The Impact of Carnosine Loading and Rehabilitation Therapy on Exercise Capacity in Multiple Sclerosis

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PROTOCOL: IMPACT OF CARNOSINE LOADING ON HOME-BASED EXERCISE THERAPY EFFECTS IN MULTIPLE SCLEROSIS

INTRODUCTION

Multiple Sclerosis (MS) is a neurodegenerative inflammatory disease of the central nervous system[1] that is characterized by central nervous system demyelination and axonal loss causing a variety of symptoms such as spasticity, tremor, paralysis, walking difficulties and cognitive abnormalities[2]. **Decreased exercise capacity, excessive (post-exercise) fatigue and reduced muscle contractile function[3, 4] are frequently occurring comorbidities that substantially affect various daily life activities** leading to an inactive lifestyle and lower quality of life[5]. This results in a disuse-related physiological profile and thus even greater muscle weakness and health risks than provided by the disease *per se*[6]. In fact, in other populations muscle weakness is an independent predictor of premature death[7]. Central mechanisms contributing to the latter symptoms include reduced motor firing rates, impaired motor unit recruitment and increased central motor conduction time[8-12]. However, **a portion of the neuromuscular dysfunction present in MS appears to reside within the affected muscle[8-10, 12-15]**.

Exercise therapy and skeletal muscle dysfunction in MS

So far, recent immune-modulatory therapies decrease MS relapse rate and disease severity[16] but they do not slow the disease process. Because such therapies, unfortunately, do not affect accumulation of MS disabilities and comorbidities and because at least some MS related impairments are inactivity related rather than a result of non-reversible tissue injury, exercise rehabilitation therapy has become an important part of overall MS treatment. Indeed, a multitude of low to moderate intensity **exercise intervention studies in MS** already reported small (+10-15%) but significant improvements in muscle strength and exercise capacity[3]. Surprisingly however, **effects are lower compared to healthy controls and some other disease populations.** In an attempt to further improve rehabilitation outcome we therefore recently performed higher intensity training (HIT) studies in an animal MS model (Experimental Autoimmune Encephalomyelitis, EAE[17]) and in MS patients[18]. Although results are promising with substantially improved (+25-30%) muscle strength and exercise capacity following 8-12w of exercise[17, 18] most MS subjects reported **higher post exercise muscle fatigue and overall perceived exertion** rates (BORG: 14.7±1.5 vs. 12.7±1.3) compared to low-to-moderate intensity training[15].

To date it is difficult to address the **underlying mechanisms of the overall therapeutic effect of exercise therapy in MS**. So far several **immune-related mechanisms** such as altered circulating cytokine levels and BDNF expression[19, 20] following an acute exercise bout and longer term exercise have been described. We recently demonstrated reduced innate markers of inflammation[21] and a rapid exercise-induced mobilization of dendritic cells that is possibly mediated by a Flt3L- and MMP-9-dependent processes[22] following exercise in MS. This may indicate a negative feedback mechanism for the immune system's ability to induce tissue damage and inflammation following exercise. The above described neuro-immune mechanisms unfortunately remain largely **speculative** supporting the above mentioned hypothesis that indeed **part of the neuromuscular dysfunction could be situated in the affected muscle**. In this respect, we and others, have reported **altered muscle contractile characteristics** and muscle fibre composition (shift to glycolytic fibre types) as well as cross-bridge (Ca^{2+} handling) abnormalities in MS[8, 14, 15]. Furthermore, **abnormal muscle energy metabolism in MS** has been demonstrated involving reduced Krebs cycle and complex I and II activities[12, 23], overproduction of reactive oxygen species (ROS[24]), increased basal AMP-activated protein kinase phosphorylation[25] and delayed phosphocreatine resynthesis after exercise[12, 13, 23, 25, 26]. This suggests higher basal and exercise related energy expenditure and increased exercise-induced intramyocellular lactate accumulation, and thus greater perceived muscle fatigue, as recently evidenced by increased basal[27] and exercise-induced (see Figure 1, Keytsman & Eijnde et al. *Manuscript in preparation*) serum

lactate concentrations. Thus, **impairments in both (I) muscle contraction and (II) energy supply seem to attenuate adequate exercise therapy outcome in MS.** Consequently, any strategy that improves muscle contraction and/or intramyocellular energy homeostasis during exercise training may therefore be an interesting tool to investigate the underlying mechanisms of the overall therapeutic effect of exercise therapy in MS and, as such, may help to further optimize exercise rehabilitation protocols.

