

## **Beta-alanine supplementation augments muscle carnosine content and attenuates fatigue during repeated isokinetic contraction bouts in trained sprinters**

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## Abstract

Carnosine ( $\beta$ -alanyl-L-histidine) is present in high concentrations in human skeletal muscle. The ingestion of  $\beta$ -alanine, the rate-limiting precursor of carnosine, has been shown to elevate the muscle carnosine content. We aimed to investigate, using proton magnetic resonance spectroscopy (proton MRS), whether oral supplementation with  $\beta$ -alanine during 4 weeks would elevate the calf muscle carnosine content and affect exercise performance in 400m sprint-trained competitive athletes. Fifteen male athletes participated in a placebo-controlled, double-blind study and were supplemented orally for 4 weeks with either 4.8g/day  $\beta$ -alanine or placebo. Muscle carnosine concentration was quantified in soleus and gastrocnemius by proton MRS. Performance was evaluated by isokinetic testing during 5 bouts of 30 maximal voluntary knee extensions, by endurance during isometric contraction at 45% MVC and by the indoor 400m running time.  $\beta$ -Alanine supplementation significantly increased the carnosine content in both the soleus (+47%) and gastrocnemius (+37%). In placebo, carnosine remained stable in soleus while a small and significant increase of +16% occurred in gastrocnemius. Dynamic knee extension torque during the fourth and fifth bout was significantly improved with  $\beta$ -alanine but not with placebo. Isometric endurance and 400m race time were not affected by treatment. In conclusion, 1) proton MRS can be used to non-invasively quantify human muscle carnosine content; 2) muscle carnosine is increased by oral  $\beta$ -alanine supplementation in sprint-trained athletes; 3) carnosine loading slightly but significantly attenuated fatigue in repeated bouts of exhaustive dynamic contractions; 4) the increase in muscle carnosine did not improve isometric endurance or 400m race time.

## Keywords

Buffer capacity, ergogenic supplements, nuclear magnetic resonance (NMR), exercise performance, track-and-field

## Introduction

Carnosine ( $\beta$ -alanyl-L-histidine) is a cytoplasmic dipeptide synthesized from the precursors L-histidine and  $\beta$ -alanine by carnosine synthetase. Carnosine is present in relatively high concentrations in skeletal muscle (5-10 mM) and other excitable tissues, such as nervous tissue. Given that the pKa value of the imidazole ring is 6.83 (4; 42), carnosine acts as a physicochemical buffer in myocytes. This function is consistent with the fact that carnosine is present in higher concentrations in glycolytic than in oxidative muscle fibers (7; 8), with the highest levels found in animals that perform frequent sprint exercises (e.g. greyhounds, thoroughbred horses), explosive flight behaviors (e.g. pheasants) and prolonged hypoxic dives (e.g. whales) (16; 40). In addition, it has been shown that carnosine causes an increase in the  $\text{Ca}^{2+}$ -sensitivity of vertebrate skeletal muscle. Given that the evidence for the latter is solely based on *in vitro* studies with skinned muscle fibers of frogs and rats (11; 26), its physiological relevance *in vivo* remains to be established.

Several studies have demonstrated, by a pre-exercise metabolic alkalosis intervention, the importance of intramyocellular pH regulation in performance during supramaximal exercise (29), probably via an indirect effect of proton accumulation on contractile function. Whilst carnosine will function as a buffer over the physiological pH range, and has been estimated to account for approximately 10% of the total buffering capacity in human vastus lateralis (19), the importance of buffering and pH control in muscle to exercise performance is still debated. By using a newly developed nutritional strategy to raise carnosine concentrations in human muscle resulting in altered buffering capacity (18; 19), there is now a means to undertake studies equating muscle buffering capacity to performance. In Harris et al. (18) a daily oral intake of ~6g  $\beta$ -alanine for 4 weeks was shown to increase the carnosine content by 60% in the vastus lateralis of untrained humans, whilst 10 weeks supplementation increased this by ~80% (19). The pronounced effect of  $\beta$ -alanine supplementation is consistent with evidence that the intramyocellular synthesis of carnosine is rate-limited by the availability of  $\beta$ -alanine (3; 9; 19) and not by histidine which is present in muscle in high concentrations relative to its  $K_m$  for carnosine synthase (20). Thus the use of  $\beta$ -alanine supplementation offers researchers a technique to investigate the role of carnosine in muscle contraction, as well as its ergogenic potential.

Several recent studies have suggested that muscle carnosine content is a determining factor in high-intensity dynamic and isometric exercise performance (19; 39; 41). Suzuki and colleagues (41) observed a positive correlation between the vastus lateralis carnosine content

and the power output generated at the end of a 30-s all-out cycling sprint (Wingate-test) in untrained men. Additionally, Hill et al (19) supplemented untrained males with  $\beta$ -alanine for 10 weeks which resulted not only in a substantial increase in the carnosine content of the vastus lateralis, but also in an increased time to exhaustion in a cycling test performed at 110% of  $W_{max}$  (power output at maximal heart rate), where the expected endurance time is approximately 2.5 min. In the current study the ergogenic potential of supplementary  $\beta$ -alanine is evaluated in sprint-trained subjects, in which there is added interest because sprint-trained athletes are reported to have a higher muscle carnosine content than untrained subjects or endurance trained athletes (31). A carnosine content which is initially high could mitigate against the effectiveness of  $\beta$ -alanine supplementation to further elevate muscle carnosine. By analogy, the latter 'ceiling' phenomenon is known to limit the effectiveness of creatine supplementation in subjects with high initial muscle creatine content (13; 17).

