

## Different effects of casein and soyabean protein on gastric emptying of protein and small intestinal transit after spontaneous feeding of diets in rats

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The effects of dietary casein and soyabean-protein isolate (SPI) on gastric emptying and small intestinal transit were observed in rats fed on an 80 g casein or 80 g SPI/kg diet. After a 24 h fast, rats were given 2 g of both the test diets containing 10 g guanidinated casein/kg diet as a marker protein. The amounts of the marker protein remaining in the stomach of the rats fed on the casein or SPI diet were similar and decreased to about 50% after 20 min. The emptying rate then slowed, especially in the casein group, so that the amount leaving the stomach after 1 h in the SPI group was slightly higher ( $P < 0.05$ ). The small intestinal transit of chyme was estimated by a bolus injection of colloidal carbon suspension or of colloidal carbon and  $^3\text{H}$ -labelled polyethylene glycol through an implanted duodenal catheter 6 min before death. The average value of transit at 12, 20, 40 and 60 min after feeding of SPI diet was about 25% faster than that after casein diet. The transit velocity of the SPI group was also faster than that of the non-protein group 40 min after feeding. These findings reveal that SPI enhances the small intestinal transit of the liquid phase of chyme. There was no correlation between the gastric emptying of homoarginine and small intestinal transit. This result suggests that the small intestinal transit of lumen contents is controlled by the dietary protein regardless of the gastric emptying of protein.

### Gastric emptying: Small intestinal transit: Dietary protein

Dietary protein has various effects on gastrointestinal functions; for example, digestive fluid secretion (Green *et al.* 1973) and gut motility (Daniel *et al.* 1990), both of which may affect the absorption rate of nutrients. Previously we reported that casein was absorbed much faster than soya-bean-protein isolate (SPI) and the absorption rates were markedly decreased 40 min after spontaneous feeding of diet containing both the proteins at a low level in conscious rats (Hara & Kiriyama, 1991). The aim of the present study was to examine the effects of these proteins on gastric emptying and small intestinal transit under the physiological conditions adopted in the previous study.

The present study was designed to measure the gastric emptying of dietary protein and small intestinal transit of the liquid phase of chyme in the same rat during spontaneous feeding of a low-casein diet, a low-SPI diet, or a non-protein diet. Gastric emptying was observed using guanidinated casein as a marker in order to exclude the effect of endogenous proteins. Small intestinal transit was measured by a direct challenge of colloidal carbon suspension or of colloidal carbon and radiolabelled polyethylene glycol (PEG) through an implanted duodenum catheter (Summers *et al.* 1970).

### EXPERIMENTAL METHODS

Gastric emptying was estimated using homoarginine (Expts 1 and 2), and small intestinal transit of the liquid phase was estimated as the 'leading edge' using carbon suspension

Table 1. *Composition (g/kg diet) of diets*

Diet...	250 g Casein/kg	80 g Casein <sup>¶</sup> /kg	80 g SPI <sup>¶</sup> /kg	Non-protein
Casein*	250	94	—	—
SPI*	—	—	96	—
Sucrose	629	785	783	879
Maize oil†	50	50	50	50
Mineral mixture‡	40	40	40	40
Vitamin mixture§	10	10	10	10
Vitamin E	1	1	1	1
Choline chloride	20	20	20	20

SPI, soya-bean-protein isolate.

\* Nitrogen contents of casein and SPI were 137 and 134 g/kg respectively, as evaluated by the Kjeldahl method.

† Retinyl palmitate (7.66  $\mu$ mol/kg diet) and ergocalciferol (0.0504  $\mu$ mol/kg diet) were added to the maize oil.

‡ The mineral mixture is identical to the mineral mixture 12 (MM2) described by Ebihara *et al.* (1979), providing (mg/kg diet): calcium 4491, phosphorus 2997, potassium 3746, magnesium 375, iron 38.0, iodine 0.31, manganese 81.1, zinc 25.9, copper 15.3, sodium 4342, chlorine 6678, selenium 0.27, molybdenum 1.12, chromium 0.49, bromine 0.35, vanadium 0.22, tin 1.05, arsenic 1.20, silicon 15.7, nickel 3.00, fluorine 2.71, cobalt 0.20.

§ The vitamin mixture was prepared in accordance with AIN-76 mixture (American Institute of Nutrition, 1977) except vitamin K as menadione and L-ascorbic acid were added to give 5.81  $\mu$ mol/kg (Bieri, 1977) and 284  $\mu$ mol/kg (Harper, 1959) diet respectively.

|| Vitamin E (granulated, Yuvela; Eisai Co., Tokyo) supplied 423  $\mu$ mol DL- $\alpha$ -tocopheryl acetate/kg diet.