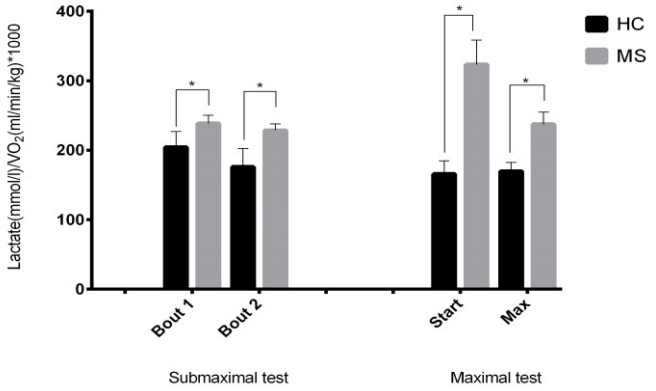


Figure 1. *Serum lactate concentrations (means±SE's) during submaximal (two 6-min exercise bouts [25% of W_{max}] and a 6-min rest interval) and maximal graded exercise testing (cycling) in healthy controls (HC, n=17) and multiple sclerosis (MS, n=20) patients (Pilot data taken from Keytsman & Eijnde et al. Increased exercise-induced lactate accumulation in MS. Manuscript in preparation).*

Muscle carnosine loading

Carnosine (β-alynyl-L-histidine) is found in high concentrations in mammalian skeletal muscle[28, 29]. Its **physiological role** is related to **(I) contractile function** in general and more specific to Ca^{++} handling[29]. In addition, **(II)** carnosine has also been shown to **buffer muscle pH** resulting from exercise–induced acidosis[29] and it has been suggested to **affect mitochondrial respiration**[30] and to **protect against exercise-induced oxidative stress[31]**. Here, carnosine reduces the production of thiobarbituric acid reactive substances and malondialdehyde due to lipid peroxidation[28, 29]. **High (or elevated) muscle carnosine content may thus be advantageous to improve muscle contractile properties and/or myocellular energy supply during exercise intervention.** Because skeletal muscle carnosine synthesis is mainly determined by β-alanine availability[32], exogenous (dietary, 2-6g/d, 4-10w) intake of β-alanine has been successfully applied to increase muscle carnosine content (+80%, **carnosine loading**) in various rodent disease models[33-35] and healthy volunteers[36-38]. In untrained and aged subjects β-alanine supplementation has recently been shown to specifically improve maximal power output (+13-30%) in exercise types lasting 1-4min[39]. Interestingly, trained muscles load more efficiently with carnosine than untrained muscles[40]. Consequently, β-alanine is rapidly becoming a popular **ergogenic substance** in the (clinical) exercise/sports community.

Carnosine's **therapeutic effect** has already been shown in Alzheimer's[41] and Parkinson's[28] disease. In these neurological disorders **carnosine treatment** (~1.5g/d β-alanine) improved a number of neurological symptoms[42]. Interestingly, recent **pilot data (Figure 2) from the laboratories suggested substantially reduced (~60%) skeletal muscle carnosine content in EAE rats. In MS patients m. vastus lateralis carnosine content was reduced by ~20%.** Though, 12 weeks of (high intense) exercise training did not remediate this (PRE-POST p>0.05, Figure 2). Hence, carnosine treatment in MS could be a valid **new approach** to further **(I) improve exercise rehabilitation therapy in MS** and **(II) investigate the effects of MS on skeletal muscle.** The carnosine loading potential of β-alanine supplementation in EAE and MS patients, as well as its associated (therapeutic) effects on muscle contractile functioning and cellular respiration, however, have not been investigated thoroughly yet. In this regard, we have recently explored the effect of β-alanine and carnosine supplementation on muscle carnosine content during EAE. Here, β-alanine and carnosine intake delayed paralysis onset and peak as well as improved recovery. Furthermore (Figure 3) and compared to controls muscle carnosine concentrations substantially increased in these animals (Figure 4). These findings in an animal MS model warrant further exploration of the effects of muscle carnosine loading in MS.

