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Research Context

The present master thesis fits within the framework of neurological rehabilitation. Neurological disorders such as Multiple Sclerosis (MS), stroke and Parkinson Disease cause an array of complex and heterogeneous symptoms including cognitive malfunctioning, autonomous dysregulation and motor impairments. These heterogeneous symptoms often lead to an impaired functional capacity and a more sedentary lifestyle resulting in loss of exercise capacity and muscle strength. Physical rehabilitation and exercise therapy are known to be essential for reducing or slowing down progression of impairments. More recently evidence favors the use of higher training intensities such as high-intensity interval training (HIIT) to improve endurance capacity and muscle characteristics in MS patients.

More specifically, this master thesis is focused on muscular impairments in MS, which might negatively influence rehabilitation outcomes following HIIT. MS patients present intramuscular disturbances with studies reporting fewer type 1 fibers, enzymatic changes, decreased fiber cross-sectional area and smaller fiber size of all fiber types. Moreover, previous studies showed impairments in buffer and oxidative capacity, and contractility, which are related to muscle carnosine functions. Given these intramuscular dysfunctions, potentially reduced carnosine concentrations in MS may, in part, contribute to the impaired exercise capacity, reduced muscle strength and exercise fatigue during HIIT exercise. Therefore, the present study investigated muscle carnosine content in an animal MS model (Experimental Autoimmune Encephalomyelitis, EAE) and MS patients. Moreover, the effect of exercise training was investigated on carnosine levels in EAE and MS.

This master thesis (part two) is a trio-thesis conducted by master students Bennet Boonen, Jorn Keirsmaekers and Kristien Leonaers. It was completed as a part of the second master year at the Hasselt University. The students were involved in different parts of the data selection. They helped with strength measurements, maximal endurance tests and the exercise training program in MS. Data-processing and statistical analysis were performed independently by the students. The research protocol and method were also written independently, using provided articles with similar research protocols. Furthermore, the students were involved in other ongoing studies at the REVAL centre with the same baseline measurements and exercise protocol. The writing process of this article (introduction, method, results, discussion and conclusion) was done under

the supervision of promotor prof. dr. Bert Op 't Eijnde and co-promotors dr. Inez Wens and drs. Keytsman Charly. The contribution of each student was equal during the development and writing of the thesis, with additional suggestions and feedback from co-promotor drs. Keytsman Charly.

This master thesis is part of the PhD of Keytsman Charly with the topic: "Multiple sclerosis: associated cardiometabolic risks and impact of exercise therapy" with protocol number 4.84/cardio14.11.

Abstract

Background Patients with Multiple Sclerosis (MS) show intramuscular impairments (reduced muscle contractile function, increased oxidative stress and exercise-induced acidosis), which are related to the functions of carnosine. Though muscle carnosine concentrations were never investigated in MS.

Objectives This study aimed to investigate muscle carnosine content in an animal MS model (Experimental Autoimmune Encephalomyelitis, EAE) and MS patients compared to healthy controls (HC). Moreover, we explored the impact of exercise therapy on muscle carnosine levels in both animals and MS patients.

Methods 80 female Lewis rats were randomized into an exercise (EX, n=40) or a sedentary group (SED, n=40). After a 14-day habituation period both groups were divided into an EAE (EAE_SED, n=20 and EAE_EX, n=19) or HC group (HC_SED, n=20 and HC_EX, n=20). EX rats performed 10 days of treadmill running, while SED rats were placed on a stationary treadmill (day 1-10). At day 17, m. tibialis anterior muscle biopsies were collected in all rats to assess carnosine, anserine and taurine concentrations.

MS patients (n=24, EDSS 2.9±0.3) and HC (n=22) were compared at baseline. Moreover, a subgroup of MS patients was divided into an exercise group (MS_EX, n=11), performing 12 weeks HIIT, or a sedentary group (MS_SED, n=6). M. vastus lateralis carnosine and taurine concentrations were assessed at baseline in all human participants. Measurements were repeated after 12 weeks in the MS subgroup only.

Results Lower muscle carnosine concentrations (p = 0.000) were observed in EAE_SED (3.21±0.20 mmol/kg WW) compared to HC_SED (2.34±0.12 mmol/kg WW) and in EAE_EX (3.24±0.16 mmol/kg WW) compared to HC_EX (2.16±0.18 mmol/kg WW). In humans, lower (p = 0.025) muscle carnosine concentrations were observed in MS (2.89±0.27 mmol/kg WW) compared to HC (3.75±0.27 mmol/kg WW). Treadmill running in rats (10 days) and HIIT (12 weeks) in MS did not alter metabolite contents (p > 0.05).

