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Muscle carnosine in experimental autoimmune encephalomyelitis and multiple sclerosis

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ABSTRACT

Background: Muscle carnosine is related to contractile function (Ca^{++} handling) and buffering of exercise–induced acidosis. As these muscular functions are altered in Multiple Sclerosis (MS) it is relevant to understand muscle carnosine levels in MS.
Methods: Tibialis anterior muscle carnosine was measured in an animal MS model (EAE, experimental autoimmune encephalomyelitis, n = 40) and controls (CON, n = 40) before and after exercise training (EAE_{EX}, CON_{EX}, 10d, 1 h/d, 24 m/min treadmill running) or sedentary conditions (EAE_{SED}, CON_{SED}). Human m. vastus lateralis carnosine of healthy controls (HC, n = 22) and MS patients (n = 24) was measured.
Results: EAE muscle carnosine levels were decreased ($p < .0001$) by ~ 40% to ~ 64% at 10d and 17d following EAE induction (respectively) regardless of exercise ($p = .823$). Similarly, human MS muscle carnosine levels were decreased (− 25%, $p = .03$).
Conclusion: Muscle carnosine concentrations in an animal MS model and MS patients are substantially reduced. In EAE exercise therapy does not restore this.

1. Introduction

Multiple sclerosis (MS) is a neurodegenerative autoimmune disease of the central nervous system (Pugliatti et al., 2006), in which inflammatory and demyelination processes also result in physical inactivity, decreased exercise capacity, excessive (post-exercise) fatigue and reduced muscle contractile function (Dalgas et al., 2008; Savci et al., 2005). These frequently occurring secondary symptoms substantially affect various daily life activities, ultimately leading to an impaired quality of life (Ellis and Motl, 2013). Central mechanisms (de Haan et al., 2000; Kent-Braun et al., 1997; Rice et al., 1992; Sharma et al., 1995; van der Kamp et al., 1991) such as reduced motor firing rates, impaired motor unit recruitment and increased central motor conduction time clearly contribute to the above described disuse-related physiological profile (Ng and Kent-Braun, 1997). A portion of the neuromuscular dysfunction in MS however, probably also resides within the skeletal muscle (de Haan et al., 2000; Garner and Widrick, 2003; Kent-Braun et al., 1997, 1994; Rice et al., 1992; Sharma et al., 1995; Wens et al., 2014).

We, and others, already reported altered muscle fiber composition (shift to glycolytic fibers), disturbed muscle contractile characteristics and cross-bridge (Ca^{2+} handling) abnormalities in muscles of MS patients (de Haan et al., 2000; Garner and Widrick, 2003; Wens et al., 2014). Furthermore, reduced Krebs cycle and complex I and II activities (Kent-Braun et al., 1997; Kumleh et al., 2006), overproduction of reactive oxygen species (ROS) (Haider et al., 2011), increased basal AMP-activated protein kinase phosphorylation (Hansen et al., 2015) and delayed phosphocreatine resynthesis after exercise (Campbell et al., 2013; Hansen et al., 2015; Kent-Braun et al., 1997, 1994; Kumleh et al., 2006) suggest muscular mitochondrial dysfunction in MS and higher basal and exercise-related energy expenditure. In keeping with this, increased intramyocellular lactate accumulation, leading to increased basal serum lactate concentrations (Amorini et al., 2014), have been shown to cause greater (perceived) muscle fatigue in MS patients. It thus seems that MS patients do not only exhibit central neurological abnormalities but also impairments in both muscular contraction mechanisms and energy supply. In keeping with this, exercise rehabilitation therapy, that amongst others induces muscular acidosis, has become an important part of overall MS treatment (Motl and Gosney, 2008).

