Role of Gastric Digestion in the Absorption of Slowly Digestible Peptide, Oligo-L-Methionine, in Rats (43541)

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Abstract. **Absorption of a slowly digestible peptide, oligo-L-methionine (OM), added to a low casein diet was faster than absorption of OM added to a low soybean protein isolate diet in early stages of feeding in chronic portal-cannulated rats. In the present study, the gastric digestion of ^{14}C-labeled OM in rats fed a casein-based diet was higher than that in rats fed an soybean protein isolate-based diet 30 min and 3 hr after feeding. In rats with chronic bile-pancreatic juice diversion from the proximal small intestine, the higher gastric solubilization of OM in the stomach of the casein group was also observed, but the contents of soluble digest of OM in the stomach were lower than those in the normal rats. The portal absorption of OM in the casein group was higher than in the soybean protein isolate group both 30 min and 4 hr after feeding in the bile-pancreatic juice-diverted rats, and the difference of the portal absorption between the diet groups corresponded to the difference of the amount of solubilized OM in the upper small intestine; this, in turn, depends on pepsin digestion in the stomach. These findings suggest that the difference between the two diet groups in the ability to digest OM in the stomach can at least partly explain the higher portal absorption of OM in the casein group in the early stages of feeding.** [P.S.E.B.M. 1993, Vol 202]

We observed that the body weight gain in rats fed a low casein diet supplemented with oligo-L-methionine (OM) was higher than that in rats fed a low soybean protein isolate (SPI) diet supplemented with OM (1). The insoluble oligopeptide is enzymatically synthesized and contains 6–11 polymerized L-methionine, with 7–9 polymers the major components. We demonstrated that OM was only slowly digested, and OM in early stages of feeding was absorbed faster in a casein-based diet than in a SPI-based diet. Absorption of OM was evaluated by differences between the portal and venous concentrations of methionine derived from OM, and disappearance of OM from the digestive tracts (2, 3). The findings reveal that effects of casein on the gastrointestinal functions are different from those of SPI. The aim of the present studies was to examine the role of gastric digestion of OM for the absorption of OM in the early stages of feeding. The behavior of OM in the digestive tract may be a model of the study of the digestion of slightly or slowly digestible dietary proteins, such as legume proteins.

We measured the gastric digestion of OM by its solubilization in the stomach after feeding of test diets in normal and bile-pancreatic juice (BPJ)-diverted rats. The latter's BPJ was chronically diverted from the proximal small intestine in order to abolish the contribution of BPJ to the OM digestion in the stomach and upper small intestine. We also observed *in vitro* digestibility of OM by pepsin.

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Materials and Methods

Diets. Table I presents diet compositions. Casein and SPI diets were made to contain 8% net protein (protein content = $N \times 6.25$). That is, the test diet contained 9.4 g/100 g diet of casein (ALACID; New Zealand Dairy Board, Wellington, New Zealand) or 9.6 g/100 g diet of SPI (Fujipro, R; Fuji Oil Co., Osaka, Japan). The SPI used in this experiment contained no or only slight trypsin inhibitor protein. Three percent [^{14}C]OM was added to the test diets. The radiolabeled

OM was synthesized enzymatically from L-methionine containing L-[*methyl*-^{14}C]-methionine (37MBq/6 g L-methionine; American Radiolabeled Chemicals Inc., St. Louis, MO) by papain (7, 8). The water-soluble fractions were completely washed out from the synthetic products, as confirmed by a ninhydrin-negative reaction of the washing solution.

Animals. *Experiment 1: Gastric digestion of OM in normal rats.* Male Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan) weighing 50 g were fed the stock diet for 7 days. After a 24-hr fast, rats were divided into four groups of seven rats and were fed 1 g of the casein and SPI diets containing 3% [^{14}C]OM. The rats of two groups were sacrificed by withdrawal of the total blood under pentobarbital anesthesia (Nembutal, sodium pentobarbital: 50 mg/kg body wt; Abbott Co., North Chicago, IL) 30 min after feeding; the other two groups were sacrificed 3 hr after feeding (9). Cardia and pylorus were immediately ligated and the stomach was removed. The gastric content was washed out with saline and quickly frozen by liquid nitrogen and freeze-dried.

