

## Effect of carnosine on the gastric secretion in rats

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### Abstract

Iron plays an important role in metabolism, and iron deficiency leads to various clinical abnormalities. One of the main causative factors of iron deficiency is the poor absorption of dietary iron. It is well established that the bioavailability of non-heme iron is enhanced by vitamin C, citric acid and the meat factor. However, the factor associated with improving iron absorption in meat has yet to be identified. Thus, we investigated the effect of carnosine, a well-known neuropeptide, on iron solubility. Pylorus-ligated rats were sacrificed 6 h after carnosine administration, and the gastric juice of each rat was collected and analyzed for volume, pH, acidity and pepsin activity.

The results indicate that carnosine promotes gastric juice and increases pepsin activity and pepsin output and suggest that carnosine enhances iron absorption by increasing iron solubility.

### Introduction

Iron deficiency is the most frequently occurring nutritional disorder in the world. One of the main causative factors of iron deficiency is the poor absorption of dietary iron. Dietary iron is classified into two types, heme iron and non-heme iron. Heme iron contained red meat, poultry and fish has a high intestinal absorption efficiency, because it directly enters mucosal cells. Non-heme iron is the main form of dietary iron, and its intestinal absorption is influenced by the composition of other foods. The absorption rate of vegetable iron is only about 1-2 %<sup>1)</sup>. Therefore, the enhancement of non-heme iron absorption is important for improving iron status.

It is well established that the bioavailability of non-heme iron from foods is enhanced by vitamin C, citric acid and the meat factor<sup>2)</sup>. Vitamin C and citric acid maintain iron in a more soluble form and prevent it from binding to inhibitors by their chelating properties<sup>2)</sup>. On the other hand, red meat, poultry and fish also enhance non-heme iron absorption. However, the factor associated with improving iron absorption in these foods has yet to be clarified.

Carnosine is a well-known neuropeptide consisting of alanine and histidine ( $\beta$ -alanyl-L-histidine). It is normally produced in the animal body and is found at millimolar concentrations in the brain, innervated tissues, eye lenses, and skeletal muscle tissues. Carnosine acts as a natural antioxidant with hydroxyl-radical-scavenging and lipid-peroxidase activities<sup>3)</sup>. Furthermore, the pharmacological activity of the carnosine-metal complex has been clarified in recent years<sup>4)</sup>.

Thus, to investigate the effect of carnosine on the solubility of iron, we investigated gastric secretion in pylorus-ligated rats administered carnosine.

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## Experiment

Eight-week-old male Wistar rats were purchased from Japan SLC, Inc. and were housed in stainless steel cages. The cages were placed in an air-conditioned room with 12 h of light.

The rats were restricted food intake but they had free access to drinking water for 18 h. The abdomen of each rat was opened and the pylorus was ligated under ether anesthesia. After closure, 50, 100 or 200 mg of carnosine or saline was immediately administered into the gut through a tube. The rats were sacrificed 6 h after the carnosine administration, and the gastric juice of each rat was collected and analyzed for volume, pH, acidity and pepsin activity.

Acidity was determined by titrating of the gastric juice against 0.1 mol/L NaOH to pH 7.0<sup>5)</sup>. Pepsin activity was determined by the Anson-Mirsky revised method using bovine hemoglobin as a substrate<sup>5)</sup>. One gram of hemoglobin was added to 10 ml of 0.3 mol/L HCl solution, then the solution was diluted to 50 ml as the hemoglobin substrate. Gastric juice was diluted 50-fold with 0.04 mol/L HCl solution to produce the sample solution. The hemoglobin substrate and 0.5 ml of the sample solution were stored at 37 °C. The sample solution was added to 2 ml of the hemoglobin substrate, and the solution was mixed. Then, the solution was incubated at 37 °C for digestion. After 10 min, the solution was added to 5 ml of 5% trichloroacetic acid and mixed. The sample solution was added to 5% trichloroacetic acid, and then hemoglobin solution was added as a blank. After 30 min, the solution was centrifuged, and 1 ml of supernatant was added to tube. The supernatant was added to 5 ml of 0.5 mol/L sodium hydroxide and phenol reagent. Color optical density was determined using a tyrosine standard at 640 nm after 60 min. Acidity and pepsin activity were expressed as mEq/L and tyrosine  $\mu\text{g}/\text{ml}/\text{min}$ , respectively. Pepsin activity was calculated using

$$\text{Pepsin activity} = (A - B) \times 50 \times \frac{7.5}{0.5} \times \frac{1}{10} = (A - B) \times 75,$$

where, A is the concentration of tyrosine in the sample, and B is the concentration of tyrosine in the blank.

