

## Ingestion of the soluble dietary fibre, polydextrose, increases calcium absorption and bone mineralization in normal and total-gastrectomized rats

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We previously demonstrated that feeding a highly fermentable and water-soluble dietary fibre, guar-gum hydrolysate (GGH) increased intestinal absorption of insoluble Ca salts in total-gastrectomized rats. In the present study, we examined the effects of feeding a less fermentable and water-soluble fibre, polydextrose (PD), on Ca absorption and bone mineralization in the normal and total-gastrectomized rats in comparison with the effects of GGH. Apparent Ca absorption was severely lowered by gastrectomy, and PD feeding (50 g/kg diet) partially restored the reduction of Ca absorption similarly to GGH feeding (50 g/kg diet). PD feeding also increased the Ca absorption in normal rats, but not GGH feeding. Femur Ca concentration was reduced with gastrectomy. Feeding PD for 21 d increased the bone Ca concentration in both normal and gastrectomized rats, but GGH feeding did not. In rats fed PD, pH of the caecal contents was lower than in rats fed fibre-free and GGH diets; however, soluble Ca concentration in the caecal contents was not different between the diet groups. Short-chain fatty acid concentrations were much lower in the PD groups than in the GGH groups. We also examined *in vitro* Ca absorption by using everted sacs of the small intestine. Addition of PD to the serosal medium of the ileal sacs increased Ca absorption, but addition of GGH did not. These results suggest that the small intestine rather than the large intestine is responsible for the increase in Ca absorption in rats fed PD, and suggests that the mechanism for the increase by PD may be different from that by GGH.

### Calcium absorption: Bone mineralization: Polydextrose: Dietary fibre

It is known that osteopenia is induced after gastric resection in patients (Koga *et al.* 1979; Nilas *et al.* 1985). Absence of gastric acid after this operation may impair insoluble Ca absorption because gastric acid is the most important factor for solubilization of insoluble Ca salts. We have previously shown that total gastrectomy markedly decreased insoluble Ca absorption and bone Ca in rats (Ohta *et al.* 1998). The reduction of the Ca absorption after gastrectomy was partially restored by feeding a highly fermentable, low viscosity dietary fibre, namely guar-gum hydrolysate (GGH; Takahashi *et al.* 1994), and we suggested that fermentation in the large intestine be involved in the restoration of Ca absorption (Hara *et al.* 1999). We also demonstrated that GGH feeding ameliorated the reduction of Ca absorption by partial nephrectomy in rats, and that the large intestine is fully responsible for the amelioration of the Ca absorption impaired by renal failure (Hara *et al.*

1996). The large intestine has high capacity for Ca absorption (Karbach & Feldmeier 1993; Ohta *et al.* 1997), and solubilization of Ca salts by acids generated through microbial fermentation in the large intestine has been proposed as the mechanism responsible for the increase in Ca absorption observed following ingestion of highly fermentable, indigestible materials (Younes *et al.* 1996). However, some food saccharides are known to enhance the small intestinal absorption of Ca (Armbrecht & Wasserman 1976; Goda *et al.* 1993; Suzuki *et al.* 1998). The mechanisms for the beneficial effects of the indigestible saccharides are not fully determined.

The aim of the present study was to examine effects of feeding polydextrose (PD) on Ca absorption and bone mineralization of normal and gastrectomized rats in comparison with those of feeding GGH. PD is a less fermentable dietary fibre with a low viscosity, and is a

**Abbreviations:** GGH, guar-gum hydrolysate; PD, polydextrose.

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widely distributed dietary fibre source. Some studies on the physiological effects of PD showed that the soluble fibre retarded lipid transport into lymph (Ogata *et al.* 1997), but did not influence glucose absorption (Bamba *et al.* 1993). The effects of PD on mineral absorption have not been evaluated.

## Experimental methods

### *Animals and diets*

Rats used in the experiments were housed individually in stainless-steel cages with mesh bottoms. The cages were placed in a room with controlled temperature (22–24°C), relative humidity (40–60%) and lighting (lights on 08.00–20.00 hours).

This study was approved by the Hokkaido University Animal Committee, and animals were maintained in accordance with the Hokkaido University guidelines for the care and use of laboratory animals.