¶ Guanidinated casein (10 g/kg; Expts 1 and 2) and 2 g indigo carmine/kg (Expt 2) were added to both 80 g protein/kg diets for feeding.

(Expts 1, 2 and 3) and as the 'geometric centre' by radiolabelled PEG (Expt 3), and diet 'front' was estimated using indigo carmine (Expt 2).

#### *Diets*

Compositions of diets containing (g/kg) 250 casein, 80 casein, 80 SPI, and the non-protein diet are shown in Table 1. Test diets were prepared by the addition of 10 g guanidinated casein/kg and 2 g indigo carmine/kg (only Expt 2) to an 80 g casein/kg diet or an 80 g SPI/kg diet in order to measure the gastric emptying rate and to estimate the diet 'front' in the small intestine respectively. Casein (ALACID; New Zealand Dairy Board, Wellington, New Zealand) and SPI (Fujipro R; Fuji Oil Co, Osaka) diets were made to contain 80 g net protein/kg (where protein content was estimated as nitrogen  $\times$  6.25), by adding either 94 g casein material or 96 g SPI material/kg, as shown in Table 1. N contents of both materials were estimated by the Kjeldahl method. Indigo carmine (sodium indigotin disulphonate) was purchased from Wako Pure Chemical Industries (Osaka). Guanidinated casein, whose lysine residues were converted to homoarginine, was prepared by guanidination of  $\epsilon$ -amino groups in casein with 1-guanyl-3,5-dimethyl pyrazole nitrate (Habeeb, 1960) which was synthesized from aminoguanidine nitrate and acetylacetone (Wako Pure Chemical Industries). The conversion efficiency of lysine to homoarginine was 98%.

#### *Animals and analyses*

Male Sprague-Dawley rats (Japan SLC Inc., Hamamatsu) were housed individually in separate cages. After a 24 h fast, rats weighing 220–240 g were anaesthetized by an intraperitoneal injection of pentobarbital sodium (40 mg/kg body-weight; Abbott Co., North Chicago, USA). A duodenal catheter made of silicone tubing (Silascon No. 00; Dow Corning, Kanagawa; i.d. 0.5 mm, o.d. 1 mm), was implanted into the duodenum 10 mm distal to the pylorus and secured by a purse-string suture. The catheter was tunnelled

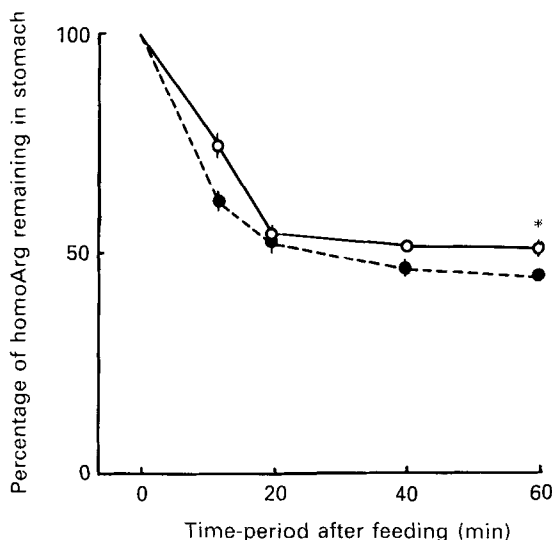


Fig. 1. Gastric emptying of homoarginine in guanidinated casein after feeding an 80 g casein/kg test diet (○) or an 80 g soya-bean-protein isolate (SPI)/kg test diet (●). The values are means with their standard errors represented by vertical bars for six rats except 20 min in the casein group ( $n$  7) and the SPI group ( $n$  5). Mean values were significantly different from those for SPI group ( $*P < 0.05$ ). For details of diets, see Table 1, and for details of procedures, see pp. 60–62.

subcutaneously to the back of neck. On the third day after implantation of the catheter we observed normal eating and growth, showing that the operative procedure did not interfere with the normal function of the gastrointestinal tract.