Conclusion This study demonstrates decreased muscle carnosine concentrations in both EAE animals and MS patients, which could not be restored by an exercise intervention.

1. Introduction

Multiple sclerosis (MS) is a chronic, auto-immune disease of the central nervous system, caused by interaction of environmental, genetic and infectious factors [27]. It is characterized by axonal and neuronal damage, inflammation and demyelination in both brain and spinal cord [14]. MS causes heterogeneous clinical symptoms associated with the affected areas of the central nervous system. These include visual disturbances [12], muscle spasticity [43], ambulation difficulties and balance/coordination problems [10]. Furthermore, impairments in aerobic capacity [35], [39], muscle weakness [11], [34], [49], [61] and fatigue [4], [32], [33] are present. This array of symptoms often leads to a more sedentary lifestyle, which results in a vicious circle of disuse-related loss of exercise capacity and muscle strength [55].

The latter is caused by a decreased motor unit firing rate, deficits in motor unit recruitment and a higher central motor conduction time [17], [40], [46]. Furthermore, MS patients present a loss of muscle mass [11], [17], [24], [30], [49]. Previous studies also reported fewer type I fibers and enzymatic changes suggesting that the muscle depends on anaerobic rather than aerobic oxidative energy supply [30]. Other changes are reduced peak Ca^{2+}-activated force [24] and smaller fiber size of all fiber types in MS [30], [61]. Similar changes in skeletal muscle characteristics were observed in an animal MS model (EAE, Experimental Autoimmune Encephalomyelitis), with studies reporting reduced fiber cross-sectional area of all fiber types, decreased muscle mass and diminished peak work [18], [62]. As such, MS negatively influences muscle characteristics, in which intramuscular changes may explain part of the muscle weakness and fatigue experienced by these individuals.

Several studies investigated the effect of aerobic exercise on muscle characteristics in EAE animals and showed that exercise did not improve muscle fiber atrophy [9], [62], however one study was able to increase skeletal muscle mass [42]. Furthermore, high-intensity running was able to delay paralysis in EAE, while lower intensities had no effect on disease onset [9]. On the other hand, studies in MS patients demonstrated improvements in muscle contractile characteristics and exercise capacity by using both low-to-moderate intensity cardiovascular [48], [50] or resistance training [15], [38]. Though, high-intensity interval training (HIIT) in MS, showed greater improvements in muscle fiber cross-sectional area, physical activity levels, muscle strength and endurance capacity compared to low-to-moderate intensity exercise [60]. Moreover, two other studies used high intense resistance training in MS patients, leading to significant improvements in health-

related quality of life, fatigue and muscle strength [31], [36]. As such, high intense resistance training and HIIT seem to be an efficient and safe way to improve exercise capacity and muscle characteristics in mild-to-moderately impaired MS patients. Though, in MS, feelings of leg exertion at (sub)maximal intensities are reported to be higher and may persist longer compared to healthy controls (HC) [13], [16]. Therefore, strategies to attenuate subjective perceived exertion during high intensity exercise in MS could further improve rehabilitation outcomes.

Increasing carnosine storages in skeletal muscles is often used to delay (muscle) fatigue during HIIT exercise bouts [6]. Carnosine is a dipeptide, synthesized by L-histidine and beta-alanine (β-alanine), which plays a role in several physiologic functions related to muscle contractility and fatigue. For instance, carnosine acts as a buffer in muscle pH regulation [26]. It also increases Ca^{2+}-release from the sarcoplasmic reticulum and sensitivity of the contractile apparatus [19], [20], [21], which increases contractility. Finally, carnosine serves as an antioxidant and reduces reactive oxygen species, which contribute to fatigue during vigorous exercises [2], [45]. As described above, muscle fatigue in MS may be caused by impairments in these intramuscular carnosine functions. Previous studies have shown that MS patients have a greater intracellular decline in pH during exercise and higher serum lactate concentrations compared to HC [1], [49]. Furthermore, a change in peak Ca^{2+}-activated force is seen in MS [24]. So far, several authors already suggested that intramuscular carnosine concentrations are reduced in Amyotrophic Lateral Sclerosis [53], [54], Parkinson's disease [5] and other neuromuscular diseases [5], [8]. Furthermore, reduced serum carnosinase activity was found in MS patients [59]. As such, potentially reduced muscle carnosine concentrations in MS may, in part, contribute to the impaired exercise capacity, reduced muscle contractile function and exercise fatigue during HIIT exercise. However, muscle carnosine concentrations were never investigated in MS or EAE.