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So far, small but significant improvements in exercise capacity and muscle contractile characteristics following various types (modality, frequency) of short to longer term regular exercise therapy have been reported in both a frequently used animal MS model, notably Experimental Autoimmune Encephalomyelitis (EAE) (Wens et al., 2015b) and in MS (Kjolhede et al., 2012; Motl et al., 2008; Petajan et al., 1996; Sallis et al., 1986; Schulz et al., 2004; Stuifbergen et al., 2006). In an attempt to further improve muscle contractile and energy metabolic responses to exercise, our laboratory recently performed high (er) intensity training studies in MS showing substantially improved (+25–50%) muscle strength and exercise capacity following 8–12w of exercise (Wens et al., 2015a, 2015b). Although promising, most MS subjects however reported higher post-exercise muscle fatigue and overall perceived exertion rates (BORG: 14.7 ± 1.5 vs. 12.7 ± 1.3) compared to regular intensity training (Wens et al., 2014). Consequently, any strategy that attenuates this may further improve therapy outcome.

Carnosine, a dipeptide composed of β-alanine and L-histidine, is found in high concentrations in mammalian skeletal muscle (Boldyrev et al., 2013). Together with anserine or ophidine/balenine, the methylated analogs that possess the same bioactivity and are exclusively found in animals, carnosine forms the histidine-containing dipeptides (HCD). The physiological role of carnosine is related to contractile function in general and more specific to Ca^{++} handling (Derave et al., 2010). Interestingly, carnosine has also been shown to buffer exercise–induced acidosis (Derave et al., 2010), to affect mitochondrial respiration (Boldyrev, 2007) and to protect against exercise-induced oxidative stress (Dawson et al., 2002). The functional role of carnosine in skeletal muscle is thus closely related to several of the above described muscle alterations seen in MS. So far, altered tissue carnosine concentrations were shown in other neurological diseases such as Amyotrophic Lateral Sclerosis (ALS) (Stuerenburg and Kunze, 1999) and Parkinson's Disease (Bellia et al., 2014; Boldyrev et al., 2013). Moreover, reduced serum carnosinase activity was already found in patients with MS (Wassif et al., 1994), indicating that several of the above described muscle alterations like impaired exercise capacity, excessive (post-exercise) fatigue and reduced muscle contractile function in MS (Dalgas et al., 2008; Savci et al., 2005; Wens et al., 2014) may, in part, be related to reduced muscle carnosine levels. This, however, has not been investigated in animal MS models and/or MS yet. Finally, it is important to note that although the overall impact of exercise on muscle carnosine content in healthy controls and disease is contradictory (Baguet et al., 2011; Derave et al., 2007; Kendrick et al., 2008, 2009; Mannion et al., 1994; Suzuki, 2004), increased muscle carnosine has been shown to reduce exercise-induced fatigue and hereby improve exercise performance during high-intensity intermittent exercise (1–4 min) (Blancquaert et al., 2015; Derave et al., 2010, 2007). Therefore, the first aim of this study is to investigate muscle carnosine levels in both EAE animals and MS patients. We hypothesize that muscle carnosine concentrations in EAE and MS are reduced compared to healthy controls. Secondly, the effect of exercise on muscle carnosine levels in EAE rats is explored.

2. Methods

Muscle carnosine content was measured in both an animal MS model, notably Experimental Autoimmune Encephalomyelitis (EAE, Part I), and MS patients (Part II). The analyzed muscle samples originate from studies that were previously performed and published by our laboratories (Keytsman et al., 2017; Wens et al., 2015b).

2.1. Part I

In a first phase, rodent muscle carnosine and anserine levels were investigated using the EAE animal MS model (Constantinescu et al., 2011). Briefly, this model is characterized by the induction of EAE on day 0, an inflammatory period with no clinical symptoms (day 0 to day 10) and gradual hindquarter paralysis (day 11 to day 14) followed by almost full recovery (day 15 to day 17).