The rats were fed on a 250 g casein/kg diet during the 10 d recovery period after surgery. After a 24 h fast, rats were given 2 g of the casein test diet, the SPI test diet, or the non-protein diet. The rats were killed by decapitation at 20, 40 and 60 min (Expt 1) and 12 min (Expt 2) after feeding of the test diets. To measure the 'geometric centre' (Expt 3) rats were killed at 40 min after feeding. Carbon suspension in 10  $\mu$ l saline (9 g sodium chloride/l; Expts 1 and 2) or the carbon and 2 mg 1,2- $^3$ H labelled PEG 4000 (37 kBq/2 mg; Expt 3; NEN Research Products, Boston, USA) in 30  $\mu$ l saline was given through the implanted duodenal catheter 6 min before the rats were killed to measure the small intestinal transit.

The pylorus, cardia, and terminal ileum were ligated, and the stomach and small intestine were immediately removed and clipped at the leading edge of the markers, and also clipped to divide the small intestine into eight segments of equal length. The length of the total small intestine and the distance the 'leading edge' of carbon (black) and indigo carmine (blue, Expt 2) had travelled from the pylorus were measured. To calculate the 'geometric centre' (Expt 3) the radioactivity in the eight segments of the small intestine was estimated without loss of contents.

The radioactivity was measured by a liquid-scintillation system (LSC-700, Aloka, Tokyo) after solubilization of each segment, including the contents, with Protosol (NEN Research Products). All gastric contents were collected and proteins and peptides were precipitated with trichloroacetic acid (TCA; 100 g/l final concentration). After washing out TCA with diethyl ether, the proteins and peptides were hydrolysed by 6 M-hydrochloric acid (110°, 24 h) and the amino acids were analysed by high-performance liquid chromatography (HPLC) as phenyl thiocarbamyl (PTC) derivatives with phenyl isothiocyanate (Bidlingmeyer *et al.* 1984; Cohen *et al.* 1986) (Tokyo Kasei Kogyo). The

HPLC was constructed from a Mini-Solvent Delivery System M-600 (Waters Assoc., Milford, USA) and PICO-TAG column (15 × 3.9 mm, Waters Assoc.).

#### *Calculation and statistical analyses*

The gastric emptying of dietary protein was estimated as the homoarginine of guanidinated casein remaining in the stomach expressed as a percentage of the homoarginine given in the meal. The gastric emptying of the amino acids glutamic acid plus glutamine, proline, and leucine were also evaluated at the same time. Small intestinal transit was evaluated as the distance travelled in 6 min by the 'leading edge' of carbon expressed as a percentage of the total length of the small intestine, and as the 'geometric centre' of the labelled PEG. In Expt 2 we also estimated the distance the leading edge of chyme travelled in 12 min as a diet 'front' measured by indigo carmine.

The statistical analyses were performed by one-way and two-way ANOVA ('time' and 'diet'). Significant differences between means in Table 4 were determined by Duncan's multiple-range test. Linear regression analysis was performed between the gastric emptying of homoarginine and the small intestinal transit ('leading edge'). The significance of the correlation and the difference between means in Fig. 1, and Tables 2 and 3 were determined by Student's *t* test.

#### RESULTS

The results of Expts 1 and 2 are combined and presented in Figs. 1 and 2, and Tables 2 and 3. As shown in Fig. 1, the percentage of homoarginine remaining in the stomach decreased to about 50% by 20 min after feeding of both casein and SPI test diets. The emptying rates of both the groups from 20 to 60 min slowed, especially in the casein-fed group. The percentage of homoarginine remaining was significantly lower in the SPI group at 60 min.

The gastric emptying of the amino acids glutamic acid plus glutamine, proline, and leucine, which are mainly found in both casein and SPI, is shown in Fig. 2. The curves of these amino acids showed the same tendency as homoarginine, but there were no significant differences between the casein and SPI group at any time after feeding.

In Table 2, the transit of the intestinal chyme of the SPI group estimated by the 'leading edge' was faster than that of the casein group. The values at all time-points were almost the same within each group. The values of the transit at 40 and 60 min and overall for all four time-periods in the SPI group were significantly higher than those of the casein group. In contrast, the values of the diet 'front' at 12 min after feeding were similar in both the groups and the 'front' of both the groups had reached the lower ileum (Table 3).

Table 4 shows the values of the 'leading edge' and the 'geometric centre' estimated by PEG distribution in the small intestine at 40 min after feeding of both the casein and the SPI diet, and the non-protein diet as the control group. The faster transit rate of the SPI group compared with the casein group was recorded again, and the transit rate of the SPI group was also faster than that of the non-protein group, in both the indications of small intestinal transit. The correlation coefficient between the values of the 'leading edge' and that of the 'geometric centre' was 0.95 ( $n$  17,  $P < 0.01$ ).

