The Acute Administration of Carnosine and Beta-Alanine Does Not Improve Running Anaerobic Performance and has No Effect on the Metabolic Response to Exercise[*]

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An increase in muscle carnosine content, following its chronic supplementation, has been shown to improve anaerobic performance. In addition, carnosine can affect plasma glucose concentration and insulin response. However, it is not clear whether the acute ingestion of carnosine can have the same effects. Aim of this study was to investigate the acute effects of carnosine ingestion on anaerobic intermittent performance and the responses of blood insulin, glucose, bicarbonate and lactate concentrations to exercise. Twelve healthy, young, active participants (BMI 23.5 ± .6, age: 22 ± 2 years) underwent in two separate occasions (double-blind, randomized, crossover design) the running-based anaerobic test (RAST), consisting of 6×35-m sprints interspersed with 10 s rest after acute (4 hours before the test) ingestion of either 1 g of L-carnosine and 1 g of β-alanine or placebo. None significant difference was found between the acute ingestion of carnosine and the placebo conditions in terms of running performance (30.0 ± .8 and 29.8 ± .8, $p = .302$), perceptual response to exercise (RPE), blood lactate, insulin (23.8 ± 13.0 and 19.5 ± 9.0 μU·ml^{-1}, $p = .329$), blood glucose (109 ± 23 and 104 ± 12 mg·dl^{-1}, $p = .969$), and blood bicarbonates (16 ± 2 and 16 ± 2 mEq·l^{-1}, $p = .277$). In conclusion, the acute ingestion of carnosine had no effect on performance, perceptual response to exercise, blood lactate concentration, insulin, glucose, and bicarbonates responses to exercise compared to a placebo treatment. It is not clear whether these results may be attributed to an insufficient dose of carnosine or to a lack of acute effect *per sé*.

Keywords: Carnosine; Performance; Administration; Metabolic Response; Running

Introduction

The dipeptide carnosine is found in various tissues, especially in skeletal muscle (Hobson et al., 2012; Harris et al.). Carnosine is recognized to have, in addition to antioxidant properties (Alhamdani et al., 2007), buffering effects on acidosis (Hobson et al., 2012), as well as stimulating actions on the immune system and on various neurotransmitters (L-carnosine lowers neural activities of sympathetic nerves and facilitates those of parasympathetic nerves) (Nagai et al., 2012). Although carnosine is synthesized endogenously, its tissue concentration is influenced by diet (Baguet et al., 2010). Indeed, exogenous supplementation of β-alanine (precursor and limiting factor in the synthesis of carnosine) has been shown to increase muscle carnosine concentration in vitro (Margolis et al., 1985) and in animal models (Hill et al., 2007).

At physiological pH, carnosine exerts a strong buffering action (stabilization of the degree of acidity) that is of fundamental importance during muscle activity, usually associated with an acidification of the intracellular compartment (Smith, 1938).

Carnosine is found in muscles of both type I and type II, but its concentration is highest in type II fibres (Baguet et al., 2010). Carnosine levels seem to be correlated to exercise performance: Suzuki et al (Suzuki et al., 2002) observed a positive correlation between carnosine content in the vastus lateralis and the power generated at the end of a 30 second cycle ergometer test (Wingate test) in untrained men. Hill et al. (Hill et al., 2007) demonstrated that β-alanine administration to active men can produce a significant increase of carnosine levels in skeletal muscle, which was related to an improvement of exercise performance. In particular, β-alanine was administered to untrained men for 10 weeks, producing not only a remarkable increase of carnosine content in the vastus lateralis, but also a significant prolongation of the time for exhaustion during a cycling test performed at 110% of energy production and maximal heart rate, where the endurance time was estimated to be about 2.5 minutes (Hill et al., 2007). The role played by carnosine, or by β-

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alanine, is of interest not only for untrained subjects: the two compounds have a positive effect on strength in trained athletes, especially sprinters, rowers and body-builder as well (Baguet et al., 2010; Derave et al., 2010). Recent studies have described other biological roles for carnosine as an antioxidant, antidiabetic (reduction of glycosylation) (Derave et al., 2010; Hipkiss, 2009), or anti-aging (elongation of chromosomal telomeres) agent (Shao et al., 2004). In particular, since carnosine content in the diaphragm of streptozotocin (STZ)-diabetic rats was lower than in wild type rats (Buse et al., 1980), it was hypothesized that carnosine may be involved in the control of glucose metabolism. In animal models peripheral administration of a small amount of carnosine (.005 to 5 nmol/300 g body weight [BW] by intraperitoneal injection [IP]), or administration of a diet containing .001% carnosine, reduced 2 DG-hyperglycemia, producing an increase in plasma insulin levels, a decrease in plasma glucagon levels, and suppression of the activity of sympathetic nerves innervating the adrenal glands and liver (Nagai et al., 2003; Yamano et al., 2001). In addition, in animal models of diabetes, the ability of carnosine to inhibit the adrenergic system, with positive effects on glucose metabolism, was demonstrated after both chronic and acute supplementation (Aldini et al.).