### *Apparent absorption of calcium in normal and gastrectomized rats (Experiment 1)*

Male Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan), weighing about 100 g (5-weeks-old), were given free access to deionized water and the semi-purified stock diet shown in Table 1 for an acclimatization period of 6–8 d, and were divided into two groups in Experiment 1. Rats in one group (*n* 27) were subjected to total gastrectomy with Roux-en-Y reconstruction (Lambert,

1965), and rats in the second group (*n* 21) were subjected to laparotomy (normal group) under anaesthesia (40 mg pentobarbital sodium/kg body weight; Abbott, North Chicago, IL, USA). In the case of gastrectomized rats, the stomach was removed after ligation of blood vessels, the cut edge of the oesophagus was end-to-side anastomosed to the upper jejunum 8 cm distal from the ligament of Treitz, and a 2 cm segment of the duodenum including the ampulla of Vater was transposed to the jejunum 5 cm from the position of oesophagojejunal anastomosis. After the operations, the rats were deprived of food and water for 24 h, then were fed cows' milk for 3 d followed by an Fe-free basal diet for 12–14 d. Five gastrectomized rats were killed because of surgical damage during recovery and test period.

The normal and gastrectomized rats were divided into three subgroups each using a randomized block design based on body weight after the recovery period. The rats of one subgroup were fed the Fe-free basal diet for a further 21 d. Rats in the other two subgroups were fed the test diet containing GGH (50 g/kg diet; GuarFiber, Meiji Seika Kaisha, Ltd, Tokyo, Japan) and the test diet containing PD (50 g/kg diet; Litesse<sup>®</sup>, Culter Foods Science, Tokyo, Japan) for 21 d after a recovery period. GGH was prepared by partial hydrolysis with  $\beta$ -1,4-mannanase, having an average relative molecular mass of 15 000. PD is a random-bonded polyglucose resistant to digestive enzymes, and the average relative molecular mass of PD is 1500. These fibre materials were added as sources of dietary fibre to the fibre-free basal diet at the expense of the whole diet (basal diet–fibre source, 95:5). Ca in the diet (0.75 mol (3.0 g) Ca/kg diet) was supplied as a water-insoluble Ca salt, CaCO<sub>3</sub>. The Ca content of the test diets was the minimum level required by normal rats of the same strain (Hara *et al.* 1996). Vitamin B<sub>12</sub> (25.6 nmol/kg body weight per d) and Fe (32.2  $\mu$ mol/kg body weight per d) as FeCl<sub>2</sub> (Wako Pure Chemical Industries, Tokyo, Japan) were supplied subcutaneously every 5 d during the recovery and test periods. Body weight and food intakes were measured every day.

Faeces were collected continuously for the last 3 d during feeding of the test diets to evaluate Ca excretion and apparent absorption of Ca. The faeces excreted in the 3 d period were sampled and freeze-dried.

At the end of the experiment, the rats were killed under pentobarbital anaesthesia. The right femur and the caecum were removed. The femur was freeze-dried and weighed. The caecum was removed without loss of its contents, the contents were collected, frozen immediately with liquid N, and stored at –40°C until subsequent analyses. The caecal wall was washed with saline and weighed. The weight of the contents was evaluated by the difference between the weight of the caecum with and without its contents.

### *In vitro calcium absorption in everted sacs of the small intestine (Experiment 2)*

Male Sprague-Dawley rats, weighing about 100 g, were given free access to tap water and the semi-purified stock diet shown in Table 1 for an acclimatization period of more than 7 d and were then starved for 24 h.

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

	Test diets*
Casein†	250
Maize oil‡	50
Mineral mixture (Ca and Fe free)§	27
Calcium carbonate	75
Vitamin mixture¶	10
Granulated vitamin E**	1.0
Choline bitartrate	4.0
Sucrose	to make 1 kg

\* The composition of the stock diet fed during the acclimatization and recovery periods was the same as that of the test diet except for the Ca and Fe concentration. Guar-gum hydrolysate (GuarFiber; Meiji Seika Kaisha Ltd, Tokyo, Japan) and polydextrose (Litesse<sup>®</sup>; Culter Food Science, Tokyo, Japan) were added to the test diet (50 g/kg diet). Crystallized cellulose (Avicel PH102; Asahi Chemical Industry Co. Ltd, Tokyo, Japan; 50 g/kg diet) was added to all the test diets. Fibre sources were added to the test diets at the expense of the whole diet.

