Absorption of Oligo-L-[^{35}S]methionine after Feeding of a Low Casein or a Low Soybean Protein Isolate Diet in Rats (43390)

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Abstract. **We studied the absorptive properties of oligo-L-methionine (OM), which is an enzymatically synthesized and slowly digestible peptide. Previously, we demonstrated that when OM was added to a low casein diet, the improvement of the body weight gain was higher than when OM was added to a low soybean protein isolate (SPI) diet and we suggested that the difference in the supplementary effect of OM depends on its absorptive rate. In the present study, the OM absorption estimated by the portovenous difference in radioactivity derived from ^{35}S-labeled OM was higher in the casein diet than in the SPI diet in early stages of feeding after fasting. Absorbed OM was quantified by subtracting the radioactivity of [^{35}S]OM remaining in the whole gut from the ingested [^{35}S]OM, 90 and 180 min after feeding casein and SPI diets containing 3% [^{35}S]OM. We also estimated the absorptive efficiencies by subtracting the amount of radioactivity remaining in the intestines from the amount of [^{35}S]OM emptied from the stomach as percentages of the emptied OM. Both the amount of absorbed OM and absorptive efficiencies of OM were higher in the casein group than in the SPI group, and the higher absorptive efficiency in the casein group indicates a higher digestibility for OM when rats are fed a 3% OM diet after fasting. The digestibility of [^{35}S]OM measured by fecal excretion of radioactivity of OM during normal feedings for diets containing 0.3% [^{35}S] OM for 7 days was about 80% in the casein group and 60% in the SPI group. We conclude that the different supplementary effects of OM in the low casein and SPI diets depend on the difference in OM digestibility. The difference in the digestibility of OM may partly depend on the faster absorption rate of OM in the early stages of feeding.** [P.S.E.B.M. 1992, Vol 200]

The body weight gain of rats fed a low casein diet supplemented with oligo-L-methionine (OM), which is a slowly digestible peptide (six to 10-L-methionine peptides mixture), was higher than that of rats fed a low soybean protein isolate (SPI) diet supplemented with OM (1). We observed previously that methionine absorption in the early stages of feeding of diets containing OM as determined by portovenous (PV) difference of methionine concentration is faster in casein-based diets than in SPI-based diets (2). These findings suggest that dietary casein is a more potent stimulant of the digestive system of stomach, small intestine, and/or pancreas than SPI.

Analysis of the phenomenon and the elucidation of the mechanisms are useful for evaluating the function of dietary protein and the difference between protein sources in the digestive system. Oligo-L-methionine is used as a probe for monitoring the functions of dietary components in the lumen.

The purpose of the present study was, first, to distinctly separate the PV difference due to OM from the PV difference originating from dietary protein using radiolabeled OM. The time course of PV difference is useful, but it is not quantitative without the blood flow rate, which is difficult to measure in individual rats.

The second purpose of this study was to quantify the amount of absorbed OM in the corresponding period observing the PV difference. Lastly, our purpose was to determine the digestibility of radiolabeled OM over a long term period under normal feeding conditions.

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The slowly digestible methionine peptide prevents a threonine imbalance from occurring when free methionine is added to a low protein diet (1). This physiological property may make OM practically useful.

Materials and Methods

Diet. Diet compositions are shown in Table I (3–6). The diets for the portal absorption experiments of [^{35}S]OM and estimation of the amount of [^{35}S]OM remaining in gastrointestinal tract were isonitrogenous. The protein level of the casein and SPI test diet was 8% (protein = nitrogen content × 6.25), and 3% OM was added to the test diets. The level of OM was adopted to get enough increments of the portal concentrations of methionine in the early stages of feeding. Diets for the experiment concerning the fecal excretion of [^{35}S]OM contained the same level of sulfur-containing amino acids, to which 8% casein diet or 10% SPI diet with 0.3% [^{35}S]OM was added. These test diets were used in the experiment observing the different supplementary effects of OM on the body weight gain mentioned above.