For the quantification of the human muscle carnosine concentrations, there are currently only two techniques used, *i.e.* the determination of imidazole dipeptides by high-performance liquid chromatography (HPLC) or by ion-exchange chromatography of extracts of muscle. Both of these require muscle biopsy samples (8; 41). However, proton Magnetic Resonance Spectroscopy (MRS) could provide a valuable non-invasive alternative (30; 36). The unique properties of the imidazole protons of the histidine subunit of carnosine make them visible in the far downfield region (7 and 8 ppm; left from the water peak) of the proton spectrum of human muscle (30). A second aim of this study was therefore to explore the possibility to quantify the carnosine content in the soleus and gastrocnemius muscles of the human calf through proton MRS.

The present study was conducted on well-trained 400m sprint runners. We hypothesized that muscle carnosine content would be substantially elevated by 4 weeks of oral  $\beta$ -alanine supplementation, despite the possibility of a high initial carnosine content in this population. Because carnosine is known to act as a proton buffer in the physiological pH range, and because intramyocellular acidosis has been proposed as a cause of fatigue during high-intensity contractions, we further aimed to investigate the effect of the potentially elevated muscle carnosine content on several aspects of muscle performance and fatigue. This was investigated through 1) an isokinetic test of repeated bouts of maximal exhaustive knee extensions, 2) an isometric test of the static endurance of the knee extensors, and 3) an indoor 400m race. Finally, we aimed to evaluate the use of proton MRS as a non-invasive tool to monitor muscle carnosine concentration in humans.

## Materials and methods:

*Subjects.* Fifteen male track-and-field athletes, each with a personal record (PR) on 400m below 53 s were recruited from Flemish athletics clubs. Their official PR on 400m were on average ( $\pm$  SD)  $50.45 \pm 1.60$  s and ranged between 47.49 s and 52.64 s. All athletes were actively competing at regional or national (Belgium) level and were preparing for the indoor competition season during the course of the study (months November/December). In the randomization, the number of athletes from the same club/trainer was matched for each group, in order to minimize the differences in training amount and intensity. The athletes of the placebo ( $n = 7$ ) and  $\beta$ -alanine ( $n = 8$ ) groups performed on average 5.4 and 5.6 training sessions per week, respectively, during the study period. The relative percentage of total training time spent on power/speed/resistance/endurance was 25/23/24/28 and 27/25/23/25 in placebo and  $\beta$ -alanine, respectively. None of the subjects was taking any other oral ergogenic supplements during the study or had taken supplements in the 3 months prior to the study. The subjects gave their informed consent and the study was approved by the local Ethics Committee (Ghent University Hospital, Belgium). The subjects' weight, height and age was  $70.7 \pm 5.7$  kg,  $183 \pm 4$  cm and  $18.4 \pm 1.5$  years for placebo and  $74.1 \pm 7.2$  kg,  $184 \pm 8$  cm and  $23.8 \pm 4.2$  years for  $\beta$ -alanine, respectively. All subjects completed all experiments and there were no complaints of side-effects of the supplements.

*Study design.* A placebo-controlled, double-blind study was performed. Subjects were supplemented orally for 4-5 weeks with either placebo (maltodextrin) or  $\beta$ -alanine (Carnosyn™, National Alternatives International, San Marcos, USA). Supplements were provided in capsules of 400mg and were administered each day as 6 divided doses, with at least 2h in between ingestions. Daily doses consisted of 2.4g/day during the first four days, 3.6g/day during the subsequent four days, and from then on 4.8 g/day until the end of the study. All measurements were performed in the week prior to the start of the supplementation (PRE) and during the last week of supplementation (POST). As for the muscle torque measurements, a familiarization session was performed one week prior to the PRE-measurements.

### *Proton MRS.*

All the MRS measurements were performed on a 3 Tesla whole body MRI scanner (Siemens Trio, Erlangen) equipped with a knee-coil. Single voxel point resolved spectroscopy (PRESS) with the following parameters was used : TR = 2000 ms, TE = 30 ms, voxel size = 40 mm x 30 mm x 12 mm, NEX = 256, 1024 data points, spectral bandwidth of 1200 Hz and a total

acquisition time of 8.40 minutes. A 500 ml spherical container filled with an aqueous solution of 50 mM carnosine (Sigma) was used as an external reference for the absolute quantification. The full-width-half maximum (FWHM) of the water signal was minimized to be in the range of 20-30 Hz during shimming.

The following equation was used to determine the concentration of C2-H carnosine *in vivo*:

$$C_m = \frac{C_r S_m F_{mT_1} F_{mT_2}}{S_r F_{rT_1} F_{rT_2}} \quad (1)$$

Where  $C_m$  is the carnosine concentration *in vivo*,  $C_r$  is the concentration of the external reference phantom prepared (50 mM),  $F_{mT_1}$  and  $F_{mT_2}$  are correction factors for the  $T_1$  and  $T_2$  relaxation of the metabolites of interest.  $S_m$  and  $S_r$  are the estimated signal peak areas obtained by the curve fitting performed in the frequency domain. Data processing included 5 Hz apodization (line broadening), phase correction, base line correction and Fourier transformation. The correction factors  $F_{T_1}$  and  $F_{T_2}$  were calculated as follows:

