ORIGINAL ARTICLE

Effects of sprint training combined with vegetarian or mixed diet on muscle carnosine content and buffering capacity

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Abstract Carnosine is an abundant dipeptide in human skeletal muscle with proton buffering capacity. There is controversy as to whether training can increase muscle carnosine and thereby provide a mechanism for increased buffering capacity. This study investigated the effects of 5 weeks sprint training combined with a vegetarian or mixed diet on muscle carnosine, carnosine synthase mRNA expression and muscle buffering capacity. Twenty omnivorous subjects participated in a 5 week sprint training intervention (2–3 times per week). They were randomized into a vegetarian and mixed diet group. Measurements (before and after the intervention period) included carnosine content in soleus, gastrocnemius lateralis and tibialis anterior by proton magnetic resonance spectroscopy (^{1}H-MRS), true-cut biopsy of the gastrocnemius lateralis to determine in vitro non-bicarbonate muscle buffering capacity, carnosine content (HPLC method) and carnosine synthase (CARNS) mRNA expression and 6×6 s repeated sprint ability (RSA) test. There was a significant diet × training interaction in soleus carnosine content, which was non-significantly increased ($+11\%$) with mixed diet and non-significantly decreased (-9%) with vegetarian diet. Carnosine content in other muscles and gastrocnemius buffer capacity were not influenced by training. CARNS mRNA expression was independent of training, but decreased significantly in the vegetarian group. The performance during the RSA test improved by training, without difference between groups. We found a positive correlation ($r = 0.517$; $p = 0.002$) between an invasive and non-invasive method for muscle carnosine quantification. In conclusion, this study shows that 5 weeks sprint training has no effect on the muscle carnosine content and carnosine synthase mRNA.

Keywords Muscle carnosine · Muscle buffering capacity · Sprint training · Vegetarian diet

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Introduction

Muscle buffering capacity is an important mechanism related to high-intensity exercise performance. A positive correlation exists between muscle buffering capacity and high-intensity exercises performance (Bishop et al. 2004; Edge et al. 2006b; Nevill et al. 1989). Cross-sectional studies have shown that athletes who are regularly involved in anaerobic high-intensity sprint-type activities have a higher muscle buffering capacity than endurance athletes or untrained subjects (Sahlin and Henriksson 1984; Parkhouse et al. 1985; Edge et al. 2006b). The in vivo muscle buffering capacity is a dynamic buffering system that includes not only the in vitro muscle buffer capacity, but also the

bicarbonate/CO_2 buffer system, and the transsarcolemmal ion fluxes (Juel 1998). Despite the limited number of studies, it is generally accepted that training can lead to an increased in vivo muscle buffer capacity (Sharp et al. 1986; Troup et al. 1986; Juel 1998). This increase could possibly be explained by an increased lactate/H^+ co-transport; due to an increased expression of monocarboxylate transporters and Na^+/H^+ exchange proteins (Bishop et al. 2008; Juel et al. 2004; Iaia et al. 2008; Juel 1998). The in vitro muscle buffering capacity is the physicochemical buffer capacity, including muscle proteins, inorganic phosphate and histidine-containing dipeptides (HCD), of which carnosine, with a pKa of 6.83, is the main buffer in humans (Parkhouse and McKenzie 1984; Hirakoba 1999; Hultman and Sahlin 1980). For in vitro muscle buffering capacity, there is less consistency about what kind of training can increase the muscle buffering capacity. Some researchers find an increased in vitro muscle buffering capacity following a chronic training program in humans (Bell and Wenger 1988; Edge et al. 2006a; Gibala et al. 2006; Weston et al. 1997) others find no effect (Edge et al. 2006c; Harmer et al. 2000; Mannion et al. 1994; Nevill et al. 1989; Pilegaard et al. 1999) and Bishop et al. (2008, 2009) indicate even a decrease of buffering capacity due to some types of training. There is still no consensus on what specific training modality leads to an increased in vitro muscle buffering capacity. To find an answer to this question, it is necessary to know which component(s) of the in vitro muscle buffering capacity are sensitive to training stimuli. The metabolite carnosine has been suggested as a candidate (Juel 1998; Parkhouse and McKenzie 1984).

Carnosine (beta-alanyl-L-histidine) is a cytoplasmic dipeptide present in relatively high concentrations in the human skeletal muscle (Harris et al. 1990; Derave et al. 2010). It is synthesized from the precursors L-histidine and beta-alanine by carnosine synthase. Parkhouse et al. (1985) showed a positive correlation ($r = 0.69$) between muscle carnosine content and the in vitro muscle buffering capacity. The increased muscle buffering capacity of sprinters, compared with marathon runners or untrained subjects, is associated with their higher skeletal muscle carnosine concentrations. According to Harris et al. (1990), carnosine could contribute to ~7% of the total in vitro muscle buffering capacity, in humans, although this is probably an underestimate.