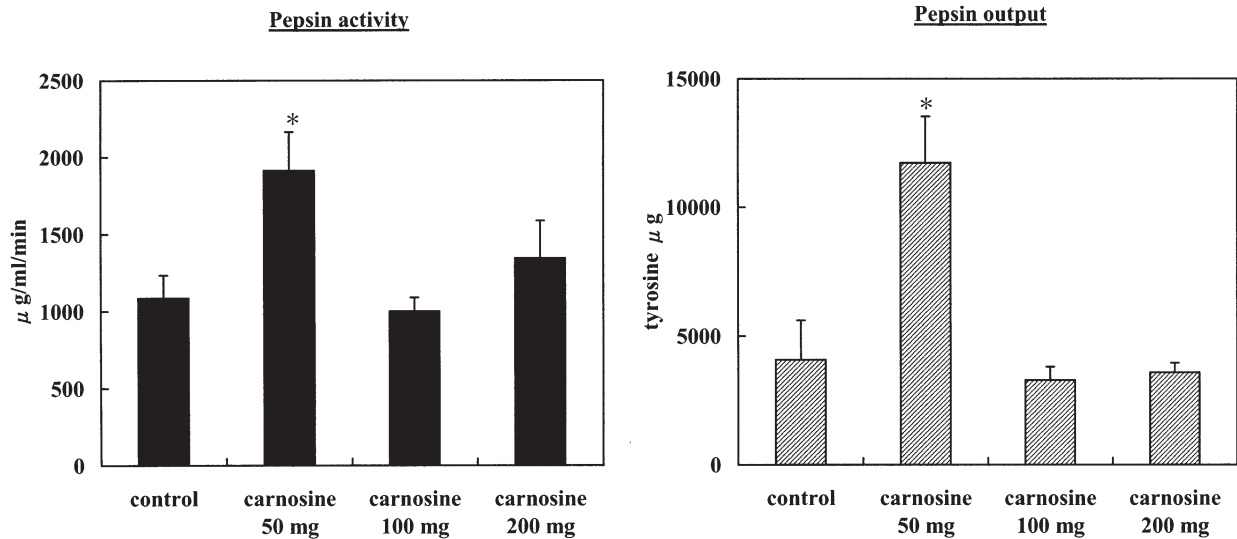
Pepsin output is calculated by pepsin activity  $\times$  volume<sup>5)</sup>.

## Results and Discussion

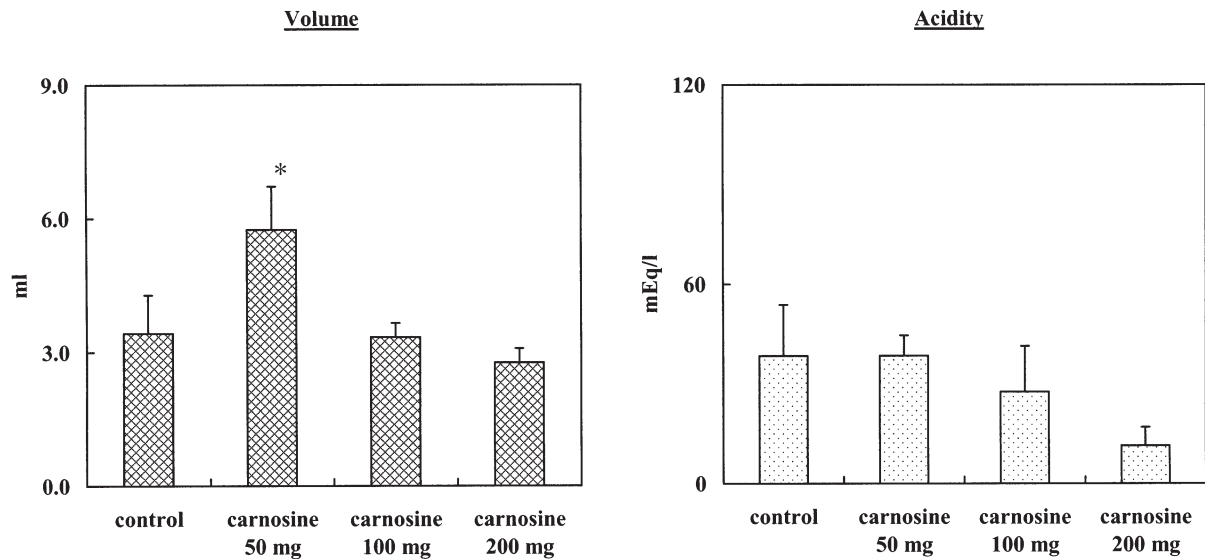
The pepsin activity and pepsin outputs in the control and carnosine-administered rats are shown in Fig. 1. The rats administered 50 and 200 mg of carnosine had increased pepsin activities compared with the control rats. Pepsin activity in the 50 mg-carnosine-administered rats was significantly higher than that in the control rats. However, no significant differences were observed between the 100 and 200 mg-carnosine-administered rats and the control rats, indicating that 50 mg of carnosine may increase pepsin activity.