$$F_{T_1} = \frac{1}{1 - \exp(-T_R / T_1)} \quad (2)$$

$$F_{T_2} = \frac{1}{\exp(-T_E / T_2)} \quad (3)$$

$T_1$  and  $T_2$  relaxation parameters of *in vitro* carnosine were measured and were found to be  $560 \pm 15$  ms,  $66.22 \pm 9.3$  ms, respectively. Thus, for the pulse sequence used (TR = 2000 ms), the correction term in equation 2 amounts to 0.9718 and can therefore be neglected. Note that this correction factor for *in vivo* carnosine would be larger than that of *in vitro* due to shorter  $T_1$  of the carnosine *in vivo*.  $T_2$  *in vivo* used in correction was 39 ms as was previously measured (30). Signal intensities were also corrected for the differences in coil loading and temperature difference between the phantom and the human body. Load correction was carried out using the following formula:

$$S_c = S_u 10^{A/20} \quad (4)$$

where  $S_c$  is the corrected,  $S_u$  is the uncorrected signal intensities and A is the difference between radiofrequency (RF) power required for 180 degree pulse *in vivo* and that for phantom, measured in decibels (38). The correction factor used for the temperature difference was 6% (44).

*Isokinetic and isometric muscle fatigue protocol.* An isokinetic and isometric test was performed on a Biodex isokinetic dynamometer (System 3, Biodex Medical Systems, New York, USA) to evaluate the contractile performance of the knee extensors. The dynamometry was preceded by a standardized warm-up consisting of an ascent and descent of a stairs (11m altitude) and two 50-m runs at moderate pace. The isokinetic test was performed on the right leg. This consisted of 5 x 30 maximal voluntary isokinetic knee extensions at a constant angular velocity of 180°/s. Each contraction was initiated from a position of 90° knee flexion and was continued to the point of full knee extension. After each extension the lower leg was passively returned to the start position at 90°/s (~1.5s for full cycle). Each bout of 30 contractions was separated by a 1 min recovery period. Subjects were encouraged during the first three contractions to make sure that they were contracting maximally from the start of each bout. Subjects received visual feedback of their produced peak torque. Peak torque during each contraction (1-30) was measured and used to calculate the average peak torque during each bout of exercise. Subsequently, the isometric test was performed on the left leg. The knee angle was fixed at 45° and the maximal static voluntary contraction (MVC) torque was determined. The highest torque of three 3s- attempts, separated by 30s of rest, was considered as MVC. Subjects were then asked to contract isometrically with their knee extensors at a target torque of 45% of MVC for as long as possible to determine isometric endurance.

*400m race.* The time to complete 400m running was evaluated in an indoor 300m flat athletics track (Flanders Sports Arena, Ghent, Belgium). Time was recorded by an infra-red light based electronic device at the start and finish (400m). Subjects were asked to warm-up according to their personal routine during approximately 45 minutes. Following warm-up and 2-3 min prior to the start, a capillary blood sample was obtained from a finger tip and analyzed for lactate with Lactate PRO test strips (Arkray Inc, Kyoto, Japan). Also at 90s and 180s following finish, blood lactate was measured. The maximal lactate accumulation was considered as the higher of the two post-sprint lactate values.

*Statistics.* A 2x2 GLM repeated measures analysis of variance (RM ANOVA) was used to evaluate the muscle carnosine content, 400m run time and lactate accumulation and isometric endurance, with 'group' (placebo vs.  $\beta$ -alanine) as between-subjects factor and time (PRE vs. POST) as a within-subjects factor (SPSS statistical software, SPSS Inc, Chicago, USA). The isokinetic contraction bouts were evaluated with a 2x2x5 RM ANOVA, with 'bout' as an additional within-subjects factor. In case of significant interaction, ANOVA was repeated at a

lower level on each half of the data. Values are presented as means  $\pm$  SD and significance was assumed at  $P < 0.05$ .

## Results

*Muscle carnosine content.* Typical proton MRS spectra and voxel localization of the soleus and gastrocnemius muscle are shown in Figure 1. In carnosine phantoms and in human soleus muscle the C4-H and C2-H peaks have very similar size. However, in gastrocnemius the C4-H peak was markedly and consistently smaller than the C2-H peak in all subjects (Figure 1D), which appears to agree with the findings of others, publishing a spectrum from a human gastrocnemius muscle (37; 46). Our additional experiments revealed that the test-retest reproducibility was good for the C2-H but poor for C4-H peak (Özdemir M et al., unpublished observations). This high variation of the C4 resonance and the low C4-C2 ratio in gastrocnemius both suggest marked orientation-dependent effects on this resonance (24), and led to the current C2-based method for carnosine quantification. In the soleus muscle (Figure 2A), the absolute carnosine concentration as measured by MRS prior to supplementation was  $7.25 \pm 1.47$  and  $7.76 \pm 1.36$  mmol/L in the placebo and  $\beta$ -alanine groups, respectively. As a result of supplementation, the carnosine content of the soleus increased by 47% ( $P < 0.0001$ ) in the  $\beta$ -alanine group to  $11.39 \pm 1.38$  mmol/L, whereas it remained stable at  $7.85 \pm 1.04$  mmol/L (+8%,  $P = 0.41$ ) in the placebo group. In the gastrocnemius, the baseline carnosine concentration was ~25% higher than in soleus ( $P < 0.05$ ) being  $8.56 \pm 1.88$  and  $10.16 \pm 1.91$  mmol/L in the placebo and  $\beta$ -alanine groups, respectively. The gastrocnemius carnosine content (Figure 2B) increased significantly in both groups over the 4 week supplementation period, but the increase was significantly more pronounced in the  $\beta$ -alanine group (+ 37%;  $P < 0.0001$ ) than in the placebo group (+16%;  $P = 0.005$ ). In subjects receiving  $\beta$ -alanine the carnosine content of the soleus increased by at least 28% and maximally 89% and in the gastrocnemius between 22% and 53% (see Figure 2C). As shown in Figure 2D, the increase in carnosine in the  $\beta$ -alanine supplemented subjects was not dependent on the initial muscle carnosine content. The lack of negative correlation ( $r = +0.18$ ) suggests that there was no ceiling effect, and that a maximal carnosine content may not have been attained. The highest carnosine concentration measured was 18.5 mM observed in the gastrocnemius muscle of a subject given  $\beta$ -alanine supplementation, which was a 40% increase over baseline.

