Baguet A, Bourgois J, Vanhee L, Achten E, Derave W. Important role of muscle carnosine in rowing performance. J Appl Physiol 109: 1096–1101, 2010. First published July 29, 2010; doi:10.1152/japplphysiol.00141.2010.—The role of the presence of carnosine (β-alanyl-L-histidine) in millimolar concentrations in human skeletal muscle is poorly understood. Chronic oral β-alanine supplementation is shown to elevate muscle carnosine content and improve anaerobic exercise performance during some laboratory tests, mainly in the untrained. It remains to be determined whether carnosine loading can improve single competition-like events in elite athletes. The aims of the present study were to investigate if performance is related to the muscle carnosine content and if β-alanine supplementation improves performance in highly trained rowers. Eighteen Belgian elite rowers were supplemented for 7 wk with either placebo or β-alanine (5 g/day). Before and following supplementation, muscle carnosine content in soleus and gastrocnemius medialis was measured by proton magnetic resonance spectroscopy (1H-MRS) and the performance was evaluated in a 2,000-m ergometer test. At baseline, there was a strong positive correlation between 100-, 500-, 2,000-, and 6,000-m speed and muscle carnosine content. After β-alanine supplementation, the carnosine content increased by 45.3% in soleus and 28.2% in gastrocnemius. Following supplementation, the β-alanine group was 4.3 s faster than the placebo group, whereas before supplementation they were 0.3 s slower (P = 0.07). Muscle carnosine elevation was positively correlated to 2,000-m performance enhancement (P = 0.042 and r = 0.498). It can be concluded that the positive correlation between baseline muscle carnosine levels and rowing performance and the positive correlation between changes in muscle carnosine and performance improvement suggest that muscle carnosine is a new determinant of rowing performance.

carnosine (β-alanyl-L-histidine) is a cytoplasmic dipeptide synthesized from the precursors L-histidine and β-alanine by carnosine synthetase. It is present in relatively high concentrations in human skeletal muscles (~5 mM in wet weight or ~20 mmol/kg in dry weight) (19). Several functions such as proton buffer (pKₐ = 6.83) (4, 12), calcium regulator (5, 15, 28), antioxidant (7), antiaging (16), inhibitor of protein glycosylation (23), wound healing (32) and inhibitor of protein-protein cross-linking (23) have been attributed to carnosine, making it a multifunctional dipeptide. It has already been shown that increased muscle carnosine levels can improve contractile behavior and reduce fatigue through several mechanisms. Possible ways by which high carnosine levels could improve performance are by increasing the non-bicarbonate muscle buffering capacity (1, 42), by increasing the sensitivity of calcium release channels (5) and/or the calcium sensitivity of the contractile apparatus (15), by diminishing the reactive oxygen species accumulation (8, 26), and by vasodilation (35). A performance improvement linked to muscle carnosine may likely be explained by one or a combination of the previous factors. More details on the metabolism of carnosine and its relation to exercise performance and training are discussed in several reviews (13, 39).

The dietary availability of β-alanine is rate limiting for carnosine synthesis in muscle, and muscle carnosine content can increase, in both untrained and trained subjects, following 4 to 10 wk of β-alanine supplementation (4.0–6.4 g/day) (2, 14, 21, 24, 25). Therefore, β-alanine supplementation studies make it possible to determine the role of muscle carnosine in exercise performance. Several studies have attempted to determine the type of exercise that can be improved by β-alanine supplementation and, hence, could be limited by the muscle carnosine content. Only one study examined a competition event (400-m running) in trained athletes and found no ergogenic effect, despite elevated muscle carnosine content and reduced fatigue in repeated contraction bouts (14). Sweeney et al. (43) also demonstrated that β-alanine supplementation did not influence the performance during repeated sprints (2 sets of 5 5-s sprints) in untrained subjects. Van Thienen et al. (44) found improved sprint capacity at the end of a 2-h simulated cycling race in moderately trained subjects and in other studies constant-load exercise tests of ~2.5 min (22) and incremental exercise tests (41) showed improvement in untrained subjects. From these available studies it can be speculated that high-intensity efforts may need to take several minutes to experience a positive influence of β-alanine. Baguet et al. (1) found that β-alanine reduced the acidosis, in untrained subjects, during a 6-min cycling exercise at an intensity of 50% between the ventilatory threshold and maximal oxygen uptake (50%Δ). It can be expected that performance in sport competition events of this exercise intensity and duration are improved, as acidosis has been proposed as a cause of fatigue (3). In this context, rowing is an ideal model to study, because the official race distance (2,000 m) takes about 5.5–8 min depending on sex, category, boat type, and environmental conditions.