The pepsin output in the 50 mg-carnosine-administered rats was significantly higher than that in the control rats. Although there were no significant differences in pepsin output between the control and the 100 and 200 mg-carnosine-administered rats, the pepsin outputs in the 100 and 200 mg-carnosine-administered rats were lower than that in control rats. This indicates that 50 mg of carnosine increases pepsin output.

The volumes and acidities of the gastric juices in the control and carnosine-administered rats are shown in Fig 2. The volume in the 50 mg-carnosine-administered rats was significantly high compared with that in the control rats. The 100 mg-carnosine-administered rats exhibited a similar volume to the control rats. On the other hand, the gastric juice volume in the 200 mg-carnosine-administered rats was lower than that in the control rats, indicating that the rats administered with 50 mg of carnosine have an increased secretion of gastric juice.



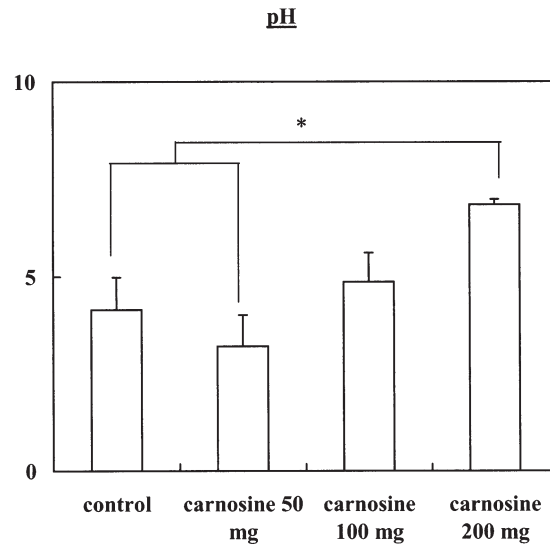
**Fig. 1** The pepsin activity and pepsin output in the control and carnosine-administered rats.  
\*Significant differences were observed between the control and carnosine-administered rats ( $P < 0.05$ ).



**Fig. 2** The volume and acidity of gastric juice in the control and carnosine-administered rats.  
\*Significant differences were observed between the control and carnosine-administered rats ( $P < 0.05$ ).

Gastric juice acidity in the 50 mg-carnosine-administered rats exhibited a similar volume to the control rats. Acidities in the other carnosine-administered groups were lower than that in the control rats. However, there were no significant differences in acidity between the control and carnosine-administered rats, indicating that carnosine does not affect acidity.

The gastric juice pHs in control and carnosine-administered rats are shown in Fig. 3. The gastric juice pH in the 50 mg-carnosine-administered rats was lower than that of the control rats, although no significant difference in pH was observed between the 50 mg-carnosine-administered and control rats. On the other hand, the pH in the 200 mg-carnosine-administered rats was significantly higher than those in the 50 mg-carnosine-administered and control rats. This result indicates that the rats administered 50 mg of carnosine decrease pH in the gut compared with the



**Fig. 3** The pH of gastric juice in the control and carnosine-administered rats.  
\*P<0.05.

rats administered 200 mg of carnosine.

These results indicate that 50 mg of carnosine enhances gastric juice and increases pepsin activity and pepsin output, although this reaction does not depend on carnosine dose. It is assumed that this result due to buffer action of carnosine. It is suggested that carnosine enhances iron absorption by increasing iron solubility.

### References

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