It has been suggested that training effects on in vitro muscle buffer capacity are related to an increase in carnosine (Juel 1998; Parkhouse and McKenzie 1984). However, there is still no consensus about the impact of training intervention on the muscle carnosine content. One study from Suzuki et al. (2004) found a doubling in muscle carnosine due to 8 weeks sprint training when untrained subjects performed two times a week 1 or 2 Wingates (30 s all-out sprints) during 8 weeks. Most other studies, however, did not find an

effect of a chronic training program on the muscle carnosine content (Kendrick et al. 2008; Kendrick et al. 2009; Mannion et al. 1994). In the study of Kendrick et al. 2009, for example, 14 Vietnamese students undertook a 4 week isokinetic one-leg training program. The muscle carnosine content of the trained leg did not increase significantly ($+2.10 \pm 4.79$ mmol/kg dw). The inconsistency between the currently available studies may possibly be due to the type of training and the selected training protocol.

In the current study, a similar training protocol as in the study of Suzuki et al. (2004) will be used. Therefore, an increase in muscle carnosine can be expected following this training program. Since beta-alanine is the rate limiting precursor of carnosine synthesis (Bakardjiev and Bauer 1994; Dunnett and Harris 1999; Hill et al. 2007) and since it is a natural constituent of meat, fish and poultry (Abe 2000; Boldyrev 2007), this training effect will probably occur only if sufficient substrate for carnosine synthesis is present. Except from the minimal traces of beta-alanine recently found in vegetable oils (Sanchez-Hernandez et al. 2011), beta-alanine containing dipeptides are virtually absent in plants and therefore, chronic vegetarians have lower muscle carnosine content than omnivorous subjects (Harris et al. 2007; Everaert et al. 2010). It can thus be expected that a combination of the above-mentioned training program with a vegetarian diet will probably not lead to an increase in muscle carnosine. Secondly we expected that, if carnosine is an important buffer and increases dramatically, the in vitro muscle buffer capacity and therefore, possibly also the performance during repeated sprint exercise would be more pronounced when training with a mixed diet, compared to a vegetarian diet. To our knowledge, the interaction of diet and sprint training on muscle buffer capacity is unexplored. Third, we aim to investigate whether training induces an upregulation of the carnosine synthase (CARNS) transcript, as this is a likely mechanism by which a training-induced increase in muscle carnosine would take place. Finally the current study will also make a direct comparison between the invasive and non-invasive techniques for measuring muscle carnosine.

Materials and methods

Subjects

Twenty healthy non-vegetarian students (11 males and 9 females) volunteered to participate in this study. They gave their informed consent and the study was approved by the local Ethics Committee (Ghent University Hospital, Belgium). All subjects were physically active (weekly 2–3 h of sport), but were not involved in sport competition or organized training. After the baseline measurements, the

subjects were allocated to a vegetarian (VD) and mixed diet (MD) group, matched for gender, age and muscle carnosine concentration at group level. As a result of an injury, there was a drop out of one man in the VD group. The MD group consisted of five females and five males and the VD group was composed of four females and five males. The subjects' age, weight and height were 21.5 ± 1.7 years, 69.9 ± 10.3 kg and 1.72 ± 0.07 m for MD and 20.8 ± 1.4 years, 60.4 ± 7.3 kg and 1.74 ± 0.11 m for VD group, respectively (all parameters were not significantly different, except weight; $p = 0.02$).

Study design

Diet

Both groups were asked to follow a specific diet for 5 weeks. All subjects of the MD group followed a beta-alanine-rich diet, which included the daily intake of meat or fish. The ovo-lacto-vegetarians on the other hand did not eat meat and fish, but eggs and dairy products were allowed. In addition, all subjects completed a food diary during three, randomly selected, days. The diaries were analyzed with the Becel nutrition software (Becel institute, BINS V3.0). To prevent a creatine deficiency in the VD group, subjects from both groups, were supplemented with 1 g/day of creatine monohydrate (100% Creapure TM 1000 mg; VerdePharma, Belgium). The beta-alanine consumption by meat and fish consumption was calculated as described before (Baguet et al. 2009).

Training protocol

Both groups completed the same sprint training program for 5 weeks. The training frequency was two times a week (minimum 1 day rest in between) during the first 2 weeks and three times a week (Monday, Wednesday and Friday) from week 3. The training program consisted of alternating cycling and running efforts. During the cycling training sessions, subjects performed 1 or 2 (with 20 min rest in between) 30 s all-out cycling sprints (Wingate test) on a mechanically braked cycle ergometer (Monark ergomedic 894Ea, Peak bike). Resistance was set to 7 and 8% of the bodyweight for females and males, respectively. During the running sessions, the students performed 1 or 2 (with a passive rest period of 20 min in between) maximal runs of 200 m.

Measurements

Proton MRS (¹H-MRS)

Before and after 5 weeks sprint training, the carnosine content was measured in three skeletal muscles (soleus, gastrocnemius lateralis and tibialis anterior) of the lower leg by proton magnetic resonance spectroscopy (¹H-MRS), as previously described (Baguet et al. 2010a). The subjects lay in supine position on their back and the lower leg was fixed in a holder with the angle of the ankle at 20° plantar flexion. All the MRS measurements were performed on a three Tesla whole body MRI scanner (Siemens Trio, Erlangen) equipped with a knee-coil. Single voxel point-resolved spectroscopy (PRESS) sequence with the following parameters was used: repetition time (TR) = 2,000 ms, echo time (TE) = 30 ms, number of excitations = 128, 1,024 data points, spectral bandwidth of 1,200 Hz, and a total acquisition time of 4.24 min. The average voxel size of the soleus, gastrocnemius lateralis and tibialis anterior was 40 × 12 × 28 mm, 40 × 10 × 28 mm and 40 × 13 × 24 mm, respectively. Following shimming procedures, the linewidth of the water signal was on average 26.9, 27.5 and 30.6 Hz for soleus, gastrocnemius lateralis and tibialis anterior, respectively. A 500-ml spherical container filled with an aqueous solution of 20 mM carnosine (Sigma-Aldrich) was used as an external reference for absolute quantification. The following equation was used to determine the carnosine concentration in vivo, based on the C2-H resonance at 8 ppm:

$$[C_m] = [C_r] \times (S_m \times V_r \times C_{T1r} \times C_{T2r} \times T_m)/(S_r \times V_m \times C_{T1m} \times C_{T2m} \times T_r)$$