Beta-alanine is usually administered orally for a period of 1 - 4 weeks (Jagim et al.), since chronic assumption seems to be necessary to ensure carnosine increase inside the muscle fibres (Derave et al., 2007). However, some evidences suggest that few hours after the ingestion of carnosine the dipeptide is already available and ready to exert its function inside the muscle fibers (Begum et al., 2005; Gardner et al., 1991). If this assumption was valid, it would be interesting for athletes who perform high intensity exercise because they might avoid the 3 - 4 weeks periods of β-alanine supplementation. The only study on the acute effect of carnosine on performance failed to show any improvement due to the ingestion of carnosine (Kraemer et al., 1995). However, it is likely that the dose administered to the participants was too low.

In a preliminary study (di Pierro et al., 2011) it has been shown that the oral ingestion of l g of carnosine few hours before a heavy training session in volleyball players might have decreased the blood lactate production and increased the spontaneous total amount of work.

Furthermore, other than the intracellular pH buffering capacity, the increase in Ca^{2+} sensitivity of the contractile fibres represents an alternative mechanism through which muscle carnosine can improve muscle performance (Dutka & Lamb, 2004). This ergogenic effect of muscle carnosine might be useful in those maximal efforts where the low levels of pH do not represent the limits for the maintenance of the contractile properties of the muscles.

The aim of this study was to evaluate whether the acute ingestion of 1 g of L-carnosine and 1 g of β-alanine can improve performance during a running anaerobic sprint test (RAST) (Zagatto et al., 2009) compared to a placebo condition. Furthermore, the response of blood insulin, glucose, bicarbonate and lactate concentrations to the RAST, following the acute ingestion of carnosine, was also investigated.

A recent meta analysis (Hobson et al., 2012) showed that the supplementation of β-alanine can improve exercise performances with a duration comprised between 60 and 240 seconds. However, some Authors (Suzuki et al., 2002) suggested that towards the end of a 30-sec Wingate test, a higher content of

muscle carnosine may be important for the preservation of a higher power output compared to a control condition. The use of the RAST (due to its intermittent feature) could be a better choice to investigate whether carnosine can increase exercise performance towards the end of an effort lasting about 30 seconds. We hypothesize that, at least, during the last 35 m-sprint of the RAST, carnosine can have a beneficial effect on the final time compared to a placebo condition.

Materials and Methods

Subjects

Participants were recruited in the Milan area in Italy, according to the following inclusion criteria: males aged between 20 and 30 years old, no smokers, absence of chronic diseases or chronic drug treatment. All subjects signed a written informed consent prior to participation according to the Declaration of Helsinki. All the procedures used complied with the Good Clinical Practice (GCP) principles.

Twelve healthy male subjects were enrolled (age: 22 ± 2 years, body mass: 74.1 ± 7.1 Kg, stature: 178 ± 5 cm, body mass index: 23.5 ± .6 kg/m²): all participants were on a stable diet and had normal glucose tolerance (according to ADA). They were physically active and used to exercise on average 4 ± 2 times a week for 2.0 ± .6 hours and 2 ± 2 times a week for 1.6 ± .8 hours at vigorous and moderate intensities, respectively. The participants recruited for this study were soccer players, basketball players and track and fields athletes, accustomed with repeated all out intermittent sprints. On a separate day, prior to the beginning of the data collection, a familiarization session with the RAST used in the present study was done.

Study Design

The study was conducted according to a double blind, randomized, crossover, counterbalanced, placebo controlled design. The randomization, the preparation of treatment and placebo tablets were done by person not involved in the study. The treatment and placebo conditions were revealed to the authors of the present study only after the conclusion of the statistical analysis.

