Low plasma carnosinase activity promotes carnosinemia after carnosine ingestion in humans

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Submitted 13 February 2012; accepted in final form 9 April 2012

Adequate glycemic and blood pressure control are the most effective therapeutic modalities in diabetic patients to delay the onset of microvascular complications (36a). Nonetheless, an increasing number of patients will continue to develop microvascular complications despite these therapeutic measures. Ample evidence indicates that susceptibility to develop diabetic nephropathy, the most common cause of renal failure in the Western world, is genetically determined (23). One of the genes that have recently been linked to diabetic nephropathy is carnosine dipeptidase-1 (CNDP1), which encodes the serum carnosinase enzyme (16). Diabetic nephropathy is strongly associated with a (CTG)5 polymorphism in the CNDP1 gene, affecting serum carnosinase secretion (30). Diabetic patients homozygous for (CTG)5 have a lower risk to develop diabetic nephropathy and have lower plasma carnosinase activity (16). Although the association between the CNDP1 polymorphism and diabetic nephropathy have been confirmed in an independent study in European Americans (11), other studies did not show an association in type 1 diabetic patients (37) or showed that the association in type 2 diabetic patients is sex specific (25). Inconsistent findings may be explained by differences in ethnicity (22) or, alternatively, by assuming that protection from diabetic nephropathy afforded by (CTG)5 homozygosity in CNDP1 may be masked by additional risk haplotypes (9, 22).

Glycation and oxidative stress (glycoxidative stress) and accelerated formation of advanced glycation end products (AGEs) during hyperglycemia have been implicated in the development of diabetic complications (8). Carnosine (β-alanyl-l-histidine) is a versatile dipeptide, mainly present in neuronal tissue and skeletal muscle, that has the propensity to suppress several aspects of glycoxidative stress, such as inhibition of AGE formation (14), quenching of reactive aldehydes (3), and suppression of oxidative stress (18). Moreover, it has been shown that carnosine inhibits angiotensin-converting enzyme (ACE) (15), albeit only at high concentrations. The influence of carnosine on blood pressure control has thus far not been demonstrated.

In humans, circulating carnosine is readily degraded by the highly active serum carnosinase enzyme, which is secreted from the liver into the plasma (30). Therefore, plasma carnosine concentrations in fasted subjects are in general below the detection limits of current quantitative carnosine assays. Also, the presence of carnosine in plasma (carnosinemia) after dietary intake of pure carnosine or meat, which is a rich source of carnosine, is controversial. Both Asatoor et al. (5) and Gardner et al. (12) could not detect carnosine in plasma after administration of a high dose of carnosine (~60 mg/kg body wt and 4 g, respectively). Cooling of the samples after blood withdrawal, however, resulted in the detection of a small amount of plasma carnosine in one subject in the latter study of Gardner et al. (12). Despite the use of EDTA-containing blood tubes, which have been shown to inhibit the hydrolysis of carnosine in plasma, no plasma carnosine was detected after oral supplementation with 450 mg carnosine (38). In line with this, both Harris et al. (13) and Yeum et al. (38) could not detect any carnosine in plasma after the ingestion of beef.
Supplementation-induced Carnosinemia in Humans

METHODS

Subjects. Twenty-five subjects (age: 20–31 yr, body weight: 70.9 ± 9.8 kg), both male (n = 15) and female (n = 10), participated in this study. All subjects were in good health, and none of the participants was vegetarian. The study protocol was approved by the local ethical committee (Ghent University Hospital, Ghent, Belgium), and written informed consent was obtained from all participants before the study.

Study design. Heparinized plasma was obtained before the experiments and on the morning before the supplementation to quantify plasma carnosinase protein content and activity. After an overnight fast (at least 8 h), an indwelling catheter was inserted in an antecubital vein, and blood was withdrawn before and 20, 30, 40, 60, and 120 min after oral supplementation of 60 mg/kg body wt carnosine (mean SD: 4,209 ± 577 mg) dissolved in 330 ml water. Blood samples for the determination of carnosine were collected in precooled (4°C) EDTA-containing tubes and immediately centrifuged (4°C) to separate the plasma. The anticoagulant EDTA was chosen for its ability to chelate Zn²⁺, which is essential for the catalytic activity of carnosinase (38). Plasma samples were deproteinized with sulfosalicylic acid (35%) and stored immediately at −20°C until further analysis. Urine was collected in EDTA-coated tubes before and 45, 90, 135, 180, and 240 min after carnosinase supplementation. Subjects were allowed to drink water and received a carnosinase-free meal after blood collection.