2.1.1. Animals

Eighty female Lewis rats (age 6–7 weeks, body weight 100–120 g, Harlan CPB, Zeist, The Netherlands) were individually housed (12 h/12 h light/dark cycle; temperature of 22 °C; relative humidity of 22–24%) in the animal facilities at Hasselt University. Rats were fed *ad libitum* with water and normal rat pellets (Carfil RN-01-K12, Harlan). The animal Ethics Committee of Hasselt University approved the study protocol that complied with the national/European legislation and the National Research Council's guide for the care and use of laboratory animals.

2.1.2. Study design

Following acclimatization and adaptation (day − 21 to − 15), animals were randomized into two subgroups: a sedentary group (SED, n = 40) and an exercise group (EX, n = 40). In order to induce comparable levels of stress, SED animals were seated on the stationary treadmill (1 h) on a daily basis (day −14 to 0). Daily food intake and body weight were registered. At day 0, SED and EX groups were subdivided into a healthy control group (CON_{SED} n = 20; CON_{EX} n = 20) and an EAE group (EAE_{SED} n = 20; EAE_{EX} n = 20). Briefly, EAE was induced by a single percutaneous injection in both footpads (100 µl/foot) under isoflurane anesthesia and consisted, per animal, of 24 µl purified myelin basic protein (MBP, 25 mg/ml) in combination with 25 µl 7RA heat-killed *Mycobacterium Tuberculosis* (20 mg/ml, Difco), 120ùl complete Freunds adjuvant (CFA, Difco) and 31 µl phosphate-buffered saline (PBS) (Polfliet et al., 2002). Hereafter, EX rats exercised daily for 1 h/day, during 10 consecutive days, until progressive hindquarter paralysis prevented this. Treadmill performance/intensity was visually monitored daily by the researchers and did not appear to be different between the control and EAE groups.

At day 10 (D10), just before onset of hindquarter paralysis, treadmill training was terminated and 10 animals of each group were evaluated. The remaining rats (n = 10 per group) endured hindquarter paralyses (EAE group, day 11–14) and were evaluated after (almost full) recovery on day 17 (D17). All animals were anaesthetized using an intraperitoneal injection of pentobarbital sodium (5 mg 100 g-1 BW) and m. tibialis anterior (TA) of the right hind limb was dissected and freed of connective tissue and visible blood. The mid-part of each muscle was snap-frozen using liquid nitrogen, and stored at − 80 °C until further analysis were performed. All animals were sacrificed after muscle sampling.

Throughout the study course, animals were carefully monitored on daily basis. Animals did not display severe discomfort immediately following immunization. Paralysis and typical signs of EAE occurred 11 days post-immunization, which is the typical time frame for this animal model. If animals exhibited signs of severe pain (high clinical score for 2 consecutive days), distress, were unable to reach food or when humane endpoints were reached, euthanasia to relief pain was performed. However, none of the animals included in the study underwent euthanasia for one of those reasons.

2.2. Part II

2.2.1. MS subjects

Twenty-two healthy controls (HC) and twenty-four patients with MS (aged > 18 years; mean EDSS 3.1 ± 1.5, range 0→6, median 2.5) were included following written informed consent. Subjects were excluded if they participated in other studies, had (in case of MS) an acute exacerbation 6 months prior to the start of the study or had an EDSS score > 6. Phenotypes of MS (RR, relapsing remitting; PP, primary progressive; SP, secondary progressive) and the duration of the disease were inventoried. The study was approved by the local Ethical

Table 1
Rat muscle carnosine and anserine concentrations.

	CON_{SED}		CON_{EX}		EAE_{SED}		EAE_{EX}	
	D10	D17	D10	D17	D10	D17	D10	D17
Carnosine	2.5 ± 0.6	2.2 ± 0.4	2.5 ± 0.6	2.4 ± 1.1	1.5 ± 0.6	0.8 ± 0.4	1.4 ± 0.3	0.9 ± 0.5
Anserine	2.2 ± 0.7	2.5 ± 0.4	2 ± 0.5	2.3 ± 1.0	3 ± 0.7	3.4 ± 1.0	3.5 ± 0.7	2.9 ± 0.6

