis together with our promotor.

1. Dalgas U, Stenager E, Ingemann-Hansen T. Multiple sclerosis and physical exercise: recommendations for the application of resistance-, endurance- and combined training. Multiple sclerosis 2008; 14: 35-53.

THE EFFECT OF ACUTE EXERCISE ON THE SKELETAL MUSCLE ENERGY METABOLISM IN MULTIPLE SCLEROSIS PATIENTS.

THE MS-HIIT STUDY

Prepared in accordance with the guidelines of 'Neurology: The Official Journal of the American Academy of Neurology': <http://www.neurology.org/site/misc/auth2.xhtml>

ABSTRACT

Objective: To examine the phosphorylation patterns of adenosine monophosphate-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) in the skeletal muscle of MS patients versus healthy controls (HC).

Methods: Twenty-six MS patients and 15 HC were selected for baseline comparison of skeletal muscle AMPK and mTOR phosphorylation patterns, muscle fiber cross-sectional area (CSA), fiber distribution, VO₂peak and muscle strength (part one). Nine MS patients and seven HC executed an endurance exercise bout (part two). Muscle samples were taken before and after exercise to examine skeletal muscle AMPK and mTOR phosphorylation patterns. Exercise caloric expenditure, intensity and Borg-RPE were comparable between groups.

Results: In part one, MS subjects had a greater skeletal muscle AMPK concentration at rest ($p = 0,000$), compared to the HC. Skeletal muscle AMPK phosphorylation was independently related to MS ($r = 0.87$, $p < 0.001$), this relation was absent for skeletal muscle mTOR phosphorylation ($r = 0.42$, $p = 0.72$). In part two, skeletal muscle AMPK concentrations at rest ($p = 0,008$) and after intervention ($p = 0,005$) were significantly different. Unlike the skeletal muscle mTOR concentrations at rest, those after intervention were significant ($p < 0.05$). In MS the relative change in skeletal muscle mTOR phosphorylation after intervention correlated significantly with VO₂peak ($r = -0.67$, $p < 0.01$) and lean tissue mass ($r = -0.73$, $p < 0.01$). There was a significant correlation between the relative change in skeletal muscle AMPK phosphorylation and relative change in skeletal muscle mTOR phosphorylation after intervention ($r = 0.69$, $p = 0.058$).

Conclusion: Significant differences in skeletal muscle AMPK and mTOR phosphorylation patterns were present in the skeletal muscle of MS patients.

INTRODUCTION

Multiple sclerosis is characterized by the onset of a highly variable clinical course in early adulthood. Most of the patients diagnosed with MS demonstrate a relapse-remitting pattern, which is marked by an up-down course of the different symptoms. Some typical symptoms include: fatigue, limb paresis, sensory and visual disturbances. These symptoms are the result of an auto-immune disease, which induces axonal demyelination and eventually full retrograde degeneration, characterized by a progressive slowing or complete blockage of the nerve's conduction speed.¹

In general, MS patients have an inactive lifestyle which often leads to a decrease in aerobic performance and functional muscle strength.² Earlier research found that the skeletal muscle oxidative capacity in MS patients is reduced and observed rather distinct strength impairments in the lower extremities.^{3,4} Exercise interventions are thus implemented to counteract these anomalies.

Both strength and endurance training at moderate intensity seem to have beneficial effects on the physiological and psychological profile of MS patients and are well tolerated. Even though endurance and strength training in the treatment of MS patients are therefore strongly encouraged, little is known about the effects of these modalities at the molecular level of the skeletal muscle.² Knowledge about the molecular reactions to endurance and strength exercise is mandatory to optimize such interventions. For example, when suboptimal molecular reactions to endurance and strength exercises are discovered in MS patients, it might be suggested to alter exercise intensity, volume, and/or duration.

A critical adaptation in responses to exercise is an increase in mitochondrial content which is regulated by the mitochondrial biogenesis cascade.⁵ The main regulator of mitochondrial biogenesis is PGC-1 α because it promotes the expression of some mitochondrial genes.⁶ Adenosine monophosphate-activated protein kinase (AMPK) is capable of phosphorylating PGC-1 α directly.⁷

AMPK plays a central role in the conservation of energy homeostasis by monitoring the intracellular ATP levels. AMPK phosphorylates downstream targets and that leads to an increase or decrease in ATP-producing or utilizing pathways.⁸

AMPK consists of three subunits, the catalytic α - subunit which has two isoforms ($\alpha 1$, $\alpha 2$) and two regulatory subunits β ($\beta 1$, $\beta 2$) and γ ($\gamma 1$, $\gamma 2$, $\gamma 3$).⁹ AMPK- α contains a kinase domain where phosphorylation of Thr-172 takes place, this is required for the activation of AMPK and the AMPK- β has a glycogen binding domain.^{10,11} The γ - subunit contains two Bateman domains and each Bateman domain is comprised out of a pair of cystationine- β -synthase (CBS) domains.¹² The γ -subunit thus contains four CBS domains, but only three of them are nucleotide binding sites. Two of these sites are able to bind with exchangeable nucleotides but with different affinities. The tight binding site, which is 30-fold stronger than the weaker, can bind AMP/ADP/ATP, NADH, but only AMP binding leads to allosteric activation. The weak binding site can bind AMP/ADP/ATP and protects against

dephosphorylation. The fourth site contains a non-exchangeable AMP- nucleotide.^{13,14} AMPK is acutely activated by exercise.¹⁵

The mammalian target of rapamycin (mTOR) is a protein kinase that responds to signals from metabolic stress, nutrients and hormones. mTOR, both functionally and individually, consists of two protein complexes: mTOR complex one and mTOR complex two. mTOR complex one is mainly composed of regulatory-associated protein of mTOR, raptor. And mTOR complex two consists mainly of rapamycin-insensitive companion of mTOR, rictor. In contrast to mTOR complex one, very little is known about mTOR complex two.¹⁶

Through this mechanism it controls cell growth and proliferation. mTOR is the key regulator of the synthesis of proteins through phosphorylation of some of its many downstream components: mainly eukaryotic initiation factor 4E-binding protein (4E-BP1) and S6 ribosomal protein kinase (S6K1). Both downstream targets impact the translation initiation of mRNA which is essential for protein synthesis.¹⁷

There is strong evidence that resistance exercise acutely increases skeletal muscle mTOR phosphorylation.^{18,19, 20, 21, 22, 23}

Therefore it is important to examine skeletal muscle AMPK and mTOR phosphorylation patterns in the skeletal muscle of patients with MS at baseline and after an acute exercise bout. We expect that the oxidative capacity and muscle strength in MS patients will be lower from those seen in healthy control subjects.² We expect significant differences in skeletal muscle APMK and mTOR phosphorylation patterns in MS patients.

METHODS

Study design

The study consisted of two parts. First, a comparison of basal skeletal muscle metabolism phosphorylation patterns were made between MS patients and healthy controls (part one). Skeletal muscle AMPK and mTOR concentrates were measured before and after an acute endurance exercise bout in MS patients and in healthy controls (part two).

Participants

All MS patients were recruited from the Rehabilitation and MS Centre Overpelt and from existing databases of subjects who already took part in other studies. Healthy controls, for the both parts, were recruited by students. In part one groups were matched for gender, age and body mass index (BMI). In part two groups were matched for gender, age and BMI and the Expanded Disability Status Scale (EDSS). For part one, 26 MS patients and 15 healthy control subjects were selected. In the second part, nine MS patients and seven healthy controls participated.