Two kinds of [^{35}S]OM were synthesized enzymatically from 3 g of L-methionine (Ajinomoto Co., Tokyo, Japan) containing 31.5 MBq of L-[^{35}S]methionine (44.4 × 10^{12} Bq/mmol; ICN Biochemicals, Inc., Irvine, CA) for the first two experiments (portal absorption and OM remaining in the lumen) and from 12 g of L-methionine containing 83 MBq of L-[^{35}S]methionine for the last experiment (fecal excretion of OM) (7,8). The synthesized OM was a mixture of 6 to 10 L-methionine peptides, which was estimated by mass spectrometry (JMS-01SG-2; Nihondenshi Co., Tokyo, Japan), and high-performance liquid chromatography as methionione sulfone derivatives.

Animals. *Experiment 1: Portal absorption of oligo-L-[^{35}S]methionine.* Male Sprague-Dawley rats, weighing 230–250 g (Japan SLC, Hamamatsu, Japan), were fed a 25% casein-sucrose diet (stock diet) for 4 days and operated on in order to implant portal and venous catheters under sodium pentobarbital anesthesia (Nembutal; Abbott, North Chicago, IL). The venous catheter (Silascone No. 00; Dow Corning, Kanagawa, Japan) was placed in the superior vena cava through the jugular vein. The portal catheter (polyethylene tube, SP 28; i.d. 0.4 mm, o.d. 0.8 mm; Natsume Seisakusho, Tokyo, Japan) was inserted directly into the portal vein as described previously (9). Both the catheters were led subcutaneously to the back region of the neck and blood was collected from both under an unrestrained condition. After a 2-day recovery period on the stock diet and a 24-hr fast, the rats were given 2 g of the casein and SPI-based diet containing 3% radiolabeled OM for 30 min. Before the feeding, 40 μl of portal and venous blood were collected through the portal and venous catheters, after which 240 μl of both types of blood were collected from each at 20, 40, 60, 120, and 180 min after feeding.

Experiment 2: Gastrointestinal residue of oligo-L-[^{35}S]methionine. Male Sprague-Dawley rats, weighing 230–250 g, were first fed the stock diet for 7days and then, after a 24-hr fast, were given 2 g of the casein and SPI-based test diet containing 3% radiolabeled OM. After 90 and 180 min, rats were sacrificed by decapitation and the stomach, small intestine, and cecum were immediately removed with their contents. the

Table I. Diet Composition

	Stock diet (%)	Experiment 1 and 2		Experiment 3	
		Casein diet[a] (%)	SPI diet[a] (%)	Casein diet[b] (%)	SPI diet[b] (%)
Casein[c]	25.0	9.4	—	8.0	—
SPI[c]	—	—	9.6	—	10.0
Sucrose	62.9	78.5	78.3	79.9	77.9
Corn oil[d]	5.0	5.0	5.0	5.0	5.0
Mineral mixture[e]	4.0	4.0	4.0	4.0	4.0
Vitamin mixture[f]	1.0	1.0	1.0	1.0	1.0
Vitamin E[g]	0.1	0.1	0.1	0.1	0.1
Choline chloride	2.0	2.0	2.0	2.0	2.0

[a] Test diet for portal absorption (Expt 1) and gastrointestinal remaining (Expt 2) of oligo-L-[^{35}S]methionine(OM). Radiolabeled OM (3%) was added to these diets.
[b] Test diet for the digestibility of [^{35}S]OM under the normal feeding condition. Radiolabeled OM (0.3%) was added to these diets.
[c] Casein (ALACID; New Zealand Dairy Board, Wellington, New Zealand) and soybean protein isolate (SPI; Fujipro R; Fuji Oil Co., Osaka, Japan) contained 13.7% and 13.4% nitrogen as evaluated by the Kjeldahl method.
[d] Retinyl palmitate (7.66 μmol/kg diet) and ergocalciferol (0.0504 μmol/kg diet) were added to the corn oil.
[e] Mineral mixture is identical with MM2 described by Ebihara *et al.* (5).
[f] The vitamin mixture was prepared in accordance with AIN-76 mixture (6), except vitamin K as menadione and L-ascorbic acid were added to give 5.81 μmol/kg (7) and 284 μmol/kg (8) diet, respectively.
[g] Vitamin E (granulated; Yuvela; Eisai Co., Tokyo, Japan) supplied 423 μmol of all-rac-α-tocopheryl acetate in kg diet.

small intestine was divided into two parts of equal length, jejunum and ileum. The contents of each were washed out with saline and freeze-dried.