*Repeated bouts of maximal isokinetic knee extensions.* The average knee extension torque produced during 5 bouts of 30 dynamic (isokinetic) contractions is shown in Table 1. We performed a 2x2x5 ANOVA (with 'group', 'PRE/POST' and 'bout' as factors) on these data

and we found a significant 3-way interaction ( $P = 0.05$ ). Therefore, the data were further analyzed with 2-way ANOVA's in both groups separately. In the placebo group, a significant PRE/POST x bout interaction was found and post-hoc analysis revealed a significant PRE/POST effect in the first two bouts, but not in the subsequent three bouts. In the  $\beta$ -alanine group, however, a significant PRE/POST main effect was observed, indicating that average torque was higher in all five bouts POST- vs. PRE-supplementation. This effect is further illustrated in more detail in figure 3. The peak knee extension torque of each contraction during bouts 4 and 5 is consistently higher POST  $\beta$ -alanine supplementation compared to PRE (+ 6.1% in bout 4 and +3.8% in bout 5), whereas in the placebo group the POST- and PRE-values overlap (+ 1.0% in bout 4 and -0.8% in bout 5).

*Isometric endurance.* The maximal duration of a sustained isometric (static) knee extensor contraction at 45% MVC was  $173 \pm 55$  s and  $201 \pm 48$  s at baseline and increased to  $187 \pm 64$  s and  $220 \pm 61$  s following supplementation of placebo or  $\beta$ -alanine, respectively. ANOVA revealed a significant PRE/POST main effect ( $P = 0.03$ ) but no significant group main effect or interaction ( $P = 0.66$ ).

*400-m race.* The mean time to complete a 400-m run on the indoor athletic track was  $52.17 \pm 1.83$  s and  $51.11 \pm 1.66$  s at baseline for placebo and  $\beta$ -alanine subjects, respectively. Times decreased on average by  $\sim 0.7$  s to  $51.44 \pm 1.57$  s and  $50.36 \pm 1.43$  s, respectively, following supplementation in both groups. The improved run time was significant but similar in both groups, as illustrated by the main PRE/POST effect ( $P = 0.02$ ) and the lack of PRE/POST x group interaction ( $P = 0.98$ ) in a 2x2 ANOVA. The individual changes in run time are shown in Figure 4A (placebo) and 4B ( $\beta$ -alanine). Likewise, the blood lactate accumulation 180 s following the 400-m sprint was significantly increased by  $\sim 1.2$  mmol/L in both groups following supplementation (Table 2). The individual changes in maximal blood lactate concentrations following the 400-m are shown in Figure 4C (placebo) and 4D ( $\beta$ -alanine). The changes in gastrocnemius carnosine content did not significantly correlate with changes in 400m running speed ( $r = 0.08$ ), in maximal lactate accumulation ( $r = -0.22$ ) or with isometric endurance ( $r = 0.12$ ).

## Discussion

The present data on muscle carnosine concentration, obtained by proton MRS, agree closely with data from muscle biopsy studies of the vastus lateralis showing that the muscle carnosine content can be substantially increased by  $\beta$ -alanine supplementation (18; 19). The subjects in this study, however, were sprint-trained competitive athletes rather than untrained subjects and the muscles studied were calf muscles (soleus and gastrocnemius) rather than thigh muscles (vastus lateralis). The magnitude of the increase in muscle carnosine content induced by 4.8 g/day  $\beta$ -alanine supplementation in the current study, namely +47% in soleus and +37% in gastrocnemius, is comparable yet slightly lower than in a previous report on untrained subjects (+64% in vastus lateralis) following 4 weeks of 4-increasing-to-6.4 g/day  $\beta$ -alanine supplementation (19).

As illustrated in Figure 2D, even the subjects with a very high initial carnosine content ( $> 12$  mmol/L) were still able to elevate their concentrations by an additional 4-5 mmol/L. This suggests a lack of any 'ceiling effect' and that the human muscle carnosine content is far from maximal. Furthermore, this is supported by the finding that following 4 weeks of  $\beta$ -alanine supplementation, an additional 6 weeks of supplementation caused an additional 20% increase in muscle carnosine (19). In this respect, carnosine is clearly different from creatine, because maximal muscle creatine content is already obtained after 1 week of oral creatine supplementation and subjects with high initial levels are often non-responders (6; 17).