This study aims to investigate muscle carnosine content in EAE animals and MS patients compared to HC. We hypothesize that EAE animals and MS patients have lower intramuscular carnosine concentrations. Moreover, we aim to explore the impact of exercise therapy on muscle carnosine content in EAE and MS.

2. Materials and methods

2.1. Animals

2.1.1. Rats

In the present study, 80 female Lewis rats (age 6-7 weeks, body weight ~100g, Charles River) were housed in the animal facilities at Hasselt University. They were constantly exposed to a light/dark cycle (12 hours/12 hours) with a temperature of 22°C and a relative humidity of 22-24% for adaptation to the environment. Food and drinking water was given ad libitum. The experimental protocol was approved by the Animal Ethics Committee of Hasselt University in accordance with the national and European legislation. The National Research Council's guide for laboratory animals was followed.

2.1.2. Study design overview

After acclimatization (day -21 to -15), all animals (n=80) were randomly assigned into an exercise (EX, n=40) or sedentary group (SED, n=40). Researchers involved in this experiment, were not blinded to group allocation. Before the start of the experiment, both groups underwent a habituation period of 14 days (day -14 to -1), in which EX animals were familiarized to treadmill running. Animals achieved 1h running at a running speed of 18m/min (25° inclination) at the end of the habituation period. This was reached by gradually increasing running duration and speed. If necessary, EX rats were motivated to run by means of electrical shocks (<1s, low intensity). On the other hand, SED animals were placed on a stationary treadmill on a daily basis and were not encouraged by means of electrical shocks.

After habituation (day 0), the SED and EX group were divided into a healthy control group (HCSED, n=20 and HCEX, n=20) and EAE group (EAESED, n=20 and EAEEX, n=20). Following this subdivision, the EAE group received an EAE-induction by a single percutaneous injection in both footpads (100μl/foot) under isoflurane anesthesia [44]. Per animal, the injection consisted of 25μl 7RA heat-killed mycobacterium tuberculosis combined with 24μl purified myelin basic, 120μl complete Freunds adjuvant and 31 μl phosphate-buffered saline.

From day 1 to 10 EX animals exercised 1h/day on a treadmill, until running was prevented by progressive hindquarter paralysis (~day 11). After 10 consecutive days of physical exercise, all animals remained sedentary (day 11-17), enduring hindquarter paralysis (EAE group) or recovery

(HC group). EAE animals were examined daily (~ 8h30) and clinical symptoms were scored on a 6-point scale (0-5) [44]. Hindquarter paralysis was expected to develop 12 to 14 days after EAE-induction, however if running was prevented (score ≥4: complete paralysis of hind limbs and midriff) before day 11, the intervention was immediately ended. These animals were excluded from the study and euthanized. After recovery (day 17), m. tibialis anterior muscle biopsies were collected from all animals to investigate muscle carnosine, anserine and taurine content. Finally, all rats were euthanized by an intracardial injection of pentobarbital sodium. Throughout the study, well-being of the animals was continuously monitored. The present study protocol was performed previously [22], [62].

Figure 1: (Adapted with permission from dr. Wens Inez) Animal study design [62].
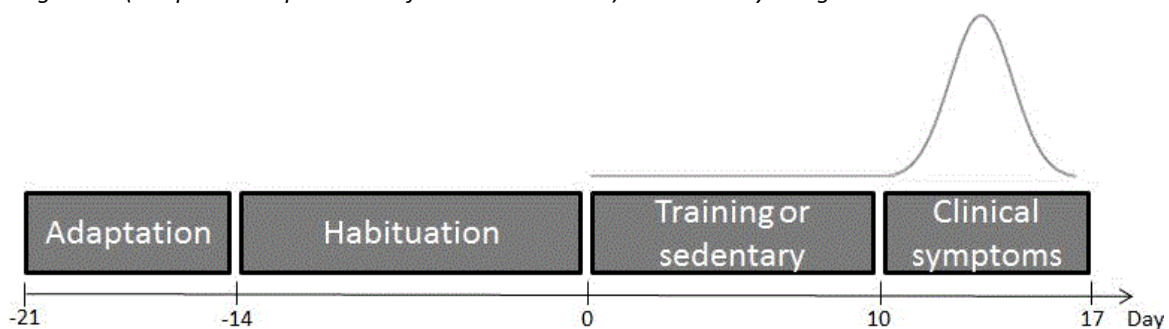
Following an adaptation period (day -21 to -14), animals were familiarized to treadmill running or placed on a stationary treadmill (day -14 to -1). After EAE induction (day 0), the rats were subjected to training or remained sedentary (day 1-10). After recovery (day 11-17) muscle biopsies were taken from all rats at day 17. Finally, all rats were sacrificed.