Experiment 3: Fecal excretion of oligo-L-[^{35}S]methionine. After a 2-day feeding of a stock diet, rats (male Sprague-Dawley), weighing 60–65 g, were fed a casein-based or SPI-based diet containing 0.3% radiolabeled OM for 7 days. Feces of individual rats were collected and freeze-dried every day.

Analyses. Methionine concentrations were measured in portal and venous plasma as phenyl thiocyanate derivatives, with phenyl isothiocyanate (Tokyo Kasei Kogyo, Tokyo) (10, 11) by high-performance liquid chromatography constructed with a Mini-Solvent Delivery System M-600 (Waters Association, Milford, MA) and a PICO-TAG column (15 × 3.9 mm; Waters). Formic acid (99%) was added to the freeze-dried gastrointestinal contents in order to dissolve the OM, and the radioactivity of the OM solution was measured.

0.3 *N* Sodium hydroxide (4 ml) was added to the freeze-dried feces, which were then homogenized by sonication and incubated at 30°C for 2 hr in order to dissolve only the proteins. The precipitate of the sodium hydroxide solution was washed with an additional 2 ml of sodium hydroxide solution and 99% formic acid was added to the washed precipitate in order to dissolve the OM. The radioactivity of the solution of the sodium hydroxide and formic acid 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) using a liquid scintillation system (LSC700; Aloka, Tokyo, Japan).

Calculation and Statistics. The portal absorption was estimated as portovenous differences (PV difference) in methionine concentrations (total methionine absorption) or radioactivity (methionine absorption derived from OM by conversion of radioactivity to μmol of methionine). All values are presented as mean ± SE. The data were analyzed by one-way (Table IV and Fig. 4) and two-way (diet, time; Tables II and III and Figs. 1–3) analysis of variance and the significant differences were determined by the least significant difference and Student's *t* test.

Results

Figure 1 presents PV difference of total methionine based on the measurements of methionine concentration, which is the sum of dietary protein and OM absorption. The values for 60 min after feeding of the casein-based diet were significantly higher than those for the SPI-based diet. In Figure 2, the profiles of PV difference in methionine derived from OM closely resembled those for total methionine absorption, but there is no significant difference at any point in time between the casein and SPI groups. The concentrations estimated from the radioactivity of portal and venous

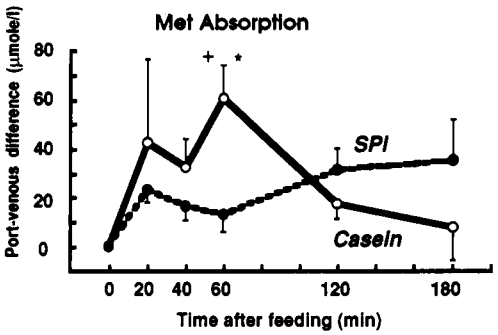

Figure 1. Portovenous difference of methionine concentration after feeding 2 g of 8% casein or 8% SPI diet containing 3% oligo-L-methionine. All values are evaluated by subtracting the initial value (0 min) individually. Values are mean ± SE (n = 4). Asterisks represent the significant difference between casein and SPI groups at the same time (* $P < 0.05$) or from the initial value within the same group (+ $P < 0.05$).

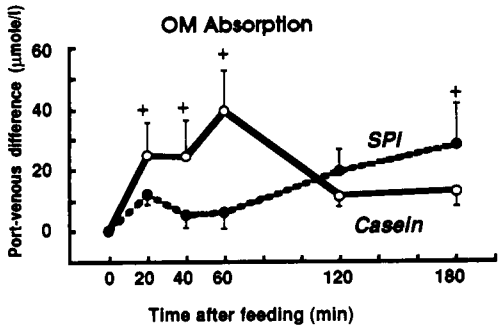

Figure 2. Portovenous difference of methionine concentration derived from oligo-L-[^{35}S]methionine calculated from the radioactivities of methionine fraction of high-performance liquid chromatography after feeding 2 g of 8% casein or 8% SPI diet containing 3% of oligo-L-[^{35}S]methionine. All values are evaluated by subtracting the initial value (0 min) individually. Values are mean ± SE (n = 4). Asterisks represent the significant difference between casein and SPI groups at the same time (* $P < 0.05$) or from the initial value in the same group (+ $P < 0.05$).