A further aim of this study was to evaluate the potential ergogenic effect of muscle carnosine loading in trained sprinters. The results show that whilst elevated muscle carnosine content lead to an improvement in muscle torque during repeated bouts of intense dynamic contractions it did not apparently affect 400m running performance. It has long been suggested that the acidotic environment that is created by carboxylate anion formation during anaerobic work limits muscle contractility (5) and there is a vast amount of literature suggesting that pre-exercise alkalosis promotes performance in brief high-intensity exercise (27; 34). Several papers with a similar study population (runners) and exercise type (running race between 300m and 800m distance) as with the present study have investigated the effect of induced metabolic alkalosis through oral pre-exercise ingestion of sodium bicarbonate or sodium citrate. The outcome of these studies, however, has been equivocal, with two studies reporting a positive effect (12; 45) and other studies reporting no effect (21; 23; 43). In the current study, the increase in muscle carnosine content in the  $\beta$ -alanine supplemented group will inevitably give rise to an increase in the intramyocellular buffer capacity. The lack of

difference (interaction term of 0.98) in 400m race time between placebo and  $\beta$ -alanine would indicate either that the gain in intracellular buffer capacity in this case was insufficient to bring about an increase in performance over and above the limitations of the test, or that decreasing pH is not a limiting factor to 400m performance in trained individuals. The apparent contradiction with earlier data by Goldfinch et al. (12), reporting a 1.5s improvement in 400m race time following bicarbonate ingestion, may in part relate to differences in training status since the male subjects in the Goldfinch study had average baseline 400m times of 58.5s (74% of current world record speed), compared to 51.6 s (84% of current WR speed) in the present study. Moreover, we must be cautious when interpreting the negative outcome of the performance part of the present study. As it takes several weeks of supplementation to induce muscle carnosine loading and because the wash-out period is unknown, it was not possible to adopt a paired cross-over design. In an unpaired experimental design, the study population of 7-8 subjects per group possibly did not generate enough statistical power. Thus, presently we can not exclude that still higher elevations of muscle carnosine content (e.g. by using a longer supplementation period) or a study with a more powerful statistical design may still demonstrate an increase in sprint performance with  $\beta$ -alanine supplementation.

Dynamic knee extension torque was significantly increased in each of the 5 bouts of 30 maximal contractions by  $\beta$ -alanine supplementation, whereas in the placebo group only the first two bouts were improved. These data for the first time show that in trained athletes performing repeated exhaustive contractions muscle fatigue can be slightly but significantly attenuated in the later (bouts 4 and 5) stages of exercise by carnosine loading. These data markedly resemble those of the first study to address the effect of muscle creatine loading on muscle fatigue (14). In a nearly identical protocol, muscle torque was found to be increased following creatine supplementation in most of the 5 x 30 maximal contractions (14).

Although still debated (25), most researchers agree that the rise in extracellular  $K^+$  and the intramyocellular accumulation of inorganic phosphate ( $P_i$ ),  $ADP_i$  and protons ( $H^+$ ) are important initiating factors of fatigue, with reduced sarcoplasmic reticulum (SR)  $Ca^{++}$  release and reduced myofibrillar  $Ca^{++}$  sensitivity as important consequences. The effects are ultimately a reduction in power output (2). Carnosine does not affect SR  $Ca^{++}$  release (11), but has been shown *in vitro* to increase myofibrillar  $Ca^{++}$  sensitivity, particularly in fast-twitch fibers (11; 26). Furthermore, carnosine may attenuate fatigue by its physicochemical buffering

capacity, thereby alleviating the detrimental effects that intracellular  $H^+$  accumulation may have, as recently shown (29; 33).

However, despite the effect of  $\beta$ -alanine supplementation on force production during repeated dynamic contractions, no effect on isometric endurance was observed. The lack of any effect is in contrast to the preliminary report of the findings of Ponte et al (32) where again endurance at 45% MVC was studied. However, in the study of Ponte et al (32) contractions of the knee extensors were performed with a knee angle of  $90^\circ$  (compared to  $45^\circ$  in the current study) resulting in endurance times close to the 80 seconds predicted by the Rohmert curve for 45% MVC (1; 35), where the circulation is essentially occluded. With this model, peak accumulation of carboxylate anion (principally lactate and pyruvate), measuring  $H^+$  formation, occurs at 45% MVC and at a rate of  $\sim 1.2$  mmol/kg dm/s (0.4 mmol/L/s) (1). The longer mean endurance times observed in the present study suggest a lesser degree of circulatory impairment with the model used, a lower rate of carboxylate anion accumulation and a smaller fall in pH. This would limit the possibility of any increase in carnosine exerting a measurable effect on endurance and could explain the difference in findings. Alternatively, the added effect of muscle carnosine loading in this case is relatively small because the sprint-trained subjects already have a high initial carnosine content and endogenous muscle buffer capacity.

The current study is possibly the first to apply the technique of proton MRS to non-invasively quantify and monitor the absolute carnosine concentrations in human skeletal muscle with  $\beta$ -alanine supplementation. Others have shown that the downfield C2-H and C4-H imidazole resonances of the proton spectrum of human muscle belong to the histidine subunit of carnosine ( $\beta$ -alanyl-L-histidine) (30; 36; 46). Pan et al. (30) indirectly estimated the absolute carnosine concentration in the forearm muscle of two volunteers from comparison with the estimated creatine concentration, and reported values of 5.13 and 8.06 mM. In the present study, the absolute quantification of the muscle carnosine content was obtained by relating the C2-H imidazole peak at 8 ppm (Figure 1) of the proton spectrum of the muscle with that of a phantom (50 mM model solution of carnosine). The resulting absolute concentrations are  $\sim 7$  mM for soleus and  $\sim 9$  mM for gastrocnemius at baseline in this population.

