Does low serum carnosinase activity favor high-intensity exercise capacity?

Audrey Baguet,1 Inge Everaert,1 Benito Yard,2 Verena Peters,2 Johannes Zschocke,3 Ana Zutinic,4 Emile De Heer,4 Tomasz Podgórska,5 Katarzyna Domaszewska,5 and Wim Derave1

1Department of Movement and Sports Sciences, Ghent University, Ghent, Belgium; 2Department of Medicine, University Medical Center Mannheim, University of Heidelberg, Mannheim, Germany; 3Division of Human Genetics, Medical University Innsbruck, Innsbruck, Austria; 4Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands; and 5Departments of Biochemistry and Physiology, University School of Physical Education, Poznan, Poland

Submitted 5 November 2013; accepted in final form 1 January 2014

Carnosine is present in high concentrations of ~5 mmol/l in the skeletal muscle cytosol, where it plays an important homeostatic role in contractility and hence, exercise performance. Studies on chronic β-alanine supplementation have shown that it can markedly elevate human muscle carnosine content (14) and improve high-intensity exercise performance (15, 17). The proton-sequestrating capacity of carnosine can aid in preventing exercise-induced acidosis (3), but its role is probably more diverse, as it is also involved in the calcium release and sensitivity of the contractile apparatus (10) and could possibly also exert antioxidant effects in exercise-induced oxidative stress (6).

Exercise-mediated acidosis and oxidative stress are not only restricted to the muscle cells but also may be observed in the circulation. Hence, interventions that acutely suppress acidosis in the circulation by pre-exercise sodium bicarbonate or citrate ingestion (5) or acutely suppress oxidative stress by N-acetyl-cysteine administration (21, 25) can also improve exercise performance. Circulating carnosine may therefore also exert ergogenic effects through a similar mechanism of attenuating acidosis and/or oxidative stress in the blood. In this line of reasoning, Suzuki et al. (27) investigated the effect of acute pre-exercise ingestion of a chicken breast extract (CBEX), which contained 0.4 g carnosine and 1.1 g anserine, the naturally occurring methylated analog of carnosine. Although the authors could demonstrate a positive effect of CBEX ingestion on exercise-induced acid-base balance, this did not lead to improved performance of an intense, intermittent exercise protocol (27). The problem is that serum CN1 is highly active, and even relatively high dietary doses of carnosine are rapidly degraded. In the study of Suzuki et al. (27), carnosinemia increased to only 2 μM. Everaert et al. (11) showed

Address for reprint requests and other correspondence: W. Derave, Dept. of Movement and Sports Sciences, Ghent Univ., Watersportlaan 2, B-9000 Ghent, Belgium (e-mail: Wim.derave@ugent.be).

http://www.jappl.org 8750-7587/14 Copyright © 2014 The American Physiological Society
recently that only people with a low CN1 activity and protein content show measurable increases in carnosinemina to maximum values of 20-200 μM.

Therefore, one could expect that a low CN1 activity is beneficial to athletes who are involved in high-intensity and sprint-type exercise. Likewise, we hypothesize that people with low CN1 activity may be more responsive to the potential ergogenic effects of acute carnosine supplementation before high-intensity exercise. These research questions are explored in the current study through, respectively, 1) a cross-sectional comparison of the serum CN1 activity and content of different types of elite athletes and controls and 2) a dietary intervention study on the effect of acute carnosine supplementation in healthy volunteers with high or low serum CN1 activity on acid-base balance and high-intensity exercise performance.

MATERIALS AND METHODS

Cross-sectional Part

A total of 235 subjects (150 men and 85 women) participated in this cross-sectional study. The study population consisted of a non-specifically trained control group (n = 154) and a group of Belgian elite athletes (n = 81), participating in track-and-field, gymnastics, judo, short track, rowing, triathlon, and swimming. All athletes were or had been competing at an international level. The “explosive group” included athletes from explosive track-and-field disciplines, short track, judo, gymnastics, and short-distance swimming. The “middle- to long-distance group” consisted of middle- and long-distance runners, long-distance swimmers, triathletes, and rowers. All subjects gave their informed consent, and the study was approved by the Local Ethics Committee (Ghent University Hospital, Belgium).