Data are expressed as means ± SD and represent rat m. tibialis anterior carnosine and anserine concentrations (mmol/kg WW) 10 (D10, n = 10/group) and 17 (D17, n = 10/group) days after acute experimental autoimmune encephalomyelitis induction (EAE) or healthy control (CON) under sedentary (CON_{SED}, EAE_{SED}) or exercise (treadmill running, 1 h/d, 18 m/min, CON_{EX}, EAE_{EX}) conditions. Main effects for group (CON vs. EAE, p = 0000) and time (D10 vs. D17, p = .005) were present for carnosine concentrations. For anserine concentrations, a main group effect (CON vs. EAE, p = 0000) was found.

Committee of the Jessa hospital and Hasselt University and complied with the Declaration of Helsinki. This study was registered at ClinicalTrials.gov (NCT02466165).

2.2.2. Muscle biopsies

Muscle biopsies were obtained from MS patients (n = 24) and HC (n = 22) from the middle part of the m. vastus lateralis (Bergström needle technique), by an experienced medical doctor. Biopsies were sampled from the weakest leg, as assessed by a preceding isometric muscle strength test performed on an isokinetic dynamometer (System 3, Biodex, ENRAF-NONIUS, New York, USA). The biopsied leg of HC was randomized, since muscle strength associated with each leg (left vs. right or dominant vs. nondominant) is equal in healthy persons (McCurdy and Langford, 2005; Siqueira et al., 2002). The sample was freed from connective tissue and snap-frozen with liquid nitrogen and stored at − 80 °C, until further analysis.

2.3. Muscle dipeptide and free amino acid content

Dipeptide and free amino acid concentrations were determined by high-performance liquid chromatography (HPLC), as previously described (Everaert et al., 2013). Muscle samples (15 mg wet weight, WW) were deproteinized using 35% sulfosalicylic acid and centrifuged (5 min, 16,000 g). Deproteinized supernatant (5 µl sample) was mixed with AccQ Fluor Borate buffer (75 µl) and reconstituted Fluor Reagent (20 µl) from the AccQTag chemistry kit (Waters). The derivatized samples were applied to a Waters HPLC system comprised of an XBridge BEH column (4.6 × 150 mm, 2.5 µm) and fluorescence detector (excitation/emission wavelength: 250/395 nm). Carnosine and anserine were assessed in Part I (animals) and carnosine, taurine, serine, glutamine and histidine were measured in Part II (human subjects). To compare muscle biopsy quality between healthy controls and MS, some reference muscle amino acid (serine, histidine and glutamine, in Part II) concentrations, expressed as total areas under the curve (tAUC), were compared between groups. In case of normal serine, histidine and glutamine concentrations, low carnosine concentrations could not be due to poor biopsy quality.

2.4. Statistical analysis

All data were analyzed using SPPS v. 22.0 (IBM). Normality of data distribution was evaluated using the Shapiro-Wilk test. In animals, ANOVA was used to evaluate the (main and interaction) effect of 'group' (CON vs. EAE), 'activity' (SED vs. EX) and 'time' (D10 vs. D17) on carnosine and anserine concentrations and. For human analysis, group comparison was performed using unpaired student's *t*-tests in case of normality and non-parametric independent *t*-tests (Wilcoxon) otherwise. All data are presented as means ± SD and the threshold for statistical significance was set at p < .05.

3. Results

3.1. Part I

3.1.1. Rat muscle carnosine and anserine concentrations

Muscle carnosine concentrations were significantly lower (main group effect, CON vs. EAE, p < .0001 range: − 40% → − 64%) in EAE compared to CON and D10 to D17(main time effect, D10 vs. D17, p = .005, range: − 4% → − 46%)(Table 1). However, exercise had no influence on carnosine concentrations (main activity effect, SED vs. EX, p = .823). No interaction effects were found.