Determination of carnosine and β-alanine by HPLC. One hundred microliters of deproteinized EDTA-containing plasma and urine were dried under vacuum (40°C). Dried residues were resolved with 40 μl of coupling reagent: methanol-triethylamine-H₂O-phenylisothiocyanate (7:1:1:1) and allowed to react for 20 min at room temperature. Samples were dried again and resolved in 100 μl sodium acetate buffer (10 mM, pH 6.4). The same method was applied to the standard solutions of β-alanine (Sigma) and carnosine (Flamma, dissolved in deionized distilled water). Derivatized samples (20 μl) were applied to a Waters HPLC system with an Hypersilica column (4.6 × 150 mm, 5 μm) and UV detector (wavelength: 210 nm). The column was equilibrated with buffer A (10 mM sodium acetate adjusted to pH 6.4 with 6% acetic acid) and buffer B (60% acetonitrile-40% buffer A) at a flow rate of 0.8 ml/min at room temperature. The limits of detection and quantification were 3 and 10 μM, respectively.

Determination of carnosine by liquid chromatography-electrospray ionization/multistage mass spectrometry. Analyses of plasma carnosine of one subject were performed using a validated liquid chromatography (LC)-electrospray ionization (ESI)/multistage mass spectrometry (LC-ESI-MS/MS) method (26). Briefly, aliquots of 100 μl EDTA-plasma samples were spiked with H-Tyr-His-OH as an internal standard (final concentration: 20 μM), deproteinized by perchloric acid (final concentration: 700 mM) and centrifuged at 18,000 rpm for 10 min. Supernatants were then diluted 1:1 with mobile phase A [CH₃CN-H₂O-heptafluorobutyric acid, 90:10:0.1 (vol:vol:vol)], filtered through 0.2-μm filters, and then injected into a ThermoFinnigan Surveyor LC system equipped with a quaternary pump and connected through an ESI to a TSQ Quantum Triple Quadrupole Mass Spectrometer (ThermoFinnigan Italia, Milan, Italy). Chromatographic separations were done by reverse-phase elution with a Phenomenex Synergi polar-RP column (150 × 2 mm inner diameter, particle size: 4 μm, Chempak Analytica, Anzola Emilia, Italy) protected by a polar-RP guard column (4 × 2 mm inner diameter, 4 μm) kept at 25°C. Separations were done by gradient elution from 100% phase A to 80% phase B (CH₃CN in 12 min at a flow rate of 0.2 ml/min (injection volume: 10 μl); the composition of the eluent was then restored to 100% phase A within 1 min, and the system was reequilibrated for 6 min. Quantitations were performed in multiple reaction monitoring (MRM) mode at a multiplier voltage of 2.00 kV, and the following MRM transitions of [M + H]⁺ precursor ion → product ions were selected: H-Tyr-His-OH (IS) mass to charge ratio (m/z) 319.2 → 156.5 + 301.6 (collision energy: 25 eV); and carnosine: m/z 227.0 → 110.6 + 156.5 (collision energy: 25 eV).

Determination of plasma carnosinase activity and protein content. At baseline, mean plasma carnosinase activity and protein content were quantified based on the activity and protein content of two different blood collections. Plasma carnosinase activity was determined according to the method described by Teufel et al. (35). Briefly, the reaction was initiated by the addition of substrate (l-carnosine) to a heparin plasma sample stopped after 10 min of incubation at 37°C by the addition of 1% SSA. Labeled histidine was derivatized with O-phthalaldehyde, and the maximum increase was used to determine the maximum activity. Fluorescence was measured by excitation at 360 nm and emission at 460 nm. Intra- and interassay variations were 7% and 25%, respectively. The lowest carnosinase activity detectable was 0.117 μmol·ml⁻¹·h⁻¹.