blood 60 min after the feeding of casein-based diet were significantly higher than those following the feeding of the SPI-based diet: portal values, 67.7 ± 12.4 (casein) vs 19.6 ± 2.2 (SPI) μmol/liter, $P < 0.05$; venous values, 28.4 ± 0.6 (casein) vs 13.7 ± 2.2 (SPI) μmol/liter, $P < 0.02$. These observations reveal that the OM absorption of the rats fed the casein-based diet is higher than that of the rats fed the SPI-based diet 60 min after feeding. The areas under the curve of PV difference derived from OM from 0 to 60 min are 1376 ± 516 and 400 ± 117 (μmol/liter × min) in the casein and SPI groups, respectively.

Table II shows the percentage of radiolabeled OM remaining in each part of the gut 90 and 180 min after the feeding of the casein and SPI test diet containing 3% ^{35}S-labeled OM. The value of OM remaining in the stomach at 180 min was more than 60% in all groups, which indicates that the rate of gastric emptying of OM is very slow. The amount of OM remaining in the

Table II. Residue of Radiolabeled Oligo-L-methionine in the Gastrointestinal Tract[a]

Time after feeding	8% Casein diet (%)	8% SPI diet (%)
90 Min		
Stomach	71.20 ± 2.60†	70.20 ± 1.30
Jejunum	1.43 ± 1.19	1.23 ± 0.71
Ileum	5.32 ± 0.55	12.93 ± 0.61*‡
Cecum	0.16 ± 0.06	0.15 ± 0.07
Absorption	23.50 ± 0.60*	15.50 ± 1.00
180 Min		
Stomach	61.60 ± 1.80	67.30 ± 5.60
Jejunum	0.18 ± 0.06	1.67 ± 0.60
Ileum	4.88 ± 0.58	5.37 ± 0.37
Cecum	1.42 ± 0.64	3.05 ± 1.00†
Absorption	32.00 ± 2.20*‡	22.60 ± 3.80

[a] Each value was expressed as percentage of the amount of radiolabeled OM ingested for 30 min. The food intakes for 30 min were 1.97 ± 0.01 (90 min) and 1.97 ± 0.01 (180 min) in the casein group and 1.95 ± 0.04 (90 min) and 1.86 ± 0.10 (180 min) in the SPI group. Absorption values were estimated by subtracting the sum of OM remaining in the total gut from the amount of OM ingested. Asterisks represent the significant difference between the casein and SPI groups (*$P < 0.05$), and between 90 and 180 min in the same group (†$P < 0.05$; ‡$P < 0.01$). All values presented mean ± SE ($n = 5$).

Table III. Absorptive Efficiencies of Radiolabeled Oligo-L-methionine Emptied from the Stomach[a]

Time after feeding	Casein diet (%)	SPI diet (%)
90 min	77.6 ± 4.4*	52.1 ± 3.1
180 min	83.0 ± 2.3**	69.7 ± 2.8

[a] The values represent the amount of OM absorbed as percentages of the radiolabeled OM emptied from the stomach. Asterisks represent the significant difference between the casein and SPI groups (*$P < 0.02$; **$P < 0.01$). All values are mean ± SE ($n = 5$).

stomach significantly decreased from 90 min to 180 min in the casein group, but not in the SPI group. The OM remaining in the distal small intestine in the SPI group at 90 min was significantly larger than that in the casein group, and the level of the SPI group decreased to the same levels as the casein group at 180 min. Absorbed OM, which was calculated by subtracting the sum of the amount of OM remaining in the total gut from the intake of OM calculated as a percentage of the amount of ingested OM, was significantly higher in the casein group than in the SPI group at 90 and 180 min.

The absorptive efficiencies of OM at 90 and 180 min shown in Table III, which were the absorbed OM as percentages of the amount of OM emptied from the stomach, were also higher in the casein group than in the SPI group. The difference between the absorptive efficiencies of the rats fed the casein-based test diet and those fed the SPI-based test diet was smaller at 180 min than at 90 min.