Muscle anserine was significantly higher in EAE compared to CON (main group effect, CON vs. EAE, p = 0000, range: +36% → +40%). No main activity and time effects as well as interaction effects were detected.

3.2. Part II

3.2.1. Subject characteristics

Subject characteristics (Table 2) did not differ between HC and MS. Relapsing-remitting MS was diagnosed in 16 patients, whilst 6 patients suffered from primary-progressive MS and 2 patients from the secondary- progressive form. Mean disease duration was 13 ± 8 years (range 1→26 y).

3.2.2. Muscle carnosine and taurine content

In MS, muscle carnosine (3.8 ± 1.2 mmol/kg WW vs. 2.9 ± 1.3 mmol/kg WW, p = .03) and muscle taurine (8.4 ± 3.3 mmol/kg WW vs. 6.5 ± 2.9 mmol/kg WW, p = .04) levels were lower compared to HC (Fig. 1). Total areas under the curve of muscle serine (5.1 ± 2.7 vs. 4.3 ± 1.1, p = .12), histidine (6.2 ± 1.6 vs. 5.5 ± 1.5, p = .07) and glutamine (59.7 ± 30.3 vs. 61.1 ± 28.6, p = .43) did not differ between groups (data not shown).

4. Discussion

Compared to healthy controls the present study clearly shows reduced muscle carnosine levels in both human MS and a frequently used

Table 2
Baseline subject characteristics.

	HC	MS
Age (years)	48.7 ± 11.8	52.7 ± 8.9
Gender (f/m)	14/8	13/11
Weight (kg)	69.5 ± 13.8	71.6 ± 12.4
Height (m)	1.69 ± 0.1	1.71 ± 0.1
BMI	24.1 ± 2.4	24.5 ± 3.5
Disease duration (years)	/	13 ± 8
EDSS	/	3.1 ± 1.5

Data are expressed as means ± SD and represent subject characteristics (BMI: body mass index) of healthy controls (HC, n = 22) and MS patients (n = 24). EDSS, Expanded Disability Status Scale.

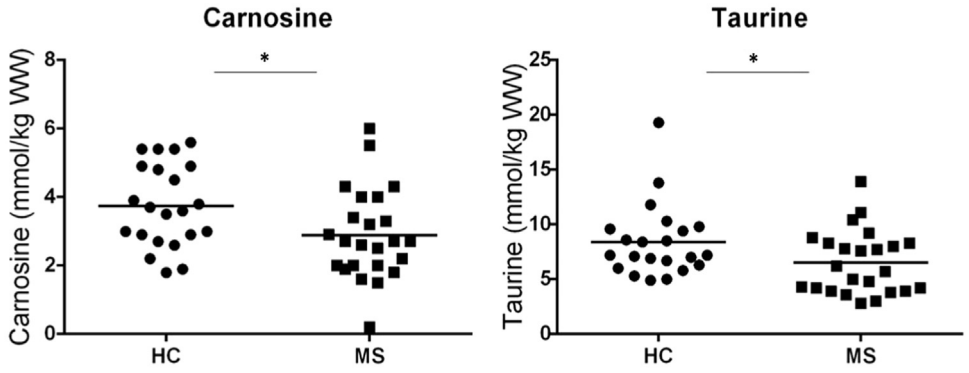

Fig. 1. Muscle carnosine and taurine content of healthy controls and multiple sclerosis. Data represent m. vastus lateralis carnosine and taurine concentrations (mmol/kg WW) of healthy controls (HC, n = 22) and MS patients (n = 24). *p < .05 between HC and MS.

animal MS model. Furthermore, in animals, exercise did not restore carnosine concentrations. In MS, lower muscle carnosine was paralleled by reduced taurine.