2.1.3. Muscle biopsies

M. tibialis anterior of one hind limb was dissected in all animals. Any visible blood, connective or fat tissue was removed from the biopsies. The mid-parts of m. tibialis anterior were then snap frozen in liquid nitrogen and stored at -80°C until further analyses were performed.

2.2. Humans

2.2.1. Subjects

Twenty-four MS patients and 22 HC, aged >18 years, were recruited by local advertisement and from MS rehabilitation centers in Belgium (Rehabilitation and MS center Overpelt and MS clinic Melsbroek). Patients with MS were diagnosed according the McDonald criteria (EDSS range 1-5) and HC were matched for age, BMI, gender, weight and height. All participants were included following written informed consent.

Subjects were excluded if they participated in another study at the same time, had an acute MS exacerbation <6 months prior to the start of the study, were physically active (> one exercise

session/week), had other diseases (cardiovascular, cancer, pulmonary and/ or renal) or contra-indications to perform high intensity exercise.

The study was approved by the Medical Ethical Committee of the Jessa hospital (Hasselt, Belgium) and Hasselt University (NCT02466165, December 2014). All study procedures followed the Declaration of Helsinki.

2.2.2. Study design overview

At baseline, a maximal endurance capacity test and maximal isometric contractions of knee flexors and extensors were performed in all subjects, as described elsewhere [61]. After at least 48 hours muscle biopsies of the m. vastus lateralis were collected to investigate muscle carnosine, taurine, histidine, glutamine and serine content.

In addition, a subgroup of MS patients was enrolled in an exercise group (MS_{EX}, n=11), performing a 12-week exercise intervention program, or a sedentary control group (MS_{SED}, n=6), receiving usual care. Baseline measurements of these subgroups (MS_{EXpre} and MS_{SEDpre}) were repeated after 12 weeks (MS_{EXpost} and $MS_{SEDpost}$). The remaining MS and HC subjects were not enrolled in the exercise intervention program and were only assessed at baseline.

2.2.3. Exercise intervention program

MS patients enrolled in the EX group (MS_{EX}, n=11) completed 12 weeks of HIIT in combination with moderate-to-high intensity resistance training, as previously described [23], [60]. Subjects performed 5 training sessions per 2 weeks and each session started with a 5min warm-up on a cycle ergometer, followed by HIIT and resistance training.

HIIT was also performed on a cycle ergometer and consisted out of a 'work' and 'rest' phase. The work phase was a short period high-intensity effort, which was followed by one minute of recovery (rest phase). This process was repeated five times each HIIT session. During the first 6 weeks, exercise duration was gradually increased from 5x1min to 5x2min. Furthermore, the maximal endurance test at baseline was used to calculate 100% of the maximal workload of each patient. This value was extrapolated to the corresponding heart rate (~80-90% of the maximal heart rate) to define exercise intensity used during the work phase of the interval sessions. During the second 6 weeks, exercise intensity increased to 100-120% of the maximal workload (~90-100% of initial maximal heart rate), while exercise duration remained stable (5x2min).

Following a short resting period, the MS subgroup performed moderate-to-high intensity

resistance training. Exercises were done unilaterally to reach appropriate workloads as MS patients display bilateral strength differences between both legs [56]. Number of repetitions was gradually progressed from 1x10reps to 2x20reps at maximal achievable load. Subjects were continuously supervised for safety reasons and standardized encouragements were provided to reach highest individual performances.

2.2.4. Muscle biopsies

Muscle biopsies from the middle part of the m. vastus lateralis of the weakest leg (baseline maximal isometric strength test) were collected using the Bergström needle technique. Muscle biopsies were collected by an experienced medical doctor at baseline and after the 12-week intervention period. To avoid interference, the second biopsy was executed at least two centimeters from the previous biopsy site. HC and part of the MS group (not enrolled in MS subgroup) only underwent baseline biopsies. Muscle samples were snap frozen in liquid nitrogen and, until further analysis, stored at -80°C.

2.3. Metabolite measurements

Muscle metabolite concentrations were determined using high-performance liquid chromatography (HPLC) in both rats and humans. First, muscle samples were deproteinized by using 35% sulfosalicyclic acid and centrifuged (5min, 14.000g). Then, deproteinized supernatant was mixed with AccQ Fluor Borate buffer and reconstituted Fluor Reagent (5:75:20) from the AccQTag chemistry kit (Waters). The derivatized samples were applied to a Waters HPLC system with a Hypersilica column (4.6 x 150 mm, 2.5µm) and ultraviolet detector (excitation/emission wavelength: 250/395nm).