Participants were included if: (1) they were diagnosed with MS from the age of 18, (2) they had an EDSS score between 0 and 6. Healthy subjects were included if they were ≥ 18 years.

Participants were excluded if: (1) they had Diabetes Mellitus, (2) they had a history of cancer, (3) they had aids, (4) they had more than two serious exacerbations two years before the start of the study, (5) they participated in other studies regarding physical activity, (6) they had issues regarding their psychological health, (7) they were pregnant, (8) they had other conditions that formed a possible contraindication for physical activity.

All participants signed an informed written consent. The study was approved by the Ethical Committee (characteristic CME 2011/311, date: 01/03/2011) according to the convention of Helsinki.

Outcome measures

For part one, primary outcome measures were skeletal muscle AMPK and mTOR phosphorylation at rest. Secondary outcome measures were muscle fiber cross-sectional area (CSA), muscle fiber distribution, VO₂peak and muscle strength. Primary outcome measures, for part two, were skeletal muscle AMPK and mTOR phosphorylation. Secondary outcome measures were total calorie expenditure, intensity (heart rate, power) and Borg scale of perceived exertion (Borg-RPE).

Interventions

Preliminary testing

Body composition. Measurements of body composition were carried out to characterize the participants and to match the MS patients with healthy subjects. The BMI was determined by measuring body length and weight ($\text{BMI} = \text{weight [kg]} / \text{height}^2 [\text{m}^2]$). Dual-energy X-Ray absorptiometry was used to calculate the total percentage of fat mass and lean tissue mass. To standardize the procedure subjects were instructed to remove jewellery or metal objects and only wore light clothing.

EDSS. The scores on the EDSS were given to us by the neurologist of the patients.

Medication. The medication use of the subjects was registered. This was analysed and then subdivided into two categories: immunomodulating and immunosuppressive medication.

Maximal cycling test. Participants executed a cardiopulmonary test on a cycling ergometer (electronically braced Ebike®, Acertys healthcare, Belgium). Pedal frequency was set at 70 rpm and resistance was systematically increased during the test. Female MS patients started at 20 W and there was an increase of 10 W every minute. Male MS patients started at 30 W and there was an increase of 15 W every minute. Healthy female subjects started at 30 W and healthy male subjects at 40 W, there was an increase of 15 W and 20 W every minute, respectively. Subjects cycled until they reached complete fatigue. This 1-minute stage exercise protocol has been shown to have a higher sensitivity for the detection of changes in the VO_2 -peak.²⁴ During the test following parameters were measured (Oxygen Alpha, Jaeger, Mijndhardt, Bunnik, Netherlands): 1) oxygen uptake (VO_2), 2) carbon dioxide output (CO_2), 3) volume of expired air (VE), which served as a foundation to determine the respiratory exchange ratio can ($\text{RER} = \text{CO}_2 \text{ output} / \text{O}_2 \text{ uptake}$). A 12-lead ECG apparatus was used to monitor the heart rate (HR) during the cycling test.

Muscle strength. The maximal voluntary isometric muscle strength of the knee-extensor (weakest leg) was determined by an isokinetic dynamometer (System 3, Biodex, ENRAF-NONIUS, New York, USA). The test was performed from a sitting position with the seat inclined to 85° and the hands firmly wrapped around the handlebars. Thighs, hips and shoulders were strapped with a safety belt for stability. The axis of the knee was aligned with the transverse axis of the Biodex. After a short warm-up session, the maximal isometric muscle strength of the extensors under a knee angle of 45° was measured twice, with 90 seconds of rest. The highest value was counted as the maximal isometric muscle strength (Nm). The seat settings, instructions and guidance was carried out and standardized, by the same researcher.

Muscle biopsy. All participants arrived in a non-fasting condition at the REVAL research facility centre on different days. Upon arriving their last meal was inventoried, after which only water intake was allowed. A single biopsy ($\pm 100\text{mg}$) was taken from the m. vastus lateralis of the weakest leg, under local anesthesia (1% Linisol). A Bergström needle modified for manual suction was used.

After sampling, biopsies were cleared from blood and other tissue. Subsequently, it was embedded in Tissue Tek O.C.D., frozen in isopentane, cooled by liquid nitrogen and stored at -80°C for further analysis in the laboratory.

ELISA protocol . The analysis of skeletal muscle AMPK and mTOR was conducted according to the manufacturing guidelines. The PathScan Phospho-AMPK α (Thr172) Sandwich Elisa Kit #7959 and the PathScan Phospho-mTOR(Ser2481) Sandwich Elisa Kit #7978 (Cell Signaling Technology) were used.

The test procedures were the same for AMPK and mTOR but in certain steps other substances were used. The procedure started with adding 100 μ l of Sample Diluent to a microcentrifuge tube. Then 100 μ l of cell lysate was transferred to the same microcentrifuge tube, which was shaken for a few seconds. 100 μ l of each diluted cell lysate was added to the proper well, the plate was sealed with tape and incubated for 2 hours at 37°C.

After 2 hours, the tape was removed and wells were washed 4 times with 200 μ l 1x Wash Buffer for each well. 100 μ l of Detection antibody was added to each well. The plate was again sealed with tape and incubated for 1 hour at 37°C after which the 4 times wash procedure was repeated.

100 μ l of HRP-linked secondary antibody was added to each well. The plate was sealed with tape and incubated for 30 minutes at 37°C. The 4 times wash procedure was repeated. Then 100 μ l of TBM Substrate was added to each well. The sealed plate was incubated for 10 minutes at 37°C, thereafter 100 μ l of STOP solution was added to each well. This substance was shaken for a few seconds.

Results were interpreted by spectrophotometric determination with an absorbance at 450nm.

Muscle fiber analysis. From the obtained muscle samples serial transverse sections (9 μ m) were cut at 20°C and stained by means of ATPase histochemistry, after preincubation at pH 4.4, 4.6 and 10.3. A Leica DM2000 microscope (Leica, Stockholm, Sweden) and a Leica Hi-resolution Color DFC camera (Leica, Stockholm, Sweden), together with image-analysis software (Leica Qwin ver. 3, Leica, Stockholm, Sweden), were used to visualize and analyse the serial sections. Fiber mask at the stained sections were made by this software, which afterwards was fitted manually to the cell borders of the selected fibers. The fibers used were the ones that were cut perpendicular to their longitudinal axis.

For types I, IIa and IIx muscle fiber CSA and fiber distribution was calculated.

Endurance exercise bout

The endurance exercise bout consisted of three periods of six minute continued cycling on a Technogym cycle ergometer bike at 70% of their W_{max}, during which the heart rate was monitored with a Polar heart rate monitor. Workload was lowered when the initial exercise intensity could not be maintained. Three minutes of rest were given between sets, during which subjects had to rate their

subjective perceived effort on a twenty-item Borg-RPE scale. Intensity was expressed as a percentage of the maximum heart rate ($[\text{HR}_{\text{exercise}}/\text{HR}_{\text{max}}]*100 = \text{HR}\%$) and as a percentage of the maximum power ($[\text{W}_{\text{exercise}}/\text{W}_{\text{max}}]*100 = \text{W}\%$). The total amount of calories was also calculated. The healthy controls exercised at the same intensity, but their exercise duration was shorter to generate equal caloric expenditure.