A 2,000-m rowing race has a specific profile, in which anaerobic alactic and lactic, as well as aerobic capacities are stressed to their maximum (45). According to Körner and Schwanz (27), a 2,000-m rowing race can be divided into three main phases, which are time bound and hence distance bound. We distinguish the start phase, the steady-state phase, and the final sprint (27). During the start phase, which takes ~30–45 s, energy supply from alactic and lactic anaerobic metabolism dominates. The steady-state phase, which takes ~3–4.5 min, is characterized by a predominantly aerobic energy supply. In the final sprint (~last 250 m, which takes 45–60 s), energy supply by the lactic anaerobic metabolism is gaining importance again (30, 37). A complete and detailed

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physiological description of rowing was made by Hagerman (17) and by Secher and colleagues (40).

The purpose of the present study was to find out if it is an advantage for rowers to have high muscle carnosine levels. To answer this question, we did both a cross-sectional and intervention study. The main question in the cross-sectional part was whether the speed over various distances was correlated with the muscle carnosine concentration within a group of rowers. The aim of the intervention study was to investigate if β-alanine can be ergogenic for a 2,000 m all-out test in elite rowers.

MATERIALS AND METHODS

This study consisted of two parts. The first part is a cross-sectional study, which examined whether there is a relationship between the baseline muscle carnosine content and how fast a rower is at four different distances (100 m, 500 m, 2,000 m and 6,000 m). A total of 19 Belgian elite rowers volunteered to participate in the cross-sectional study. Fifteen of them rowed all distances, and the four others rowed only the 2,000 m. Part 2 is an intervention study, in which 18 of the 19 rowers from the cross-sectional study were supplemented for 7 wk. The athletes gave their informed consent, and the study was approved by the local ethics committee (Ghent University Hospital, Belgium).

Cross-Sectional Part

Subjects. Nineteen (18 males and 1 female) Belgian elite rowers took part in this cross-sectional study. All rowers were competing at national or international level. Fifteen of the 19 rowers are among the Belgian national squad for international competitions. The four other rowers are the fastest Belgian subelite athletes. None of them were taking any ergogenic supplement during the study or had taken supplements 3 mo before the measurements. Age, weight, and height were 23.2 ± 4.4 yr, 84.2 ± 7.8 kg, and 188.0 ± 4.4 cm, and they performed on average 10.2 ± 2.9 training sessions/wk.

Study design. 1H-MRS. The carnosine content was measured in two skeletal muscles (soleus muscle and gastrocnemius medialis muscle) of the lower leg by proton magnetic resonance spectroscopy (1H-MRS), as previously described (14). The subjects were lying 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 3-T whole body MRI scanner (Siemens Trio, Erlangen) equipped with a knee coil. Single-voxel point-resolved spectroscopy was used with the following parameters: 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 and gastrocnemius muscle. T1 was measured using a TE of 30 ms and TR set to 1,500, 2,000, 2,500, 3,000, 3,500, 4,000, 5,000 and 6,000 ms. T2 was measured using a TR of 4,000 ms and TE was set to 30, 45, 60, 75, 90, and 105 ms for gastrocnemius and 30, 60, 90, 120, 150, and 200 ms for soleus. For each measurement 128 averages were acquired. The T1 relaxation times were found to be 1,488 ± 377 and 1,771 ± 225 ms in soleus and gastrocnemius, respectively, which are similar values as mentioned in Ramadan et al. (34), a study performed at 7 T. The T2 relaxation times were found to be 152 ± 28 and 106 ± 50 ms in soleus and gastrocnemius, respectively, which is slightly higher than in Ramadan et al. (34), which is expected, since T2 decreases with increasing field strength.

PERFORMANCE TESTS. Rowing performance was measured for four different distances (100 m, 500 m, 2,000 m, and 6,000 m). This standardized test protocol is used by the Belgian rowing league and includes a 100- and 500-m test on Monday, a 2,000-m test on Wednesday, and a 6,000-m test on Friday. All tests were performed on a rowing ergometer (Concept2 Model D PM4 indoor rower) in controlled laboratory conditions. The 100-m and 500-m tests were performed on the same day, and all tests were completed within the same week, with 1 day recovery in between. Fifteen subjects rowed the four different distances, and the 2,000 m was completed by all 19 rowers. In a planned post hoc analysis the four 500-m splits of the 2,000-m ergometer test were analyzed between rowers with the lowest and rowers with the highest carnosine content. To make this distribution, all male rowers (n = 18) were ranked from the lowest to the highest carnosine content. The first nine rowers, who had the lowest carnosine contents, were called the “low carnosine group,” and the nine rowers with the highest carnosine content belonged to the “high carnosine group.”

