BIOAVAILABILITY OF CARNOSINE FROM BEEF MEAT IN MINI-PIGS

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Introduction

Carnosine (*β*-alanyl-*L*-histidine) is a cytoplasmic dipeptide found in skeletal muscle at concentrations ranging from 2 to 25 mM (Chan and Decker, 1994). Various physiological actions have been ascribed to carnosine including pH buffering, antioxidant activity, reactive aldehydes scavenging, prevention of protein crosslinking, and involvement in the degradation of carbonylated proteins (Guitto *et al.*, 2005). Through these activities, carnosine has several potential health benefits for humans and it could be considered as a bioactive food component. Indeed, it was reported that some dietary carnosine can cross the gut barrier and appear into human plasma after consumption of beef meat (Park *et al.*, 2005).

In this study, we have investigated the quantitative significance of carnosine absorption in mini-pigs following ingestion of beef meat coming from three different muscles differing in their carnosine concentration.

Materials and Methods

Four Pitman-Moore mini-pigs (21-22kg), 6-month old, were surgically fitted with catheters in the portal vein and the abdominal aorta, and an ultrasonic flow probe around the portal vein. Three meats were tested on each animal: grilled sirloin, grilled neck-brisket and braised shoulder containing 7.77, 2.92 and 1.93mg of carnosine/g of muscle, respectively. Sampling sessions were separated by at least 2 days. Before sampling, the mini-pigs were deprived of food from 17.00 to 11.00. Base-line arterial and portal blood samples were simultaneously withdrawn at 10.00, 10.30 and 11.00 during the post-absorptive period (PA). Then, at 11.00, the mini-pigs were offered a meal exclusively of meat (30 g of proteins per meal). Arterial and portal blood samples were then taken at different times (11.30, 12.00, 13.00, 14.00, 15.00, 16.30 and 18.00) during the post-prandial period (PP). Blood samples (5mL) were collected in cold syringes with heparin. Haemoglobin concentration and packed cell volume were immediately determined (ABL510 System, Radiometer, Copenhagen). Blood samples were centrifuged at 3000g for 10 min at 4 °C. Plasma (300 μL) spiked with homocarnosine as an internal standard (30μL, 10μM) was vortexed with sulfosalicylic acid (5% w/v, final concentration) for 1 min, then left for 15 min at room temperature and centrifuged at 10000g for 15 min at 4°C. The resulting supernatant was stored at -80°C prior analysis. Portal vein blood flow was continuously recorded during the sampling session.

Arterial plasma carnosine concentrations were determined by RP-HPLC (Perkin Elmer, France) using pre-column derivatisation with O-phthaldehyde reagent (column: C18-HDO 250 x 4.6 mm, 5 µm; Uptisphere, Interchim), adapted from the method of Maynard *et al.* (2001). The portal net flux of carnosine in plasma (μmole/h), noted PNF_{carn}, is calculated as followed: $PNF_{carn} = ([Carn_{port}] \times (Hb_{art}/Hb_{port}) - [Carn_{art}]) \times PPF$, where $[Carn_{port}]$ and $[Carn_{art}]$ are carnosine concentrations (μM) in arterial and portal plasma, Hb_{art} and Hb_{port} are haemoglobin concentrations (g/dL) in arterial and portal whole blood and PPF is portal plasma flow (L/h). Portal plasma flow (L/h) is calculated as followed: $PPF = PBF \times (100 - PCV_{port})/100$, where PBF is portal blood flow (L/h) and PCV_{port} is packed cell volume in portal blood (%).

Data were statistically analysed using repeated measures analysis of variance through SAS/PC Program.

Results and Discussion

Average portal blood flow during the PA period was 49 ± 2mL.min^{-1}.kg^{-1}. The time-variations in PBF throughout the PP period were not affected by the nature of meat.