All participants underwent two RAST on two different occasions: on the first day, half of the participants (selected by a computer-assisted simple randomization procedure) took 4 tablets containing 250 mg of L-carnosine + 250 mg of β-alanine (DDM Carnosina, Omeopiacenza s.r.l., Pontenure—PC, Italy) 4 hours before the test, whereas the other half of participants took 4 tablets of placebo (containing no active ingredients but with the same appearance of L-carnosine and β-alanine tablets), and vice versa on the second day of test (2 weeks apart). Two weeks seems to be a more than sufficient washout period when carnosine is acutely administered to human subjects (Suzuki et al., 2006). The dose of carnosine administered to the participants was similar to a previous study (di Pierro et al., 2011) and in accordance to the prescriptions of the manufacter.

The Running-Based Anaerobic Sprint Test (RAST)

A standardized 10 min warm-up, consisting of 5 min running at a moderate pace followed by 5 accelerations over a distance of 40 m, interspersed with 1 min of passive recovery, was completed by each participants prior to the beginning of the RAST,

followed by five min recovery.

Starting from a standing position, on a track and field 400 m track, each participant sprinted all-out over a distance of 35 m for six times (forth and back) interspersed by 10 seconds of passive recovery. Performance time was electronically recorded using photocells gates at a height of 1.3 meters and positioned .5 meters ahead of the start line. The 35 m were measured as the distance between the two photocells gates. During all the sprints the athletes were verbally encouraged by the researchers, in order to obtain the maximal performance. The RAST has been shown to be a valid and reliable (for Total Effort Time ICC = .90) test for assessing both anaerobic power and predicting short-distances performances in running (Zagatto et al., 2009).

The outcome measures were a) time to complete each section of 35 m b) total time (the sum of the six partial times over the 35 m sections), c) ratings of perceived exertion (RPE) and muscle pain (PAIN) immediately after the completion of the test using the validated CR 10 Borg scale (Borg, 1998). Thirty minutes after the end of the RAST test RPE and PAIN were assessed again. RPE collected at this time point was used to assess the internal load through the session RPE-based method (Foster et al., 2001; Impellizzeri et al., 2004). Finally, the delayed onset of muscle soreness (DOMS) was evaluated at 24 and 48 hours post RAST.

Blood Analysis

Blood samples for lactate evaluation was collected from an ear lobe at 3, 5, 7, and 9 minutes after the completion of the test and analyzed using a portable blood lactate enzymatic analyzer (GM-7, Analox Instruments, Hammersmith, London, UK). Two different blood samples were drawn from an antecubital vein two hours prior to the beginning of the RAST test and 1 minute after the completion of the test, respectively, for the analysis of blood glucose, insulin and bicarbonates.

All blood samples (20 ml each) were collected in test tubes containing EDTA and placed on ice until plasma or serum were separated by centrifugation at 4°C (within 1.5 hours from sampling). All plasma and serum aliquots were frozen at −60°C for later analysis. All assessments were carried out in duplicate. Plasma glucose was measured with a glucose analyzer (Beckman Instruments, Fullerton, CA). Free-insulin was assessed by a highly specific two-site monoclonal antibody-based immunosorbent assay (ELISA; Dako Diagnostics, Cambridgeshire, UK).

Aliquots of blood were taken before centrifugation for measurement of bicarbonate, enzymatically determined using a commercial kit.

Statistical Analysis

The normality of data distribution was preliminary checked by the Shapiro-Wilk's test. Data are expressed as mean ± Standard Deviation (SD). Differences between the two treatments (carnosine and placebo) in terms of performance (total time given by the sum of each 35 m run), perceived exertion and pain at the end of the test, and session RPE at 30 minutes after the completion of the test, were tested using the Student paired *t*-tests.

A series of two way (condition × time) fully repeated measures ANOVAs were used to test the differences in glycemia, insulin and bicarbonates levels prior to and after the RAST test (PRE and POST test, main factor time) in the two conditions (treatment with carnosine or placebo, main factor condition).

A series of two way (condition × time) fully repeated measures ANOVAs were used to test the differences between the two conditions (treatment with carnosine or placebo, main factor condition) either for the times to complete each bout of the RAST and the time course of blood lactate after the RAST (main factors time).

Differences in DOMS between the two conditions (treatment with carnosine or placebo, main factor condition) at 24 and 48 hours after the RAST (main factor time) were tested by mean of a two way (condition × time) fully repeated measures ANOVA.

Significant differences detected by ANOVAs were adjusted using the Bonferroni method.

The level of significance was set a priori at $\alpha < .05$.

Results

None of the participants reported any side effects due to the administration of 1 g of Carnosine + 1 g of β-alanine.

No significant differences were found in performance and RPE, pain, and session RPE immediately and 30 minutes after the RAST respectively (**Table 1**).

