JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Quantitation of Carnosine in Humans Plasma after Dietary Consumption of Beef

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Carnosine (β-alanyl-L-histidine) is a dipeptide found in the muscle foods that has been postulated to be a bioactive food component. The objective of this research was to determine the concentration of carnosine in human plasma after ingestion of beef. Nine males and nine females were recruited for the study. Food devoid of meat products was given to the subjects so that they did not consume carnosine for 48 h prior to the test. Subjects fasted for 12 h and then had blood withdrawn prior to a meal containing 200 g of ground beef. Additional blood samples were collected over the following 24 h and carnosine concentrations were determined by HPLC. The cooked ground beef used in the study contained 52% water, 24% protein, 22% fat, and 124 mg of carnosine/100 g of beef. No plasma carnosine was detected in subjects before the consumption of the beef. Carnosine was detected in plasma 15 min after beef consumption. Plasma carnosine concentrations continued to increase with a maximum (32.7 mg of carnosine/L of plasma) being recorded 2.5 h after consumption. Carnosine concentrations then decreased until no carnosine could be detected at 5.5 h postconsumption. These results indicate that dietary carnosine is absorbed into human plasma after the consumption of beef. Since carnosine has several potential health benefits, evidence of its bioavailability suggests that it could be a bioactive food component.

KEYWORDS: Carnosine; antioxidant; beef; functional foods

INTRODUCTION

Carnosine is a β-alanylhistidine dipeptide found in skeletal muscle and nervous tissue at concentrations ranging from 2 to 25 mM (*1*). Carnosine was first detected in beef extract by Gulewitsch and Amiradzibi (*2*) at the beginning of the 20th century. Since then, the combination of carnosine and the related dipeptides anserine (β-alanyl-L-1-methylhistidine), ophidine (β-alanyl-L-3-methylhistidine), and homocarnosine (γ-aminobutyriyrylhistidine) has been found at concentrations of up to 50 mM in the skeletal, cardiac, and nervous tissues of vertebrates (*1*, *3*−*5*). Although several roles of carnosine have been postulated, its precise functions in biological systems are still obscure. Carnosine was reported to serve as a buffer in muscle to offset the production of lactic acid during exercise (*4*, *6*). Carnosine also chelates copper (*7*, *8*), cobalt (*9*), and zinc (*10*). Researchers have found that carnosine can act as a neurotransmitter in the brain (*11*) and carnosine also activates various enzymes, including muscle calpain II (*12*), myofibrillar-ATPase (*13*), and phosphorylase b (*14*).

Oxidative reactions have been postulated to promote or exacerbate heart disease and cancer. To protect against these oxidative reactions, tissues contain both water- and lipid-soluble antioxidants that help control the activity of reactive oxygen species (*15*). Dietary antioxidants have been postulated to play an important role in the control of diseases linked to reactive oxygen species. Boldyrev and co-workers (*16*, *17*) were the first to report that carnosine has membrane-protecting properties. Since then, the antioxidant activity of carnosine and anserine has also been demonstrated in systems containing phosphatidylcholine liposomes (*18*), linoleic acid emulsions (*19*), and skeletal muscle microsomes (*8*). Researchers found that carnosine was capable of inhibiting lipid oxidation catalyzed by iron (*17*, *18*), copper (*8*, *19*), hydrogen peroxide-activated hemoglobin (*18*), singlet oxygen (*20*), lipoxygenase (*18*), peroxyl radicals (*19*), and hydroxyl radicals (*21*). Carnosine is thought to inhibit oxidative reactions by inactivating reactive oxygen species (*22*) and chelating pro-oxidant metals (*8*). Carnosine has been shown to inhibit the oxidation of low-density lipoprotein, suggesting that it could help protect cardiovascular disease (*23*).

Carnosine could potentially act as a bioactive antioxidant, since it has been suggested to be is absorbed intact (without hydrolysis of the peptide bond) through the intestine into the blood (*24*). While carnosine has been detected in human blood

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10.1021/jf047934h CCC: $30.25
Published on Web 05/03/2005

(*25*), very little is known about the kinetics of its absorption after consumption of a carnosine-containing meal. Therefore, the objective of this research was to determine how consumption of ground beef influenced blood plasma carnosine concentrations in human subjects.