The carnosine content was 25% higher in gastrocnemius compared to soleus. These data agree with previous studies, where higher carnosine levels were found in rat gastrocnemius vs. soleus (28) and in fast-twitch vs. slow-twitch fibers of the camel (10), horse (7) and human (15; 19; 22). When adopting a probable fiber type distribution (% type I/II) of 88/12 in soleus

and 47/53 in gastrocnemius (19) and a type I/II carnosine concentration ratio of 0.60, we calculated that carnosine content should be 25% higher in gastrocnemius compared to soleus, which is exactly the same as what we actually measured. Thus, the difference in carnosine content between the two muscles is probably entirely explained by the difference in their fiber type composition. This observation may be promising for the future development of MRS-based techniques to non-invasively determine muscle fiber type composition and fiber compartmentalization, in humans. We believe that it is correct to state that we provide physiological validation of the use of proton MRS in the quantification of human muscle carnosine concentrations, based on the acceptable absolute values of carnosine concentrations, the magnitude of  $\beta$ -alanine induced increase in carnosine content and the difference in carnosine content between muscle types found in this study.

It can be concluded from this study that 1) proton MRS can be used to non-invasively quantify human muscle carnosine content; 2) muscle carnosine content can be substantially elevated by oral  $\beta$ -alanine supplementation in sprint-trained athletes; 3) muscle carnosine loading slightly but significantly attenuates fatigue in repeated bouts of exhaustive dynamic contractions; and 4) 4 weeks of supplementation with  $\beta$ -alanine at the prescribed dose used in this study does not result in a significant improvement in 400m run performance. This latter result may be a consequence of a too little increase in intramyocellular pH buffering capacity for an improvement to be detected within the limits of the experimental procedures used, or alternatively may indicate that in trained athletes 400m running performance is not limited by intracellular pH decrease.

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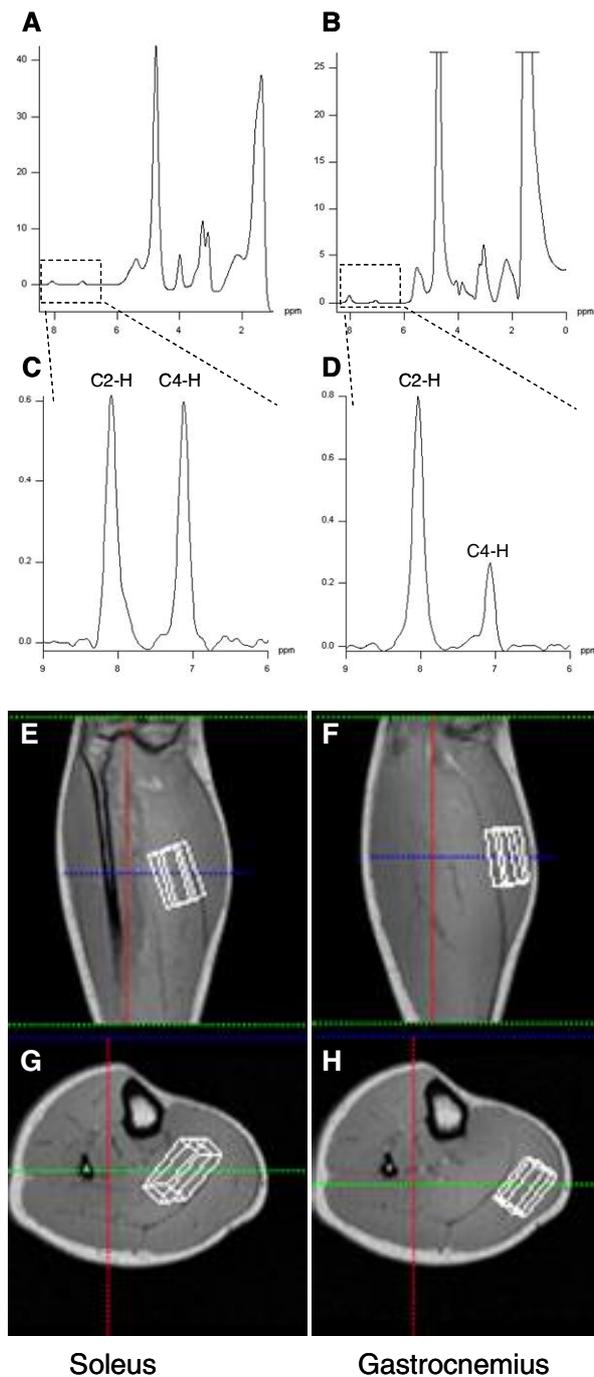
**Table 1:** Average knee extension torque during 5 bouts of 30 dynamic contractions. The P-values for the main effects (PRE/POST and bout) and for the interaction of the 2x5 ANOVA are shown and underlined in the case of significance. \* Significant difference vs. PRE.