After the RAST test blood insulin significantly increased (main factor time, $p < .001$) both in the carnosine (pretest 6.0 ± 2.5 vs posttest 23.8 ± 13.0 μU·ml^{-1}) and placebo condition (pretest 5.2 ± 2.0 vs posttest 19.5 ± 9.0 μU·ml^{-1}) but without significant interaction (condition × time, $p = .329$). Similarly, after the RAST blood glucose significantly increased (main factor time, $p < .001$) both in the carnosine (pretest 79 ± 9 vs posttest 109 ± 23 mg·dl^{-1}) and placebo condition (pretest 74 ± 10 vs posttest 104 ± 12 mg·dl^{-1}) but without significant interaction (condition × time, $p = .969$).

Despite no significant interaction (condition × time, $p = .277$), blood bicarbonates showed in both conditions (carnosine pre test 30 ± 2 vs post test 16 ± 2 mEq·l^{-1} and placebo pre test 29 ± 2 vs post test 16 ± 2 mEq·l^{-1}) a significant decrease (main factor time, $p < .001$).

During the RAST the time to complete each bout of 35 m progressively increased in the two conditions (main factor time, $p < .001$) but without significant interaction (condition × time, $p = .855$) (**Figure 1**).

No significant interaction (condition × time, $p = .509$) was detected for the blood lactate response monitored after the completion of the RAST (**Figure 2**).

Table 1.

The acute effects of carnosine administration, compared to a placebo treatment, on the running-based anaerobic sprint performance (RAST), rating of perceived exertion (RPE), pain, session RPE, blood glucose, insulin and bicarbonate. Data are mean ± SD.

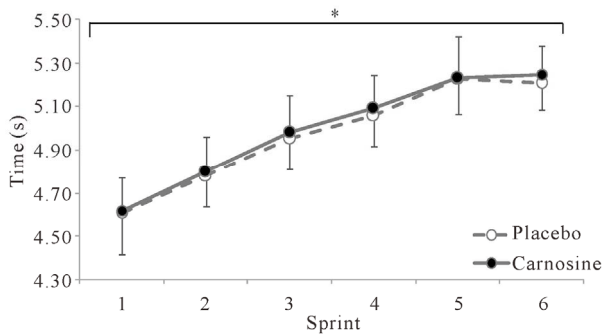|	Carnosine	Placebo	*p* value
Performance (s)	30.0 ± .8	29.8 ± .8	.302
RPE (a. u. 0 - 10)	6.5 ± 1.2	7.0 ± 1.3	.195
Pain (a. u. 0 - 10)	4.0 ± 2.6	3.4 ± 2.1	.173
Session RPE (a. u.)	107 ± 23	106 ± 28	.703
Blood glucose (mg/dl)	109 ± 23	104 ± 12	.969
Insulin (μU/ml)	23.8 ± 13.0	19.5 ± 9.0	.329
Bicarbonate (mEq/l)	16 ± 2	16 ± 2	1.0

Figure 1.
The acute effects of carnosine administration, compared to a placebo treatment, on the six sprint bouts of the RAST test; *Significant ($p < .05$) main effects of time.
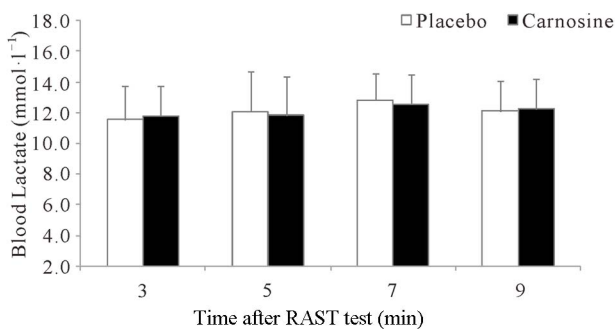

Figure 2.
Time course of blood lactate concentration in the two conditions (carnosine and placebo) following the running-based anaerobic sprint test (RAST).

The RAST caused a very small and similar increase in DOMS in the two conditions (condition × time, $p = .755$) with a significant decrease towards zero (main factor time, $p < .05$) at 48 hours post test (carnosine 1.2 ± 1.3 vs $.6 \pm 1.0$ at 24 and 48 hours respectively and placebo 1.0 ± 1.0 vs $.4 \pm .7$ at 24 and 48 hours respectively).