Where $[C_m]$ is the carnosine concentration in vivo; $[C_r]$ is the concentration of the external reference phantom (20 mM); S_m and S_r are the estimated signal peak areas of the muscle and reference phantom, respectively, obtained by curve fitting performed in the frequency domain and were also corrected for differences in coil loading between phantom and the muscle; V_m and V_r are the volumes of the voxels in vivo and in the phantom, respectively; C_{T1m}, C_{T2m}, C_{T1r} and C_{T2r} are correction factors for the T1 and T2 relaxation times in vivo and in the phantom, respectively; T_m and T_r are the temperatures in vivo and in the phantom, respectively. The formulae used to calculate the correction factors are:

$$C_{T1} = (1 - e^{(-TR/T1)})$$

$$C_{T2} = e^{(-TE/T2)}$$

For the determination of T1 and T2 relaxation times, the carnosine signal was measured with a PRESS sequence by varying TR and TE, respectively, and the resulting peak areas were fitted to mono-exponential functions. The T1 and T2 relaxation times of in vitro carnosine were determined and were found to be 2616 ± 20 ms and 250 ± 29 ms, respectively. For the determination of T1 and T2 relaxation times in vivo five healthy subjects (2 males and 3 females; age: 21 to 26 year) were scanned for the soleus, five (3 males and 2 females; age: 21 to 25 year)

for the gastrocnemius lateralis and five for the tibialis anterior muscle (5 females; 22 to 26 year). T1 was measured using a TE of 30 ms and 8 TRs varying from 1,500 ms to 6,000 ms, while T2 was measured using a TR of 4,000 ms and 6 TEs varying from 30 ms to 200 ms. For each measurement 128 averages were acquired. The T1 relaxation times were found to be $1,488 \pm 377$ ms, $1,771 \pm 225$ ms and $1,692 \pm 432$ ms in soleus, gastrocnemius lateralis, and tibialis anterior, respectively, which are similar values as mentioned in Ramadan et al. (2010), a study performed at 7 T. The T2 relaxation times were found to be 152 ± 28 ms, 106 ± 50 ms and 64 ± 32 ms in soleus, gastrocnemius lateralis and tibialis anterior, respectively, which is slightly higher for soleus and gastrocnemius, than in Ramadan et al. (2010), which is expected, since T2 decreases with increasing field strength.

Muscle biopsy

Before and after 5 weeks sprint training muscle biopsies were taken from the right gastrocnemius lateralis at rest, with 14 Gauge true-cut biopsy needle (Bard Magnum Biopsy gun; Bard, Inc., New Jersey, USA). Under ultrasonographic guidance (Ultrasonography Pro Sound SSD-5000, ALOKA Co., Ltd., Tokyo, Japan. with probe UST-5545, frequency 5–13 MHz), two muscle samples ($\sim$15 mg) were taken following local anesthesia (lidocaine 1%, Linisol®). The samples were then immediately frozen in liquid nitrogen and stored at $-80°C$ until subsequent analysis.

In vitro muscle buffering capacity; titration method (β_m in vitro)

The first muscle sample was freeze-dried and 2.78 ± 0.43 mg was homogenized in a solution containing sodium fluoride (NaF 10 mM) at a dilution of 10 mg dw/ml homogenizing solution. The pH was measured with a microelectrode (MI-415-4 cm pH combination electrode, Microelectrodes inc., USA) connected to a pH meter (WTW series, inoLab 730, VWR Belgium). Sodium hydroxide (NaOH) was added to the homogenates to adjust the initial pH to 7.2. Thereafter, the homogenate was titrated to a pH of 6.1 by serial addition of 4 µl hydrochloric acid (HCl 10 mM). The number of moles H^+ (per kg dw) required to change the pH from 7.1 to 6.5 was calculated and normalized to the whole pH unit. The titration was performed twice and the average value of the two measurements was used.

High performance liquid chromatography (HPLC) analysis

An aliquot of 100 µl of the homogenate, to calculate β_m in vitro, was deproteinized by adding sulfosalicylic acid (35%, 9:1) and centrifuged at 14,000 g for 5 min. The supernatants (100 µl) were then dried under vacuum (40°C). Dried residues were resolved with 40 µl of coupling reagent: methanol-triethylamine-H_2O-phenylisothiocyanate (7:1:1:1) and allowed to react for 20 min at room temperature. The samples were dried again and resolved in 100 µl of sodium acetate buffer (10 mM, pH 6.4). The same method was applied to standard solutions of carnosine (Sigma-Aldrich) with distilled water. The derivatized samples (20 µl) were applied to a Waters HPLC system with an Atlantis HILIC Silica column (4.6 × 150 mm, 5 µm) and UV detector (wavelength 254 nm). The column was equilibrated with buffer A [10 mM sodium acetate adjusted to pH 6.4 with 6% acetic acid] and buffer B [60% acetonitrile—40% buffer A].