Statistical analysis

SPSS version 22.0 for Windows was used for the statistical analysis. Data are expressed as means $\pm$ SD. For both studies, normality was checked for all the variables using a Shapiro-Wilk test. Non parametric testing was used, as the data were not normally distributed. For the first part of the study, an independent samples Mann-Whitney U test, with significance set at $p < 0.05$, was used to assess differences between MS and control populations for both skeletal muscle AMPK and mTOR phosphorylation at baseline. To examine the relations between AMPK or mTOR phosphorylation and subjects characteristics (age, gender, body weight and body height, BMI, presence/absence of MS), a multivariate linear regression model was used. The Spearman correlation test was used to assess for possible univariates. Statistical significance was set at $p < 0.05$ for the regression models and correlation analysis. For the second part of the study, the same tests were used, except the regression models, to assess changes in skeletal muscle AMPK and mTOR phosphorylation after exercise compared to baseline.

RESULTS

Participants

For the baseline comparison, 29 MS patients and 18 healthy controls were recruited. At baseline biopsy procedure, two MS subjects failed to produce a usable muscle sample which led to drop-out. After data analysis, four subjects were excluded from the study because their sample could not be used for analysis. This all led to a total of 26 MS patients and 15 healthy subjects (see Fig. 1). The subject characteristics are summarized in Tables 1.A and 1.B. An overview of medication used at the time of the study is shown in Tables 2.A and 2.B.

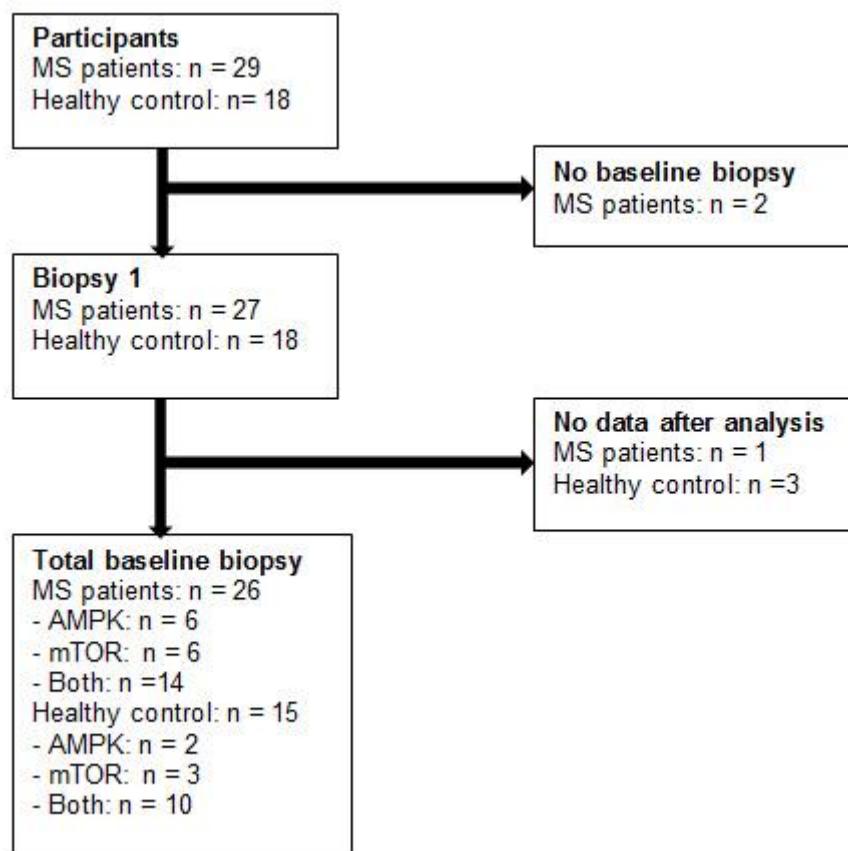


Figure 1: Flowchart part 1

Table 1.A: Subject characteristics AMPK part 1

AMPK	MS patients	Healthy subjects
Characteristic	Mean (S.D.)	Mean (S.D.)
Age (years)	47.7 (8.97)	48.5 (7.48)
Body height (m)	1.68 (0.06)	1.73 (0.08)
Body Weight (kg)	73.02 (14.10)	71.67 (11.40)
BMI (kg.m ⁻²)	25.68 (4.22)	23.97 (3.31)
Total fat mass (g)	25975 (8024)	22932 (9118)*
Total fat free mass (g)	45737 (8860)	48622 (8896)*
Fat free mass of weakest leg (g)	7379 (1570)	8389 (1946)*
VO2peak (ml/min)	1901.11 (582.95)*	3143.43 (1019.35)***
Muscle strength (Nm)	127.72 (39.40)	125.50 (31.23)****
CSA type I (μm ²)	4039.27 (1222.37)	4830.98 (1244.13)
CSA type IIa (μm ²)	3738.35 (1451.75)	5210.40 (1557.89)
CSA type IIx (μm ²)	3069.44 (1249.98)*	3589.14 (914.17)**
Percentage type I (%)	45.78 (8.12)	47.25 (14.34)
Percentage type IIa (%)	34.62 (11.55)	34.81 (12.72)
Percentage type IIx (%)	19.99 (9.66)*	20.61 (12.75)**
N =	20	12

* Missing values of one subject

** Missing value of two subjects

*** Missing value of five subjects

**** Missing value of seven subjects

Table 1.B: Subject characteristics mTOR part 1

mTOR	MS patients	Healthy subjects
Characteristic	Mean (S.D.)	Mean (S.D.)
Age (years)	46.55 (10.21)	46.46 (7.87)
Body height (m)	1.70 (0.09)	1.73 (0.07)
Body weight (kg)	75.05 (12.15)	73.62 (14.24)
BMI (kg.m ⁻²)	25.89 (4.00)	24.72 (4.68)
Fat mass (g)	26454 (8120)*	25555 (12447)*
Fat free mass (g)	47994 (9067)*	47792 (7504)*
Fat free mass of weakest leg (g)	7786 (1711)*	8229 (1703)*
VO2peak (ml/min)	1920.60 (660.54)	2882.50 (1198.04)***
Muscle strength (Nm)	128.22 (44.75)	131.02 (35.47)***
CSA type I (μm ²)	4186.74 (847.75)*	4958.81 (1383.72)
CSA type IIa (μm ²)	3917.53 (1323.02)*	5166.81 (1588.34)
CSA type IIx (μm ²)	3142.09 (1292.06)**	3547.11 (1187.32)**
Percentage type I (%)	41.15 (10.00)*	48.41 (12.87)
Percentage type IIa (%)	35.97 (11.77)*	32.66 (11.39)
Percentage type IIx (%)	23.28 (9.17)**	21.43 (11.81)**
N =	20	13

* Missing values of one subject

** Missing value of two subjects

*** Missing value of five subjects

Table 2.A: Subject medication AMPK part 1

AMPK	MS patients	Healthy subjects
Medication	Number of subjects	Number of subjects
Immunomodulating	14	0
Immunosuppressive	4	0
N =	20	12

Table 2.B: Subject medication mTOR part 1

mTOR	MS patients	Healthy subjects
Medication	Number of subjects	Number of subjects
Immunomodulating	12	0
Immunosuppressive	4	0
N =	20	13

For the second part of the study, a portion from the subjects of the first part was included, to undergo an endurance exercise bout. A first drop-out of subjects took place after the AMPK and mTOR analysis of the muscle samples. For four subjects, something went wrong in the process of the analysis. After the data of the analysis were available, only subjects of which both AMPK and mTOR data were available, were included. This resulted in a total of nine MS patients and seven healthy controls. An overview of drop-out is illustrated in Figure 2. The subject characteristics are summarized in Table 3. An overview of subjects their medication is shown in Table 4.