MATERIALS AND METHODS

Chemicals. L-Carnosine and *o*-phthaldehyde were obtained from Sigma Chemical Co. (St. Louis, MO). Analytical grade diethyl ether, perchloric acid, and all other chemicals (analytical grade) were obtained from Fisher Scientific (Fair Lawn, NJ). Water was purified through a Millipore Q-Plus purification train (Millipore Corp., Bedford, MA).

Ground Beef Patty Preparation and Cooking. Ground beef labeled at 25% fat was purchased from a local retailer. Ground beef was shaped into 100 g patties, vacuum packaged, and stored at −80 °C. Patties were thawed at refrigeration temperatures for 36 h. The patties were cooked by broiling in a standard kitchen oven 14 cm below the heating element for 5 min on each side, resulting in an internal temperature of 74 °C. After cooking, patties were immediately consumed by the subjects.

Proximate Analyses. Proximate analysis was performed on both raw and cooked beef patties. Moisture content was determined by heating in an oven at 105 °C for 1.5 h when samples reached a steady weight (*27*). Ash content was determined by heating the beef solids in a muffle furnace at 550 °C for 6 h. Fat content was determined upon extraction of dried sample in diethyl ether in a Tecator HT 1043 and HT2 1046 Soxtec (automated Soxhlet) system. Protein content was estimated as 6.25 × nitrogen content determined using the Kjedahl procedure (*28*).

Human Subjects Study Design. All human subject experiments were approved by the Institutional Review Board of the Human Subjects Compliance Department at the University of Massachusetts−Amherst.

All subjects were given a detailed oral explanation of the study, including the risks, benefits, and procedures. Those subjects who consented to participate answered a basic medical history questionnaire to ensure there was no history of chronic disease. Following a review of the medical history questionnaire, subjects were asked to attend an information session and read and sign an informed consent form, which explained the risks and benefits of the study.

Subjects. Nine females and nine males between 18 and 25 years of age were recruited from the University of Massachusetts student population. Criteria used for selection included (a) body mass index (BMI) from 18 to 26 kg/m^2, (b) no history of cardiovascular disease, diabetes, hypo- or hyperthyroidism, or any other chronic disease, (c) not on a weight reduction program and no evidence of disordered eating, and (d) nonsmoking.

Blood Draws. To minimize blood carnosine concentration prior to the feeding trial, subjects were provided with food devoid of muscle foods for 48 h prior to the test. Subjects fasted overnight for a minimum of 8 h. Blood was collected in the morning by a registered nurse who inserted a cannula into the antecubital vein in the arm. All blood was drawn into heparinized vacutainers. After the initial blood draw (1 h before beef consumption), all subjects ate two ground beef patties (total of 200 g of uncooked ground beef). Additional blood samples were collected over the following 24 h (0.25, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, and 24 h). The subjects were provided with food devoid of muscle foods during the 24 h of blood sampling.

Carnosine Analysis in Plasma. Blood samples (approximately 15 mL) were immediately place on ice after collection and within 1 h were centrifuged at 2000*g* for 10 min at 4 °C to isolate plasma. Plasma (5 mL) was vortexed with 0.36 M perchloric acid for 30 s and then heated in a boiling water bath for 5 min. The samples were centrifuged at 2000*g* for 10 min, and the resulting supernatant was stored at −80 °C. Samples were filtered through 0.45μm syringe filters, after which the carnosine in 20 μL of the filtrate was derivatized with 120 μL *o*-phthaldehyde by incubation at room temperature for 2 min. Carnosine concentrations were determined by high performance liquid chromatography (HPLC) by a modified method of Teahon and Rideout (*29*). Separations were performed using a 5 μm Hypersil ODS high-resolution end-capped HPLC column (250 mm × 4.6 mm) from Alltech Associate Inc. (Deerfield, IL). The mobile phase consisted of 1 part methanol and 3 parts sodium acetate (0.3 M, pH 5.5) at 1.5 mL/min. Column temperature was maintained at 30 °C, and eluted compound were detected using a fluorescence detector with excitation and emission wavelengths at 310 and 375 nm, respectively. Eluted carnosine was identified and quantitated using an authentic standard.