Numbers in each group and age characteristics are shown in Table 1. Heparinized venous blood samples were collected from all subjects to quantify plasma CN1 protein content and activity. Plasma CN1 activity was determined, according to the method described by Teufel et al. (28). Briefly, the reaction was initiated by addition of substrate (L-carnosine) to a heparin plasma sample and stopped after 10 min of incubation at 37°C by adding 1% sulfosalicylic acid (SSA). Liberated histidine was derivatized with o-phthalaldehyde, and the maximum increase was used for determining the maximum activity. Fluorescence was measured by excitation at 360 nm and emission at 460 nm. The intra- and interassay variations were 7% and 25%, respectively.

Plasma CN1 protein content was measured by ELISA. Human CN1 ELISA was developed by coating high absorbant microtiter plates (Greiner BioChemica, Flacht, Germany) overnight with 100 μl goat polyclonal anti-human CN1 (1 μg/ml; R&D Systems, Wiesbaden, Germany). The plates were washed extensively and incubated with 5% wt/vol dry milk powder to avoid unspecific binding. For each sample and standard, serial dilutions were carried out. The plates were placed on a shaker for 1 h and subsequently, washed extensively with PBS (11.18 g/l Na2HPO4, 1.86 g/l KCl, 2.04 g/l KH2PO4, 81.9 g/l NaCl, 1:10). Hereafter, anti-human carnosinase monoclonal antibody (clone ATLAS; Abcam, Cambridge, MA) was added for 1 h, followed by extensive washing. Horseradish peroxidase-conjugated goat antirabbit IgG (sc-2030; 1:1,000 in PBS) was added for 1 h, and the plates were washed. After addition of peroxidase substrate (deep-blue POD; Roche Diagnostics, Mannheim, Germany), the reaction was stopped after 15 min by addition of 50 μl of 1 M H2SO4 and read in an ELISA reader at 450 nm. CN1 protein concentrations were assessed in the linear part of the dilution curve.

Interventional Part

Subjects. Twelve trained men volunteered to participate in this double-blind, placebo-controlled, crossover study. The subjects’ age, weight, and relative maximal oxygen consumption were 21.6 ± 2.2 years, 74.5 ± 5.4 kg, and 59.7 ± 5.5 ml·kg⁻¹·min⁻¹, respectively. They gave their informed consent, and the study was approved by the Local Ethics Committee (Ghent University Hospital).

Study design. The participants attended the laboratory on six separate occasions. During the first visit, a graded exercise cycling test until exhaustion was performed (Excalibur; Lode, Groningen, The Netherlands). Throughout this cycling test, starting at 150 W and with a ramp rate of 25 W/min, the subjects maintained a constant pedal cadence of 80 rpm until volitional exhaustion. The maximal power output at the end of the test was called Wmax. The second visit was reserved for the familiarization of the cycling capacity test at 110% of the Wmax (CCT 110%). Visits three to six were reserved for the execution of the CCT 110% tests following carnosine or placebo (maltodextrin) supplementation. Each condition (carnosine or placebo) was performed twice, in a random order, with 1 wk time in between. The average of the two tests/condition was used for further analysis. Figure 1 illustrates the time schedule of visits three through six.

CCT 110%. The warm-up of the CCT 110% test consisted of 5-min cycling at 100 W, followed by 2-min stretching. The test started with 15 s cycling at 80% Wmax, followed by 15 s at 95%, after which, the subjects cycled as long as possible at 110% Wmax.

Supplementation. About 1 h 45 min after the ingestion of a standardized, carnosine-free breakfast (four slices of bread with strawberry jam, banana, 33 g fruit juice), subjects were supplemented with 20 mg/kg body wt pure carnosine (Flamma, Bergamo, Italy; average dose of 1.491 ± 108 mg) or placebo (maltodextrin). The capsules were swallowed with water 35 min before the start of the CCT 110% test. The subjects were allowed to drink water ad libitum throughout the experiment.