† ALACID; New Zealand Dairy Board, Wellington, New Zealand.

‡ 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 was prepared as established by the AIN-76 Workshop held in 1989 (Reeves, 1989), without Ca and Fe. It provided (mg/kg diet): P 2997, K 3746, Mg 375, I 0.32, Mn 10.0, Zn 34.7, Cu 6.00, Na 4279, Cl 6542, Se 1.05, Mo 1.00, Cr 0.50, B 0.50, V 0.25, Sn 2.00, As 1.00, Si 20.0, Ni 1.00, F 2.72, Co 0.20, Fe (100 mg/kg diet) was added to the stock diet fed during the acclimatization period.

|| The Ca concentration was 3.0 g/kg diet in the test diets. For the stock diet, CaCO<sub>3</sub> was added at 11.25 g/kg diet (4.5 g Ca/kg diet).

¶ The vitamin mixture was prepared in accordance with the AIN-76 mixture (American Institute of Nutrition, 1977) except that menadione and L-ascorbic acid were added at 5.81  $\mu$ mol/kg (American Institute of Nutrition, 1980) and 284 mmol/kg (Harper, 1959) diet respectively.

\*\* Vitamin E granules (Juvella; Eisai Co., Tokyo, Japan) supplied 423  $\mu$ mol all-*rac*- $\alpha$ -tocopheryl acetate/kg diet.

To prepare everted sacs, the rats were anaesthetized with sodium pentobarbital and killed. Immediately, three consecutive segments of 3 cm each were dissected from the upper (jejunum) and lower (ileum) half of the small intestine. The intestinal segments were everted with a plastic rod and ligated with surgical silk at one end. An artificial serosal fluid (0.8 ml) was instilled from the other end, which was then ligated. The serosal fluid was 30 mM-Tris-HCl buffer, pH 7.4, containing 125 mM-NaCl, 4 mM-KCl, 10 mM-glucose and 1.25 mM-CaCl<sub>2</sub>, saturated with a mixed gas (O<sub>2</sub>-CO<sub>2</sub> (95:5, v/v)) and warmed to 37°C. The sacs were transferred to individual flasks containing 30 ml gassed (O<sub>2</sub>-CO<sub>2</sub> (95:5, v/v)) and warmed artificial mucosal fluid (30 mM-Tris-HCl buffer, pH 7.4, containing 125 mM-NaCl, 4 mM-KCl, 10 mM-glucose and 10 mM-CaCl<sub>2</sub>). PD and GGH were added to the mucosal fluid up to 50 g/l. Sacs were incubated for 30 min in a water bath at 37°C shaken at 110 r.p.m. We observed linear increases in Ca absorption by the gut sacs for 30 min (data not shown). After collection of the serosal fluid, length of sacs between both ligations were measured to calculate Ca absorption rate by the sacs.

#### Analytical methods

Freeze-dried faeces were milled to very fine powder. The powdered faeces (about 70 mg) and dried right femur were carefully wet-ashed in a mixture of 10 M-HNO<sub>3</sub> and 2.3 M-HClO<sub>4</sub> without drying up. The caecal contents diluted with nine volumes deionized water were homogenized by means of a Teflon homogenizer. Amounts of total Ca in the homogenates were measured after the samples had been wet-ashed in the same way as for the faeces. Soluble Ca was assayed in the supernatant obtained upon centrifugation (30 000 g for 20 min) of the homogenate. Ca concentrations in the ashed solutions were measured by atomic absorption spectrophotometry (AA-6400F; Shimadzu Corporation, Kyoto, Japan) after adequate dilution with water after addition of strontium chloride solution (final concentration 57 mmol/l). Although we assayed a relatively small sample of dried faeces (70 mg), the CV of the measurement was 3.4%. Recovery of Ca in the diet was 97.3 (SE 0.7)%. The Ca concentration of the artificial serosal fluid in the everted sac was measured by a commercial kit (Calcium-C test; Wako Pure Chemical Industries, Osaka, Japan).