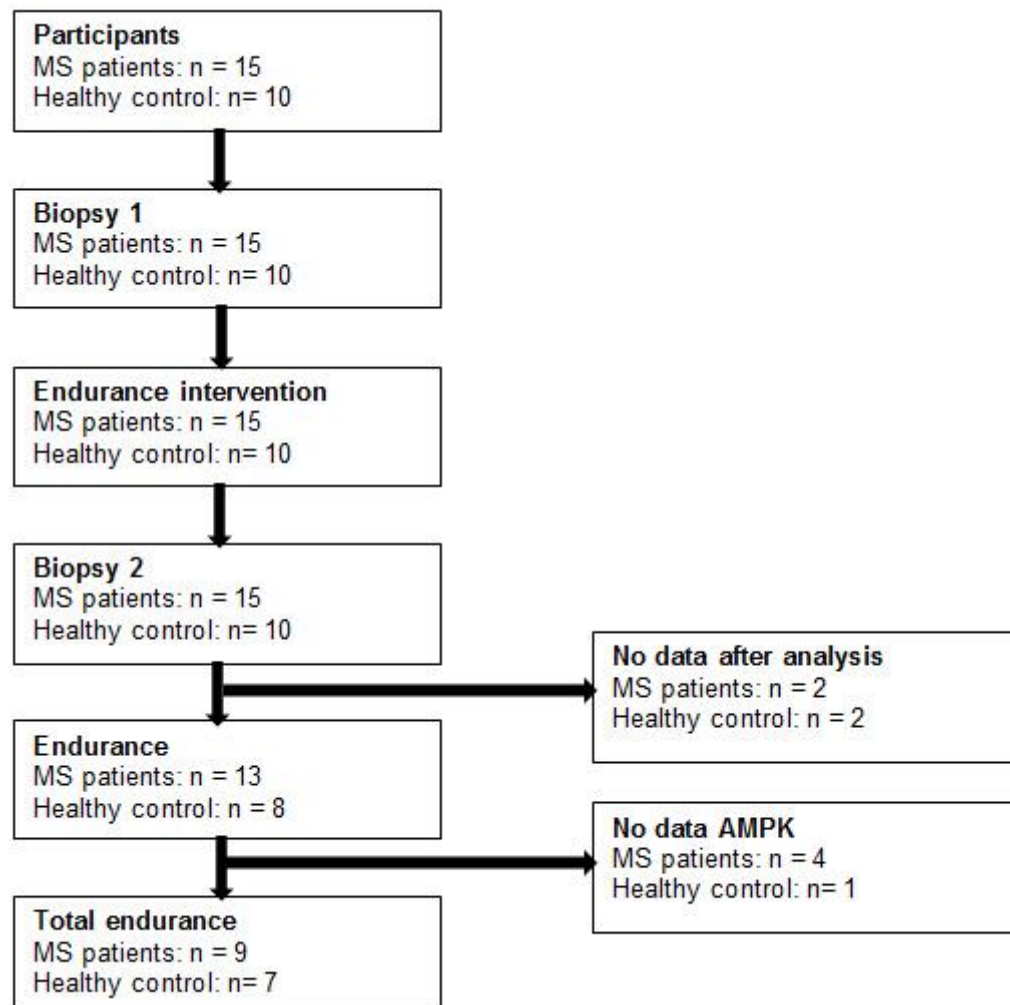


Figure 2: Flowchart part two 2

Table 3: Subject characteristics part 2

	MS patients	Healthy subjects
Characteristic	Mean (S.D.)	Mean (S.D.)
Age (years)	47.77 (9.41)	47.43 (9.68)
Body height (m)	1.66 (0.05)	1.76 (0.06)
Body weight (kg)	69.69 (13.57)	69.14 (10.79)
BMI (kg.m ⁻²)	25.35 (4.73)	22.15 (2.53)
Total body fat mass (g)	24537 (8857)	16799 (5883)*
Total body fat free mass (g)	43958 (7509)	50214 (8422)*
VO2-peak (ml/min)	1953.08 (648.44)	3143.43 (1019.35)
EDSS	2.56 (1.01)	/
N =	9	7

*Missing value of 1 subject

Table 4: Subject medication part 2

	MS patients	Healthy subjects
Medication	Number of subjects	Number of subjects
Immunomodulating	6	0
Immunosuppressive	1	0
N =	9	7

Interventions

Part 1

Skeletal muscle AMPK phosphorylation at baseline. Subjects were matched for age ($p = 0.797$), body weight ($p = 0.781$), body height ($p = 0.136$) and BMI ($p = 0.387$). For an overview see Table 1.A. The healthy controls had a significant higher cross sectional area of type IIa muscles ($p = 0.006$).

More importantly, the MS patients had a lower VO₂peak value compared to the healthy controls ($p = 0.002$). But in contrast, a greater skeletal muscle AMPK concentration at rest was observed within the MS population ($p = 0.000$) (Fig. 3). No significant results were found for muscle distribution and muscle strength ($p > 0.05$).