Measurements. Capillary blood samples (70 μl) were taken from the fingertip to determine blood gas analysis (ABL90 Flex; Radiometer, Brønshøj, Denmark) at rest, following warm-up, and 2.5 and 5 min following the CCT 110%. These samples were analyzed for pH, lactate, and bicarbonate levels.

Venous blood samples were collected in the fasted state and after the CCT 110%. They were analyzed for carnosine concentration, CN1 protein content, tiobarbituric acid reactive substances (TBARS), ferric reducing ability of plasma (FRAP), and creatine kinase activity (CK).

Plasma carnosine levels were analyzed as described by Peters et al. (24). Precooled (4°C) EDTA tubes were centrifuged immediately at 4°C to separated plasma. Plasma samples were deproteinized with SSA (35%) and stored immediately at −20°C until further analysis. Carnosine concentrations were measured fluorometrically by HPLC.

Plasma CN1 protein content was measured as described above (see Cross-sectional Part). The 12 subjects were ranked from lowest to highest CN1 protein content. In post hoc comparison, the first six subjects, who had the lowest CN1 protein content, were called the “low CN1 group,” and the six with the highest CN1 belonged to the “high CN1 group.”

Allantoin was determined in 250 μl deproteinized serum, in accordance with the methodology developed by Grootveld and Halliwell.

Table 1. Characteristics of the study population

<table>
<thead>
<tr>
<th>Controls</th>
<th>Explosive</th>
<th>Middle to Long Distance</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of men</td>
<td>89</td>
<td>31</td>
<td>30</td>
</tr>
<tr>
<td>Number of women</td>
<td>65</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>Total number</td>
<td>154</td>
<td>45</td>
<td>36</td>
</tr>
<tr>
<td>Male mean age, yr</td>
<td>23.5 ± 5.5</td>
<td>22.6 ± 6.7</td>
<td>24.2 ± 4.7</td>
</tr>
<tr>
<td>Male age range, yr</td>
<td>18–47</td>
<td>16–49</td>
<td>17–40</td>
</tr>
<tr>
<td>Female mean age, yr</td>
<td>24.5 ± 7.0</td>
<td>24.1 ± 5.2</td>
<td>32.8 ± 9.5*</td>
</tr>
<tr>
<td>Female age range, yr</td>
<td>19–50</td>
<td>18–35</td>
<td>22–45</td>
</tr>
</tbody>
</table>

*P < 0.05; significantly different from untrained controls within the same gender.
(13). Thereafter, the probes were centrifuged (4,000 g, 10 min, 4°C), 250 µl of the supernatant was transferred to a glass test tube, and 250 µl of 0.12 M NaOH was added. Next, the probes were boiled for 20 min in a water bath at 100°C and mixed with 250 µl of 1 M HCl and 50 µl 2,4-dinitrophenol and warmed again for 5 min to the same temperature. The mobile phase consists of trisodium citrate and sodium acetate (pH 4.75). Measurements were done by HPLC with UV detection (UV Series 1050; Hewlett-Packard, Palo Alto, CA) at 360 nm.

TBARS measurement is based on methodology proposed by Buege and Aust (8). Plasma (50 µl) was mixed with 50 µl of 0.01% butylated hydroxytoluene, 300 µl of 20% acetic acid, and 300 µl of 0.8% TBA and placed in 2 ml polypropylene screw-cap microcentrifuge tubes. After shaking, the tubes were put in a water bath for 60 min at a temperature of 100°C. Subsequently, all probes were brought to room temperature, shaken vigorously, and centrifuged for 10 min at 4,000 g at 4°C. The supernatant (200 µl) was pipetted on an ELISA plate. Measurements were performed at 532 nm with a multimode microplate reader (Synergy 2, SIAFRT; BioTek, Winooski, VT).

Determination of FRAP was based on methodology elaborated by Benzie and Strain (4) and modified by Janaszewska and Bartosz (18). Plasma (10 µl) was mixed with 30 µl deionized water on an ELISA plate. Then, 300 µl reaction reagent (37°C), consisting of 250 µl of 300 mmol/l acetate buffer (pH 3.6), 25 µl of 10 mmol/l 2,4,6-tripirydyl-s-triazine in 40 mmol/l HCl, and 25 µl of 20 mmol/l FeCl3·6 H2O solutions, was added to all wells and mixed carefully. After 6 min of incubations, the color of the solutions was read on a multimode microplate reader (Synergy 2, SIAFRT; BioTek) at 593 nm.