Concentrations of short-chain fatty acids (acetic, propionic and butyric acids) in the homogenate of the caecal contents were evaluated by a method described previously (Hara *et al.* 1994). Individual short-chain fatty acids were measured by GLC (Shimadzu GC-14A, with a prepacked glass column (1600 mm × 3 mm, SP-1220 + H<sub>3</sub>PO<sub>4</sub> (15%+1% respectively) on 80–100 mesh Chromosorb W-AW DMCS; Shimadzu Corporation) after adding phosphoric acid (final concentration 0.67 mol/l).

#### Calculations and statistical analysis

The apparent absorption of Ca was calculated as follows: apparent Ca absorption (%) = 100 × (total Ca intake – faecal Ca excretion)/total Ca intake.

The rate of Ca absorption by the everted sacs was expressed as the net increase in the amount of Ca in the artificial serosal fluid per cm intestinal segment per h.

The results were analysed by two-way ANOVA (Gastrectomy × Dietary fibre) in Experiment 1 and one-way ANOVA in Experiment 2. Duncan's multiple range test was used to determine whether mean values were significantly different (Duncan, 1995;  $P < 0.05$ ). These statistical analyses were done by the GLM procedure of the Statistical Analysis System program (version 6.07, SAS Institute Inc., Cary, NC, USA).

## Results

### Apparent calcium absorption in normal and gastrectomized rats (Experiment 1)

Food intakes were similar in all groups (Table 2), however, body-weight gains were lower in each diet group in gastrectomized rats than the corresponding diet group in normal rats. In the gastrectomized rats, the mean value for the PD group was higher than that of the basal-diet group. Faecal dry weight excreted for the last 3 d was higher in gastrectomized rats fed PD than in rats of the other groups.

Apparent Ca absorption for the last 3 d of the test period was strikingly lower in the gastrectomized rats than in the normal rats fed the basal diet, and the reduced absorption caused by gastrectomy was doubled in rats fed GGH and PD. The Ca absorption of the PD group was higher than those of the other two groups in the normal rats (Fig. 1).

Femur dry weights and Ca concentrations in the femurs were lower in the all gastrectomized groups than in the normal groups (Fig. 2). Femur Ca concentrations were higher in the PD group than in the basal and GGH group for the normal and gastrectomized rats respectively.

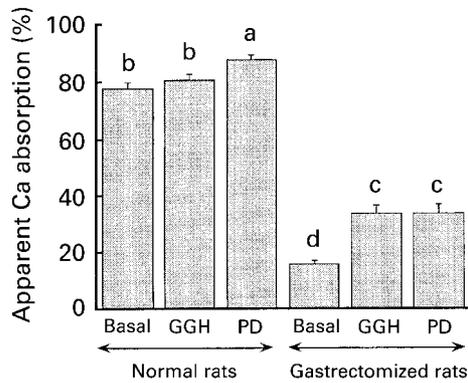
Total Ca pool in the caecal contents was much higher,

**Table 2.** Body-weight gain (g/d) and food intake (g/d) during feeding of test diets for 21 d and faecal dry weight (g/3 d) for the last 3 d in the test period of normal (laparotomized) and gastrectomized rats fed diets with and without addition of guar-gum hydrolysate (GGH) or polydextrose (50 g/kg diet)\*

Diet	n	Body weight gain		Food intake		Faecal dry weight	
		Mean	SE	Mean	SE	Mean	SE
		(Mean values with their standard errors)					
Normal							
Basal	7	2.48 <sup>ab</sup>	0.090	10.6	0.01	2.13 <sup>b</sup>	0.058
GGH	7	2.66 <sup>ab</sup>	0.054	10.6	0.02	2.32 <sup>b</sup>	0.060
Polydextrose	7	2.77 <sup>a</sup>	0.107	10.6	0.01	2.29 <sup>b</sup>	0.107
Gastrectomized							
Basal	7	1.90 <sup>d</sup>	0.118	11.0	0.51	2.42 <sup>b</sup>	0.137
GGH	8	2.22 <sup>cd</sup>	0.146	10.3	0.52	2.36 <sup>b</sup>	0.192
Polydextrose	7	2.42 <sup>bc</sup>	0.211	11.0	0.46	2.97 <sup>a</sup>	0.174
Statistical significance (ANOVA) of effect of:							
Gastrectomy (GX)		$P = 0.017$		NS		$P = 0.002$	
Dietary fibre (DF)		$P < 0.001$		NS		$P = 0.018$	
GX × DF		NS		NS		NS	

<sup>a,b,c,d</sup>Mean values within a column with unlike superscript letters were significantly different ( $P < 0.05$ ; NS  $P \geq 0.05$ ; Duncan's multiple range test).