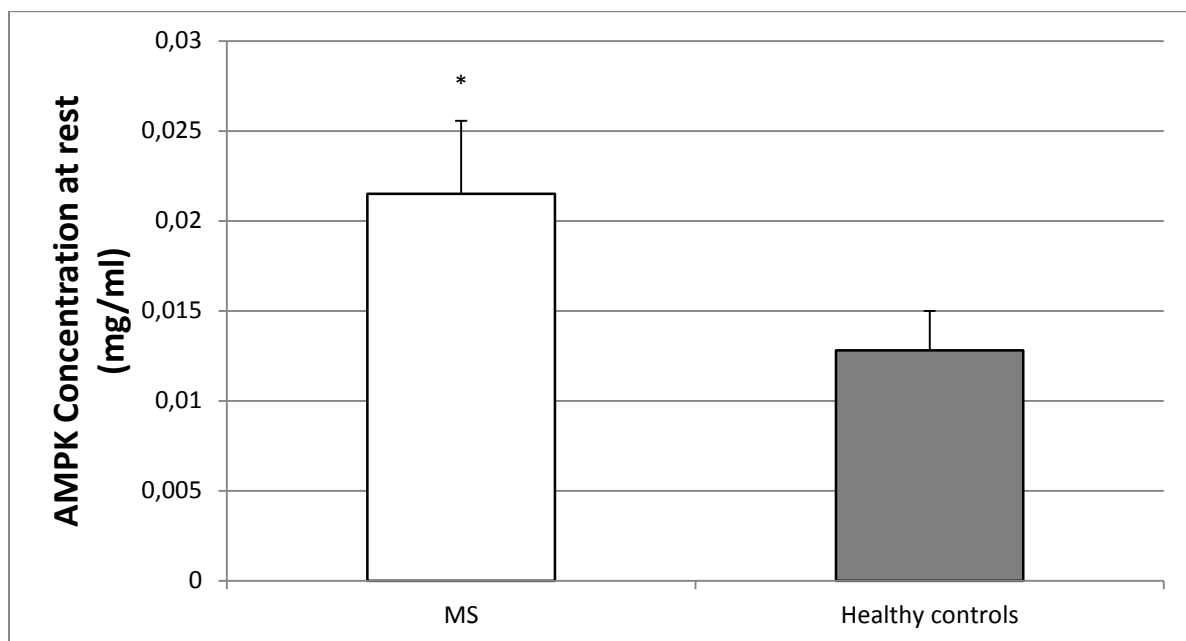


Figure 3: AMP-activated protein kinase (AMPK) expressed in milligram per millilitre (mg/ml), for both Multiple sclerosis patients (MS) and healthy subjects (Healthy controls) at baseline AMPK skeletal muscle biopsy testing. *Significantly different from healthy subjects ($P < 0.05$). Data are mean $\pm$ SEM, MS $n = 20$ and healthy controls $n = 12$.

Skeletal muscle mTOR phosphorylation at baseline. Subjects were matched for age ($p = 0.979$), body weight ($p = 0.760$), body height ($p = 0.334$) and BMI ($p = 0.353$). For an overview see Table 1.B. As with AMPK, the healthy controls had a significant higher cross-sectional area of type IIa muscle s ($p = 0.016$).

And again, a higher VO₂peak value was observed in the healthy controls compared to the MS patients ($p = 0.043$). A slight tendency to a higher skeletal muscle mTOR value in the MS population at rest was observed, but it was not significant ($p = 0.062$) (Fig. 4). Again no significant results were found for muscle distribution and muscle strength ($p > 0.05$).

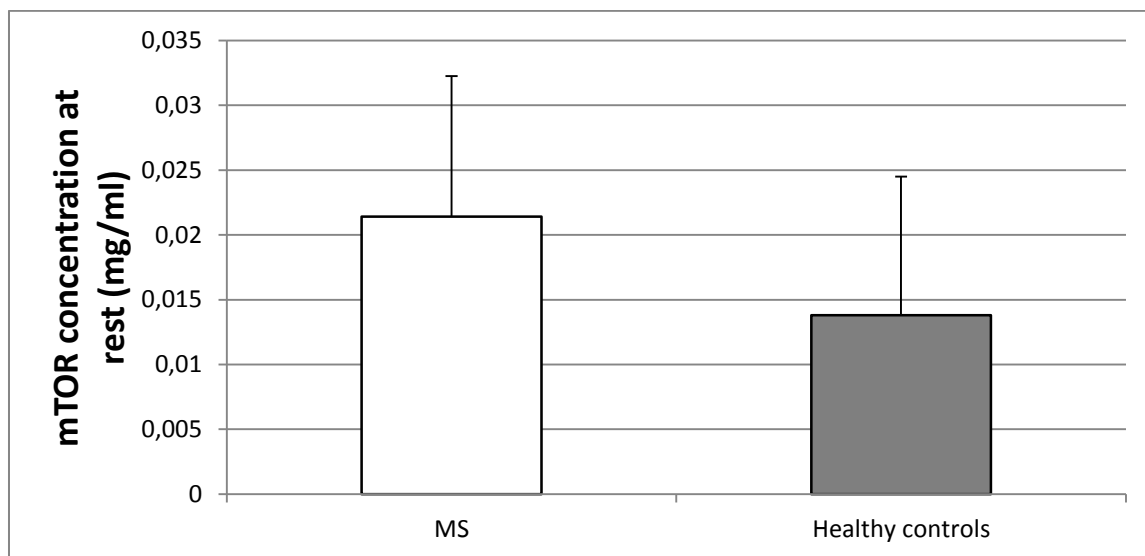


Figure 4: Mammalian target of rapamycin (mTOR), expressed in milligram per millilitre (mg/ml), for both Multiple sclerosis patients (MS) and healthy subjects (Healthy controls) at baseline mTOR muscle biopsy testing. Data are mean $\pm$ SEM, MS $n = 20$ and healthy controls $n = 13$.

Univariate correlations and regression analysis. A near significant correlation was found for skeletal muscle AMPK phosphorylation and VO₂peak ($r = -0.359$, $p = 0.072$, $n = 26$). This would suggest a negative relationship between skeletal muscle AMPK phosphorylation and VO₂peak, meaning a higher VO₂peak value resulting in a lower skeletal muscle AMPK phosphorylation or vice versa. No significant correlations were found with skeletal muscle mTOR phosphorylation at baseline.

Skeletal muscle AMPK phosphorylation was independently related to the presence of MS ($r = 0.87$, $p < 0.001$), while no independent relations were discovered for skeletal muscle mTOR phosphorylation ($r = 0.42$, $p = 0.72$).

Part 2

Subject characteristics. Subjects were matched for age ($p = 0.758$) and body weight ($p = 0.758$). The MS patients and healthy controls had a significant difference in body height ($p < 0.05$) but not in BMI ($p > 0.05$). The total fat free mass was significantly different ($p < 0.05$), it was higher for MS patients compared with the healthy controls. VO_{2peak} and W_{peak} were higher for the healthy controls ($p < 0.05$). For an overview see Table 3.

Exercise parameters. The groups were perfectly matched for total calorie expenditure ($p = 1,000$), exercise Borg-RPE ($p = 0.837$) and exercise HR% ($p = 0.918$). A significant difference ($p < 0.05$) was found between groups. Results are displayed in Table 5.

Table 5: Exercise parameters

	MS patients	Healthy subjects
Parameters	Mean (S.D.)	Mean (S.D.)
Exercise power (W)	95.89 (40.51)	166.93 (58.28)
Kilocalories (kcal)	123.01 (47.64)	117.00 (48.35)
Exercise HR (bpm)	147.33 (26.17)	155.86 (16.48)
Exercise Borg-RPE	12.74 (3.78)	12.93 (2.97)
Exercise HR percentage (%)	89.44 (10.32)	90.29 (5.15)
N =	9	7

Abbreviations: HR = heart rate

Skeletal muscle AMPK phosphorylation. Skeletal muscle AMPK concentrations at rest ($p = 0.008$) and after the intervention ($p = 0.005$) were significantly different ($p < 0.05$) between the MS population and the healthy subjects (Fig. 5). In the MS patients skeletal muscle AMPK concentration elevated when comparing baseline values and values after intervention. No difference after intervention was seen in the skeletal muscle AMPK concentration of the healthy controls. The absolute change in skeletal muscle AMPK phosphorylation ($= AMPK_{rest} - AMPK_{exercise}$) was not significantly different ($p = 0.055$). We did see increase of 15% in the relative change of skeletal muscle AMPK phosphorylation in MS patients, this was not seen in the healthy controls (Fig. 6).