Figure 3 presents the changes in the excretion of

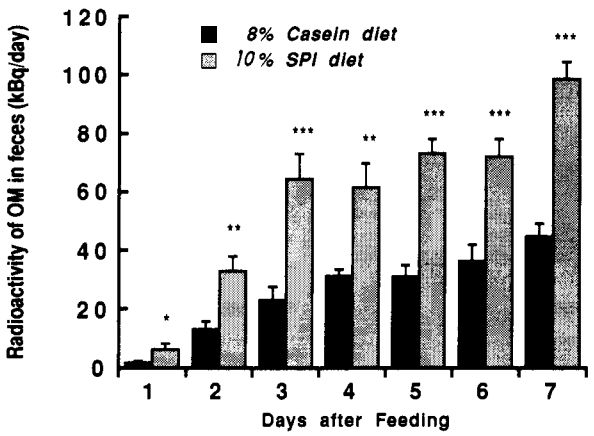

Figure 3. The changes in fecal excretion of radiolabeled oligo-L-methionine in rats fed a low casein or a low SPI diet containing 0.3% oligo-L-[^{35}S]methionine for 7 days (1 kBq = 0.123 mg of OM). Values are mean ± SE ($n = 9$). Asterisks represent the significant difference between the casein and SPI groups (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

OM in feces as radioactivity (conversion rate, 1 kBq = 0.123 mg of OM) in the rats fed the test diets containing 0.3% ^{35}S-labeled OM. The excretions gradually increased until the third day and then reached plateau levels in both the casein and SPI groups, which demonstrates that it takes 3 days to pass the ingested OM through the whole gut and to excrete the indigestible OM in feces. On the first day, the amount of OM radioactivity in the feces of the SPI group was 4-fold higher than that in the casein group. Finally, it was 2-fold higher in the SPI group on the seventh day. We calculated the digestibility from the amount of OM ingested from the second to fourth day and the amount of OM excreted from the fifth to seventh day. The amounts of OM absorbed were estimated by subtracting the OM excretion from the OM intake, as presented in Table IV. Digestibility of OM in the casein-based diet was significantly higher than that in the SPI-based diet.

In Figure 4, the fecal excretion of radiolabeled compounds other than OM, which may be mainly mucosal and pancreatic proteins labeled with ^{35}S *in vivo*, was significantly higher in the casein group than in the SPI group during a 7-day period (Fig. 4, Total).

Discussion

In the present study, we confirm, using [^{35}S]OM, that there is a higher absorptive rate of OM added to a low casein diet over that of OM added to a low SPI diet in early stages of feeding. By using radiolabeled OM, we were able to separate exactly the PV differences originating from OM from those originating from dietary proteins. Portovenous differences of OM in the casein group, shown in Figure 2, decreased after 120 min, which may be due to the retardation of OM emptying from the stomach, as shown in Table II. In contrast, the PV difference of the SPI group gradually increased until 180 min after the feeding, even though

Table IV. Digestibility (%) of ^{35}S-Labeled Oligo-L-methionine (OM) in Rats Fed a Low Casein or a Low SPI Diet[a]

	8% Casein diet	8% SPI diet
OM intake (mg/3 days)	68.9 ± 2.1	78.3 ± 3.1*
OM excretion in feces (mg/3 days)	13.8 ± 1.1	30.5 ± 1.4**
OM absorbed (mg/3 days)	55.3 ± 5.0**	47.8 ± 2.0
Digestibility (%)	80.4 ± 3.0***	61.1 ± 0.9

[a] The amount of OM intake was estimated from Days 2 to 5 and the amount of OM excreted was estimated from Days 5 to 7. Details are presented in Materials and Methods. Asterisks represent the significant difference between the casein and SPI groups (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). All values are mean ± SE ($n = 9$).