Experiment 2: Gastric digestion and portal absorption of OM in BPJ-diverted rats. Male Sprague-Dawley rats (Japan SLC) weighing 200–220 g were operated after a 24-hr fast for implantation of a cannula into the common bile-pancreatic duct and upper ileum under pentobarbital anesthesia. A tip of polyethylene catheter (SP 28, i.d. 0.4 mm, o.d. 0.8 mm; Natsume Seisakusyo, Tokyo, Japan) connected to silicone tubing (i.d. 0.5 mm, o.d. 1.0 mm; Dow Corning Co., Kanagawa, Japan) was used for diverting of BPJ to the small intestine at a point 45 cm distal from the ligament of Treitz. The details were described previously (10). After a 5-day recovery period on the stock diet and a 24-hr fast, rats were divided into four groups of seven rats and fed 2 g of the test diets containing 3% [^{14}C]OM. The amounts of the test diets given per body weight were similar to those in Experiment 1. Portal and aortic (arterial) blood were collected by direct puncture of a syringe with needle (23 gauge) under pentobarbital anesthesia 30 min and 4 hr after feeding. After ligations of the cardia, pylorus, and terminal ileum, the stomach and small intestine were removed with contents. The small intestine was divided into two segments of equal length. The gastric and intestinal contents were collected and dried with the same procedure used in Experiment 1.

Table I. Composition of Diets

	Stock diet	Test diets	
		Casein diet[a] (g/100 g diet)	SPI diet[a] (g/100 g diet)
Casein[b]	25.0	9.4	—
SPI[b]	—	—	9.6
Sucrose	62.9	78.5	78.3
Corn oil[c]	5.0	5.0	5.0
Mineral mixture[d]	4.0	4.0	4.0
Vitamin mixture[e]	1.0	1.0	1.0
Vitamin E[f]	0.1	0.1	0.1
Choline chloride	2.0	2.0	2.0

[a] Radiolabeled OM (3%) was added to these basal diets in concurrence with a removal of a corresponding amount of sucrose.
[b] Casein and SPI contained 13.7% and 13.4% nitrogen, respectively, as evaluated by the Kjeldahl method.
[c] Retinyl palmitate (7.66 μmol/kg of diet) and ergocalciferol (0.050 μmol/kg of diet) were added to the corn oil.
[d] The mineral mixture is identical to the mineral mixture 2 described by Ebihara *et al.* (3). It provided (mg/kg diet); Ca, 4491; P, 2997; K, 3746; Mg, 375; Fe, 38.0; I, 0.31; Mn, 81.1; Zn, 25.9; Cu, 15.3; Na, 4342; Cl, 6678; Se, 0.27; Mo, 1.12; Cr, 0.49; B, 0.35; V, 0.22; Sn, 1.05; As, 1.20; Si, 15.7; Ni, 3.00; F, 2.71; and Co, 0.20.
[e] The vitamin mixture was prepared in accordance with AIN-76 mixture (4), except vitamin K as menadione and L-ascorbic acid, which were added to give 5.81 μmol/kg (5) and 284 μmol/kg (6) of diet, respectively.
[f] Vitamin E (granulated, Yuvela; Eisai Co., Tokyo, Japan) supplied 423 μmol all-*rac*-α-tocopheryl acetate in kg diet.

Analyses. Amino acid concentrations of the portal and arterial plasma were measured by high-performance liquid chromatography after formation of phenyl thiocarbamoyl derivatives with phenylisothiocyanate (Tokyo Kasei Kogyo, Tokyo, Japan) (11, 12). The liquid chromatography system was constructed by Mini-Solvent delivery system M 600 (Waters Associates Milford, MA) and PICO-TAG column (150 × 3.9 mm; Waters Associates).