Regression analyses were performed between gastric emptying and small intestinal transit ('leading edge'). There was no correlation. The correlation coefficients were 0.045 ( $n$  12, not significant) and 0.137 ( $n$  12, not significant) at 12 and 20 min respectively.

#### DISCUSSION

Guanidinated casein was used as a marker of gastric emptying of dietary protein in order to eliminate the effect of endogenous proteins. Rogers *et al.* (1960) reported that the changes in total N remaining in the stomach after feeding of a casein diet is almost the same

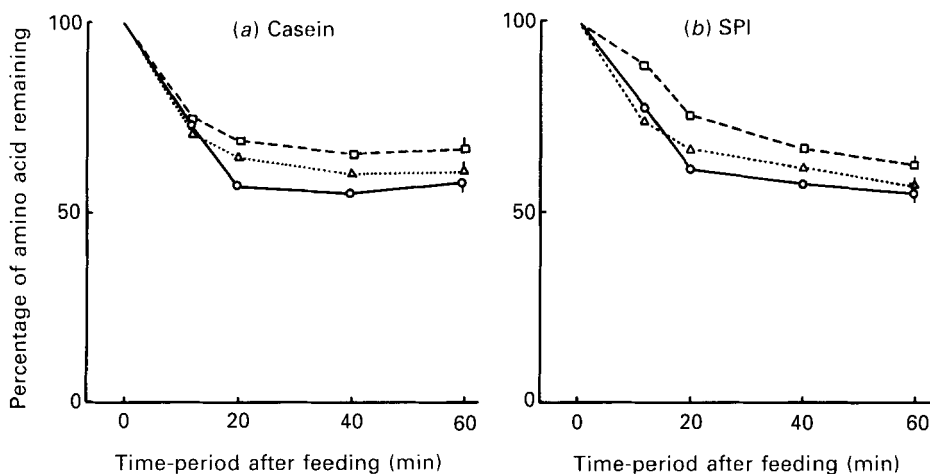


Fig. 2. Gastric emptying of glutamic acid plus glutamine (O), proline (□) and leucine (△) after feeding (a) an 80 g casein/kg test diet or (b) an 80 g soya-bean-protein isolate (SPI)/kg test diet. The values are means with their standard errors represented by vertical bars for six rats except 20 min in the casein group (*n* 7) and the SPI group (*n* 5).

Table 2. Expts 1 and 2. Small intestinal transit of chyme (%)† after feeding casein or soya-bean-protein isolate (SPI) test diets‡ to rats§

(Mean values with their standard errors for six rats except values for 20 min and 'All' which are shown in parentheses)

Time-period after feeding (min)...	12		20		40		60		All	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
80 g Casein/kg	42.9	6.2	51.3	4.6(7)	46.6	2.8	51.4	2.4	48.9	2.0(25)
80 g SPI/kg	60.1	6.1	61.5	4.7(5)	59.8**	1.7	59.4*	2.2	61.8***	2.1(23)

Mean values were significantly different from those for casein diet for the same time-period after feeding: \**P* < 0.05, \*\**P* < 0.005, \*\*\**P* < 0.001).

† Distance travelled by the 'leading edge' of colloidal carbon in 6 min as a percentage of total length of the small intestine at 12, 20, 40 and 60 min after feeding (for details, see pp. 60–62).

‡ For details of diets, see Table 1.

§ For details of procedures, see pp. 60–62.

|| The overall sum of four time-periods.

as that after feeding a soya-bean-protein diet. Our results indicated that gastric emptying of protein in the casein group was significantly slower than that of the SPI group (Fig. 1). This finding provides evidence that the much faster absorption of casein compared with SPI (Hara & Kiriya, 1991), as described previously, was not due to the difference in gastric emptying. It was also observed that the profiles of gastric emptying of glutamic acid plus glutamine, proline, and leucine, which are mainly derived from dietary proteins, were similar to that of homoarginine (Fig. 2). These results indicate that the percentage of homoarginine remaining in the stomach gives a sensitive estimate of the gastric emptying of dietary protein.

The retardation of the discharge of dietary proteins from the stomach from 20 min to 60 min after feeding was also established. The marked decrease in absorption rate of protein

Table 3. *Expt 2. The diet 'front' (%)\* at 12 min after feeding of casein or soya-bean-protein isolate (SPI) test diets† to rats‡*

(Mean values with their standard errors for six rats)

Diet	12 min after feeding	
	Mean	SE
80 g Casein/kg	83.8	0.8
80 g SPI/kg	82.4	0.8

\* Distance travelled by the leading edge of indigo carmine (blue dye) in 12 min as a percentage of the total length of small intestine.