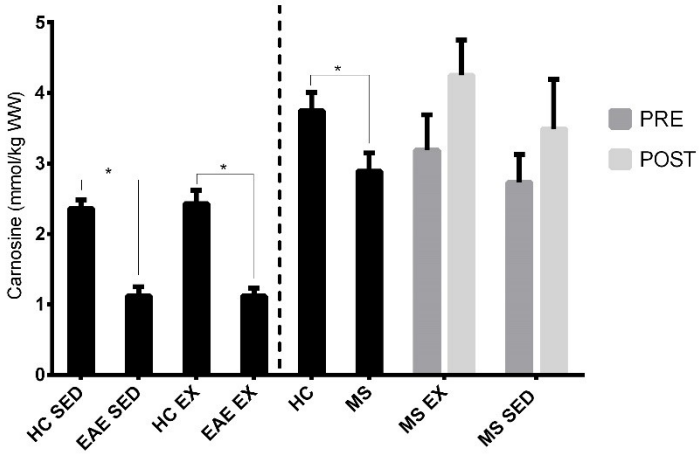
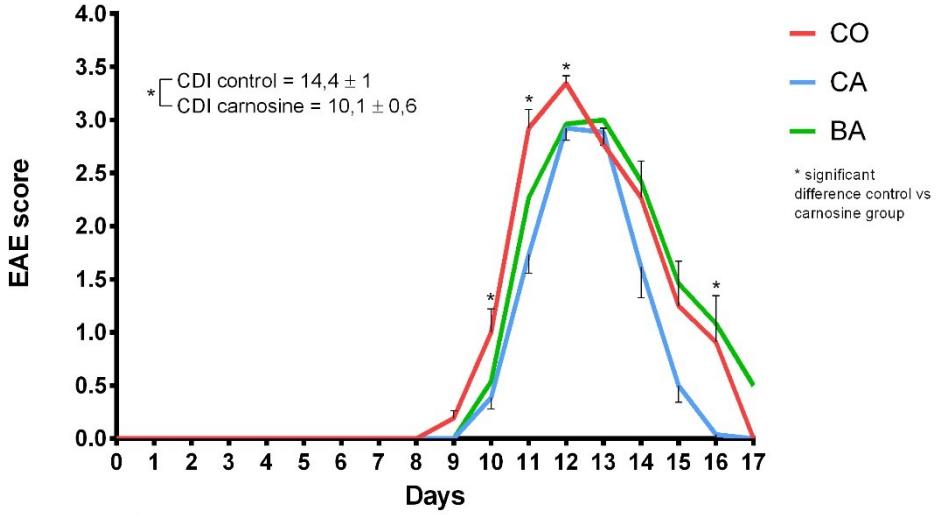


Figure 2. *Muscle carnosine concentrations (mmol/kg ww, means±SE's) in sedentary (SED, n=15) and exercised (EX, n=16) EAE rats (animal MS model), in healthy controls (HC, n=18) and in MS patients (n=16) before (PRE) and after (POST) 12w of exercise intervention. Muscle biopsies for this pilot study were provided by prof. dr. Bert Op 't Eijnde (Hasselt University) and analysed by prof. dr. Wim Derave (Ghent University). As these pilot data are believed to be pre-competitive, the data have not been published yet.*



	Control (n=13)	Carnosine (n=13)	β-alanine (n=13)
Peak scores EAE	3,8 ± 0,2	3,1 ± 0,1*	3,8 ± 0,3
Onset (days)	9,8 ± 0,2	10,4 ± 0,2	10,2 ± 0,1
Peak (days)	11,8 ± 0,1	12,5 ± 0,2*	12,4 ± 0,2
Recovery (days)	4,9 ± 0,3	3,2 ± 0,3*	4 ± 0,3
CDI	14,4 ± 1	10,1 ± 0,6*[a]	14 ± 1,2