Nm	Bout 1	Bout 2	Bout 3	Bout 4	Bout 5	PRE-POST	Bout	Interaction
<b>Placebo</b>								
PRE	167 (23)	150 (15)	143 (19)	142 (20)	144 (18)	0.169	<u>&lt;0.001</u>	<u>0.010</u>
POST	178 * (21)	158 * (16)	148 (14)	143 (13)	143 (13)			
<b>Beta-alanine</b>								
PRE	176 (22)	148 (19)	137 (18)	132 (20)	133 (21)	<u>0.011</u>	<u>&lt;0.001</u>	0.739
POST	181 * (25)	155 * (23)	143 * (22)	139 * (23)	137 * (25)			

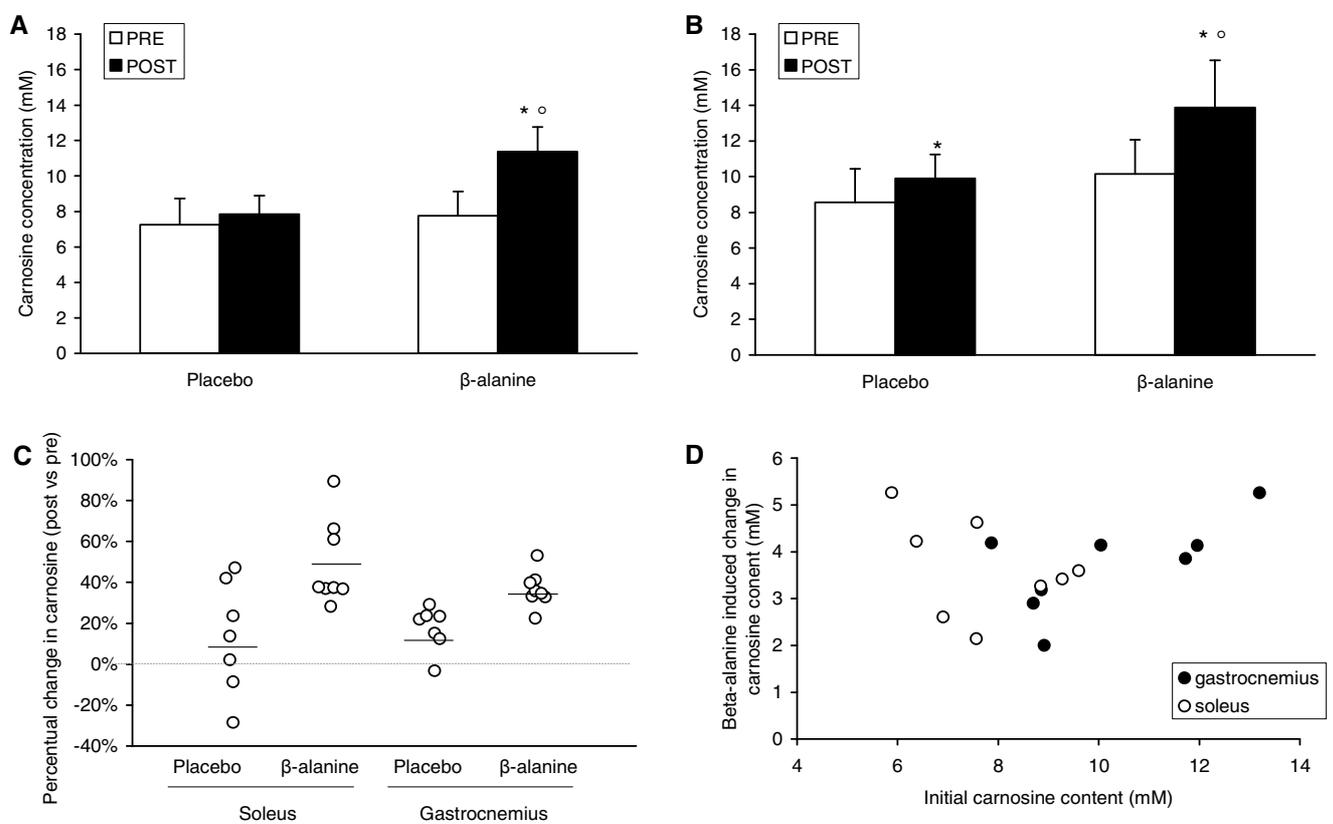
**Table 2:** Capillary blood lactate accumulation prior to the start (post warm-up) and 90 s and 180 s after the finish of a 400m run. The P-values for the main effects (PRE/POST and group) and for the interaction of the 2x2 ANOVA are shown and underlined in the case of significance. \* Significant difference vs. PRE.

Lactate (mmol/L)		PRE	POST	PRE/POST	Group	Interaction
Post warm-up	Placebo	2.5 (0.9)	3.4 (1.4)	0.208	0.554	0.457
	$\beta$ -alanine	3.1 (1.1)	3.3 (0.9)			
90 s post finish	Placebo	14.7 (1.8)	15.4 (1.4)	0.162	0.248	0.723
	$\beta$ -alanine	15.5 (0.9)	16.0 (1.2)			
180 s post finish	Placebo	14.7 (1.3)	15.9 * (0.7)	<u>0.003</u>	0.441	0.989
	$\beta$ -alanine	15.1 (1.4)	16.3 * (0.8)			