Interventional Part

Subjects. Eighteen Belgian elite rowers (17 males and 1 female) volunteered to participate in this double-blind, placebo-controlled study. At the start of this study they were randomized into an experimental and a placebo group based on their performance on a 2,000 m, muscle carnosine concentration, age, and weight. As a result of an injury, there was a drop out of one person from the β-alanine group after 5 wk. The athletes of the experimental (n = 8) and placebo group (n = 9) performed, on average, respectively, 9.5 ± 3.0 and 9.6 ± 3.5 training sessions/wk during the study. The subjects’ age, weight, and height were 24.2 ± 3.5 yr, 83.7 ± 4.8 kg, and 189.8 ± 3.9 cm for experimental and 21.7 ± 3.4 yr, 81.2 ± 10.9 kg, and 185.6 ± 5.4 cm for the placebo group, respectively (all parameters were not significantly different between groups; NS).

Study design. The rowers were supplemented orally for 7 wk with either 5 g/day (divided over 5 doses of 1 g, ingested with 2-h intervals) β-alanine (SportsControl Bétalanine Fast, Special Slow Release Formula, Verdepharma Belgium) or placebo (maltodextrine, Verdepharma Belgium). None of the subjects reported side effects due to the supplementation. Before (Pre) and during the last week of the supplementation period (Post) the subjects underwent two tests, including the measurement of the muscle carnosine content and the rowing performance.
H-MRS. The muscle carnosine content in soleus and gastrocnemius was measured by H-MRS, as mentioned above.

TWOTHOUSAND METERS. Before the start of the 2,000 m all-out test (Concept2 Model D PM4 indoor rower), subjects received a heart rate monitor (Suunto), their feet were strapped, and the drag factor was set in accordance with their category (heavyweight male: 130; lightweight male and heavyweight female: 120). The test was preceded by a standardized warm-up, lasting 20 min. All parameters, except the remaining distance to be rowed, were covered during the test and verbal encouragement was not allowed. Both pre and post tests were conducted at the same time of the day for each subject to account for any possible circadian rhythm effects.

LACTATE MEASUREMENTS. A capillary blood sample was obtained from a hyperemic ear lobe and analyzed for lactate with Lactate PRO test strips (Arkray, Kyoto, Japan) after warm-up, immediately after the 2,000 m all-out and after 3 min of recovery.

Statistics. All correlations were evaluated by Pearson correlations. A general linear model repeated-measures ANOVA was used to evaluate the muscle carnosine content, 2,000-m row time, and lactate accumulation, with “group” (placebo vs. β-alanine) as between-subjects factor and “time” (Pre vs. Post) as a within-subjects factor (SPSS statistical software, SPSS 16.0, Chicago, IL). Values are presented as means ± SD, and significance was assumed at .05. A one-tailed P value was used for the effect of β-alanine on the muscle carnosine content and on the performance improvement.

RESULTS

Cross-Sectional Part

Baseline muscle carnosine and performance. The average muscle carnosine contents in soleus and gastrocnemius medialis were 3.27 ± 0.59 and 4.75 ± 0.83 mM, respectively. Compared with a previous study of untrained subjects, the muscle carnosine content of the soleus and gastrocnemius muscles of the rowers were not significantly different from these of a control population (2), when calculated with the same T1 and T2 relaxation parameters, as newly determined in the present study (see MATERIALS AND METHODS) [soleus (P = 0.535): 3.16 ± 0.48 mM; and gastrocnemius (P = 0.632): 4.61 ± 0.89 mM] (2). Moreover the ratio (gastrocnemius/soleus) was nearly identical in rowers (1.48 ± 0.30) and control subjects (1.49 ± 0.34) (P = 0.93) (2). The performances on row tests of 100, 500, 2,000, and 6,000 m were evaluated. Figure 1 shows the correlation between the mean baseline muscle carnosine and the rowing speed at various distances. The higher the mean baseline muscle carnosine concentration, the higher the speed of a 100 m (P = 0.018), 500 m (P = 0.007), 2,000 m (P = 0.006), and 6,000 m (P = 0.003) all-out. All correlations of muscle carnosine, for the individual muscles and mean of the muscles, for the various distances are presented in Table 1.