Table 1. Proximate Composition of Raw and Cooked Beef Patty[a]

component (g/100 g of beef)	raw beef	cooked beef
water	54.7 ± 0.2	51.6 ± 1.0
protein	14.6 ± 0.8	23.9 ± 1.5
fat	28.8 ± 1.4	22.4 ± 0.7
ash	0.7 ± 0.1	0.7 ± 0.1
total	98.7	98.6

[a] Data represent mean (n = 3) ± standard deviation.

Analysis of Carnosine in Ground Beef. One part of beef was homogenized with 2 parts of 5 mM phosphate buffer (pH 7.0) for 60 s and homogenate was centrifuged at 2500*g* for 10 min according to the method of Chan et al. (*30*). The supernatant (10 mL) was vortexed with 0.36 M perchloric acid for 60 s and then heated in a boiling water bath for 5 min. The solution was centrifuged at 2000*g* for 15 min and the resulting supernatant was filtered using a 0.45 μm syringe filter. Filtrate extract (20 μL) was derivatized with 120 μL of *o*-phthaldehyde by incubation at room temperature for 2 min. Carnosine concentrations were determined by the same modified HPLC method of Teahon and Rideout (*29*) mentioned above for carnosine analysis in serum.

Statistics. All analyses of beef samples were made in triplicate and reported as means ± standard deviation. SAS (Cary, NC) was used to analyze for main effects of all data using the general linear model (PROC GLM). PROC ANOVA with Duncan's multiple range test was used to separate these treatment means, and significance was set at the 5% level.

RESULTS AND DISCUSSION

Composition of Beef Patties. The proximate composition in raw and cooked beef patties is shown in **Table 1**. The fat content (28.8%) in the raw patties was slightly higher than the 25% fat indicated on the retail label. Broiling caused a decrease in water and fat content that resulted in an increase in protein concentration. The proximate composition of broiled regular ground beef cooked to a medium degree of doneness (71 °C) reported in USDA Handbook 8-13 is water = 55.1, protein = 23.0, lipid = 20.9, and ash = 0.9. The lower water and higher lipids seen in the patties used in this experiment is likely due to cooking to a higher internal temperature (74 °C) and the higher starting lipid content of the beef [28.8% in this experiment versus 26.6% in USDA Handbook 8−13 (*31*)].

There is limited data on carnosine concentrations in beef. Crush (*1*) reported a beef leg muscle carnosine and anserine concentration of 150 and 50 mg/100 g of tissue, respectively. Carnegie et al. (*32*) used HPLC to determine beef dipeptide concentration and found 333 and 52 mg/100 g of tissue of carnosine and anserine, respectively. These researchers used topside, rump, and shin muscle. It is unclear whether these muscles were analyzed together or separately, because but no specifics were given on how the muscle was processed. Plowman and Close (*33*) reported carnosine and anserine concentrations in beef shoulder and rib steak and found 341 and 58 mg/100 g of tissue of carnosine and anserine, respectively. Again no details were provided on whether these muscles were analyzed together or separately. More recently, Purchas et al. (*34*) reported carnosine concentrations in check, cardiac, and *semitendinosus* muscle as 42.9, 32.6, and 452.6 mg/100 g of tissue.

Table 2. Carnosine Concentrations in Raw and Cooked Beef Patties

	carnosine concentration		
	mM	mg/g of beef	mg/200 g of beef
raw beef	1.22	1.24	248.54
cooked beef	1.19	1.34	267.06

[a] Data represent mean ($n = 3$) ± standard deviation.

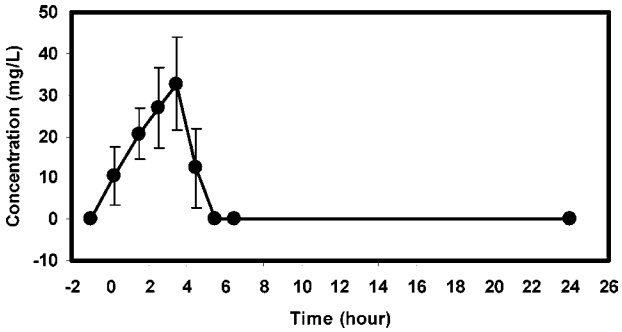

Figure 1. Change in carnosine concentrations in human plasma following consumption of 200 g of ground beef that was cooked to an internal temperature of 74 °C. Beef was consumed at time 0. Data represent mean ($n = 18$) ± standard deviation.