Intramuscular carnosine concentration was the measure of primary interest in both rats and humans. Secondary outcome measures were anserine and taurine.

Anserine is the methylated analog of carnosine which is exclusively found in all animals, with the same bioactivity as carnosine [8]. Taurine, a sulfur-containing b-amino acid, is also present in mammalian skeletal muscles being highest in type 1 fibres [25].

In addition, some reference metabolites (serine, histidine and glutamine) were measured in human subjects to verify that potential lowering of carnosine concentrations was not due to poor quality of the biopsy in case of normal serine, histidine and glutamine concentrations.

2.4. Statistical analysis

All data were analysed using IBM SPSS Statistics 22 software. First normality was checked using the Shapiro-Wilkinson test and equality of variances by means of the Levene's test. Both animal and human data were analysed using nonparametric statistical tests because of sample characteristics (small sample size of the groups and no normal distribution). Animal data were analysed by means of a Man-Whitney U test and the following comparisons were made: EAE_{SED} vs HC_{SED}, EAE_{EX} vs HC_{EX}, EAE_{EX} vs EAE_{SED} and HC_{EX} vs HC_{SED}. Baseline comparison between MS and HC subjects was performed using the Mann-Whitney U test. Differences between MS_{EXpost} vs MS_{EXpre} and $MS_{SEDpost}$ vs MS_{SEDpre} were analysed by a Wilcoxon Signed Ranks Test. MS_{EX} and MS_{SED} group were compared by calculating their delta values (post minus pre), followed by a Mann-Whitney U test. All data were expressed as individual values with mean bars and standard error of mean (SEM) in figures or as ±SEM in text. $P<0.05$ represents the threshold for statistical significance.

3. Results

3.1. Animals

3.1.1. Primary outcome measure: carnosine

Significantly lower muscle carnosine concentrations (p = 0.000, figure 2) were observed between EAE$_{SED}$ (1.12±0.13 mmol/kg WW) vs HC$_{SED}$ (2,36±0.12 mmol/kg WW) and EAE$_{EX}$ (1.12±0.11 mmol/kg WW) vs HC$_{EX}$ (2.43±0.19 mmol/kg WW).

No difference was found in carnosine concentrations between SED and EX of the same group (p > 0.05, figure 2).

(table 1)

Figure 2: Carnosine content in rats.
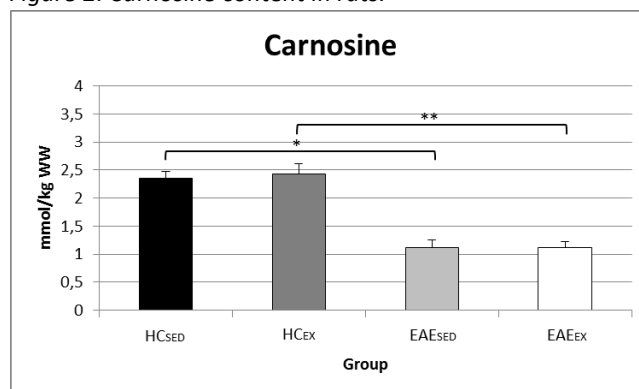
*Mean (±SEM) carnosine concentrations in m. tibialis anterior of rats. HC$_{SED}$, sedentary healthy controls; HC$_{EX}$, exercise healthy controls; EAE$_{SED}$, sedentary experimental autoimmune encephalomyelitis; EAE$_{EX}$, exercise experimental autoimmune encephalomyelitis. *, P<0.05, compared between SED groups. **, P<0.05, compared between EX groups.*

3.1.2. Secondary outcome measures: anserine and taurine

Significantly higher muscle anserine concentrations were observed between EAE$_{SED}$ (3.21±0.20 mmol/kg WW) vs HC$_{SED}$ (2.34±0.12 mmol/kg WW) (p = 0.001) and EAE$_{EX}$ (3.24±0.16 mmol/kg WW) vs HC$_{EX}$ (2.16±0.18 mmol/kg WW) (p = 0.000).

Muscle taurine concentrations were significantly higher (p = 0.001) in EAE$_{EX}$ (15.00±0.76 mmol/kg WW) vs HC$_{EX}$ (11.22±0.69 mmol/kg WW), whereas no significant difference
 (p = 0.056) was found in EAE$_{SED}$ (13.38±0.82 mmol/kg WW) vs HC$_{SED}$ (11.39±0.58 mmol/kg WW).

No differences were found in anserine and taurine concentrations between SED and EX of the same group (p > 0.05).