The soluble fraction of the freeze-dried gastric and small intestine contents were extracted with ice-cold water. In Experiment 2, the residue was dried and added to 99% formic acid to dissolve the residual undigested OM completely.

The chain length of OM was determined by high-performance liquid chromatography with C_{18} column (μBondapak C_{18}, 7.8 × 30 mm; Waters Associates) after formation of methionine sulfone derivatives by performic acid (T. Kasai and T. Tanaka, submitted for publication).

Peptic digestion products *in vitro* were evaluated as 50 g/liter of trichloroacetic acid-soluble radioactivity released from ^{14}C-labeled OM. The *in vitro* digestion of OM by pepsin was performed with the medium containing OM (0.1 g/liter) and pepsin (24.5–24,500 units/ml, P-7012; Sigma Chemical Co., St. Louis, MO) in 50 m*M* citrate buffer (pH 1.8) at 37°C for 1 hr. One unit of pepsin expresses a 0.001 increase of optical density at 280 nm/min at 37°C, using hemoglobin as substrate. Acid-soluble radioactivity after the incubation under the same condition without pepsin was very low and did not increase during incubation. The control acid-soluble radioactivity was subtracted from all data.

The radioactivity of the aqueous, formic, and trichloroacetic acid solutions of OM digest was measured in a 15-ml scintillator consisting of *p-terphenyl* and *p*-bis(*o*-methylstyryl) benzene in toluene and ethylene glycol monoethyl ether (1:1) by liquid scintillation system (LSC700; Aloka, Tokyo, Japan).

Calculation and Statistical Analyses. To evaluate the portal absorption of methionine, we adopted porto-arterial (P-A) difference of methionine concentration 30 min after feeding of the test diets (Fig. 1).

The statistical analyses were performed by two-way analysis of variance (diet and time). The significant differences between the diet groups were determined by Student's t test. All values are presented as mean ± SE.

Results

Table II presents the contents of solubilized [^{14}C] OM in the stomach of the normal rats. The contents of solubilized OM were expressed as percentages of the radioactivity of soluble fraction of OM digest in the stomach to the radioactivity of OM ingested. The values were small, but were significantly higher in the casein group than in the SPI group 30 min and 3 hr after feeding, respectively. The test diets given were consumed completely in 30 min by all the rats.

Table III shows the results of the BPJ-diverted rats. The contents of solubilized OM in the stomach of the diversion rats were smaller than the contents of the normal rats shown in Table II, but the average value in the rats fed the casein-based diet was 2-fold higher than that of rats fed the SPI-based diet 30 min after feeding. The amounts of soluble digest of OM in the absorptive site, upper and lower small intestine, were 5-fold higher in the casein group 30 min after feeding. These differences between the casein and SPI groups became small 4 hr after feeding. The intakes of the casein and SPI diets in diversion rats were 1.96 ± 0.00 g and 1.93 ± 0.02 g (n = 7), respectively.

Figure 1 presents the P-A differences of methionine concentration in the BPJ-diverted rats fed a low casein or a low SPI diet containing 3% OM. The values were significantly higher in the casein group than in the SPI group both 30 min and 4 hr after feeding.

Table IV presents the changes in the proportions of the water-soluble fraction to the water-insoluble fraction from the stomach to the upper and lower small intestine. In both the groups, the proportion was not changed in the upper small intestine in comparison with in the stomach, both 30 min and 4 hr after feeding.

We determined *in vitro* digestibility of radiolabeled OM with pepsin, and OM digestibility reached more than 70% by 0.1% pepsin containing medium (2450 units/ml) at 37°C for 60 min (pH 1.8).

Table II. Gastric Digestion of Radiolabeled OM[a]

Diet	Time after feeding: 30 min (%)	3 hr (%)
Casein	1.29 ± 0.11[b]	2.26 ± 0.12[c]
SPI	0.77 ± 0.11	1.63 ± 0.13

[a] Gastric digestion of OM was evaluated by the contents of water-soluble digest of OM in the stomach after feeding of a low casein (casein) or a low SPI diet containing 3% [^{14}C]OM in the normal rats. The values were presented as percentages of the radioactivity of OM ingested, and were means ± SE (casein group, n = 6; SPI group, n = 7). The effects of diet and time were significant by two-way analysis of variance ($P < 0.05$).
[b] Significant difference between the casein and SPI groups, $P < 0.02$.
[c] Significant difference between the groups, $P < 0.01$.