Repeated sprint ability test (6 × 6 s RSA)

Before and after the training period, all subjects performed a 6 × 6 s repeated sprint ability test (RSA) on an electromagnetically braked cycle ergometer (Lode, Excalibur sport; Groningen, The Netherlands). The RSA test was preceded by a standardized warm-up including 5 min cycling at 80 W, followed by a passive rest period of 90 s and one practice sprint of 6 s. After warming-up, the subjects rested 5 min before the start of the RSA. The RSA test consisted of six maximal 6 s-sprints with 24 s slow pedaling in between. About 5 s before the start of each sprint the subjects were asked to accelerate until the display showed $\sim$80 RPM. They were allowed to perform in standing position and strong verbal encouragement was provided. The cycle ergometer was used in the linear mode and the linear factor was set at 7 and 8% of the body weight for females and males, respectively.

Lactate measurements

Capillary blood samples (7 µl) were taken from a hyperaemic ear lobe in order to determine the lactate concentration after warm-up, after 6 × 6 s RSA and after 5 min active recovery (Analox instruments Ltd., London, UK).

RNA isolation, cDNA synthesis and real-time-PCR

From the second biopsy, total RNA was isolated using the TriPure Isolation Reagent (Roche) followed by a purification with the RNeasy Mini Kit (Qiagen). An on-column DNase treatment was performed using the RNase-Free DNase Set (Qiagen). RNA was quantified using a Nonodrop 2000C spectrophotometer (Thermo Scientific) and RNA purity was assessed using the A260/A280 ratio. Using a blend of oligo(dT) and random primers, 150 ng RNA was reversed transcribed with the iScript cDNA

Synthesis kit (Biorad), according to the manufacturer's instructions. RT-PCR was carried out on a Lightcycler 480 system (Roche) using an 8 µl reaction mix containing 3 µl template cDNA (1/10 dilution), 300 nM forward and reverse primers and 4 µl SYBR Green PCR Master Mix (Applied Biosystems, Belgium). The cycling conditions comprised a polymerase activation at 95°C for 10 min, followed by 45 cycles at 95°C for 15 s, 60°C for 30 s and 72°C for 30 s. Primer sequences of CARNS (gene ID: 57571 forward: GGCGTCAGCAAGAAGTTCGT; reverse: CCGGTGCTCTGTCATGTCAA) were designed using Primer Express software 3.0 (Applied Biosystems). Primer sequences of the reference gene EEF1A1 (gene ID: 1915, forward: CTGGCAAGGTCACCAAGTCT; reverse: CCGTTCTTCCACCACTGAT) were selected from the RTprimerDB site (http://medgen.ugent.be/rtprimerdb/). Sequence specificity was confirmed using NCBI Blast analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Since geNorm ranked EEF1A1 as the best reference gene, this was used to normalize the expression of CARNS and primers. To control the specificity of amplification, data melting curves were inspected and PCR efficiency was calculated (99.9 and 102% for CARNS and EEF1A1, respectively). Relative gene expression levels were calculated via the delta delta-Ct method (Derveaux et al. 2010).

Statistics

All correlations were evaluated by Pearson's correlations. A 2×2 general linear model repeated-measures ANOVA was used to evaluate the muscle carnosine content, the relative CARNS mRNA expression and in vitro muscle buffering capacity, with "diet group" (VD vs. MD) as between-subjects factor and "time" (pre vs. post) as a within-subjects factor. In case of a significant interaction effect, a post hoc analysis was performed. The performance improvement during the 6×6 s RSA test was evaluated with a 2×6 (diet $\times$ sprint number) general linear model repeated-measures ANOVA. An independent sample t test was used for the nutritional analysis (VD vs. MD) and a paired sample t test was used to analyze lactate values (SPSS statistical software, SPSS 16.0, Chicago, IL). Values are presented as means $\pm$ SD, and significance was assumed at $p \leq 0.05$. The normalized mRNA expressions were log transformed for further analysis.

Results

Muscle carnosine content

Before (pre) and after (post) the training period the muscle carnosine content was measured in three lower leg muscles
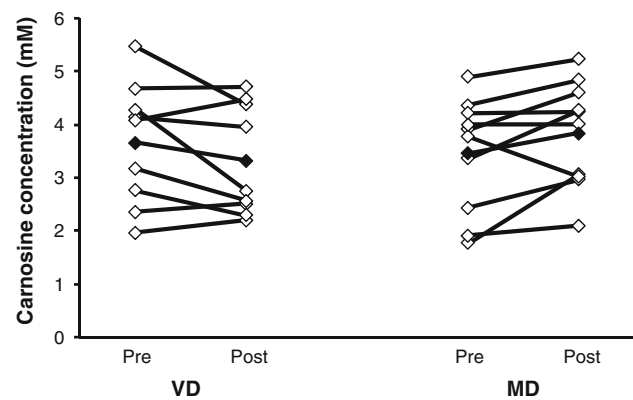
Fig. 1 Muscle carnosine content of the soleus measured by ^{1}H-MRS, before (pre) and after (post) 5 weeks sprint training combined with a vegetarian (VD) or mixed diet (MD). Individual values (*open symbols*) and group averages (*black symbols*) are shown