Literature indicates that carnosine metabolism could be altered in neuromuscular diseases. Wassif et al. (1994) already demonstrated reduced serum carnosinase activity (− 49%) in fresh blood samples from MS patients compared to healthy controls. Carnosinase causes hydrolysis of carnosine into β-alanine and L-histidine. This suggests alterations in carnosine metabolism in patients with MS. Stuerenburg (Stuerenburg and Kunze, 1999) investigated the carnosine content of skeletal muscles from patients with suggestive neuromuscular diseases and rats of various ages. Following stepwise regression modelling they reported that another neurological disease such as ALS was negatively correlated with muscle carnosine content and they suggested that this was caused by progressive denervation processes, as also seen in MS. Although altered tissue carnosine concentrations in other neuromuscular diseases such as ALS (Stuerenburg and Kunze, 1999) and Parkinson Disease (Bellia et al., 2014; Boldyrev et al., 2013) and reduced serum carnosinase activity in MS (Wassif et al., 1994) were already described, we are now the first to report substantially reduced muscle carnosine levels in an animal MS model and confirm this in MS patients.

Apart from reduced muscle carnosine in EAE we also detected increased muscle anserine concentrations. Interestingly anserine, the methylated form of carnosine, is exclusively found in animals where it possesses the same bioactivity as carnosine (Boldyrev et al., 2013). Hence, when carnosine decreases and anserine tends to increase (Table 1), the total amount of histidine-containing dipeptides (HCD) remains unchanged. In order to keep the total HCD concentration constant, it is possible that carnosine methyltransferase (CMT) is upregulated following EAE induction, leading to enhanced muscle anserine levels. Interestingly, Drozak et al. (2015) recently molecularly identified UPF0586 protein C9orf41 as the mammalian carnosine-N-methyltransferase, responsible for anserine formation in rat muscle (Drozak et al., 2015). However, to date, no activity assay for CMT is available. Quantitative PCR could be an alternative to measure the effect of EAE on CMT gene expression, but this is not always in agreement with the amount of mRNA that is effectively translated into protein. In human subjects however, the methylated analog anserine is absent. Therefore, in humans a decrease in muscle carnosine content implicates an effective reduction of the total histidine-containing dipeptide store.

Although the exact underlying mechanisms remain unclear, several contributing factors may explain reduced carnosine stores in MS muscles. In MS, central mechanisms (de Haan et al., 2000; Kent-Braun et al., 1997; Rice et al., 1992; Sharma et al., 1995; van der Kamp et al., 1991), such as impaired motor unit recruitment and delayed conduction/reaction times induce a disuse-related physiological profile (Ng and Kent-Braun, 1997). In sedentary populations and in chronic disease (e.g. ALS, osteoarthritis), lower muscle carnosine levels (Stuerenburg and Kunze, 1999; Tallon et al., 2007) have been reported that might result from an inactivity related reduction in muscle protein content. However, in the present study muscle histidine, glutamine and serine, that are also prone to inactivity, were not affected in MS muscle samples. As such, we assume that the presented decrease in muscle carnosine content was not due to poor biopsy or muscle quality. Possibly, several intramyocellular dysfunctions that are associated with MS and relate to the physiological role(s) of carnosine in muscle (Boldyrev et al., 2013), could partly be related to the present findings. However, the exact effects of muscle carnosine on, amongst others, disturbed muscle contractile characteristics and cross-bridge (Ca^{2+} handling) abnormalities (de Haan et al., 2000; Garner and Widrick, 2003; Wens et al., 2014), excessive exercise-induced acidosis (de Haan et al., 2000; Kent-Braun et al., 1997; Sharma et al., 1995) and mitochondrial dysfunction (Campbell et al., 2013; Hansen et al., 2015; Kent-Braun et al., 1997, 1994; Kumleh et al., 2006; Sharma et al., 1995) in MS have not been investigated yet.

The effect of exercise therapy on muscle carnosine content is not fully clear. With the exception of Suzuki (2004), who detected increased carnosine concentrations after an 8 week sprint interval program, most studies concerning this matter did not demonstrate positive effects after a 4–16 week isokinetic resistance training program (Kendrick et al., 2008, 2009; Mannion et al., 1994) or a 5 week sprint-training intervention (Baguet et al., 2011) on muscle carnosine concentrations in healthy subjects. The present study explored this in an animal MS model and confirmed that exercise therapy did not prevent reduction of muscle carnosine. Possibly, nutritional interventions (e.g. β-alanine supplementation) are required to exert such effects.