\* For details of diets and procedures, see Table 1 and p. 656.



**Fig. 1.** Apparent calcium absorption in normal and gastrectomized rats fed basal diet (Ba) or a guar-gum hydrolysate (GGH)- or polydextrose (PD)-containing diet (soluble fibre sources, 50 g/kg diet) 21 d after the start of feeding the test diets in Experiment 1. For details of diets and procedures, see Table 1 and p. 656. Values are means with their standard errors for seven rats except for GGH-fed gastrectomized group (eight rats). *P* values estimated by two-way ANOVA were <0.001 for Gastrectomy (GX) and Dietary fibre (DF), and 0.002 for GX × DF. <sup>a,b,c,d</sup>Mean values with unlike superscript letters were significantly different between groups (*P* < 0.05).

and soluble Ca was much lower in the gastrectomized rats than in the normal rats (Table 3). Feeding GGH and PD did not influence the soluble Ca concentration of the caecal contents in the normal rats. In gastrectomized rats the concentration in the GGH group was significantly higher than those gastrectomized rats fed the basal or PD diet (*P* = 0.005).

The weights of the caecal walls and the caecal contents were greater and pH of the contents was lower in both

fibre-fed groups than in the basal-diet group in the normal and gastrectomized rats (Table 4). Within the fibre-fed groups, pH in the PD group was lower than that in the GGH group in normal and gastrectomized rats.

Concentration of acetic, propionic and butyric acids in the caecal contents are shown in Table 5. In normal and gastrectomized rats fed PD, these major short-chain fatty acid concentrations were clearly lower than those in rats fed basal and GGH-containing diets except for butyrate in normal rats.

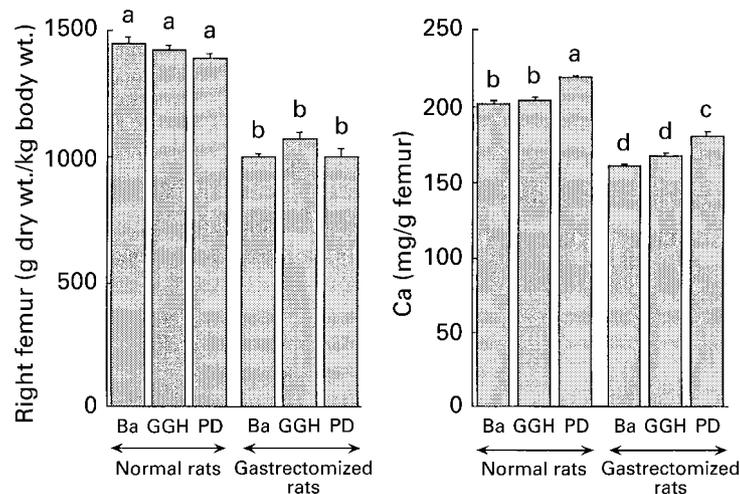
#### Calcium absorption by everted sacs of the small intestine (Experiment 2)

*In vitro* Ca absorption by everted sacs of the ileum was significantly increased (*P* < 0.05) by addition of PD to the mucosal fluid, but not by addition of GGH compared with the absorption from the no-fibre fluid (Fig. 3). The Ca absorption of the jejunal sacs changed by addition of both fibres similarly to those of the ileal sacs, but the changes were not significantly different.

#### Discussion

Body-weight gains in gastrectomized rats were lower than those in normal rats in spite of the fact that food intakes were similar between both rats. Faecal outputs were not significantly increased by gastrectomy except for rats fed PD. The results suggest that some metabolic changes, rather than impaired nutrient absorption, is involved in the growth retardation in the gastrectomized rats.