by ^{1}H-MRS. The baseline carnosine concentrations of the soleus ($p = 0.712$), gastrocnemius lateralis ($p = 0.944$) and tibialis anterior ($p = 0.969$) were not significantly different between the MD and VD group. Males had a significant higher muscle carnosine content compared to females (soleus: $p < 0.001$, gastrocnemius: $p = 0.01$, tibialis: $p < 0.001$). 5 weeks sprint training resulted in a significant interaction effect ($p = 0.020$) in the soleus (Fig. 1). The post hoc analysis showed that the carnosine content of the soleus was non-significantly increased ($+11\%$) in the MD group and non-significantly decreased (-9%) with VD. The effects of training and diet on the carnosine content of the gastrocnemius lateralis muscle were measured by both ^{1}H-MRS and by HPLC. As seen in Table 1, both methods show that the mean carnosine content of the gastrocnemius lateralis remained stable after 5 weeks sprint training, in both groups (interaction effect: ^{1}H-MRS $p = 0.879$ and HPLC $p = 0.182$). Table 1 indicates that the muscle carnosine content of the tibialis anterior did not change ($p = 0.063$) following the intervention.
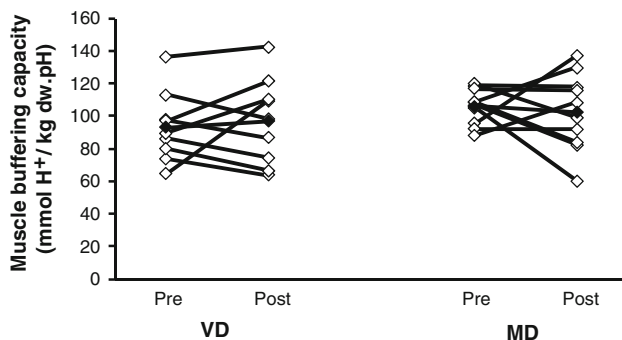
Muscle buffering capacity (β_{m})

Five weeks sprint training did not result in a change ($p = 0.514$) in in vitro muscle buffering capacity in any group. As can be observed in Fig. 2, the muscle buffering capacity was 93.3 ± 21.6 mmol H$^+$/kg dw.pH and 106.2 ± 11.9 mmol H$^+$/kg dw.pH before and 97.1 ± 26.5 mmol H$^+$/kg dw.pH and 102.7 ± 19.6 mmol H$^+$/kg dw.pH after the training period, for the VD and MD group, respectively. Figure 3 shows a rather modest, yet significant ($p = 0.047$) positive ($r = 0.343$) correlation between in vitro muscle buffering capacity and carnosine concentration of the gastrocnemius lateralis (measured by HPLC).

Table 1 Muscle carnosine content of the soleus, gastrocnemius lateralis, tibialis anterior and mean of the three muscles measured by ^{1}H-MRS, before (pre) and after (post) 5 weeks sprint training

	Group	Pre	Post	Interaction	Pre vs. post
Muscle carnosine concentration (mM) measured by ^{1}H-MRS					
Soleus	VD	3.66 (1.16)	3.32 (1.04)	**<u>0.020</u>**	0.910
	MD	3.47 (1.08)	3.84 (1.00)		
Gastrocnemius lateralis	VD	5.22 (1.69)	5.31 (1.71)	0.879	0.647
	MD	5.17 (1.57)	5.35 (1.75)		
Tibialis anterior	VD	5.27 (2.46)	5.98 (2.57)	0.417	0.063
	MD	5.23 (2.58)	5.52 (2.88)		
Mean 3 muscles	VD	4.72 (1.71)	4.87 (1.41)	0.657	0.145
	MD	4.62 (1.61)	4.90 (1.72)		
Muscle carnosine concentration (mmol/kg dw) measured by HPLC					
Gastrocnemius lateralis	VD	20.34 (4.51)	18.84 (4.82)	0.182	0.630
	MD	23.80 (7.75)	26.92 (11.58)		

Values are means $\pm$ (SD) of 9 subjects in the vegetarian (VD) and 10 subjects in the mixed diet group (MD). The carnosine content of the gastrocnemius lateralis, measured by HPLC, is shown (VD $n = 7$; MD $n = 8$)

The p values for the interaction (time $\times$ group) and for the main effect (pre/post) are shown and underlined in case of significance

Fig. 2 In vitro muscle buffering capacity of the gastrocnemius lateralis, before (pre) and after (post) 5 weeks sprint training combined with a vegetarian (VD) or mixed diet (MD). Individual values (*open symbols*) and group averages (*black symbols*) are shown
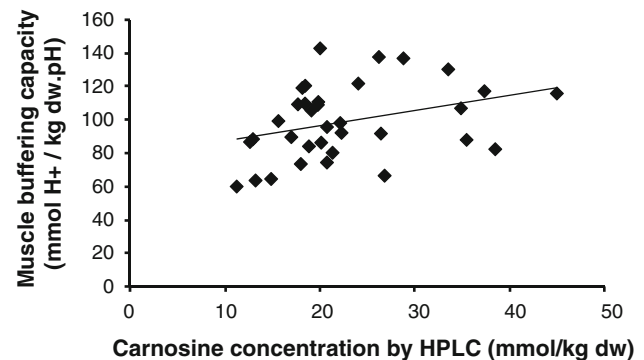