Plasma carnosinase protein content was measured by ELISA. In brief, a human carnosinase-1 (CN1) ELISA was developed by coating high-absorbent microtitre plates (Greiner BioChemica, Flacht, Germany) overnight with 100 μl of goat polyclonal anti-human CN1 (1 μg/ml, R&D, Wiesbaden, Germany). The plates were extensively washed and incubated with 5% (wt/vol) of dry milk powder to avoid unspecific binding. For each sample and standard, serial dilutions were carried out. Plates were placed on a shaker for 1 h and subsequently extensively washed with PBS-Tween. Anti-human carnosinase monoclonal antibody (clone ATLAS, Abcam) was then added for 1 h followed by an extensive wash. Horseradish peroxidase-conjugated goat anti-mouse IgG was added for 1 h, and plates were washed. After the addition of peroxidase substrate (deep-Blue POD, Roche diagnostics, Mannheim, Germany), the reaction was stopped after 15 min by the addition of 50 μl of 1 M H₂SO₄, and samples were read in an ELISA reader at 450 nm. CN1 protein concentrations were assessed in the linear part of the dilution curve. The sensitivity of the ELISA was ~20 ng/ml.

CNPD1 genotyping. A more detailed description of the CNPD1 genotype determination can be found in the study of Mooyaart et al. (24). In brief, a standard PCR protocol was used with following.
primers: 5′-FAM-GCGGGGAGGGTGAGGAGAAC-3′ (forward) and 5′-GGTAACAGACCTTCTTGAGGAATTTGG-3′ (reverse). The denaturing, annealing, and extension temperatures were 94, 60, and 72°C, respectively. After PCR amplification, fragment analysis was performed with an ABI3130 analyzer (Perkin Elmer) to determine the fragment length corresponding with the different genotypes. Each peak corresponded with the number of leucine repeats on each allele. The 157-, 160-, and 163-bp products corresponded with five, six, and seven CTG codons encoding for five, six, and seven leucine repeats, respectively.

Statistics. Data are expressed as means ± SD. Bivariate correlations and independent sample t-tests were used for statistical analysis (SPSS 17).

RESULTS

Carnosine is detectable in human plasma by HPLC. A HPLC chromatogram of EDTA-containing plasma derivatized with phenylisothiocyanate (UV detection) of a subject 30 min after 60 mg/kg body wt carnosine ingestion is shown in Fig. 1A. Spiking the sample with a carnosine standard resulted in an identical chromatogram except for a higher peak at 15.6 min, representing carnosine. If the necessary precautions to block the endogenous carnosinase activity were not taken (heparin instead of EDTA-containing tubes, not precooled, not immediately deproteinized), then the same subject at the same time point did not display a carnosine peak at 15.6 min (Fig. 1B) and the peak of β-alanine (the product of the carnosinase reaction) increased, which illustrates that the peak at 15.6 min genuinely represents carnosine. Plasma samples of one subject were analysed by an independent laboratory with the LC-ESI-MS/MS method (26), and carnosine was detected at 30 min (11.8 μM) and 45 min (3.4 μM) after carnosine supplementation (4 g), whereas at the other time points no carnosine could be detected.

Supplementation-induced carnosinemia depends on CN1 protein content and activity. Mean CN1 protein levels varied widely between subjects, from 24.38 to 148.02 μg/ml (mean ± SD: 77.82 ± 30.98 μg/ml), and CN1 activity values were situated between 2.79 and 10.90 μmol·ml⁻¹·h⁻¹ (mean ± SD: 5.95 ± 1.91 μmol·ml⁻¹·h⁻¹). Furthermore, CN1 protein content was positively correlated with the activity of the enzyme ($P = 0.004$, $r = 0.58$, $R^2 = 0.34$; Fig. 2), confirming that the activity level was largely determined by the amount of enzyme available in the plasma. Upon carnosine ingestion, 8 of the 25 subjects displayed an increase in plasma carnosine concentra-

![A](http://ajprenal.physiology.org/)

![B](http://ajprenal.physiology.org/)