Discussion

In normal rats, the solubilization of OM in the stomach in the casein group is higher than that in the SPI group. The larger contents of the solubilized OM in the stomach of the casein group were also observed in the rats in which BPJ was completely excluded from the stomach (Table III). The result provides evidence that pepsin is involved in the higher solubilization of OM in the stomach of the casein group.

The absolute levels of the soluble fractions were relatively low in both the diet groups in the normal and BPJ-diverted rats. Oligo-L-methionine may behave as a solid in the stomach because the peptide is insoluble in aqueous solutions. Solid emptying is known to be slow compared with liquid (13, 14). The relatively low contents of the solubilized OM in the stomach suggest the

Table III. Amount of Water-Soluble Digest of Radiolabeled OM in the Gastrointestinal Tract of the BPJ-Diverted Rats Fed a Low Casein and a Low SPI Diet Containing 3% [^{14}C]OM[a]

	Diet	Time after feeding: 30 min (%)	4 hr (%)
Stomach	Casein	0.43 ± 0.03[b]	0.59 ± 0.08
	SPI	0.21 ± 0.02	0.50 ± 0.08
Upper small intestine	Casein	0.0104 ± 0.0012[b]	0.0050 ± 0.0010
	SPI	0.0019 ± 0.0005	0.0026 ± 0.0008
Lower small intestine	Casein	0.0918 ± 0.0146[b]	0.0506 ± 0.0135
	SPI	0.0160 ± 0.0068	0.0341 ± 0.0106

[a] The values were presented as percentages of the radioactivity of OM ingested, and were means ± SE (casein group, n = 7; SPI group, n = 6). The effects of diet and time were significant in all the sites of gut by two-way analysis of variance ($P < 0.05$).
[b] Significant difference between the case and SPI groups, $P < 0.001$.

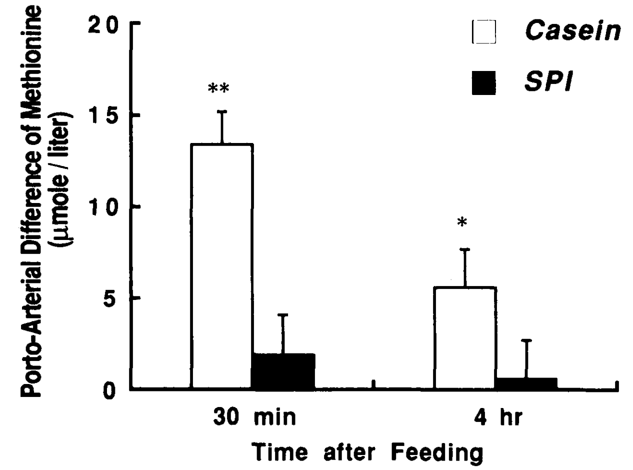

Figure 1. Portal absorption of oligo-L-methionine evaluated by porto-arterial difference of methionine concentration in the BPJ-diverted rats. The values were estimated 30 min and 4 hr after feeding of a low casein (Casein) or a low SPI diet containing OM. The values were means ± SE (casein group, $n = 7$; SPI group, $n = 6$). The effects of diet and time were significant by two-way analysis of variance ($P < 0.05$). Asterisks represent the significant difference, *$P < 0.05$ and **$P < 0.01$.

preferential emptying of the solubilized OM from the stomach.