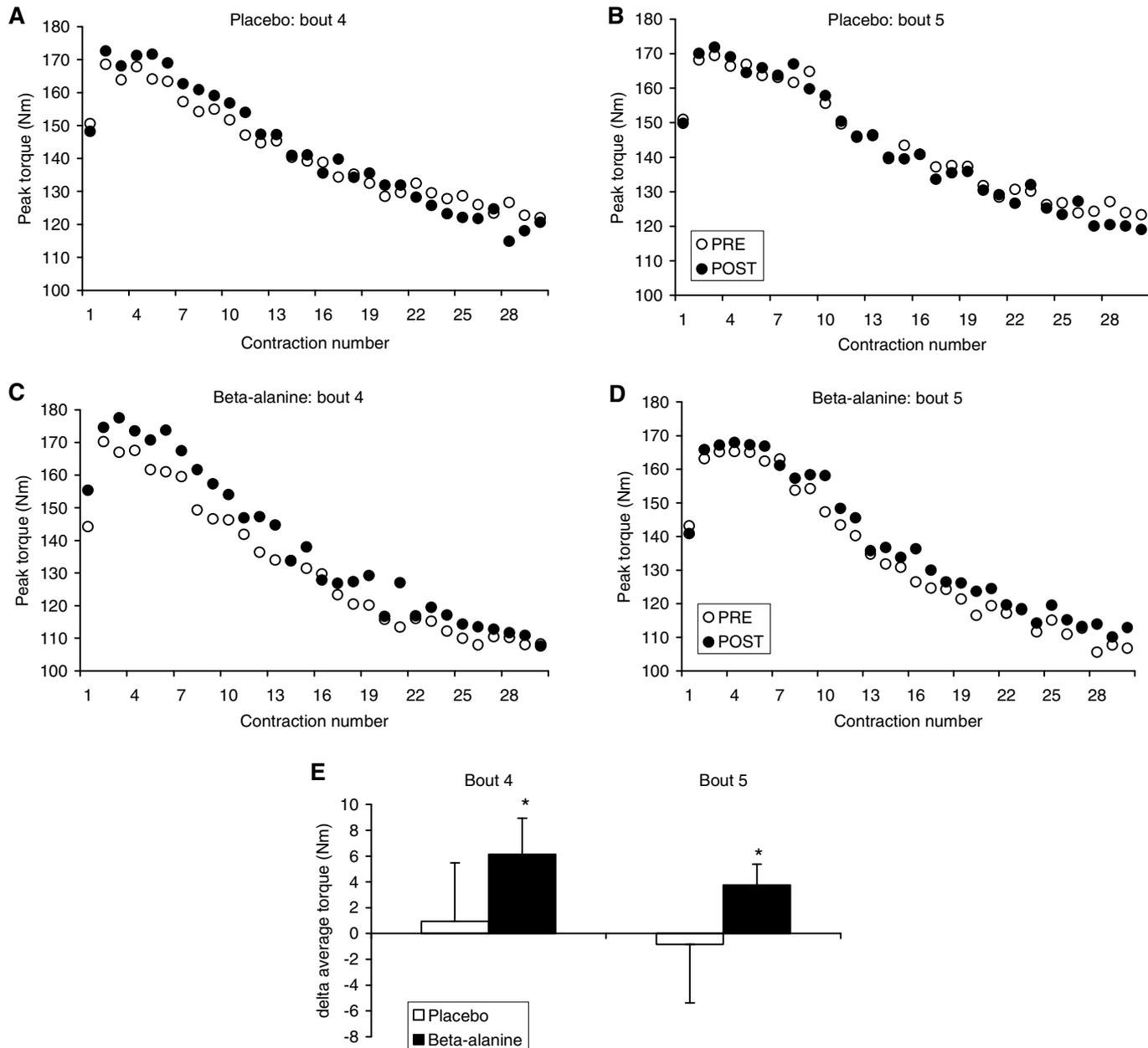
**Figure 1:** Proton MRS and MRI of the right calf muscles in a representative sprint-trained subject. A and B: Full proton spectra of the soleus (A) and gastrocnemius (B). C and D: Detailed spectra of the downfield carnosine region in soleus (C) and gastrocnemius (D). Resonances for the C2-H and C4-H imidazole protons of carnosine are at ~8 and ~7 ppm, respectively. E to H: Coronal (E and F) and transverse (G and H) images obtained by MRI in soleus (E and G) and gastrocnemius (F and H) to demonstrate the localization of the voxel.



**Figure 2:** Muscle carnosine concentration (mmol/L) in soleus (A) and gastrocnemius (B) before and after 4 weeks supplementation of placebo or  $\beta$ -alanine. The individual change in muscle carnosine content (POST vs. PRE) in the 7 placebo subjects and 8  $\beta$ -alanine subjects is shown in panel C. The horizontal line depicts the average procentual change for each group. In panel D, the absolute change in muscle carnosine concentration is shown in relation to the initial carnosine content of soleus (open symbols) and gastrocnemius (closed symbols) in each of the  $\beta$ -alanine supplemented subjects. \* Different from PRE ( $P < 0.05$ ). ° Different from Placebo ( $P < 0.05$ )



**Figure 3:** Peak knee extension torque during each contraction of bout 4 (A and C) and bout 5 (B and D) before and after 4 weeks of placebo (A and B) or  $\beta$ -alanine (C and D) supplementation. Average of 7 (placebo) and 8 ( $\beta$ -alanine) subjects. E: Delta (POST vs. PRE) average torque of all contractions in one bout, mean  $\pm$  SEM. \* Difference between POST and PRE ( $P < 0.05$ )



**Figure 4:** *Panel A and B:* Time (s) to complete a 400m run before and after 4 weeks of placebo (A) or  $\beta$ -alanine (B) supplementation. *Panel C and D:* Maximal capillary blood lactate concentration (mmol/L) following a 400m run before and after 4 weeks of placebo (A) or  $\beta$ -alanine (B) supplementation. Individual values (filled symbols) and group averages (open symbols) are shown.