The CK determination was based on a colorimetric (spectrophotometric) measurement (Liquick Cor-CK 30; PZ Cormay, Łomianki, Poland).

Statistics A one-way ANOVA analysis was used to analyze the plasma CN1 activity and content (cross-sectional part). In case of significance, a post hoc analysis was performed. For the interventional part, paired sample t-tests were used to compare pH, lactate, and bicarbonate between the placebo and carnosine condition at a point of time. A 2 × 2 repeated-measures ANOVA was used to evaluate time to exhaustion, pH, lactate, bicarbonate, and redox factors with “group” (low vs. high CN1 content) as a between-subjects factor and “condition” (placebo vs. carnosine) as a within-subjects factor (SPSS Statistical Software; SPSS, Chicago, IL). Values are presented as mean ± SD, and significance was assumed at P ≤ 0.05.

RESULTS

Cross-sectional Part

Within the whole study population, a significant (P < 0.001) positive correlation (r = 0.510) was found between plasma CN1 activity and content. When considering all groups together (control and athlete groups), women have significantly higher CN1 activity (6.91 ± 1.54 vs. 5.70 ± 1.65 µmol·ml⁻¹·h⁻¹, P < 0.001) and CN1 content (86.2 ± 36.6 vs. 69.0 ± 31.7 µg/ml, P < 0.01) than their male counterparts.

The one-way ANOVA analysis demonstrated that CN1 activity (P < 0.01) and content (P < 0.001) were significantly different among the three groups (comparing both genders). Explosive athletes have a lower CN1 activity and content than untrained controls (P < 0.01 and P < 0.001, respectively) and middle- to long-distance athletes (P < 0.01).

When making group comparisons for each gender separately, similar results were found. Figure 2 shows that plasma CN1 activity (P < 0.01 for men and P < 0.05 for women) and...
CN1 content ($P < 0.01$ for men and $P < 0.05$ for women) were significantly different among the three groups. Within a similar age range, both male and female explosive athletes (men: $4.96 \pm 1.64 \, \text{µmol}\cdot\text{ml}^{-1}\cdot\text{h}^{-1}$; women: $5.70 \pm 0.83 \, \text{µmol}\cdot\text{ml}^{-1}\cdot\text{h}^{-1}$) had a lower CN1 activity (Fig. 2A) compared with controls (men: $5.72 \pm 1.54 \, \text{µmol}\cdot\text{ml}^{-1}\cdot\text{h}^{-1}$, $P < 0.1$; women: $7.15 \pm 1.60 \, \text{µmol}\cdot\text{ml}^{-1}\cdot\text{h}^{-1}$, $P < 0.05$). Similar differences were found with regard to CN1 protein content (Fig. 2B). Both male and females explosive athletes (men: $50.8 \pm 20.5 \, \text{µg/ml}$; women: $60.6 \pm 29.9 \, \text{µg/ml}$) had a lower CN1 content compared with controls (men: $72.3 \pm 33.8 \, \text{µg/ml}$, $P < 0.01$; women: $90.7 \pm 36.1 \, \text{µg/ml}$, $P < 0.05$). Middle-to-long-distance athletes did not differ from controls, but the men had higher CN1 activity and content ($6.46 \pm 1.74 \, \text{µmol}\cdot\text{ml}^{-1}\cdot\text{h}^{-1}$, $P = 0.003$, and $78.78 \pm 27.99 \, \text{µg/ml}$, $P < 0.01$, respectively) than explosive athletes.

**Interventional Part**

The mean CN1 protein content of all subjects ranged from 26.1 to 82.9 µg/ml (mean 56.6 ± 19.9 µg/ml). The low CN1 group had a CN1 protein content of 39.6 ± 10.8 µg/ml (range: 26.1–51.0 µg/ml), whereas it was 73.7 ± 7.1 µg/ml (range: 63.0–82.9 µg/ml) for the high CN1 group. Only in three subjects did acute carnosine supplementation of 20 mg/kg body wt result in plasma carnosine levels at or above the detection limit of 0.5 µM. Two of them were categorized in the low CN1 group, and concentrations ranged between 0.5 and 1.5 µM (Fig. 3).