Figure 3*. Clinical disease scores of EAE rats (animal MS model), without (control, n=13), with carnosine (carnosine, n=13) or β-alanine (β-alanine, n=13) supplementation. CID, Cummulative Disease Index. * p<0.05 for carnosine compared to control. [a]p<0.05 for carnosine compared to β-alanine.*

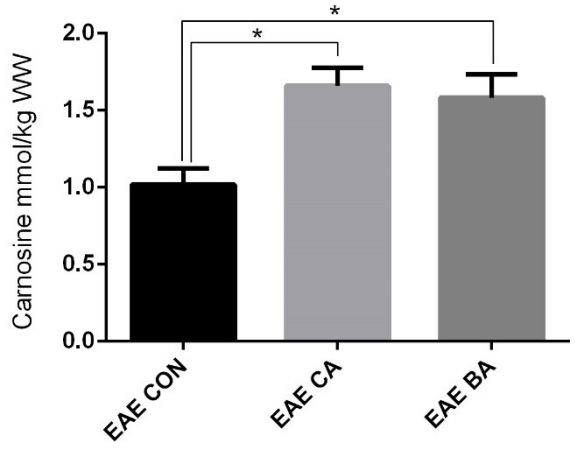


Figure 4. *Muscle carnosine concentrations (mmol/kg WW) in EAE rats (animal MS model), without (EAE CON, n=13) and with carnosine (EAE CA, n=13) or β-alanine (EAE BA, n=13) supplementation. *p<0.05 compared to control group.*

Research objectives

Increasing evidence favours exercise therapy as an efficient tool to counteract inactivity related secondary symptoms in MS. Furthermore, exercise therapy may affect MS-associated muscle contractile and energy supply dysfunctions. So far, low to moderate intensity exercise rehabilitation has shown to induce small but consistent improvements in several functional parameters. High intensity exercise training in MS seems to further improve this. However, although results are promising, impairments in both muscle contraction and energy supply probably attenuate therapy outcome. In keeping with the above described physiological role of skeletal muscle carnosine and because muscle carnosine content may be lower in MS, **the primary aim of the present project is to investigate whether carnosine loading improves exercise therapy outcome (exercise capacity, body composition) and performance in MS.** If the latter hypothesis can be confirmed, muscle carnosine loading could be a novel intervention to improve exercise capacity and muscle function in this population.

Ergogenic potential of muscle carnosine loading and exercise therapy in MS

So far, it is clear that β-alanine intake enhances exercise capacity of untrained, trained and aged individuals by improving contractile properties, maintaining higher intracellular energy levels and optimizing training adaptations. Because early fatigue of contracting musculature during rehabilitation is the predominant cause of exercise cessation, postponing exercise-induced fatigue by β-alanine supplementation will be clinically very relevant (improving exercise therapy efficiency). Consequently, we aim to research the ergogenic potential of β-alanine intake in MS rehabilitation and **hypothesize that β-alanine supplementation in combination with exercise therapy optimizes therapy outcome (exercise capacity, body composition) and performance in this population.**

METHODS

Subjects

Twenty multiple sclerosis (MS) patients, diagnosed according to the McDonald criteria, and twenty healthy controls (HC), aged >18y will be included following written informed consent. Subjects will be excluded if they experience contraindications to participate in moderate to high intensity exercise, already participate in another study, experienced an acute MS exacerbation <6 months prior to the start of the study or have an EDSS score >3. This study will be performed in accordance with the Declaration of Helsinki and will be registered at ClinicalTrials.gov.