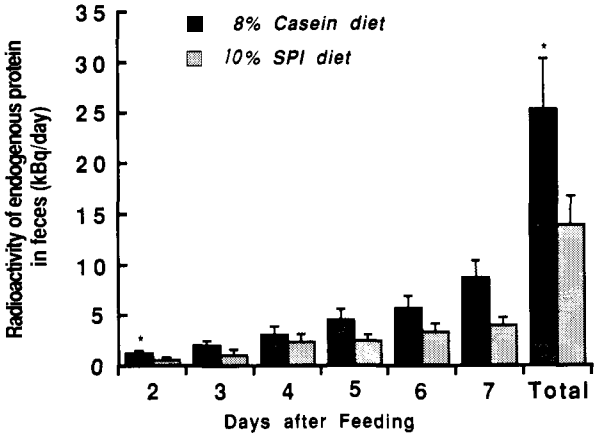

Figure 4. The changes in fecal excretion of radiolabeled proteins in rats fed a low casein or a low SPI diet containing 0.3% oligo-L-[^{35}S] methionine for 7 days. The radiolabeled protein synthesized *in vivo* is the soluble fraction of 0.3 *N* sodium hydroxide solution. On the first day, radioactivity was not detectable in the feces of entire group. Values are mean ± SE ($n = 9$). Asterisks represent the significant difference between the casein and SPI groups (* $P < 0.05$).

OM emptying from the stomach in the SPI group almost stopped 90 min after feeding (Table II). The findings suggest that OM ingested with the SPI-based diet is digested and/or absorbed more slowly in the small intestinal lumen than OM ingested with the casein-based diet.

The OM remaining in the ileum in the SPI group was larger than that in the casein group only at 90 min after the feeding, as shown in Table II, and the difference of absorptive efficiencies between the casein and SPI groups was larger at 90 min than at 180 min, as shown in Table III. These findings indicate a faster absorption of OM in the early stages of the feeding of a casein-based diet, which corresponds to the change in the PV difference of OM (Fig. 2).

The digestibilities of OM, as estimated by the fecal excretion of radiolabeled OM under the condition of normal feeding of the casein and SPI diets shown in Table IV, are comparable to the absorptive efficiencies of OM at 180 min in both the groups presented in Table III. These results indicate that the values of absorptive efficiencies of OM after fasting and refeeding reflect the digestibility of OM introduced at 0.3% to the diets under normal feeding conditions.

As shown in Figure 3, the fecal excretion of radioactivity of OM on the first day after feeding of [^{35}S]OM added to the SPI-based diet was 4-fold higher than that of the OM-supplemented casein-based diet. The difference between both the groups is larger than the difference of the OM excretion on the final day and the difference of the total digestibility of OM estimated in Table IV, which suggests that the gastrointestinal transit of OM in the SPI group is faster than that in the casein group.

Figure 4 presents the excretions of radioactive proteins labeled with ^{35}S in feces, which may consist of mucosal protein and digestive enzymes and are synthesized from ^{35}S compounds derived from OM *in vivo*. The amount of the proteins was larger in the casein group than in the SPI group. The difference in the excretion of the newly synthesized endogenous proteins between the casein and SPI groups is larger than the absorptive efficiency of OM shown in Table IV, which reveals that absorbed methionine derived from OM is more efficiently incorporated into the proteins of the digestive systems in the casein-based diet than in the SPI-based diet. Casein may play a trophic role in the digestive system.

Several mechanisms may account for the difference in absorptive efficiency between OM added to the casein and SPI diets; the gastric digestion, the response of the exocrine pancreatic secretion, activation and stability of pancreatic proteases, competition with the dietary protein on the hydrolysis of OM by the proteases, and small intestinal transit may be determinative. Dietary proteins are known to potentiate the exocrine pancreatic secretion in the rats (12, 13). We observed previously that there were relatively small differences in the response of the exocrine pancreatic secretion between a low casein and a low SPI diet (14). We also demonstrated the small intestinal transit is faster after the feeding of a low SPI diet than a low casein diet (15). Gaertner and Puigserver (16, 17) reported that oligo-L-methionine is digested by pepsin *in vitro*, which suggests that the gastric digestion of OM is one of the possible mechanisms affecting the difference in the absorptive efficiency of OM between the casein and SPI groups. More than two factors may be responsible for the different absorption rate of OM and the different supplementary effect of OM on body weight gain between

the rats fed the casein-based diet and those fed the SPI-based diet.

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