In the present study, we examined the effects of a less



**Fig. 2.** Femur dry weight and calcium concentration in the femur in normal and gastrectomized rats fed basal diet (Ba), or a guar-gum hydrolysate (GGH)- or polydextrose (PD)-containing diet (soluble fibre sources, 50 g/kg diet) 21 d after the start of feeding the test diets in Experiment 1. For details of diets and procedures, see Table 1 and p. 656. Values are means with their standard errors for seven rats except for GGH-fed gastrectomized group (eight rats). *P* values estimated by two-way ANOVA for femur dry weight were <0.001 for Gastrectomy (GX), NS for Dietary fibre (DF), and NS for GX × DF; for calcium concentration the *P* values were <0.001 for Gastrectomy (GX) and Dietary fibre (DF), and NS for GX × DF, (NS, *P* ≥ 0.05). <sup>a,b,c,d</sup>Mean values with unlike superscript letters were significantly different between groups (*P* < 0.05).

**Table 3.** The total and soluble calcium pools ( $\mu\text{mol/g}$  wet caecal contents) and soluble calcium concentration ( $\mu\text{mol/g}$  wet caecal contents) in the caecal contents of normal (laparotomized) and gastrectomized rats fed diets with and without addition of guar-gum hydrolysate (GGH) or polydextrose (50 g/kg diet) for 21 d\*  
(Mean values with their standard errors)

Diet	n	Total Ca pool		Soluble Ca pool		Soluble Ca concentration	
		Mean	SE	Mean	SE	Mean	SE
<b>Normal</b>							
Basal	7	237 <sup>d</sup>	22.2	11.2 <sup>bc</sup>	1.32	3.15 <sup>a</sup>	0.355
GGH	7	337 <sup>cd</sup>	29.7	15.7 <sup>b</sup>	4.65	2.10 <sup>ab</sup>	0.817
Polydextrose	7	400 <sup>cd</sup>	65.6	28.1 <sup>a</sup>	8.14	2.23 <sup>ab</sup>	0.500
<b>Gastrectomized</b>							
Basal	7	675 <sup>bc</sup>	46.8	0.926 <sup>c</sup>	0.115	0.323 <sup>c</sup>	0.050
GGH	8	1036 <sup>b</sup>	164	7.39 <sup>bc</sup>	2.10	1.37 <sup>bc</sup>	0.351
Polydextrose	7	1655 <sup>a</sup>	183	5.39 <sup>bc</sup>	1.28	0.564 <sup>c</sup>	0.108
Statistical significance (ANOVA) of effect of:							
Gastrectomy (GX)		$P < 0.001$		$P < 0.001$		$P < 0.001$	
Dietary fibre (DF)		$P < 0.001$		$P = 0.040$		NS	
GX $\times$ DF		$P = 0.002$		NS		NS	

<sup>a,b,c,d</sup>Mean values within a column with unlike superscript letters were significantly different ( $P < 0.05$ ; NS  $P \geq 0.05$ ; Duncan's multiple range test).  
\* For details of diets and procedures, see Table 1 and p. 656.

**Table 4.** Weight of the caecal wall (g wet weight/kg body weight) and caecal contents (g wet weight/rat), and the pH of the caecal contents of normal (laparotomized) and gastrectomized rats fed diets with and without addition of guar-gum hydrolysate (GGH) or polydextrose (50 g/kg diet) for 21 d\*  
(Mean values with their standard errors)

Diet	n	Caecal wall		Caecal contents		pH	
		Mean	SE	Mean	SE	Mean	SE
<b>Normal</b>							
Basal	7	2.67 <sup>c</sup>	0.07	3.59 <sup>c</sup>	0.19	7.26 <sup>a</sup>	0.058
GGH	7	4.31 <sup>b</sup>	0.18	8.25 <sup>b</sup>	0.43	6.93 <sup>b</sup>	0.065
Polydextrose	7	4.48 <sup>ab</sup>	0.22	12.1 <sup>a</sup>	0.86	6.29 <sup>cd</sup>	0.142
<b>Gastrectomized</b>							
Basal	7	3.06 <sup>c</sup>	0.09	3.04 <sup>c</sup>	0.21	7.07 <sup>ab</sup>	0.049
GGH	8	4.30 <sup>b</sup>	0.18	6.93 <sup>b</sup>	0.73	6.33 <sup>c</sup>	0.142
Polydextrose	7	5.17 <sup>a</sup>	0.34	9.84 <sup>b</sup>	0.98	6.06 <sup>d</sup>	0.075
Statistical significance (ANOVA) of effect of:							
Gastrectomy (GX)		NS		$P = 0.039$		$P < 0.001$	
Dietary fibre (DF)		$P < 0.001$		$P < 0.001$		$P < 0.001$	
GX $\times$ DF		NS		NS		NS	