Fig. 1. A: HPLC chromatogram of human plasma (subject from the responder group) withdrawn 30 min after the ingestion of 60 mg/kg body wt carnosine in a cooled EDTA-containing tube, which was immediately deproteinized (thick line), and the same sample spiked with standard carnosine (thin line). The peak at 15.6 min (carnosine) increased, whereas all other peaks remained the same (including β-alanine at 12.1 min). B: HPLC chromatogram from human plasma (subject from the responder group) withdrawn 40 min after the ingestion of 60 mg/kg body wt carnosine in a cooled EDTA-containing tube, which was immediately deproteinized (thick line), and in a noncooled heparin-containing tube (not deproteinized before storage, thin line). The carnosine peak at 15.6 min disappeared, and β-alanine (12.1 min), taurine (13.2 min), and the peak at 14.2 min (partially histidine) were higher in heparin- versus EDTA-containing plasma.
tion (carnosinemia), which we termed as responders (increase of >10 μM carnosine after supplementation). The increase in plasma carnosine reached its maximum concentration (mean ± SD: 73.3 ± 59.7 μM, range: 30.7–195 μM) at 30–40 min on average after supplementation and rapidly declined within 1–2 h (Fig. 3). However, the remaining 17 subjects had no measurable increment (nonresponders) in plasma carnosine after oral supplementation with a high dose of carnosine, despite the precautions that were taken to block carnosinase activity during blood collection. Post hoc analysis revealed that there was a marked difference in plasma carnosinase protein content (P < 0.001) and activity (P = 0.007) between responders and nonresponders (Fig. 4, A and B). Mean plasma carnosinase protein levels were approximately twofold higher in the nonresponder group (mean ± SD: 91.57 ± 25.37 μg/ml) compared with the responder group (mean ± SD: 44.42 ± 11.22 μg/ml, P < 0.001). Moreover, it seems that there was a clear cutoff value in plasma carnosinase protein content (Fig. 4A), as the highest value of the responders was approximately the same as the lowest of the nonresponders (55 μg/ml). Likewise, CN1 activity was ~1.5-fold higher in nonresponders (mean ± SD: 6.65 ± 1.80 μmol·ml⁻¹·h⁻¹) compared with responders (mean ± SD: 4.53 ± 1.26 μmol·ml⁻¹·h⁻¹, P = 0.007; Fig. 4B). Also, within the responder group, the amount of plasma carnosine [area under the curve (AUC)] was negatively correlated with plasma carnosinase protein levels (P = 0.059, r = −0.68, R² = 0.47) and carnosinase activity (P = 0.059, r = −0.73, R² = 0.52). Both women (n = 2) and men (n = 6) and subjects with the 5-5 (n = 3), 5-7 (n = 1), and 6-6 (n = 4) CNDP1 genotypes were represented in the responder group (Table 1). The mean age of responders (mean ± SD: 21.6 ± 0.7 yr) was significantly lower (P = 0.045) compared with the mean age of nonresponders (mean ± SD: 23.1 ± 2.6 yr).

Urinary carnosine is related to supplementation-induced carnosinemia. Urinary carnosine recovery (percentage of the ingested dose) varied largely between 0.23% and 13.27%. Responders had a significantly (P = 0.006) higher recovery (mean ± SD: 7.7 ± 3.6%) compared with nonresponders (mean ± SD: 2.9 ± 1.3%; Fig. 5A). Furthermore, a strong negative association was observed between urinary carnosine recovery (percentage of the ingested dose) and the increase in plasma carnosine (Fig. 5B).
The main finding of this study was that high carnosinase activity counteracts the presence of circulating carnosine upon an oral challenge. This likely provides a missing link in the pathophysiological mechanism between carnosine, CNDP1 genotype, and diabetic complications, as shown in Fig. 7. The mechanism of the protective effect of the Mannheim allele in diabetic nephropathy has been attributed to lower carnosinase activity (16). However, the further link with higher circulating carnosine has never been established. Carnosine has been shown to be a natural scavenger of the hyperglycemia-induced overproduction of ROS, AGEs, and reactive aldehydes and is therefore a promising candidate therapeutic molecule in reducing

### Table 1. Responders’ CN1 activity and protein content separated by CNDP1 genotype and sex

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Responders</th>
<th>Nonresponders</th>
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<td></td>
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<td>CN1 Protein Content, µg/ml</td>
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<td>82.3 ± 32.3</td>
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</tr>
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<td>67.3 ± 26.0</td>
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<tr>
<td>Female</td>
<td>6.8 ± 1.6*</td>
<td>92.5 ± 32.6†</td>
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Values for carnosinase-1 (CN1) activity and protein content are means ± SD. CN1 activity and protein content were separated by carnosine dipeptidase-1 (CNDP1) genotype and sex. The responder group contained both male and female subjects and was characterized by 5-5, 5–7, and 6-6 CNDP1 genotypes. *P < 0.05 vs. male subjects; †P < 0.1 vs. male subjects.

(percentage of the ingested dose) and plasma carnosinase activity (P = 0.001, r = −0.64, R² = 0.41; Fig. 5B) and protein content (P < 0.001, r = −0.66, R² = 0.44; Fig. 5C). There was a trend for a positive correlation between the AUCs of plasma and urinary carnosine within the responder group (n = 8, P = 0.07, r = 0.66, R² = 0.44). The kinetics of urinary carnosine 4 h after carnosine supplementation were comparable between responders and nonresponders. The majority was excreted during the first hour (responders: 45.6% and nonresponders: 58.6%), and, thereafter, the concentration slowly began to decline, and 90–95% of the increase in urinary carnosine had disappeared 4 h after carnosine ingestion.