Muscular carnosine concentrations are a good marker of the total body carnosine stores, as > 99% of the compound is found in skeletal muscle cells (Boldyrev et al., 2013). A reduction of total carnosine stores is possibly also related to the increased oxidative stress and the resulting accumulation of cytotoxic compounds, such as the reactive carbonyls acrolein and hydroxynonenal (HNE). Carnosine serves as a sacrificial sequestering agent for acrolein and HNE by forming unreactive adducts (Song et al., 2014) and as such provides an endogenous protective mechanism against protein and ultimately tissue damage induced by these reactive carbonyls (Aldini et al., 2011; Baba et al., 2013). The increased urinary elimination of carnosine-carbonyl conjugates has been demonstrated for certain metabolic conditions (metabolic syndrome, obesity, type-2 diabetes) that are characterized by increased carbonyl stress (Regazzoni et al., 2016). Interestingly, acrolein has also been implicated in the pathogenesis of MS, as it directly damages myelin, through reaction with protein and lipid components of myelin, leading to demyelination. Furthermore, acrolein has recently been identified as a promising and effective therapeutic target (Leung et al., 2011; Tully and Shi, 2013) in this population. In fact, when Leung et al. (2011) treated EAE mice with the acrolein scavenger hydralazine, myelin integrity appeared to be largely preserved indicating that acrolein removal may offer neuroprotection. Possibly,

reduced muscle carnosine content in MS results from the incapacity of endogenous carnosine synthesis to compensate for the increased 'consumption' of carnosine to detoxify and eliminate acrolein. According to this line of reasoning, nutritional strategies to improve muscle carnosine content in persons with MS are worthwhile to explore. Carnosine supplementation (~ 1.5 g/d β-alanine) has already been shown to improve a number of neurological symptoms (Boldyrev et al., 1987) in Alzheimer's (Preston et al., 1998) and Parkinson's (Boldyrev et al., 2013) disease. Clinically, nutritional interventions that normalize or even elevate the reduced muscle carnosine content could therefore be a valid new approach to improve muscle contractile properties, myocellular energy supply and/or possibly neurological symptoms in MS. Moreover, MS patients would be able to exercise more efficiently at higher intensities leading to a better clinical rehabilitation therapy outcome in these patients.

4.1. Limitations

The present study holds certain limitations that should be taken into account regarding future research. We show that EAE and MS reduce muscle carnosine, though because no nerve tissue was collected it was not possible to investigate whether this was due to neuroaxonal injury and/or muscle denervation. It is therefore warranted that in future EAE research nerve tissue is sampled to investigate peripheral nerves, motor end-plates and the extent of both neuronal and axonal damage in the spinal cord. In MS, future studies should also investigate spinal cord lesion load.

The current paper describes a reduction in muscle carnosine concentrations in an EAE group, compared to control animals. However, in order to determine whether reduced muscle carnosine levels were actually related to EAE itself, a control group with injection of Complete Freund's Adjuvant alone, causing inflammatory processes, would be appropriate. Indeed, the injection of CFA may have caused local inflammation and thus influenced m. tibialis anterior carnosine concentrations. This issue may also be addressed by unilateral immunization and comparison of muscle carnosine of the two hind limbs. Furthermore, for future carnosine related research in MS, assessment of fatigue levels and dietary habits is worthwhile.

In summary, under the conditions of the present study EAE and MS reduce skeletal muscle carnosine levels. Exercise therapy alone could not prevent this in the animal MS model. This warrants further research investigating the effect of nutritional interventions that restore muscle carnosine levels either or not in combination with exercise therapy.

Conflict of interest

The authors report no conflicts of interest.

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