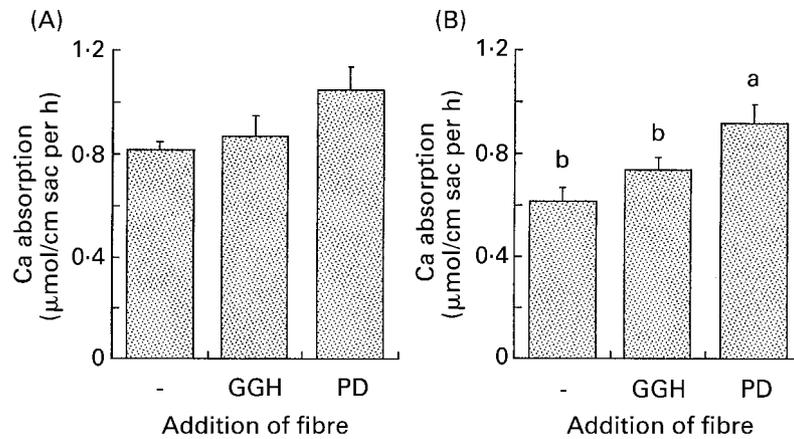
<sup>a,b,c,d</sup>Mean values within a column with unlike superscript letters were significantly different ( $P < 0.05$ ; NS  $P \geq 0.05$ ; Duncan's multiple range test).  
\* For details of diets and procedures, see Table 1 and p. 656.

fermentable and soluble dietary fibre, PD, on Ca absorption in rats. We showed that total gastrectomy strikingly reduced apparent Ca absorption (Fig. 1) and bone mineralization (Fig. 2). The results show that gastrectomy induced severe Ca deficiency in rats. Feeding PD partially restored the reduction of Ca absorption by total gastrectomy, and the effect was similar to that of GGH. Femur Ca of gastrectomized rats was also restored in the PD-fed group, but not in GGH-fed group. In normal rats, apparent Ca absorption and the bone Ca concentration in the rats fed PD were higher than those in rats fed the basal diet, but not in the rats fed GGH. These findings suggest that the mechanism for the enhancement of Ca absorption by PD feeding is different from that by GGH feeding. In the previous study, we suggested that feeding GGH restores reduced Ca absorption with gastrectomy in the large

intestine (Hara *et al.* 1999). Both the large intestine and small intestine are possibly responsible for the increases in Ca absorption with feeding PD.

Solubilization of Ca in acidified caecal contents by intestinal fermentation is a factor for increasing Ca absorption (Younes *et al.* 1996). The soluble Ca concentration in the caecum was not increased by feeding of PD both in normal and gastrectomized rats (Table 3). In contrast, GGH feeding markedly increased the soluble Ca concentration in the caecal contents of gastrectomized rats. These results reveal that the soluble Ca concentration in the caecal contents is not involved in the effect of PD on Ca absorption. Caecal pH was the lowest in normal and gastrectomized rats after feeding PD (Table 4). These results agree with a previous study on PD (Yoshioka *et al.* 1994). Lower caecal pH usually causes higher solubilization of Ca salts, however, this relationship between pH and soluble Ca was not observed in the present study. The results indicates that lower pH does not usually increase soluble Ca concentration in the caecum. The reason for the low solubilization of Ca in spite of low pH in the caecal contents is not known.