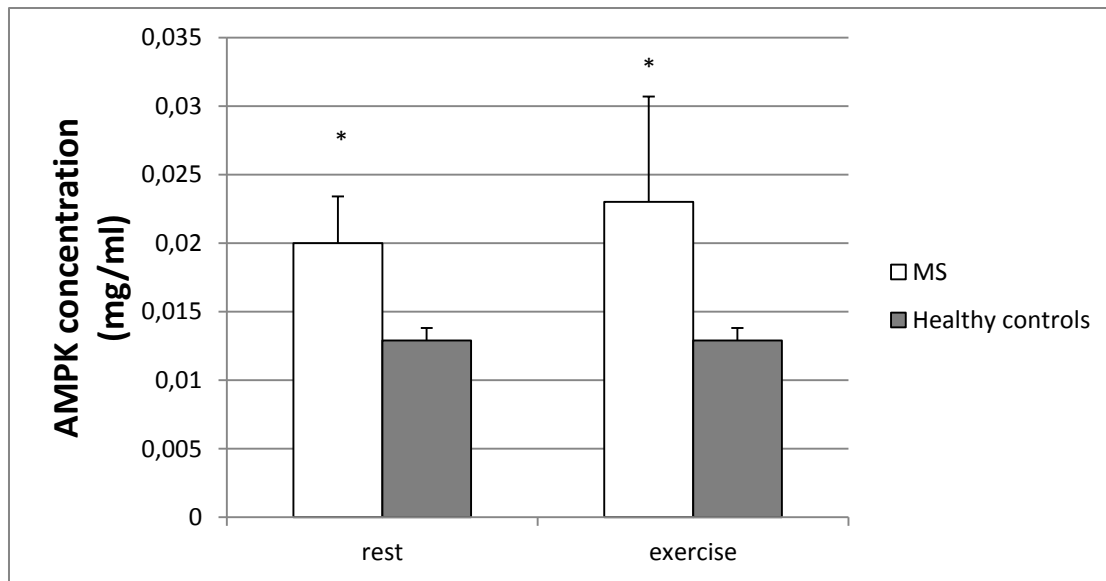


Figure 5: AMP-activated protein kinase (AMPK) , expressed in milligram per millilitre (mg/ml), for both Multiple sclerosis patients (MS) and healthy subjects (Healthy controls) at baseline (rest) and after an acute endurance bout. *Significantly different from healthy subjects ($P < 0.05$) at baseline and after an acute endurance bout. Data are mean $\pm$ SEM, MS $n = 9$ and healthy controls $n = 7$.

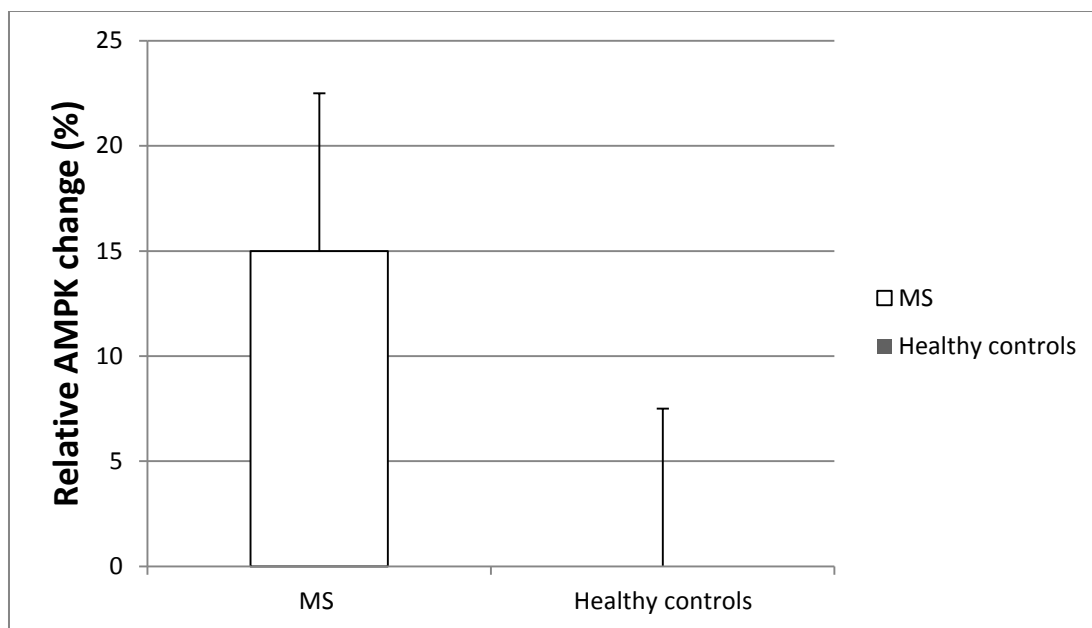


Figure 6 : Relative change in AMP-activated protein kinase (AMPK) , expressed in percentage (%), for both Multiple sclerosis patients (MS) and healthy subjects (Healthy controls) after an acute endurance bout. A relative change of 15% in AMPK is seen in MS after an acute endurance bout. Data are mean $\pm$ SEM, MS $n = 9$ and healthy controls $n = 7$.

Skeletal muscle mTOR phosphorylation. Skeletal muscle mTOR concentrations at rest were not significantly different between the MS population and the healthy subjects. Skeletal muscle mTOR concentrations after intervention were significant ($p < 0.05$) (Fig. 7). In the MS patients skeletal muscle mTOR concentrations were elevated after intervention, the concentrations of healthy subjects decreased. The absolute change in skeletal muscle mTOR phosphorylation ($= \text{mTOR}_{\text{exercise}} - \text{mTOR}_{\text{rest}}$) was not significant. A relative change of 33% in skeletal muscle mTOR phosphorylation is seen in MS after an acute endurance bout. In healthy controls skeletal muscle mTOR phosphorylation decreased after an acute endurance bout (Fig. 8).

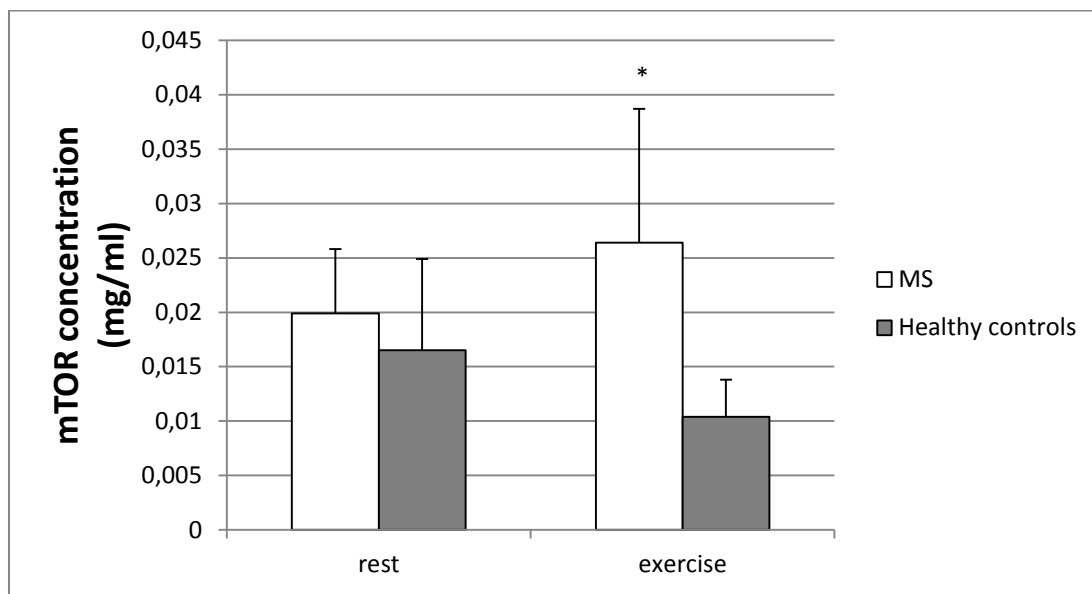


Figure 7: Mammalian target of rapamycin (mTOR), expressed in milligram per millilitre (mg/ml), for both Multiple sclerosis patients (MS) and healthy subjects (Healthy controls) at baseline (rest) and after an acute endurance bout. *Significantly different from healthy subjects ($P < 0.05$) after acute an endurance bout. Data are mean $\pm$ SEM, MS $n = 9$ and healthy controls $n = 7$ (One missing value for MS mTOR concentration in both rest and exercise).

