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

Masterthesis of Melissa Moors and Niels Vansina

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Introduction

- Multiple sclerosis is characterized by a highly variable clinical course:
  - most commonly a relapse-remitting pattern
  - typical symptoms include: fatigue, limb paresis, sensory and visual disturbances
  - resulting of an auto-immune disease, which induces axonal demyelination and eventually full retrograde degeneration
- Most MS patients have an inactive lifestyle, which leads to a decrease in:
  - aerobic performance
  - functional muscle strength
- Earlier research found a reduced skeletal muscle oxidative capacity in MS patients and observed distinct strength impairments.
- Adenosine monophosphate-activated protein kinase (AMPK) is acutely activated by exercise and conserves energy homeostasis by monitoring intracellular ATP levels. It phosphorylates downstream targets that lead to an increase or decrease in ATP-producing or utilizing pathways.
- The mammalian target of rapamycin (mTOR) controls cell growth and proliferation. There is strong evidence that resistance exercise acutely increases mTOR phosphorylation.
- **Research goal:** To examine AMPK and mTOR phosphorylation in the skeletal muscle of MS patients and healthy controls at baseline and after acute exercise.
- **Hypothesis:** We expect that the oxidative capacity and muscle strength in MS patients will be lower from those seen in healthy control subjects. We expect significant differences in skeletal muscle APMK and mTOR phosphorylation patterns in MS patients.

Methods

- **Study Design:** Cross-sectional observational study.
- **Participants:** 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 both studies were recruited by students and matched with the MS patients.

  For part one 26 MS patients and 15 healthy control subjects were selected. In the second part of the study nine MS patients and seven healthy controls participated.
- **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).
- **Study procedure:** All participants arrived in a non-fasting condition at the REVAL research facility centre on different days after which only water intake was allowed.

  A single biopsy was taken from the m. vastus lateralis of the weakest leg.

  Afterwards the participants performed an endurance exercise bout and upon completion a second muscle biopsy was taken.

  The endurance exercise bout consisted of three periods of six minute continued cycling on a Technogym cycle ergometer bike at 70% of their Wmax. Three minutes of rest was given between sets. The healthy controls exercised at the same intensity, but their exercise duration was shorter to generate equal caloric expenditure.
- **Data analysis:** For the analysis of AMPK and mTOR phosphorylation patterns the PathScan Phospho-AMPKα(Thr172) Sandwich Elisa Kit #7959 and the PathScan Phospho-mTOR(Ser2481) Sandwich Elisa Kit #7978 (Cell Signaling Technology) were used.
- **Statistical analysis:** SPSS version 22.0 for Windows was used for statistical analyses.

Results

Part 1

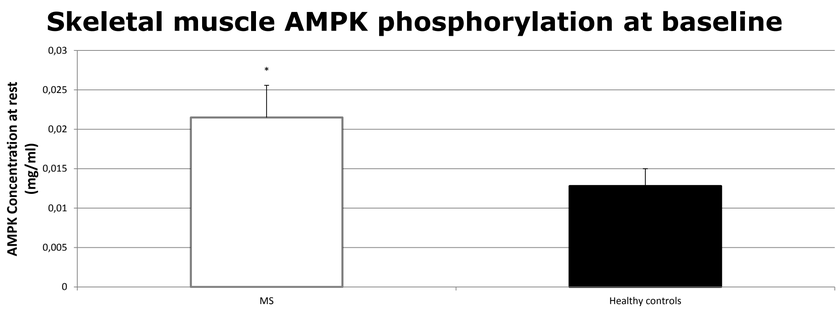

Figure 1: AMP-activated protein kinase, expressed in milligram per millilitre (mg/ml), for both Multiple sclerosis patients (MS) and healthy subjects (Healthy controls) at baseline AMPK muscle biopsy testing. *Significantly different from healthy subjects (P < 0.05). Data are mean ± SEM, MS n = 20 and healthy controls n = 12.

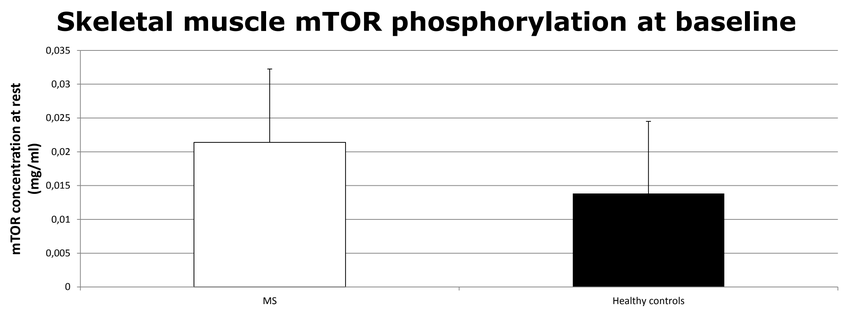

Figure 2: Mammalian target of rapamycin, expressed in milligram per millilitre (mg/ml), for both Multiple sclerosis patients (MS) and healthy subjects (Healthy controls) at baseline mTOR muscle biopsy testing. Data are mean ± SEM, MS n = 20 and healthy controls n = 13.