### Profile of β-alanine in plasma after carnosine ingestion

Figure 6 shows the profile of β-alanine in plasma after 60 mg/kg body wt carnosine supplementation separated for subjects with or without supplementation-induced carnosinemia. The total amount of β-alanine detected in plasma (AUC) was significantly higher in the responder group (P = 0.005, +30%) versus the nonresponder group. The maximum concentration was reached after 30 min for nonresponders (mean ± SD: 382.6 ± 122.9 µM) and after 40 min for responders (mean ± SD: 468.2 ± 66.0 µM); and β-alanine was almost completely removed from the plasma 2 h after supplementation. Furthermore, plasma β-alanine content was negatively correlated with plasma carnosinase protein levels (P = 0.001, r = −0.61, R² = 0.37) and activity (P = 0.019, r = −0.47, R² = 0.22).

### Side effects

During the first hour after supplementation with 60 mg/kg body wt carnosine, a total of six participants transiently suffered from both headache and paraesthesia symptoms, two subjects only from paraesthesia, and two subjects from headache. The presence of these symptoms was not related to supplementation-induced carnosinemia, CNDP1 genotype, or sex. However, subjects complaining about paraesthesia and/or headache had a higher total amount of β-alanine in urine (symptoms: 93.7 ± 66.8 mg and no symptoms: 48.1 ± 45.6 mg, P = 0.05), although the total amount of plasma β-alanine was not different compared with subjects without paraesthesia and/or headache (symptoms: 1.4 ± 0.2 mM and no symptoms: 1.3 ± 0.4 mM, P > 0.5).

### DISCUSSION

The main finding of this study was that high carnosinase activity counteracts the presence of circulating carnosine upon...
the risk to develop diabetic complications. More interestingly, Riedl et al. (31) recently showed that carnosinase activity is increased by hyperglycemia through N-glycosylation and is elevated in type 2 diabetic patients. Therefore, hyperglycemia not only directly induces glycoxidative stress but also indirectly suppresses the endogenous protective mechanism through carnosine, which probably further speeds the development of complications (Fig. 7).

The findings of this study implicate that the quantification of plasma carnosinase protein content could be a reliable tool to determine the risk for developing nephropathy in diabetic patients as there was almost no overlap between subjects with or without supplementation-induced carnosinemia with respect to plasma carnosinase protein content. Interestingly, this indirectly takes the glycemic control into account, as carnosinase protein levels are elevated under hyperglycemic conditions (31). In 2005, Jansen et al. (16) recommended investigation of the potential of CNDP1 variants in predicting the risk for developing diabetic nephropathy. However, the potential of the CNDP1 gene may be limited as it not all patients with homozygosity for the Mannheim allele were protected against diabetic nephropathy (16) and 2 subjects with supplementation-induced carnosinemia in this study were characterized by 5-5, 5-7, and 6-6 CNDP1 genotypes. Therefore, it would be interesting to investigate whether diabetic patients with low plasma carnosinase protein content will be more protected against diabetic nephropathy compared with patients with higher carnosinase protein content (irrespective of CNDP1 genotype).

Carnosinase activity and protein content were shown to be discriminating factors for supplementation-induced carnosinemia. Consequently, a potential therapeutic strategy to reduce the risk of diabetic nephropathy could be inactivation of the carnosinase enzyme. In addition to (CTG)₉ polymorphism in the CNDP1 gene (10, 16, 24), the female sex (6, 10, 29) and increasing age (29) are determinants associated with higher carnosinase activity that cannot be manipulated. In vitro experiments, however, revealed that activity of the carnosinase enzyme was markedly decreased in the presence of both homocarnosine (γ-aminobutyryl-L-histidine) (28, 29) and anserine (β-alanyl-N₁-methylhistidine) (28). However, the correlation between circulating homocarnosine or anserine levels in fasted subjects and plasma carnosinase activity in vivo is less obvious (28, 29). Importantly, a good glycemic control, either by intervention with insulin, exercise, or diet, seems to be crucial for diabetic patients as this not only directly influences oxidative and glycation stress but also indirectly affects carnosinase activity (31). Besides inactivation of the carnosinase enzyme, the development of a carnosine-analog resistant to the carnosinase enzyme would be a promising strategy to prevent patients from diabetic complications. In this light, Aldini et al. (4) recently showed that the enantiomer D-carnosine has the same protective effect as L-carnosine in obese Zucker rats, whereas it is more resistant to carnosinase activity. However, D-carnosine has a lowered bioavailability as it is less absorbed in respect to L-carnosine, and, therefore, the relevance for human use may be limited (4).