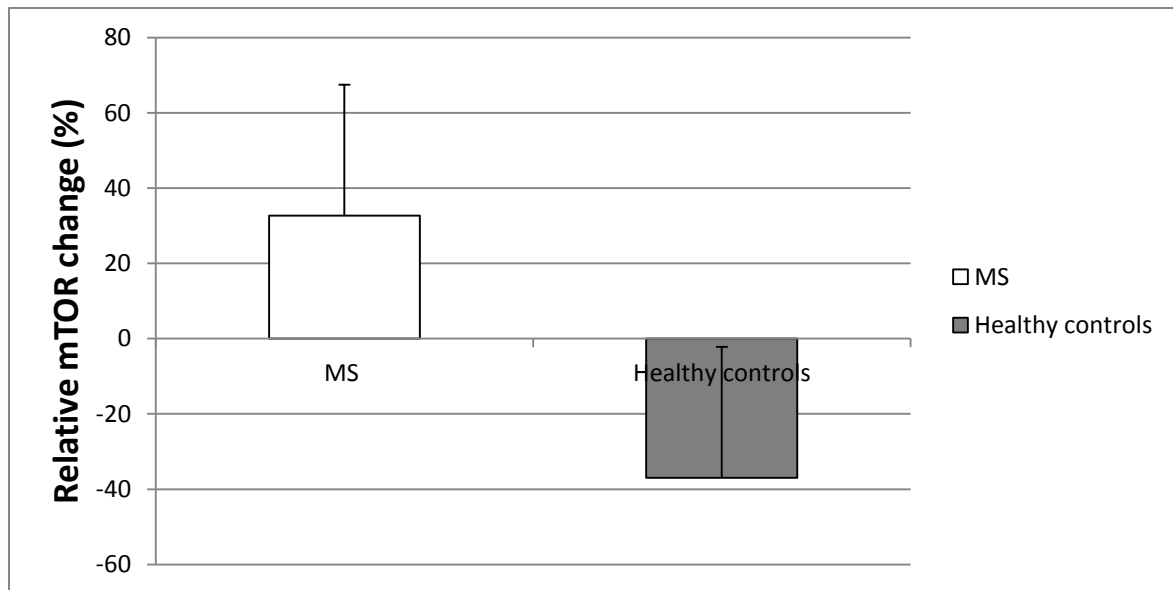


Figure 8: Relative change in mammalian target of rapamycin (mTOR), expressed in percentage (%), for both Multiple sclerosis patients (MS) and healthy subjects (Healthy controls) after an acute endurance bout. A relative change of 33% in mTOR is seen in MS after an acute endurance bout. Data are mean $\pm$ SEM, MS $n = 9$ and healthy controls $n = 7$. (One missing value for MS relative change in mTOR).

Skeletal muscle AMPK and mTOR. The absolute AMPK and mTOR change was not significant ($p = 0,054$) (Fig. 9).

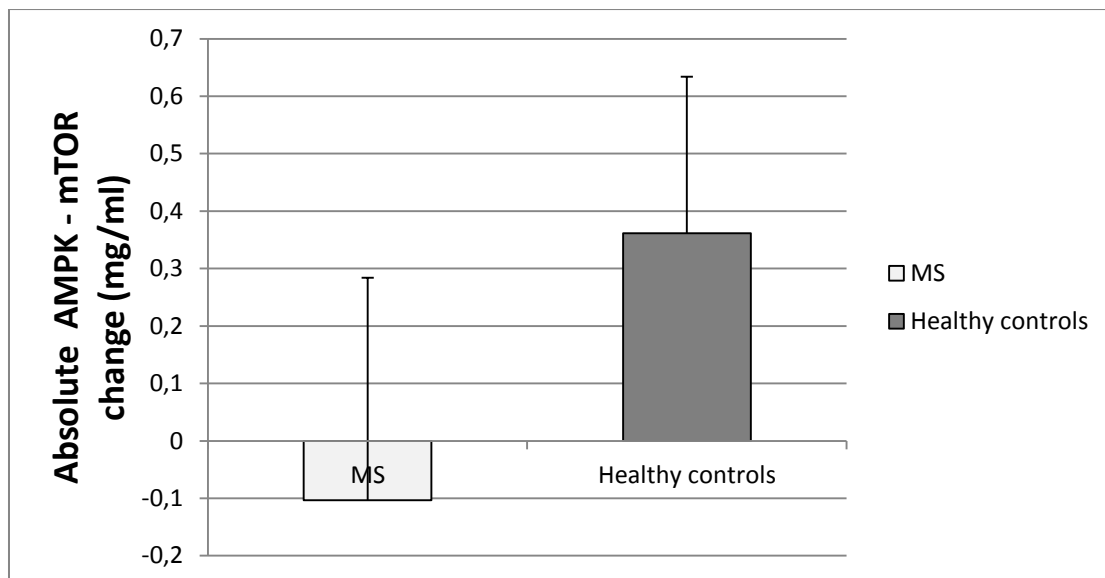


Figure 9: The absolute change of AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR), expressed in milligram per millilitre (mg/ml), for both Multiple sclerosis patients (MS) and healthy subjects (Healthy controls) after an acute endurance bout. No significant results were found ($P < 0.05$). Data are mean $\pm$ SEM, MS $n = 9$ and healthy controls $n = 7$. (One missing value for MS absolute AMPK-mTOR change).

Correlations. The relative change in skeletal muscle mTOR phosphorylation after endurance intervention correlated significantly with VO₂peak ($r = -0.67$, $p < 0.01$) and lean tissue mass ($r = -0.73$, $p < 0.01$). In total group, no correlations were found for changes in skeletal muscle AMPK phosphorylation and changes in skeletal muscle mTOR phosphorylation after endurance intervention. A significant correlation was found between the relative change in skeletal muscle AMPK phosphorylation and relative change in skeletal muscle mTOR phosphorylation after endurance intervention ($n = 9$, $r = 0.69$, $p = 0.058$). In healthy subjects this last correlation was absent ($n = 7$, $r = -0.39$, $p = 0.38$).

DISCUSSION

In the present study we found a significantly elevated resting skeletal muscle AMPK phosphorylation in patients with MS. In addition, as a result of endurance exercise changes in skeletal muscle AMPK and mTOR phosphorylation were different in MS patients versus healthy subjects. These data may suggest that the molecular adaptations in skeletal muscle cells as a result of endurance exercise are disturbed in patients with MS.