During the PA period, average $[Carn_{art}]$ and PNF_{carn} were 3.86 ± 1.05μM and -1.9 ± 4.8μmole/h, respectively. Whereas $[Carn_{art}]$ was nearly not affected by the ingestion of shoulder, it sharply increased between 30 and 60 min after the meal of sirloin and neck-brisket (Figure 1). The maximum $[Carn_{art}]$ was reached 1h, 2h and 3h after the meal of neck-brisket, sirloin and shoulder, respectively. The maximum $[Carn_{art}]$ and the area under the curve of $[Carn_{art}]$ throughout the PP period were 2 to 3 times greater for sirloin than for neck-brisket. This increase in plasma $[Carn_{art}]$ following meat ingestion is in agreement with the observations of Park *et al.* (2005) in humans. In the present study, differences observed with the $[Carn_{art}]$ pattern according to the type of meat can be related to the carnosine concentration in the ingested meat.

Portal net flux of carnosine (Figure 2) reflects carnosine absorption by the small intestine. As for $[Carn_{art}]$, PNF_{carn} was not affected by ingestion of shoulder but it significantly increased after the meal of sirloin and neck-brisket, and the

maximum PNF_{carn} was reached 1h after the meals. It returned to the basal level after 2h and 3h for neck-brisket and sirloin, respectively. Portal net release of carnosine throughout the post-prandial period, calculated from PNF_{carn} integration, was 470, 220, and 0μmole/7 h for sirloin, neck-brisket and shoulder, respectively. For sirloin and neck-brisket, it accounts for 13% of ingested carnosine.

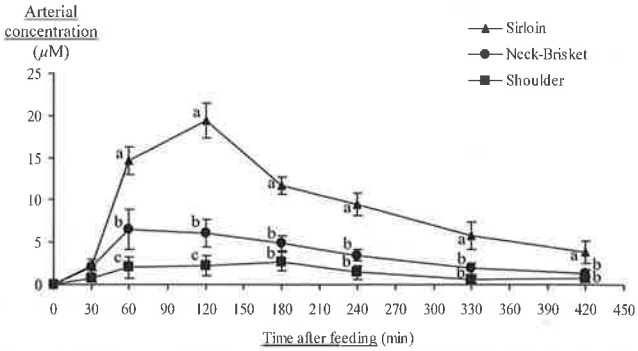

Figure 1: Increase in arterial plasma carnosine concentration (μM) in mini-pigs (n=4) after a meal of grilled sirloin, grilled neck-brisket or braised shoulder. Values are means ± standard error. For each sampling time, mean values with different letters are significantly different (P<0.05).

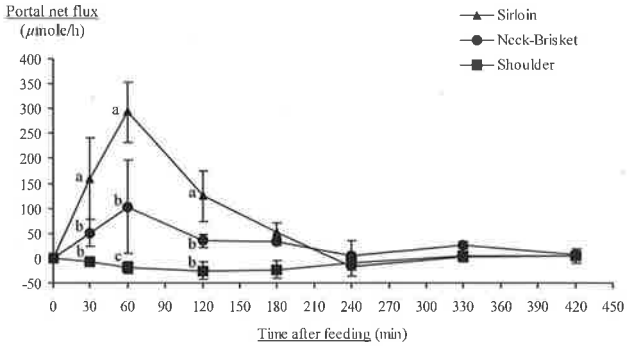

Figure 2: Increase in portal net flux of carnosine in plasma (μmole/h) in mini-pigs (n=4) after a meal of grilled sirloin, grilled neck-brisket or braised shoulder. Values are means ± standard error. For each sampling time, mean values with different letters are significantly different (P<0.05).

Conclusions

In the present study, absorption of intact carnosine was evidenced for the first time from direct measurements of portal net release. This absorption appeared highly related to carnosine concentration in food. Below 2 mg/g of meat, all dietary carnosine seems to be degraded during digestion and absorption. Above this value, carnosine bioavailability was proportional to carnosine intake.

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