Discussion

The ingestion of 1 g of carnosine and 1 g of β-alanine 4 hours prior to an all-out shuttle running test did not have any effect on performance, perceived exertion, muscle soreness and session RPE. Similarly, the response of insulin, glucose, bicarbonates and blood lactate concentration to exercise were not affected by carnosine supplementation compared to placebo.

During the RAST the time to complete each bout of 35 m increased similarly in the two conditions, with no difference in total time. Contrary to the study by Suzuki et al. (Suzuki et al., 2002) we failed to show a difference between the two conditions towards the latter fractions of an intermittent all-out exercise. The different type of exercises (that is cycling versus running) and the modality (that is continuous versus intermittent) might represent some of the causes for the discrepancy between the two studies. Moreover, we cannot exclude that the acute ingestion of carnosine failed to increase the muscle content of carnosine (see below in the Discussion). The occurrence of fatigue is the most plausible explanation for the decrease of the running speed. In particular, a decrease in muscle pH represents

one of the main factors responsible for the decline in muscle contractility (Allen et al., 2008). The high level of blood lactate observed at the end of the RAST might be an indirect marker of increased acidosis in the muscle. Therefore, it can be hypothesized that the dose of carnosine utilized in this study was not sufficient to increase the muscle content of carnosine to a level that was sufficient to exert a buffering effect on muscle cells acidosis. Alternatively, it can be hypothesized that the RAST did not determine such a decrease in muscle pH to require a higher concentration of muscle carnosine content than normally present. However, the blood lactate concentration and the level of bicarbonates after the all-out shuttle running indirectly suggest that the metabolic stress (and the hydrogen ions concentrations) was high.

Noteworthy, in the present study we failed to detect any difference in blood bicarbonates concentration between conditions. On the contrary, some authors (in spite of the administration of a lower dose of carnosine) showed such a difference and hypothesized an increased action exerted by the nonbicarbonat buffering due to carnosine and anserine administration (Suzuki et al., 2006).

The lack of difference in performance between the two conditions neither support the increase in Ca^{2+} sensitivity of the contractile fibres that represents an alternative mechanism through which muscle carnosine can improve muscle performance (Dutka & Lamb, 2004). In theory, this ergogenic effect of muscle carnosine should have been useful if the maximal effort performed was not sufficiently high to elicit low levels of muscle pH.

A further aspect that might have contributed to the lack of a positive effect of acute carnosine ingestion on performance is the duration of the exercise. According to a recent meta analysis (Hobson et al., 2012) a higher content of muscle carnosine is beneficial mostly for high intensity exercises lasting between 60 and 240 seconds. In the present study the average overall duration of the RAST was approximately 35 seconds.

Blood glucose and insulin showed an increase after the RAST compared to baseline that was similar in the two groups. It is likely that the increase in blood glucose was mediated by an increase in cathecolamines. However, as for performance, it can be hypothesized that the amount of carnosine administered was not sufficient to be of any effect on the adrenergic system.

Although in the present study a much higher dose of carnosine than in an earlier study (Kraemer et al., 1995) was used, no enhancing effect on short high intensity running performance was found.

It cannot be excluded that the acute administration of carnosine is a vain strategy for the improvement of exercise. However, future studies should aim to evaluate the effects of ingestion of carnosine at higher dosages (e.g. twofold the dose used in the present study) and to assess its efficacy using much higher stressing exercise (e.g. all-out intermittent or continuous anaerobic exercises performed for a longer time period). Furthermore, it would be interesting to test the effect of acute ingestion of carnosine in individuals (like vegetarians) who might be characterized by a lower level of muscle carnosine.

A possible limitation of this study could be the lack of evaluation of the dietary habits of subjects. In fact the content of muscle carnosine in a subject that follows a predominantly vegetarian diet could be drastically lower than a person taking meat in high amount.

Conclusion

In conclusion, the acute ingestion of 1 g of L-carnosine and 1 g of β-alanine few hours prior to an intermittent all-out running exercise does not have any effect on overall performance and on the latter bouts of the exercise. Similarly, the responses of blood insulin, glucose, bicarbonate and lactate concentrations to the RAST were not affected by the acute ingestion of carnosine compared to placebo.

Based on the present results, the acute oral administration of carnosine prior to an anaerobic running exercise lasting around 30 seconds does not seem to be an effective strategy to improve performance.

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Abbreviations

BMI: Body Mass Index;
RAST: running-based anaerobic sprint test;
RPE: perceptual response to exercise;

DOMS: delayed onset of muscle soreness;
EDTA: Ethylenediaminetetraacetic acid;
ELISA: Enzyme-Linked Immuno Sorbent Assay