The time to exhaustion and Wmax of the graded exercise test were, on average, 607 ± 43 s and 391 ± 18 W, respectively. The CCT 110% was characterized by a mean load of 420 ± 133 W and a test-retest coefficient variation (= SD/mean) of 8.6%. Each subject performed the test twice in placebo condition and twice after acute carnosine supplementation. For the whole group, the mean time to exhaustion was 128 ± 21 s following placebo and 128 ± 16 s after acute carnosine supplementation ($P = 0.85$). No significant interaction effect (high/low CN1 protein content × carnosine/placebo supplementation) could be found for the time to exhaustion of the CCT 110% test ($P = 0.440$). As a result of carnosine supplementation, the time to exhaustion of the low CN1 group changed nonsignificantly from 124.8 ± 22.1 to 127.1 ± 16.2 s ($P = 0.501$) and from 130.5 ± 22.2 to 129.1 ± 18.2 s in the high CN1 group ($P = 0.729$; Fig. 4).

Table 2 shows an overview of the blood gas analysis parameters. The blood pH at rest was 7.40 ± 0.01 and was not affected by carnosine supplementation. The CCT 110% test resulted in a pH decrease toward ~7.20, which was similar for placebo and carnosine supplementation. Even after splitting the group, according to low and high CN1, no significant effects of supplementation were found. Blood lactate concentration increased from ~1.6 mmol/l (at rest) to approximately 15–16 mmol/l at the end of the cycling test, but no differences were found between the two supplementation conditions.

Bicarbonate at rest was significantly higher following carnosine supplementation compared with placebo (Table 2). Moreover, it appeared that the six subjects with low CN1 protein content had significantly higher bicarbonate levels at rest compared with the high CN1 group, both following carnosine (low CN1: 25.84 ± 0.49 mmol/l; high CN1: 25.17 ± 0.58 mmol/l, $P < 0.1$) and placebo (low CN1: 25.48 ± 0.63 mmol/l; high CN1: 24.70 ± 0.50 mmol/l, $P < 0.05$) supplementation (Fig. 5). The bicarbonate concentration decreased significantly as a result of the cycling test, independent of the CN1 content or supplementation.

Before (in fasted state) and following the cycling test, venous blood samples were collected and analyzed for allantoin, TBARS, FRAP, and CK (Table 3). Plasma allantoin and CK levels increased as a result of exercise. Yet, none of these parameters showed a significant condition (placebo or carnosine) or interaction effect (condition × time) when comparing the low and high CN1 groups.

**DISCUSSION**

The most remarkable finding of the current study is that explosive athletes at an elite level possess lower CN1 activity and content compared with untrained controls in both genders. Carnosinase is a unique enzyme that is secreted into the serum by the liver and possibly the brain (28). Its activity is greatly determined by its content in the serum, as supported by the strong positive correlation found in the current ($r = 0.510$, $P < 0.001$) and previous studies (1, 11). Moreover, it has a marked variability in content between subjects, equaling a fourfold variation (between lowest and highest CN1 content) in the intervention study and a 17- and sixfold variation (between lowest and highest values) in CN1 content and activity, respectively, in the cross-sectional study. However, the CN1 activity is remarkably stable within subjects throughout the day (23).
Table 2. Capillary pH, lactate, and bicarbonate after acute carnosine or placebo supplementation at rest, following warm-up, and 2.5 and 5 min after CCT 110%