(table 1)

3.2. Humans

3.2.1. Baseline subject characteristics

No baseline differences were observed in subject characteristics between MS and HC. (table 2)
Both BMI and EDSS tended (p = 0.05) to be lower in MS$_{EXpre}$ compared to MS$_{SEDpre.}$ (table 3)

3.2.2. Primary outcome measure: carnosine

Muscle carnosine concentrations were significantly lower (p = 0.025, figure 3) in MS (2.89±0.27 mmol/kg WW) compared to HC (3.75±0.26 mmol/kg WW). (table 4)

A significant increase (p = 0.021, figure 4) in carnosine was observed between MS$_{EXpre}$ (4.08±0.57 mmol/kg WW) vs MS$_{EXpost}$ (4.60±0.74 mmol/kg WW), whereas no significant difference (p = 0.173, figure 4) was found between MS$_{SEDpre}$ (2.74±0.34 mmol/kg WW) vs MS$_{SEDpost}$ (3.49±0.61 mmol/kg WW). No significant difference (p = 0.546, figure 5) was found between MS$_{EX}$ (1.05±0.34 mmol/kg WW) vs MS$_{SED}$ (0.75±0.67 mmol/kg WW) when comparing delta values. (table 5)

Figure 3: Carnosine content in humans at baseline.
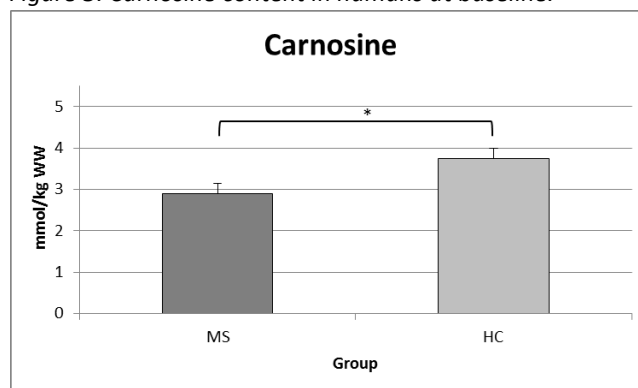
Mean (±SEM) carnosine content in m. vastus lateralis of humans. MS, multiple sclerosis; HC, healthy controls. *, P<0.05, compared between groups.

Figure 4: Carnosine content in MS subgroups.
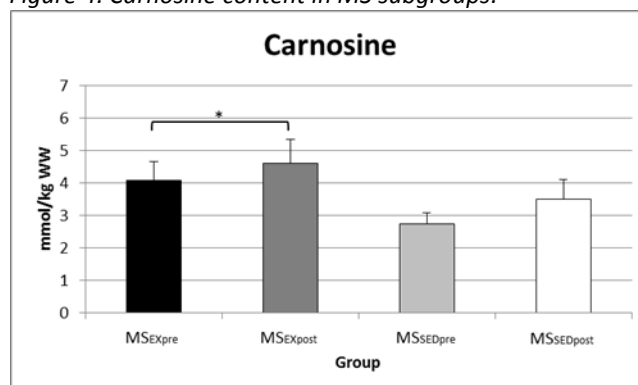
Mean (±SEM) carnosine content in m. vastus lateralis of humans. MS$_{EXpre}$, multiple sclerosis pre-exercise; MS$_{EXpost}$, multiple sclerosis post-exercise; MS$_{SEDpre}$, multiple sclerosis pre-sedentary; MS$_{SEDpost}$, multiple sclerosis post-sedentary. *, P<0.05, compared with pre-intervention within group.

Figure 5: Carnosine content delta values in MS subgroups.
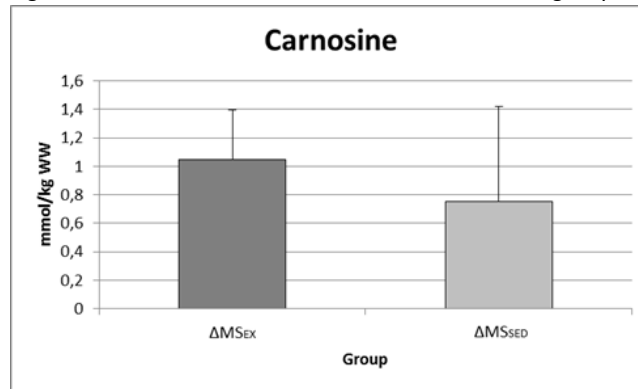
Mean (±SEM) carnosine content in m. vastus lateralis of humans. ΔMS$_{EX}$, MS$_{EXpost}$ minus MS$_{EXpre}$; ΔMS$_{SED}$, MS$_{SEDpost}$ minus MS$_{SEDpre}$. *, P<0.05, compared between groups.