Fig. 3 Correlation ($p = 0.047$; $r = 0.343$) between muscle carnosine of the gastrocnemius lateralis measured by HPLC (*x*-axis) and in vitro muscle buffering capacity (*y*-axis)

Repeated sprint ability test (6 $\times$ 6 s RSA)

The typical 6 $\times$ 6 s RSA profile showed that the mean power output per sprint decreased from sprint 1 to sprint 6 independent of group (at baseline: total mean decrease of $\sim$170 W and following training: total mean decrease of $\sim$100 W). As seen in Fig. 4a, b, 5 weeks sprint training caused an increase in the power output per sprint ($p < 0.05$) for both MD and VD group. The mean power output for all sprints together ($p < 0.001$; pre: 765 $\pm$ 196 W and post: 852 $\pm$ 228 W) increased following 5 weeks sprint training independent of the diet group ($p = 0.707$). Figure 4c shows a significant main effect of sprint number ($p = 0.010$) for the training-induced increase in power output per sprint. The subjects improved more in sprint 6 in comparison with sprint 1 (Δpost–pre:

S1 = +48.58 $\pm$ 93.92 W; S6 = +114.68 $\pm$ 51.00 W), without differences between groups ($p = 0.392$). A moderate to strong positive correlation was observed between baseline muscle carnosine content of the gastrocnemius lateralis (^{1}H-MRS) and both mean ($p = 0.004$; $r = 0.63$) and maximal ($p = 0.005$; $r = 0.62$) power output of the RSA test (pre). There was no significant correlation between performance on the RSA test (mean power output: $p = 0.707$; max: $p = 0.262$) and in vitro muscle buffering capacity. The lactate concentration before and after the training period was measured after warm-up, after the 6 $\times$ 6 s RSA test and after 5 min active recovery. For both groups, the lactate concentration following the 6 $\times$ 6 s RSA test was $\sim$1.2 mmol/l higher after the training period (pre: 7.6 $\pm$ 1.4 mmol/l and post: 8.8 $\pm$ 1.5 mmol/l; $p = 0.021$).
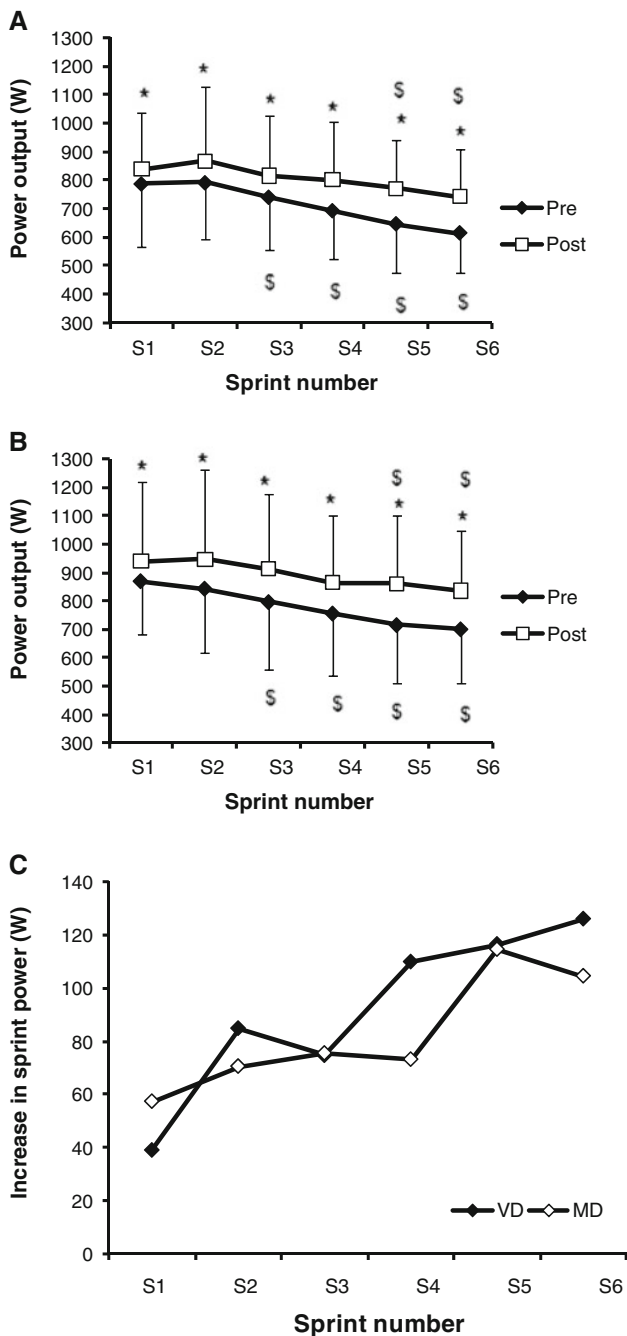
Fig. 4 Mean power output for the vegetarian (a) and mixed diet group (b) during the 6 × 6 s repeated sprint ability test (RSA) before (pre) and after (post) 5 weeks sprint training. Sprints 1–6 (S1–S6) are shown on the x-axis. Data are mean ± SD of 9 subjects in the vegetarian and 10 subjects in the mixed diet group. Different from pre (*$p < 0.05$). Different from sprint 1 (S1) ($^{$}p < 0.05$). c Increase in sprint power after 5 weeks sprint training for the vegetarian (VD) and mixed diet (MD) group