To investigate which 500-m split of the official competition distance of 2,000 m is most dependent on muscle carnosine content, a post hoc comparison was made by splitting the group in rowers with highest and lowest muscle carnosine content. This revealed that the group with high muscle carnosine was significantly faster during the second and third 500-m split (500–1,500 m), which is the slowest part of the race (Fig. 2).

Interventional Part

Muscle carnosine content. Before supplementation, the carnosine concentration in soleus was 3.45 ± 0.62 and 3.13 ± 0.58 mM for placebo and β-alanine groups, respectively (P = 0.221). The significant interaction effect (P < 0.001), due to the supplementation, shows that the carnosine content of the soleus significantly increased with 45.3% in β-alanine group,
while it remained stable (−2.8%) in placebo group (Fig. 3A). A similar significant ($P = 0.013$) interaction effect was visible in the gastrocnemius. As illustrated in Fig. 3B, the muscle carnosine content of the gastrocnemius increased by 28.2% (from 4.57 ± 0.56 to 5.86 ± 1.63 mM) in the β-alanine group and did not change in the placebo group (from 4.87 ± 1.07 to 4.69 ± 1.30 mM). In subjects receiving β-alanine, the muscle carnosine content of the soleus increased by at least 5% and maximally 71% and the highest responder in the gastrocnemius increased 55%. There was not only a positive correlation in baseline carnosine content between soleus and gastrocnemius ($P = 0.044; r = 0.481; r^2 = 0.231$), but also between the absolute increase in muscle carnosine in soleus vs. gastrocnemius following β-alanine supplementation ($P = 0.003; r = 0.681; r^2 = 0.464$). The higher the baseline muscle carnosine content was, the higher was the increase in muscle carnosine, following supplementation ($P = 0.023; r = 0.777; r^2 = 0.604$).

**Performance tests.** As can be observed on Fig. 4, the β-alanine group improved 2.7 ± 4.8 s and the placebo group slowed 1.8 ± 6.8 s on the 2,000-m all-out after 7 wk β-alanine or placebo supplementation. These changes did not reach statistical significance ($P = 0.07$). The significant ($P = 0.042$) positive correlation ($r = 0.498$) of the change in muscle carnosine and performance improvement on the 2,000 m shows that the more the muscle carnosine concentration increased, the more the performance on the 2,000 m improved (Fig. 5).

**Lactate.** The blood lactate concentration before supplementation was 1.7 ± 0.5, 12.9 ± 3.5, and 13.0 ± 2.5 mmol/l, after warm-up, immediately after the 2,000 m, and after 3 min recovery, respectively. The blood lactate concentration did not change as a result of 7 wk supplementation with β-alanine or placebo, without differences between groups.
The present study found a strong and significant positive correlation between the muscle carnosine content and performance over a rowing distance of 100, 500, and 6,000 m (Table 1). Even for the official 2,000-m distance, the correlation was strong (Table 1). As with every correlation, the causality of the relationship between muscle carnosine and performance should be evaluated. This question was addressed in the intervention part of our study by influencing the muscle carnosine, using β-alanine supplementation, and investigating if the performance improved. The muscle carnosine content increased by 45% and 28% in soleus and gastrocnemius, respectively, following 7 wk β-alanine supplementation (5 g/day), which is in line with previous reports (2, 14, 21, 22). Following supplementation, the β-alanine group was 4.3 s faster than the placebo group, whereas before supplementation they were 0.3 s slower (Fig. 4). Moreover, a strong positive correlation was found between the increase in muscle carnosine and the improvement in performance. This supports the conclusion that the above-mentioned correlation between muscle carnosine content and rowing performance is at least partially causal and relevant. Notwithstanding this finding, it is evident that other factors also play an important role.

As far as we know, except for skeletal muscle glycogen there are no precedents where correlations between baseline muscle metabolite concentrations and performance are found in a homogeneous group of elite athletes. Glycogen content strongly determines the time to fatigue in endurance events (46). However, glycogen is a rapidly fluctuating substrate as it is a fuel that declines with exercise and increases with carbohydrate ingestion (6). In contrast, carnosine is a stable compound, which is found to be characteristic for each individual (2). Muscle carnosine shows only small fluctuations (CV of 10%) over a 3-mo period (2).

The strong positive correlation between baseline muscle carnosine content and the speed during four different rowing distances (Table 1) suggests that besides the known performance-related factors (anaerobic threshold (30), absolute maximum oxygen uptake (V02max) (9, 31, 45), muscle composition (18, 29), and anthropometric profile (10)), muscle carnosine content is also a determinant of rowing performance.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

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