3.2.3. Secondary outcome measure: taurine

Muscle taurine concentrations were significantly lower (p = 0.040) in MS (6.52±0.59 mmol/kg WW) compared to HC (8.40±0.70 mmol/kg WW). (table 4)

No significant difference in taurine concentrations was observed between MS$_{EXpre}$ (9.00± 0.98 mmol/kg WW) vs MS$_{EXpost}$ (8.96±1.56 mmol/kg WW) (p = 0.182), MS$_{SEDpre}$ (6.65±1.20 mmol/kg WW) vs MS$_{SEDpost}$ (8.32±0.92 mmol/kg WW) (p = 0.116) and between the delta values of MS$_{EX}$ (51.54±0.89 mmol/kg WW) vs MS$_{SED}$ (51.67±0.95 mmol/kg WW) (p = 0.884). (table 5)

3.2.4. Reference metabolites: serine, glutamine and histidine

No significant difference in serine (p = 0.613), histidine (p = 0.244) and glutamine (p = 0.692) concentrations were found between MS and HC.

4. Discussion

The present study is the first to investigate intramuscular carnosine concentrations in an animal MS Model (EAE) and MS subjects. The results of this study show that carnosine concentrations are decreased in both EAE rats and MS patients. Moreover, this could not be restored by an exercise intervention.

Previous research demonstrated that MS patients have impairments related to intramuscular carnosine functions such as greater intracellular pH-decline during exercise [1], [49], higher serum lactate concentrations [1], [49] and a change in peak Ca^{2+}-activated force [24]. Moreover, several studies suggested that intramuscular carnosine concentrations could be reduced in neuromuscular diseases [5], [8], [54] and a decrease in carnosinase activity was already found in MS [59]. Though, the present study is the first to assess muscle carnosine concentrations in MS and to show that these are reduced in an animal MS model and MS patients. This might implicate that reduced muscle carnosine concentrations have a role in the impaired exercise capacity, reduced muscle contractile function and exercise fatigue seen in patients with MS.

The reason for the decrease in carnosine content remains unclear. It is suggested that the decline is caused by the combination of progressive denervation and reduced physical activity, which occurs in MS and other neuromuscular diseases. Though in this case, a similar decline in other muscle metabolites is expected. In this study we observed no significant differences in serine, glutamine and histidine content between MS and HC. Hence reduced muscle carnosine concentrations in MS are not caused by disuse or poor biopsy quality, but specifically MS-related.

Apart from muscle carnosine, this study investigated intramuscular anserine concentrations in healthy and EAE rats. Anserine was examined because it possesses common properties in skeletal muscles compared to carnosine and therefore it is important to investigate a possible interaction between these metabolites. The present study shows that anserine concentrations were increased in EAE rats. We suggest that the total histidine-containing dipeptide content remains equal in EAE rats as the increase in anserine content might be a mechanism to compensate altered carnosine concentrations. As anserine is not present in human muscle tissue, a similar compensation is not possible in MS patients.

Cross-sectional studies have demonstrated higher muscle carnosine concentrations in highly anaerobic trained persons compared to endurance athletes and sedentary people [41], [57], suggesting that high carnosine concentrations may be a consequence of long-term training adaptations. Several longitudinal prospective studies used exercise training to increase muscle carnosine storages, but results were inconsistent. One study found increased carnosine concentrations after 8 weeks of sprint training [56]. Recently, another study suggested slight benefits of both low-intensity (high-volume) and high-intensity cycle exercise training combined with β-alanine supplementation on carnosine loading compared to solely supplementation [7]. Though, studies using high-intensity resistance training did not find increased carnosine concentrations after 4, 10 and 16 weeks, respectively [28], [29], [37]. Moreover, 5 weeks of alternating running and cycle sprint sessions also had no effect on carnosine content [3]. The present study thus confirms the majority of these studies, as we have shown that 10 days of treadmill running and 12 weeks of HIIT did not affect carnosine content in animals and MS patients respectively. Therefore, other strategies to restore muscle carnosine content in MS should be explored.

Supplementation with β-alanine is used in previous studies to increase intramuscular carnosine concentrations. β-alanine, the rate-limiting precursor of carnosine [52], has been shown to increase carnosine storages in healthy skeletal muscles [26], leading to improvements in exercise capacity and performance. It is shown to be beneficial, especially in exercise types of short duration and high intensity [47]. Due to its potential to improve high-intensity exercise performance, β-alanine has become an interesting ergogenic aid, often used by athletes. However, the effect of β-alanine supplementation in MS was never investigated yet and deserves further research.