The ground beef used in this study contained 124 mg carnosine/100 g of tissue with no anserine detected (**Table 2**). The lower carnosine concentration could be due to the higher fat content of the beef used in this experiment (fat content of the muscle was not reported in the previous studies outlined above). Higher fat content could decrease carnosine concentrations, because carnosine is mainly found in the cytosol of skeletal muscle and the presence of adipose fat would decrease skeletal muscle carnosine concentrations. Lower carnosine and a lack of anserine could also be due to the different types of beef muscle analyzed. It is well-known that carnosine and anserine concentrations are higher in white-type (e.g. fast twitch) muscle fibers, where they are thought to act as an intercellular buffering agent (*4*, *6*). If the muscle used to prepare the ground beef used in this study was low in white fiber skeletal muscle, the carnosine concentration would be expected to be low. Carnosine concentrations increased from 124 to 134 mg/100 g during the cooking process. This is equivalent to 274 and 277 mg of carnosine/100 g of meat solids (dry weight basis) for raw and cooked beef, respectively, suggesting that no significant degradation of carnosine occurred during cooking.

Carnosine has been reported to be absorbed intact in hamsters (*35*). Perry et al. (*25*) found carnosine in human plasma, suggesting that carnosine is also absorbed intact in humans. Blood samples were obtained from all 18 subjects 1 h prior to the dietary intervention with beef, and neither carnosine nor anserine were detected in the plasma of any of the subjects (**Figure 1**). This suggested that all subjects had complied with the recommendation not to consume food products containing carnosine 48 h prior to the dietary intervention. Alternately, subjects could have consumed food products containing carnosine and sufficient time had elapsed for carnosine to be cleared from the blood by carnosinase and excretion through the kidney (*26*). Following beef consumption, blood was collected at 0.25, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, and 24 h. Carnosine increased in the plasma to 10.4 mg/L of plasma ($p < 0.5$) plasma within 15 min of beef consumption. Plasma carnosine concentrations continued to increase ($p < 0.5$) reaching a maximum (32.7 mg of carnosine/L of plasma) 3.5 h after beef consumption. Carnosine concentrations then decreased until no carnosine could be detected at 5.5 h postconsumption.

Gardner and co-workers (*26*) monitored the excretion of carnosine and β-alanine (one of the two amino acids in carnosine) in the urine of human subjects in a study where subjects fasted for a minimum of 9.5 h and then consumed a beverage containing 4 g of carnosine and 5 g of lactulose. Subjects only consumed water or orange juice during the experiment. In this experiment, both carnosine and β-alanine could be detected in the urine 1 h after ingestion of the carnosine-containing beverage. Urinary carnosine and β-alanine concentrations increased for up to 2 h postconsumption, after which concentrations decreased. Urine was sampled for 5 h, at which time both carnosine and β-alanine returned to basal levels. Gardner et al. (*26*) reported maximal urine carnosine concentrations at 2 h compared to maximal plasma carnosine concentrations 3.5 h after the dietary intervention in the present study. This difference could be due to the form of carnosine consumed, with the carnosine beverage resulting in a more rapid absorption than would occur when the carnosine was consumed in the form of beef. Gardner and co-workers (*26*) reported a plasma carnosine concentration of 183.1 mg/L for a single subject 0.5 h after ingestion of the carnosine beverage. This plasma concentration is 5.6-fold greater than the highest plasma carnosine concentration observed in our study. This difference is likely due to the 15-fold greater amount of carnosine consumed in the Gardner et al. (*26*) study. Both the current study and Gardner et al. (*26*) found that carnosine was not detected in plasma 5.0−5.5 h after the dietary intervention.

CONCLUSIONS

Cooking did not cause a decrease in carnosine concentration in ground beef. Upon ingestion of ground beef, carnosine was detected in plasma within 15 min. Plasma carnosine concentration peaked 3.5 h after beef consumption, after which it rapidly decreased until no plasma carnosine was detectable after 5.5 h. These results indicate that dietary carnosine is absorbed into plasma after the consumption of ground beef. Since carnosine has several potential health benefits, evidence of its bioavailability suggests that it could be suitable for use as a functional food ingredient.

ACKNOWLEDGMENT

This research was partially supported by a grant from the National Beef Cattlemen Association.

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Received for review December 8, 2004. Revised manuscript received March 25, 2005. Accepted April 7, 2005.

JF047934H