Study design

Following inclusion, baseline measurements (PRE) will be performed in MS patients (n=20) and HC (n=20). First, exercise capacity (maximal graded exercise test) will be evaluated. Prior to the maximal exercise test, heart function will be assessed by an experienced medical doctor, followed by measurement of whole body composition (DEXA). Furthermore, maximal strength of the back- and abdominal muscles will be assessed to evaluate core stability (important during prolonged cycle training). Hereafter, MS patients and HC will be randomly allocated to one of four intervention groups following 6 months of moderate-to-high-intensity cardiovascular exercise therapy with (MSβ, n=10; HCβ, n=10) or without ($MS_{placebo}$, n=10; $HC_{placebo}$, n=10) β-alanine supplementation. Groups not receiving β-alanine supplements, will receive placebo tablets that will be identical in taste and appearance. Following 6 months of exercise training (POST) measurements will be performed similar to baseline.

Exercise program

Moderate-to-high-intensity cardiovascular exercise therapy

All participants will perform a home-based supervised exercise training program. Participants will receive weekly training instructions using a smartphone based heart rate monitor app (Polar®). Training involves cycling, performed independently on their personal race bicycle. The exercise training program (6 months) involves 3 week cycles (week I-III). During week I, subjects will perform high volume moderate intensity cardiovascular cycle training (3x/week). Twice a week, subjects perform 3h training sessions (70-80% HR_{max}*) and once a week a 1.5h session will be executed (80-90% HR_{max}). During week II, subjects will perform low volume maximum intensity interval cycle training (3/w). High intensity interval cycle training (HIIT) will consist of 3x maximal sprints (90-100% HR_{max}) of 1.5min, interspersed with 3min rest intervals. A 5min standardized warming up and 5min cooling down will be performed. Week III involves a recovery week where subjects will perform one training session of 1.5h at an exercise intensity of 70-80% HR_{max} and one session of HIIT. Throughout the intervention program at least 3 collective tours will be organized, where all subjects will perform a training session together, to improve group dynamics and motivation.

**obtained from the maximal graded exercise test*

β-alanine supplementation

The supplementation protocol of β-alanine (Etixx® Omega Pharma Belgium NV) involves oral intake of 4 x 800mg (3.2g/day[29, 43]) daily with at least 2h apart of slow-release β-alanine during the first 12 weeks. After this loading period, subjects will receive a maintenance dose of 2 x 800mg (1.6g/day) β-alanine for the remaining study duration. Placebo groups will follow the same supplementation protocol with placebo tablets that will have identical taste and appearance. All subjects will be advised to take the tablets together with meals. This supplement is frequently used to improve sport performances in athletes. The beta-alanine supplement consists of beta-alanine, hydroxymethylcellulose, cellulose, siliciumdioxide, magnesium stearate and zinc. In some cases, taking beta-alanine can provoke a tingling feel (paresthesia) on the skin which is reversible within the hour. However, this is not dangerous and completely reversible. To prevent this paresthesia, β-alanine tablets will consist of slow-release β-alanine which, together with the spread intake throughout the day, will not provoke this discomfort.

Measurements

Exercise capacity & serum lactate. Exercise capacity will be assessed using a maximal (12-lead ECG) graded cardiopulmonary exercise test (♂: 30W+15W/min, ♀: 20W+10W/min) with pulmonary gas exchange analysis (Jaeger Oxycon®). Subjects will perform the exercise test on their personal bicycle (Cyclus2® Leipzig, Germany). VO_2, VE, RER will be monitored and 2min capillary blood samples will be obtained to analyse blood lactate concentrations (Analox®) and determine the anaerobic threshold before, during and after exercise. RER values will be evaluated to verify if the test was performed maximally (RER >1.1).

Body composition Whole body fat and lean tissue mass will be obtained using Dual Energy X-ray Absorptiometry scan (DEXA) (Hologic Series Delphi-A Fan Beam X-ray Bone Densitometer, Vilvoorde, Belgium). A calibrated analogue weight scale (Seca®) will be used to measure total body mass.

Statistical analysis

Baseline differences between groups and post-intervention differences will be analysed using an unpaired student's t-test and a 4x2 ANOVA for repeated measures, respectively (SAS Institute®). The threshold for statistical significance will be set at $p<0.05$.

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