The present study is characterized by several limitations. A rather small sample size was used in the human study, because of the explorative character (no power analysis). Researchers investigating both animals and humans were not blinded to group allocation. Furthermore, the assignment of MS patients into EX and SED groups was not randomized, which could have caused selection bias.

5. Conclusion

In conclusion, this study shows decreased muscle carnosine concentrations in both EAE animals and MS patients. Furthermore, exercise did not improve this. Other strategies to restore muscle carnosine levels in MS should be explored.

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7. Appendices

Table 1: Carnosine, anserine and taurine content in rats.

	HC$_{SED}$	HC$_{EX}$	EAE$_{SED}$	EAE$_{EX}$
Carnosine	2.36 ±0.12	2.43 ±0.19	1.12 ±0.13[*]	1.12 ±0.11[**]
Anserine	2.34 ±0.12	2.16 ±0.18	3.21 ±0.20[*]	3.24 ±0.16[**]
Taurine	11.39 ±0.58	11.22 ±0.67	13.38 ±0.82	15.00 ±0.76[**]

*Mean (±SEM) carnosine, anserine and taurine content in m. tibialis anterior of rats. HC$_{SED}$, sedentary healthy controls; HC$_{EX}$, exercise healthy controls; EAE$_{SED}$, sedentary experimental autoimmune encephalomyelitis; EAE$_{EX}$, exercise experimental autoimmune encephalomyelitis. * P<0.05, compared between SED groups. **, P<0.05, compared between EAE groups.*

Table 2: Baseline subject characteristics.

	HC	MS
Age (y)	48.7 ±2.6	52.7 ±1.9
Gender (f/m)	14/8	13/11
Weight (kg)	69.5 ±3.0	71.6 ±2.6
Height (m)	1.69 ±3.0	1.71 ±0.0
BMI	24.1 ±0.5	24.5 ±0.7

Data are expressed as means (±SEM). HC, healthy controls (n=22); MS, multiple sclerosis (n=24). y, years; f/m, females/males; kg, kilograms; m, meters; BMI, body mass index.

Table 3: Baseline MS subgroup characteristics.

	MS$_{EXpre}$	MS$_{EXpre}$
Age	58.3 ±4.7	51.3 ±2.2
Gender (f/m)	2/4	7/4
Weight (kg)	78.7 ±3.7	67.9 ±4.6
Height (m)	1.73 ±0.0	1.71 ±0.0
BMI	26.4 ±1.0	23.1 ±1.1[*]
EDSS	4.1 ±0.7	2.5 ±0.4[*]

*Data are expressed as means (±SEM). MS$_{EXpre}$, multiple sclerosis pre-exercise group (n=22); MS, multiple sclerosis pre-sedentary group (n=24); y, years; f/m, females/males; kg, kilograms; m, meters; BMI, body mass index; EDSS, expanded disability status scale. *, p=0.05, compared between groups.*

Table 4: Carnosine and taurine content in humans at baseline.

	HC	MS
Carnosine	3.75 ±0.26	2.89 ±0.27[*]
Taurine	8.40 ±0.70	6.52 ±0.59[*]

*Mean (±SEM) carnosine and taurine content in m. vastus lateralis of humans. HC, healthy controls; MS, multiple sclerosis. *, P<0.05, compared between groups.*

Table 5: Carnosine and taurine content in MS subgroup.

	MS$_{SED}$		MS$_{EX}$		MS$_{EX}$	ΔMS$_{EX}$
	Pre	**Post**	**Pre**	**Post**	**post–pre**	**post-pre**
Carnosine	2.74 ±0.34	3.49 ±0.61	4.08 ±0.57	4.60 ±0.74[*]	0.75 ±0.67	1.05 ±0.34
Taurine	6.65 ±1.20	8.32 ±0.92	9.00 ±0.98	8.96 ±1.56	1.67 ±0.95	1.54 ±0.89

*Mean (±SEM) carnosine and taurine content in m. vastus lateralis of humans. MS$_{SED}$, MS sedentary; MS$_{EX}$, MS exercise; ΔMS$_{SED}$, MS$_{SEDpost}$ minus MS$_{SEDpre}$; ΔMS$_{EX}$, MS$_{EXpost}$ minus MS$_{EXpre}$. *, P<0.05, compared with pre-intervention within group.*

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Richting: **master in de revalidatiewetenschappen en de kinesitherapie-revalidatiewetenschappen en kinesitherapie bij musculoskeletale aandoeningen**
Jaar: **2017**

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