Part 2

Exercise parameters

	MS patients
Parameters	Mean (S.D.)
Exercise power (W)	95,89 (40,51)
Kilocalories (kcal)	123,01 (47,64)
Exercise heart rate (bmp)	147,33 (26,107)
Exercise Borg-RPE	12,74 (3,78)
Exercise heart rate percentage (%)	89,44 (10,32)
N =	9

	Healthy subjects
Parameters	Mean (S.D.)
Exercise power (W)	166,93 (58,28)
Kilocalories (kcal)	117,00 (48,35)
Exercise heart rate (bmp)	155,86 (16,48)
Exercise Borg-RPE	12,93 (2.97)
Exercise heart rate percentage (%)	90,29 (5,15)
N =	7

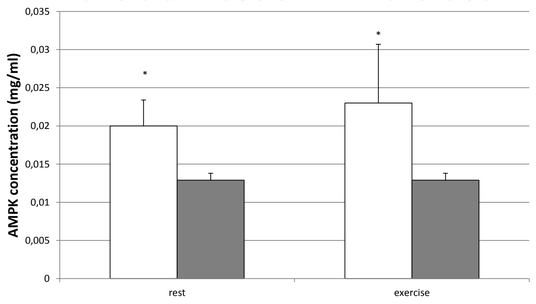

Figure 3: AMP-activated protein kinase (AMPK) , expressed in milligram per millilitre (mg/ml), for both Multiple sclerosis patients (MS) and healthy subjects (Healthy controls) at baseline (rest) and after an acute endurance bout. White = MS, grey = healthy controls. *Significantly different from healthy subjects (P < 0.05) at baseline and after an acute endurance bout. Data are mean ± SEM, MS n = 9 and healthy controls n = 7.

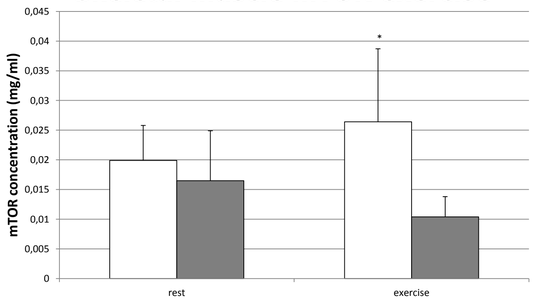

Figure 4: Mammalian target of rapamycin (mTOR), expressed in milligram per millilitre (mg/ml), for both Multiple sclerosis patients (MS) and healthy subjects (Healthy controls) at baseline (rest) and after an acute endurance bout. *Significantly different from healthy subjects (P < 0.05) after acute an endurance bout. Data are mean ± SEM, MS n = 9 and healthy controls n = 7 *(One missing value for MS mTOR concentration in both rest and exercise)*.

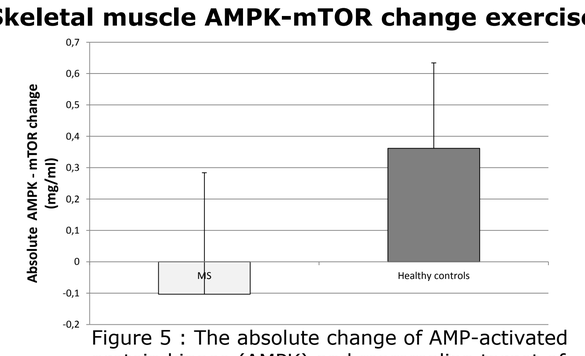

Figure 5 : The absolute change of AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR), expressed in milligram per millilitre (mg/ml), for both Multiple sclerosis patients (MS) and healthy subjects (Healthy controls) after an acute endurance bout. White = MS, grey = healthy controls. No significant results were found (P < 0.05). Data are mean ± SEM, MS n = 9 and healthy controls n = 7. *(One missing value for MS absolute AMPK-mTOR change)*.

Discussion

Part 1: Skeletal muscle AMPK/mTOR phosphorylation at baseline:

- A greater basal skeletal muscle AMPK concentration and a slight tendency to a higher skeletal muscle mTOR phosphorylation value at rest was observed in MS.
- 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 indicates that MS patients are in a constant state of stimulation of mitochondrial biogenesis. The etiology of the skeletal muscle AMPK hyperphosphorylation in MS remains speculative: it might be related to disturbances in blood by Vitamin D levels or to acute systemic inflammation. This could also 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.

Part 2: Skeletal muscle AMPK/mTOR phosphorylation as result of endurance exercise:

- Caloric expenditure was matched between groups.
- 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.
- In MS patients skeletal muscle mTOR phosphorylation increased significantly as result of exercise.

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. It could be the case that the caloric expenditure as result of exercise was too low to alter skeletal muscle AMPK phosphorylation.

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.

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 a 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.