With the assumption that dietary intake of carnosine can diminish the risk for diabetic complications, lack of carnosine in the diet, as is the case in a vegetarian diet, could be deleterious for the protection against glycation and oxidative stress. Indeed, plasma AGE content of healthy vegetarians has been reported to be higher compared with omnivores (19, 33). Although a low-fat, carbohydrate-rich vegetarian diet is often recommended for diabetic patients [for its positive impact on insulin sensitivity, blood pressure, serum lipid profile, etc. (21)], the dietary intake of carnosine, perhaps by supplementation, should not be neglected.

Carnosine is, even after meat or carnosine supplementation, hard to detect in human plasma, as a result of high carnosinase activity. Yet, when several precautions are taken (cooling, use of EDTA-containing tubes, and immediate deproteinization), when pharmacological doses of carnosine (60 mg/kg body wt) are ingested, and when subjects with low carnosinase activity are studied, plasma carnosine levels can be clearly quantified up to 1 h after ingestion. In contrast to plasma, urinary carnosine is more easily detectable, even with subjects ingesting their usual diets (0.2–18.6 μM) (1, 36). This urinary carnosine excretion is increased after carnosine or meat supplementation, with a urinary recovery of up to 14% of the ingested amount (12, 38). As there is no increase in urinary carnosine after β-alanine (2 g) and histidine (2 g) intake, Gardner et al. (12) hypothesized that the excreted carnosine is not a result of the resynthesis of β-alanine and histidine after hydrolysis in the intestine and/or plasma and that the amount of urinary carnosine is a reflection of plasma carnosine. This hypothesis was confirmed in the present study by the positive correlation between urinary and plasma carnosine in the re-

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![Fig. 6. The mean (±SD) area under the curve of plasma β-alanine (in μM) was significantly higher in the responder group (dotted line) versus the nonresponder group (solid line; P = 0.005, +30%).](http://ajprenal.physiology.org/)

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![Fig. 7. Proposed pathophysiological mechanism linking carnosine and carnosine dipeptidase 1 (CNDP1) genotype to diabetic complications. AGE, advanced glycation end products. *Effects that are now supported by the findings of the present study in humans.](http://ajprenal.physiology.org/)
Sponder group and by the strong negative relation between urinary carnosine and plasma carnosinase activity and protein content (Fig. 5, B and C). After carnosine supplementation, urinary carnosine excretion seems to be a reliable and easier measurable estimation of plasma carnosinase content, which is equally dependent on serum carnosinase activity and content.

Since the renal tubular epithelium is equipped with oligopeptide transporters with a high affinity for carnosine (17, 34), circulating carnosine can be accumulated by the kidney and may provide an additional exogenous source of protective peptides against diabetic metabolites in patients with low levels of carnosinase.

One would expect that β-alanine, the degradation product of the plasma carnosinase enzyme, would be positively related with carnosinase activity. However, the opposite is true, as the responder group showed a higher total amount of plasma β-alanine compared with the nonresponder group. This may suggest that the majority of carnosine is in fact degraded in other compartments and tissues than in the circulation. Thus, low carnosinase activity favors both carnosinemia and β-alaninemia after carnosine supplementation, although the mechanism for the latter is unclear.

It can be concluded that 1) carnosine can be detected in human plasma after oral ingestion and 2) high carnosinase activity and content potently counteract the presence of circulating carnosine. In a diabetic environment, this could impede the ability of carnosine to exert its protective effects against cytotoxic agents, leading to diabetic complications. The inhibition of the carnosinase enzyme and the development of a carnosine analog resistant to carnosinase have to be investigated as potential therapeutic strategies for reducing the risk for diabetic complications.

ACKNOWLEDGMENTS

The practical contributions of Anneke Volkaert, Sam Beelprez, Joren Biesbrouck and Fiona Albers are greatly acknowledged. The authors thank Flamma (Italy) for generously providing carnosine.

GRANTS

This work was financially supported by Research Foundation-Flanders Grants 1.5.149.08 and G.0046.09.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS


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