Table 2 Nutritional analysis

	Group		t test
Total energy intake (kcal/day)	VD	2226.84 (657.96)	0.780
	MD	2315.70 (701.20)	
Proteins (%)	VD	13.13 (3.28)	0.078
	MD	15.78 (2.86)	
Carbohydrate (%)	VD	55.08 (6.42)	0.868
	MD	54.55 (7.14)	
Lipids (%)	VD	28.00 (4.58)	0.405
	MD	26.02 (5.43)	
Alcohol (%)	VD	3.79 (3.98)	0.956
	MD	3.67 (5.18)	
Beta-alanine (g/day)	VD	< 0.001	_p < 0.001_
	MD	0.36 (0.23)	

Total energy intake (kcal/day), percentage of total energy intake of proteins, carbohydrates, lipids and alcohol and amount of beta-alanine consumption (g/day) for the vegetarian (VD) and mixed diet group (MD)

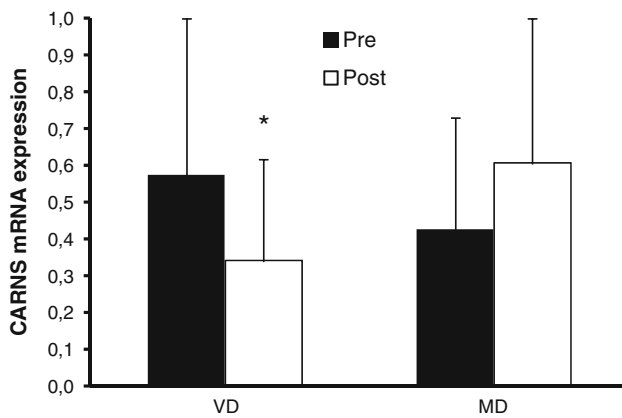Data are shown as means ± (SD) and p values of t tests are underlined in case of significance
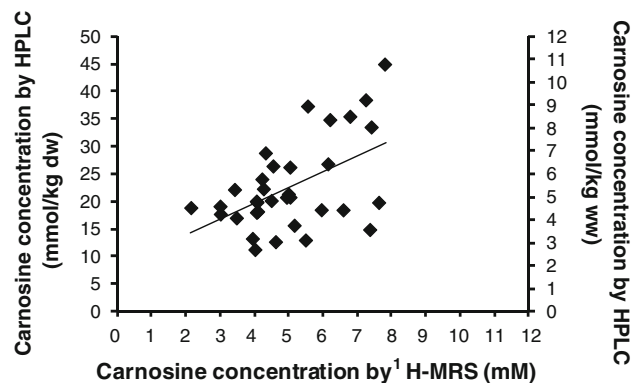
Fig. 5 Correlation ($p = 0.002$; $r = 0.517$) between the muscle carnosine concentration of the gastrocnemius lateralis measured by ^{1}H-MRS (wet weight) (x-axis) and by HPLC (primary y-axis: dry weight; secondary y-axis: wet weight) ($n = 34$)

fat and alcohol and there were no differences between MD and VD group regarding total energy intake. There was only a tendency that the VD group consumed on average less (~17%) proteins than MD group ($p = 0.078$). Due to their meat and fish consumption, the MD group consumed on average 0.36 ± 0.23 g beta-alanine a day (range: 0.067–0.812 g/day), while the VD group consumed hardly any beta-alanine.

Measurement of muscle carnosine content: comparison of ^{1}H-MRS and HPLC

The muscle carnosine concentration of the gastrocnemius lateralis muscle was measured by an invasive (HPLC

Diet registration

The analysis of the food diaries can be found in Table 2. Both groups consumed the same amount of carbohydrates,

Fig. 6 CARNS mRNA expression of the VD and MD group before (pre) and after (post) 5 weeks sprint training. (Different from pre; *$p < 0.05$)

technique using a true-cut biopsy) and a non-invasive method (^{1}H-MRS). As shown in Fig. 5, there was a significant ($p = 0.002$) positive correlation ($r = 0.517$) between the two measurement methods. The dry weight muscle carnosine content (measured by HPLC) was back-calculated into a wet weight value by multiplying the dry weight value by the percentage weight remained after freeze-drying. The wet weight carnosine concentration values of both methods (HPLC and ^{1}H-MRS) were comparable ($p = 0.56$).

CARNS mRNA expression

The baseline CARNS mRNA expression was not different ($p = 0.628$) between males (0.41 ± 0.36) and females (0.48 ± 0.33). The interaction effect (training × diet) was not significant ($p = 0.076$). Figure 6 shows that the CARNS mRNA expression of the MD group non-significantly increased ($p = 0.325$) from 0.42 ± 0.31 to 0.60 ± 0.39 and significantly decreased ($p = 0.023$) in the VD group from 0.57 ± 0.43 to 0.34 ± 0.28.