† For details of diets, see Table 1.

‡ For details of procedures, see pp. 60–62.

Table 4. *Expt 3. Small intestinal transit estimated from the 'leading edge' (colloidal carbon) and the 'geometric centre' (radiolabelled polyethylene glycol (PEG))\* after feeding casein or soya-bean-protein isolate (SPI) test diets†‡ to rats*

(Mean values with their standard errors)

Diet	Leading edge(%)		Geometric centre		n
	Mean	SE	Mean	SE	
Non-protein	37.7 <sup>b</sup>	2.2	2.52 <sup>b</sup>	0.14	6
80 g Casein/kg	32.9 <sup>b</sup>	4.5	2.38 <sup>b</sup>	0.19	5
80 g SPI/kg	50.3 <sup>a</sup>	1.2	3.45 <sup>a</sup>	0.09	6

<sup>a,b</sup> Mean values with unlike superscript letters were significantly different ( $P < 0.05$ ).

\* Estimated by an injection of carbon suspension (leading edge) or of <sup>3</sup>H-labelled PEG (geometric centre, measured by the PEG distribution in eight segments) through the duodenal catheter 6 min before killing rats at 40 min after feeding of diets.

† The details of diets, see Table 1.

‡ For details of procedures, see pp. 60–62.

in the early stages of feeding shown in our previous study (Hara & Kiriya, 1991) may have been due to the retardation of gastric emptying of protein. The reason for the retardation, especially in the casein group, even though more than 50% of the protein remained in the stomach, is not known (Fig. 1). The pattern of gastric emptying with two phases, fast and slow, observed in the present study agrees with several reported findings (Poulakos & Kent, 1973; Mangel & Koegel, 1984; Parr *et al.* 1988). Factors which affect gastric emptying are: particle size of gastric chyme, energy density and osmotic pressure in the duodenum (Hunt & Knox, 1972; Burn-Murdoch *et al.* 1978), and gut hormone secretions. Two possibilities are considered for the retardation of emptying. First, both casein and soya-bean protein have a weakly acidic isoelectric point. Therefore, those proteins remaining in the stomach after 20 min may have comprised solid curds. Solid emptying is well known to be slower than liquid emptying. Secondly, dietary protein is known to be a stimulator of cholecystikinin release (Konturek *et al.* 1973; Liddle *et al.* 1986) and this hormone is known to slow gastric emptying (Debas *et al.* 1975; Moran & McHugh, 1982; Mangel & Koegel, 1984). Thus, the retardation of gastric emptying, especially in the casein group, may also be mediated by the release of cholecystikinin.

Small intestinal transit of the SPI group is about 25% faster than that of the casein group, which may be related to the nature of the dietary protein itself because the same amount of protein travelled from the stomach up to 20 min after feeding (Fig. 1). Transit velocity of the small intestinal contents is generally determined by intestinal motility, fluid secretion into the lumen, viscosity of the chyme, and some amino acids in the lumen (Bull *et al.* 1985; Bouyssou *et al.* 1988). Opiates as well as several hormones control intestinal motility (Kinsman & Read, 1984; Fioramonti *et al.* 1988; Kromer, 1988; Spiller *et al.* 1988). Soyabean protein may affect the secretion or action of hormones or opiates. Also, the slightly digestible peptides derived from SPI possibly exert the elevation of transit.

There was no difference in the diet 'front' between the casein and SPI group, as shown in Table 3. It suggests that the first chyme excreted from the pylorus moved along the small intestine at the same speed. The chyme indicates the pattern of dietary composition to the small intestine and sets the corresponding transit speed. This hypothesis was described by Schemann & Ehrlein (1986). The other feature is that the transit velocity is decreased at the lower small intestine and the difference between diets in the proximal small intestine is reduced, which was demonstrated by Summers *et al.* (1970).

Gastric emptying and small intestinal transit were measured separately in the same rat. There are no correlations between gastric emptying and small intestinal transit while digesta is flowing copiously from the stomach, that is 12 and 20 min after feeding. These findings suggest that gastric emptying and small intestinal transit are controlled independently. Read *et al.* (1982) also report the same conclusion by different responses for gastric emptying and small bowel transit of various diets.

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