Previously, we demonstrated that the gastric emptying rate of *p*-aminobenzoic acid, as a marker of soluble component, of the rats fed a low casein diet was similar to that of the rats fed a low SPI diet in the same conditions as the present study (15). In the present study, the gastric remaining of total OM for the initial 30 min after feeding in the casein group was similar to that in the SPI group calculated from Tables III and IV (72% in the casein group and 73% in the SPI group). This result supports that the higher content of solubilized OM in the stomach of the casein group is due to the faster digestion of OM in the stomach, not due to slower gastric emptying of solubilized OM. The gastric remaining of OM 4 hr after feeding in the casein group (63%) was smaller than that in the SPI group (70%), which may contribute the faster gastric digestion of OM in the casein group.

Gaertner and Puigserver (16, 17) reported that the digestive products of polymethionine by pepsin were tri- and tetramethionine. These peptides were soluble in water (data were not shown). These small peptides are absorbed very rapidly (18, 19) and the liquid phase is known to transit faster than solid phase along the small intestine (20). So, the soluble digest of OM may disappear from the upper small intestine much faster than the insoluble intact OM which is slowly digestible and is slowly absorbed. In Table IV, the proportion of soluble fraction to insoluble fraction in the upper small intestine of the BPJ-diverted rats in the two diet groups was not decreased compared with those in the stomach, which supports the preferential emptying of the solubilized OM from the stomach and may be the reason for the relatively low contents of the soluble digest of OM in the stomach.

Absolute values of the contents of solubilized OM in the stomach of the BPJ-diverted rats in Table III were smaller than those for the normal rats shown in Table II. This result suggests that BPJ also contributes to the gastric digestion of OM. It is known that there is a duodenogastric reflux of digest (21, 22). In the normal rats, BPJ flows into the stomach. The gastric pH, 30 min after feeding of low casein and low SPI diets, was 5.04 ± 0.07 and 5.64 ± 0.11 ($n = 6$), respectively (23). In this condition, the proteases in BPJ may be able to degrade OM.

In Figure 1, the portal absorption of methionine in the BPJ-diverted rats fed the casein diet containing OM was significantly higher than in those rats fed the SPI diet containing OM. The result is similar to that in the normal rats, as we presented previously (2, 3). This result suggests that the pepsin digestion of OM in the stomach is at least partly responsible for the higher absorption of OM in the casein group.

The P-A difference of the BPJ-diverted rats fed the casein-based diet shown in Figure 1 was 5-fold higher than that in rats fed the SPI-based diet, but the values were the sum of the absorption of OM and dietary proteins. The amounts of the soluble digest of OM in the upper and lower small intestine of the casein group

Table IV. Changes in the Proportions of Water-Soluble to -Insoluble Radioactivity in Stomach and Upper and Lower Small Intestine of the BPJ-Diverted Rats[a]

	Time after feeding			
	30 min		4 hr	
	Casein (%)	SPI (%)	Casein (%)	SPI (%)
Stomach	0.596 ± 0.035	0.286 ± 0.029	0.934 ± 0.071	0.714 ± 0.102
Small intestine				
Upper site	0.530 ± 0.226	0.657 ± 0.273	2.514 ± 1.187	0.784 ± 0.235
Lower site	1.016 ± 0.205	0.223 ± 0.050	0.716 ± 0.172	0.388 ± 0.026

[a] The values were means ± SE (casein group, $n = 7$; SPI group, $n = 6$).

were also much larger than those of the SPI group, as shown in Table III. This result suggests that the larger P-A difference in the casein group of the BPJ-diverted rats is largely due to the larger amount of solubilized OM in the small intestine in the casein group.

We conclude that the gastric digestion of OM in the casein diet is higher than that in the SPI diet, which may affect the portal absorption of OM in the early stages of feeding. The difference of the absorption rate of OM between the casein and SPI group may depend partly on the different velocities of pepsin digestion in the stomach. The mechanism of the higher gastric digestion of OM in the casein group is not known, but differences in the gastric pH and degrees of the competitive inhibition by casein and SPI in peptic digestion of OM may be associated with the phenomenon. The findings in the present study suggest that the gastric digestion of slightly digestible dietary protein, for example, legume proteins, may be influenced by the other dietary proteins ingested at the same time.

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