Fermentation products in the large intestine is another effective factor that increases Ca absorption. It is believed that fermentability of PD is relatively low. Available energy from PD for human subjects (Achour *et al.* 1994) or rats (Juhr & Franke 1992) was 4–6 kJ/g PD. The value is comparable with that of cellulose in rats (Juhr & Franke, 1992). In the present study, the higher output of faeces in gastrectomized rats fed PD than in rats fed GGH (Table 2) and the low concentration of short-chain fatty acids in the caecal contents (Table 5) indicate low fermentability of PD compared with GGH. We previously suggested that caecal fermentation products, especially propionic acid, are involved in the enhancement of Ca absorption in gastrectomized rats fed GGH. Trinidad *et al.* (1996) showed propionate administration increases Ca absorption in the large intestine. In the present study, caecal propionic acid concentration was much higher in gastrectomized rats fed GGH than in any other group. However, the caecal



**Fig. 3.** Calcium absorption by everted jejunal (A) and ileal (B) sacs of acclimatized rats fed on the stock diet for more than 7 d in Experiment 2. Guar-gum hydrolysate (GGH) and polydextrose (PD) were added to mucosal medium (50 g/l). For details of diets and procedures, see Table 1 and p. 656. Values are means with their standard errors for five rats per group. *P* values estimated by one-way ANOVA were NS and 0.011 for jejunal and ileal sac respectively. <sup>a,b</sup>Mean values with unlike superscript letters were significantly different between groups (*P* < 0.05).

**Table 5.** Concentrations of short-chain fatty acids ( $\mu\text{mol/g}$  wet caecal contents) of normal (laparotomized) and gastrectomized rats fed diets with and without addition of guar-gum hydrolysate (GGH) or polydextrose (50 g/kg diet) for 21 d\*  
(Mean values with their standard errors)

Diet	<i>n</i>	Acetic acid		Propionic acid		Butyric acid	
		Mean	SE	Mean	SE	Mean	SE
<b>Normal</b>							
Basal	7	54.1 <sup>ab</sup>	4.65	22.4 <sup>b</sup>	1.55	11.5 <sup>a</sup>	0.78
GGH	7	42.8 <sup>bc</sup>	2.16	25.8 <sup>b</sup>	1.32	4.35 <sup>b</sup>	0.639
Polydextrose	7	21.1 <sup>d</sup>	2.04	12.0 <sup>c</sup>	0.90	2.51 <sup>b</sup>	0.522
<b>Gastrectomized</b>							
Basal	7	61.8 <sup>a</sup>	5.72	22.7 <sup>b</sup>	3.54	14.0 <sup>a</sup>	1.12
GGH	8	74.1 <sup>ab</sup>	9.66	50.8 <sup>a</sup>	7.09	7.83 <sup>a</sup>	3.03
Polydextrose	7	29.3 <sup>cd</sup>	2.69	19.9 <sup>b</sup>	0.89	3.94 <sup>b</sup>	0.602
Statistical significance (ANOVA) of effect of:							
Gastrectomy (GX)		<i>P</i> = 0.010		<i>P</i> = 0.003		<i>P</i> = 0.009	
Dietary fibre (DF)		<i>P</i> < 0.001		<i>P</i> < 0.001		<i>P</i> < 0.001	
GX $\times$ DF		NS		<i>P</i> = 0.019		NS	

<sup>a,b,c,d</sup>Mean values within a column with unlike superscript letters were significantly different (*P* < 0.05; NS *P*  $\geq$  0.05; Duncan's multiple range test).

\* For details of diets and procedures, see Table 1 and p. 656.

propionic acid concentration in the PD group was lower even than in the basal group in normal and gastrectomized rats. Acetic acid concentration in the PD-fed groups was also lower than that for the other two diet groups in normal and gastrectomized rats. These results suggest that caecal fermentation is not greatly involved in the increase of Ca absorption on feeding PD. We did not observe the fermentation in the colon; however, dietary fibre is mainly fermented in the caecum in rats.

As described earlier, we did not find any contribution of the caecum to the increase of Ca absorption after feeding PD in normal and gastrectomized rats. We showed that PD, but not GGH, enhanced Ca absorption of the ileal segment in Experiment 2 (Fig. 3). From these results, we speculate

that the small intestine largely contributes to the enhancement of Ca absorption by feeding PD in normal and gastrectomized rats. Feeding of GGH may increase Ca absorption in the large intestine in the gastrectomized rats by increasing fermentation products.

In conclusion, PD feeding increased Ca absorption and bone mineralization in normal and gastrectomized rats. Small intestinal absorption may be involved in these beneficial effects of PD. The PD feeding improved bone mineralization impaired by gastrectomy. Ingestion of PD also increased bone Ca concentration in normal rats, which may be relevant for decreasing the risk of osteoporosis.

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