Discussion

This is the first study that investigated the effect of sprint training in combination with a vegetarian diet on the muscle carnosine content and muscle buffering capacity. Beta-alanine, which is hardly present in vegetarian food, is the rate limiting precursor of carnosine synthesis. The hypothesis that muscle carnosine would increase after 5 weeks sprint training, provided beta-alanine was present in the diet, cannot be confirmed. The carnosine content did not significantly increase in soleus, gastrocnemius and tibialis anterior. This further isolates the findings by Suzuki et al. (2004) who showed a massive increase (+113%) in

carnosine in the vastus lateralis muscle, with a similar training program as in our study, whereas almost all other studies found no effect irrespective of various training programs (Kendrick et al. 2008; Kendrick et al. 2009; Mannion et al. 1994). The current study confirms that there is no effect of a short training period on the muscle carnosine content. It could be argued that a 5-week training program is too short, given the slow loading and unloading profile of muscle carnosine (Baguet et al. 2009). However, even at mRNA level of carnosine synthase, which could be expected to be a more early sign of increased carnosine synthesis, there is no training effect visible.

Since the carnosine synthase gene was just recently cloned (Drozak et al. 2010), this is—to our knowledge—the first study, which investigated the CARNS mRNA expression in human skeletal muscle. The enzymatic regulation of muscle carnosine probably depends mainly on CARNS, but it is still unclear how its activity and expression is regulated. The current study shows that the gender difference, which is clearly present for muscle carnosine concentration (Mannion et al. 1992), seemed to be absent in CARNS expression. Furthermore, based on the results in Fig. 6, it can be suggested that beta-alanine availability is probably an important factor in CARNS expression, as this significantly declined in the VD group. This suggestion is further supported by the significant interaction effect (diet × training) in the soleus muscle carnosine content. This interaction effect showed a non-significant increase of ~11% in the MD group and in the VD group a non-significant decrease of ~9%, which could have partially resulted from differential CARNS expression.

Carnosine's quantitative contribution to the in vitro buffer capacity of human muscle is estimated to be 7–8% (Harris et al. 1990; Hill et al. 2007). Indeed, in the current study a positive correlation was found between muscle carnosine content and in vitro muscle buffering capacity. A substantial increase in muscle carnosine content, such as the 80% increase in vastus lateralis carnosine concentration elicited by 10 weeks of oral beta-alanine supplementation, has been calculated to increase carnosine's contribution from 8.2 to 13.5% and elevate the total in vitro buffer capacity by 6% (Hill et al. 2007). Likewise, oral beta-alanine supplementation reduces the magnitude of exercise-induced acidosis in healthy volunteers (Baguet et al. 2010b). If training would induce a 113% increase in muscle carnosine content, as suggested by Suzuki et al. (2004), this would mean a sizeable 9% increase in muscle buffer capacity. Since the current study did not find an effect of training on the muscle carnosine content, we did not expect that the in vitro buffer capacity would be affected. Indeed, our results indicate no change in in vitro buffer capacity in the gastrocnemius lateralis with training and diet intervention.

Sprint training improved the performance on a RSA test in both groups. The observation that subjects improved more in the final sprints as compared to the first sprint and that higher lactate concentrations were reached post-training could suggest that the in vitro muscle buffering capacity is increased. However, this was not the case (in the gastrocnemius lateralis). Other possible explanations of the performance improvement are an increase in aerobic capacity (McMahon and Wenger 1998) or a neural effect (Ross et al. 2001). The performance enhancement could also be due to an increase in lactate/H^+ transport capacity (Juel and Halestrap 1999; Pilegaard et al. 1999; Mohr et al. 2007; Thomas et al. 2007) or to bicarbonate buffering, since in vitro buffer capacity does not include this components (Hultman and Sahlin 1980).

The finding that the performance improvement was not different between both diet groups, suggests that acute vegetarianism has no acute adverse effects on adaptations to sprint exercise. However, it should be noted that in the current study, all subjects received 1 g creatine a day, similar to dietary intake of creatine by meat consumption (Venderley and Campbell 2006), to prevent a creatine deficiency in VD group and to isolate the effect of beta-alanine deprivation. Ovo-lacto vegetarians, who do not take creatine supplements, can possibly develop a creatine deficiency (Lukaszuk et al. 2002; Burke et al. 2003) and therefore, possibly experience a decrease in performance. In addition, it should also be mentioned that the intervention only lasted 5 weeks and that the results of the current study can, therefore, not be generalized for a longer intervention period.

The current study also aimed to validate the quantification of muscle carnosine by ^{1}H-MRS, using the HPLC analysis of muscle biopsy tissue as the criterion measure. This is the first study, which compared the invasive and non-invasive methods to measure muscle carnosine, within the same study. The current study found a significant positive correlation ($r = 0.517$) between muscle carnosine measured by HPLC and ^{1}H-MRS and also the absolute (wet weight) concentrations seem to match well.

Conclusion

In conclusion, 5 weeks sprint training does not induce an increase in muscle carnosine content nor in CARNS mRNA content, whereas deprivation of dietary beta-alanine (vegetarianism) seems to slightly affect these factors negatively. Although this could in the long run signify a new disadvantageous aspect of vegetarian diet on muscle adaptations to sprint training, the current vegetarian diet intervention during 5 weeks of training did not negatively affect performance or muscle buffer capacity.

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