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>After Warm-up</th>
<th>2.5 min Postexercise</th>
<th>5 min Postexercise</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>7.400 (0.014)</td>
<td>7.387 (0.014)</td>
<td>7.201 (0.045)</td>
<td>7.211 (0.053)</td>
</tr>
<tr>
<td>Carnosine</td>
<td>7.399 (0.011)</td>
<td>7.388 (0.015)</td>
<td>7.203 (0.046)</td>
<td>7.209 (0.058)</td>
</tr>
<tr>
<td><strong>Lactate, mmol/l</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>1.7 (0.5)</td>
<td>2.4 (0.7)</td>
<td>15.5 (2.1)</td>
<td>14.5 (2.2)</td>
</tr>
<tr>
<td>Carnosine</td>
<td>1.6 (0.4)</td>
<td>2.6 (0.7)</td>
<td>16.1 (2.5)</td>
<td>14.7 (2.8)</td>
</tr>
<tr>
<td><strong>Bicarbonate, mmol/l</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>25.09 (0.67)</td>
<td>24.70 (0.85)</td>
<td>14.65 (1.30)</td>
<td>14.65 (1.66)</td>
</tr>
<tr>
<td>Carnosine</td>
<td>25.50 (0.62)</td>
<td>24.90 (0.74)</td>
<td>14.64 (1.39)</td>
<td>14.71 (1.79)</td>
</tr>
<tr>
<td><strong>p</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>0.89</td>
<td>0.71</td>
<td>0.74</td>
<td>0.82</td>
</tr>
<tr>
<td>Carnosine</td>
<td>0.66</td>
<td>0.28</td>
<td>0.17</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Data are means (SD) of 12 subjects. Carnosine, β-alanyl-L-histidine; CCT 110%, cycling capacity test at 110% maximal power output. P value is placed in boldface for significance between Placebo and Carnosine.

and also, over a 6-mo period, the test-retest is very good both for CN1 content (r = 0.92; P < 0.001) and activity (r = 0.63; P < 0.001) (S. Stegen and W. Derave, unpublished observations). To our knowledge, the current study is the first to demonstrate that the steady-state activity and content of a serum enzyme significantly differ between a specific group of elite athletes and controls. Similar to other liver-secreted blood enzymes [e.g., serum cholinesterase (29)], preliminary unpublished (S. Beeckman, A. Baguet, and W. Derave, unpublished observations), twin-derived data suggest that CN1 activity/content is largely genetically determined. This would render CN1 activity/content as a genetic selection criterion for sprint performance. The plasma CN1 content and activity are genetically determined by the leucine repeat number and possibly some single nucleotide polymorphisms of the carnosine dipeptidase 1 (CNDP1) gene (19, 26). However, larger genetic studies on cohorts of athletes will be required to identify whether the known polymorphisms of the CNDP1 and related genes are involved in talent selection and athletic endowment for sports with short, high-intensity exercise modes. Whether a low CN1 activity/content is directly or rather indirectly linked to an athlete’s predisposition for explosive disciplines is not clear yet. It can, at present, not be excluded that a metabolic characteristic of the athletes or the training regime influences CN1 secretion, even without a clear functional or ergogenic impact.

In accordance with Everaert et al. (11), it can be hypothesized that subjects with low CN1 activity and content exhibit higher levels of circulating carnosine (after supplementation or dietary ingestion), and they will therefore probably be favored during high-intensity exercise. This hypothesis was tested in the second part of the current study by comparing subjects with low and high CN1 content regarding time to exhaustion in a high-intensity cycling test after acute carnosine supplementation. However, the CCT 110% test could not be sustained longer after acute carnosine ingestion compared with placebo supplementation. Even when a distinction was made between subjects with low and those with high CN1 protein content, no differences in performance were found.