Part 1

The objective for the first part of the study was to compare basal skeletal muscle mTOR and AMPK phosphorylation between MS patients and healthy controls. In this study the subjects were matched for age, body weight, body height and BMI. However, the healthy controls had a significantly higher cross sectional area of type IIa muscle ($p = 0,006$) and VO₂peak, while maximal voluntary isometric muscle strength was comparable between groups.

Skeletal muscle AMPK phosphorylation. A greater basal skeletal muscle AMPK concentration was observed in MS patients. Moreover according to multivariate regression analysis the basal skeletal muscle AMPK phosphorylation status was independently associated with MS. These data thus indicate that an increased basal skeletal muscle AMPK phosphorylation is typically associated with MS, regardless of age and gender. This higher basal skeletal muscle AMPK concentration indicates that MS patients are in a constant state of stimulation of the mitochondrial biogenesis. However, VO₂peak is significantly lower in patients with MS. Therefore this hyperphosphorylation status could be a compensatory reaction for disturbed post phosphorylation molecular cascades in the skeletal muscle cells. However, the latter hypothesis remains to be verified in future studies. The aetiology of the skeletal muscle AMPK hyperphosphorylation in MS remains speculative. This might be related to disturbances in blood by Vitamin D levels in MS. Previous studies indeed showed that skeletal muscle AMPK phosphorylation lowers in rats as result of vitamin D supplementation.²⁵ In addition, studies have found that elevations in muscle cell interleukin-18 content in mice, and the induction of acute systemic inflammation in humans, leads to elevation in muscle AMPK α phosphorylation.^{26,27}

Skeletal muscle mTOR phosphorylation. The healthy controls had a significant higher cross sectional area of type IIa muscles, but the maximal voluntary isometric muscle strength was the same across both groups. The MS patients had a lower VO₂peak value compared to the healthy controls and a slight tendency to a higher skeletal muscle mTOR phosphorylation value at rest. Skeletal muscle mTOR phosphorylation was comparable between groups and according to multivariate regression analysis not independently associated with MS.

Part 2

Participants were correctly matched for age and weight. The total fat mass was higher in the MS patients and the total fat free mass was lower. This may be caused by the sedentary lifestyle of MS patients.² $\dot{V}O_{2peak}$ and W_{peak} were significantly different, both were lower in the MS patients. This is not surprising as many studies showed a decrease in aerobic performance and a reduced skeletal muscle oxidative capacity in MS patients.^{2,3}

The healthy controls exercised at the same intensity as the MS patients (exercise Borg-RPE and exercise HR% were comparable), but their exercise duration was shorter to generate equal caloric expenditure. Results show that the caloric expenditure was matched between groups.

Skeletal muscle AMPK phosphorylation. As result of endurance exercise the skeletal muscle AMPK phosphorylation increased further in patients with MS while, no such change was observed in healthy subjects. These data may indicate that intramuscular factors are present in MS, that lead to an abnormal molecular response to exercise. However, explaining the aetiology of such a disturbed response to exercise in MS remains difficult due to a lack of data from previous studies. However, a sedentary lifestyle, which is highly prevalent in MS, could result into a faster activation of skeletal muscle AMPK phosphorylation during exercise. On the other hand, in healthy subjects no change in skeletal muscle AMPK phosphorylation was found after endurance exercise. These data seem to contradict previous studies where healthy subjects were examined.^{28,29,30} Although, a low caloric expenditure was generated in the present study. It could be the case that the caloric expenditure as result of exercise was too low to alter skeletal muscle AMPK phosphorylation.

Skeletal muscle mTOR phosphorylation. Like we expected, skeletal muscle mTOR phosphorylation decreased in the healthy subjects as result of exercise, because AMPK activation would lead to inhibition of mTOR activation.¹⁶ This mechanism seemed to be disturbed in MS patients because skeletal muscle mTOR phosphorylation increased significantly as result of exercise. These data, may again indicate that the post skeletal muscle AMPK phosphorylation cascades are severely disturbed in MS. The overall change in skeletal muscle mTOR phosphorylation was not significantly different. We were expecting this outcome, because mTOR is most likely activated by resistance training.¹⁹

Correlations. The relative change in skeletal muscle mTOR phosphorylation after endurance intervention correlated significantly with $\dot{V}O_{2peak}$ ($r = -0.67$, $p < 0.01$) and lean tissue mass ($r = -0.73$, $p < 0.01$). A negative correlation between mTOR and $\dot{V}O_{2peak}$ could be expected because AMPK, which is activated by an endurance bout, inhibits mTOR. But a correlation between AMPK and $\dot{V}O_{2peak}$ was not found, the reason for this remains speculative. The atrophic muscles in MS patients could be responsible for the negative correlation between mTOR phosphorylation and lean tissue mass because mTOR may need to be more active in these muscles compared with non-atrophic muscles. A significant correlation was found between the relative change in skeletal AMPK phosphorylation and relative change in skeletal muscle mTOR phosphorylation after endurance intervention ($r = 0.69$, $p = 0.058$) in MS only. This increase, in both the relative change of AMPK and

relative change in mTOR activity, may indicate disordered molecular cascades in the skeletal muscle of MS patients.

This study was the first, to our knowledge, to investigate skeletal muscle AMPK and mTOR phosphorylation in MS patients both at rest and after an acute exercise bout. The results from the present study could however be far-reaching because they indicate that it might be necessary to revise current understanding of exercise physiology in MS and the training programs we implement. It seems necessary that additional studies are launched to further examine these skeletal muscle molecular cascades in MS in order to understand the aetiology of the observed disturbances and/or to verify our findings.

There were a few limitations in our study. The sample sizes of the studies were relatively small. Not all of the healthy controls underwent a preliminary maximal isometric muscle strength testing, which resulted in a small sample size to compare with the MS subjects. On the other hand, this the first study that addresses skeletal muscle AMPK phosphorylation in MS. Groups were matched for age, gender, body weight, body height. Exercise conditions were matched between groups. The timing of second muscle biopsy was matched between groups (20 min in the MS patients and 18 min in the healthy controls).

CONCLUSION

This study found a significantly elevated resting skeletal muscle AMPK phosphorylation in patients with MS. Furthermore, changes in skeletal muscle AMPK and mTOR phosphorylation were different in MS patients versus healthy subjects as result of endurance exercise. These data may suggest that, as a result of endurance exercise, the molecular adaptations in skeletal muscle cells are disturbed in MS patients.

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APPENDIX

Appendix 1

Melissa	Niels	Together
Writing - Research context - Introduction AMPK -Methods: participants, outcome measures - Methods intervention: preliminary testing, endurance exercise bout - Methods statistical analysis: part 2 - Results participants: part 2 - Results intervention: part 2 - Discussion: part 2 Data-analysis - Interpretation part 2	Writing - Introduction mTOR - Methods: study design -Methods statistical analysis: part 1 - Results participants: part 1 - Results intervention: part 1 - Discussion: part 1 Data-analysis - Interpretation part 1	Writing - Acknowledgements - Abstract -Methods intervention: muscle biopsy, ELISA protocol - Reference list Data-analysis - Enter all data in Excel

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The effect of acute exercise on the skeletal muscle energy metabolism in Multiple Sclerosis patients

Richting: **master in de revalidatiewetenschappen en de kinesitherapie-revalidatiewetenschappen en kinesitherapie bij musculoskeletale aandoeningen**

Jaar: **2014**

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