The finding that acute carnosine supplementation in a single acute dosage of 20 mg/kg body wt was not effective to improve performance or only minimally influenced the acid-base balance and redox factors probably relates to the fact that only three out of 12 subjects showed measurable carnosinemia, and even they displayed only very moderate concentrations of 0.5–2 μM after acute carnosine supplementation. This is likely explained by the high CN1 activity in human plasma and the relatively low administered dose compared with previous reports (11, 12). Everaert and colleagues (11) observed carnosinemia in the range of 20–200 μM in eight out of the 25 subjects after administration of a threefold higher (60 mg/kg dietary ingestion), and they will therefore probably be favored during high-intensity exercise. This hypothesis was tested in the second part of the current study by comparing subjects with low and high CN1 content regarding time to exhaustion in a high-intensity cycling test after acute carnosine supplementation. However, the CCT 110% test could not be sustained longer after acute carnosine ingestion compared with placebo supplementation. Even when a distinction was made between subjects with low and high CN1 content regarding time to exhaustion in a high-intensity exercise test after acute carnosine supplementation. However, the CCT 110% test could not be sustained longer after acute carnosine ingestion compared with placebo supplementation. Even when a distinction was made between subjects with low and high CN1 content regarding time to exhaustion in a high-intensity exercise test after acute carnosine supplementation. However, the CCT 110% test could not be sustained longer after acute carnosine ingestion compared with placebo supplementation. Even when a distinction was made between subjects with low and high CN1 content regarding time to exhaustion in a high-intensity exercise test after acute carnosine supplementation. However, the CCT 110% test could not be sustained longer after acute carnosine ingestion compared with placebo supplementation.
body wt) acute dose of carnosine. We reduced the carnosine dose in the current study to avoid the side effects that were encountered in the latter study (11) and to provide a dose that is representative and relevant for dietary carnosine intake, which usually will not exceed 10–20 mg/kg body wt per meal [a 150-g serving of meat will generally contain 500–1,000 mg carnosine (30)]. The strategy of lower dosing appeared to be effective in reducing the side effects (none were observed), but we must conclude that the dietary-relevant doses of carnosine resulted in very small to undetectable levels of carnosinemia (<2 μM), which are unlikely to be sufficient to alter acid-base balance markedly during high-intensity exercise.

So in our search to explain the possible advantage and ergogenic mechanism underlying the observed low serum CN1 activity and content in elite sprinters, our hypothesis can be discarded that it would favor the stability of carnosine in the human circulation upon dietary ingestion. After all, the half-life of carnosine in the human circulation is merely a few minutes, even in subjects with low CN1 activity/content (11, 12, 30). One possible alternative explanation relates to the methylated carnosine analogs, anserine and ophidine/balenine, which are present in similar doses as carnosine in the human diet (mainly in poultry and fish) but have a much longer half-life in the human blood (approximately 20–30 min) (30). Thus low CN1 activity may favor the presence and stability upon dietary ingestion of these methylated carnosine analogs, which seem to have similar biochemical properties with regard to proton sequestration and antioxidant activity (7).

Even though the effects of carnosine supplementation were only very small, we could observe a significantly elevated blood bicarbonate concentration at rest. This observation is in agreement with the hypothesis that carnosine can function as a pH buffer in the circulation and thereby, spare other buffering systems, such as bicarbonate, as observed previously following ingestion of CBEX (27). Interestingly, subjects with low CN1 activity/content exhibited moderately but significantly elevated resting bicarbonate concentrations, even following placebo supplementation, which may help to explain the possible physiological advantages associated with the low CN1 activity/content observed in the elite explosive athletes (see Cross-sectional Part).

The current data were inconclusive with regard to the function of carnosine as an antioxidant. Allantoin, the oxidative product of uric acid, has been proposed as a potential biomarker for in vivo free radical reactions (13). In the current study, plasma allantoin was elevated by high-intensity exercise, but the marker is probably not sensitive enough and the plasma carnosine concentrations too low to be able to evaluate closely possible antioxidant properties of carnosine in the current setting.

In conclusion, elite athletes who excel in short, high-intensity exercise have a low CN1 activity and protein content compared with untrained controls. Given the low intrindividual and high interindividual variation and the genetic basis for serum CN1 content/activity, this finding may have been a result of performance-related genetic selection. Acute carnosine supplementation in dietary-relevant doses does not work to improve acid-base balance nor performance during high-intensity exercise, because carnosinemia can hardly be attained, even in subjects with low CN1 activity.

ACKNOWLEDGMENTS
We thank Flamma (Italy) for generously providing carnosine.

GRANTS
Financial support for this study was provided by grants from the Research Foundation-Flanders (FWO G035213N and G024311). A. Baguet is a recipient of a post doc scholarship from the Special Research Fund (UGent).

DISCLOSURES
The authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS