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

Hiroshi Hara*, Takuya Suzuki and Yoritaka Aoyama

Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

(Received 20 September 1999 – Revised 22 March 2000 – Accepted 27 March 2000)

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 ileal intestine be involved in the restoration of Ca absorption (Hara et al. 1998). 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.

* Corresponding author: Dr Hiroshi Hara, fax +81 11 706 2504 or +81 11 716 0879, email hara@chem.agr.hokudai.ac.jp
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†, Cultor Foods Science, Tokyo, Japan) for 21 d after a recovery period. GGH was prepared by partial hydrolysis with β-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, CaCO3. The Ca content of the test diets was the minimum level required by normal rats of the same strain (Hara et al., 1996). Vitamin B12 (25.6 nmol/kg body weight per d) as FeCl2 (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.

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

<table>
<thead>
<tr>
<th>Test diets*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein†</td>
<td>250</td>
</tr>
<tr>
<td>Maize oil‡</td>
<td>50</td>
</tr>
<tr>
<td>Mineral mixture (Ca and Fe free)§</td>
<td>27</td>
</tr>
<tr>
<td>Calcium carbonate¶</td>
<td>75</td>
</tr>
<tr>
<td>Vitamin mixture¶</td>
<td>10</td>
</tr>
<tr>
<td>Granulated vitamin E**</td>
<td>1-0</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>4.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>to make 1 kg</td>
</tr>
</tbody>
</table>

* 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 (GuarFibre; Meiji Seika Kaisha Ltd, Tokyo, Japan) and polydextrose (Litesse†; Cultor 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 μmol/kg diet) and ergocalciferol (0.0504 μmol/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, CI 6542, Se 1-05, Mo 1-00, Cr 0-50, B 0-80, 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) as FeCl2 and stored at −30°C until subsequent analyses. The mineral 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 581 μmol/kg (American Institute of Nutrition, 1980) and 284 mmol/kg (Harper, 1959) diet respectively.
¶ 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 581 μmol/kg (American Institute of Nutrition, 1980) and 284 mmol/kg (Harper, 1959) diet respectively.
** Vitamin E granules (Juvela; Eisai Co., Tokyo, Japan) supplied 423 μmol α-tocopheryl acetate/kg diet.

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.
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-CaCl₂, saturated with a mixed gas (O₂–CO₂ (95:5, v/v)) and warmed to 37°C. The sacs were transferred to individual flasks containing 30 ml of artificial mucosal fluid (30 mM-Tris–HCl buffer, pH 7.4, containing 125 mM-NaCl, 4 mM-KCl, 10 mM-glucose and 10 mM-CaCl₂). PD and GGH were added to the mucosal fluid up to 1 M-KCl, 10 mM-glucose and 1.25 mM-CaCl₂, saturated with a Tris–HCl buffer, pH 7.4, containing 125 mM-NaCl, 4 mM-KCl, 10 mM-glucose and 10 mM-CaCl₂. PD and GGH were added to the mucosal fluid up to 1 M-KCl, 10 mM-glucose and 1.25 mM-CaCl₂, saturated with a Tris–HCl buffer, pH 7.4, containing 125 mM-NaCl, 4 mM-KCl, 10 mM-glucose and 10 mM-CaCl₂. The 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-HNO₃ and 2.3 M-HClO₄ 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, 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 + H₃PO₄ (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 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,
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 \( P \leq 0.005 \).

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)

\textit{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...
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. 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

Table 3. The total and soluble calcium pools (μmol/g wet caecal contents) and soluble calcium concentration (μmol/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)

<table>
<thead>
<tr>
<th>Diet</th>
<th>n</th>
<th>Mean ± SE</th>
<th>Mean ± SE</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>7</td>
<td>237 ± 7</td>
<td>11.2 ± 2c</td>
<td>3.15 ± 4a</td>
</tr>
<tr>
<td>GGH</td>
<td>7</td>
<td>337 ± 7bc</td>
<td>15.7 ± 5b</td>
<td>2.10 ± 6ab</td>
</tr>
<tr>
<td>Polydextrose</td>
<td>7</td>
<td>400 ± 7d</td>
<td>28.1 ± 8a</td>
<td>2.23 ± 6ab</td>
</tr>
<tr>
<td>Gastrectomized</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>7</td>
<td>675 ± 7c</td>
<td>0.926 ± 0c</td>
<td>0.323 ± 0d</td>
</tr>
<tr>
<td>GGH</td>
<td>8</td>
<td>1036 ± 7b</td>
<td>7.39 ± 2c</td>
<td>1.37 ± 3bc</td>
</tr>
<tr>
<td>Polydextrose</td>
<td>7</td>
<td>1655 ± 7a</td>
<td>5.39 ± 1c</td>
<td>0.564 ± 1d</td>
</tr>
</tbody>
</table>

Statistical significance (ANOVA) of effect of:
- Gastrectomy (GX) P < 0.001
- Dietary fibre (DF) P < 0.001
- GX × DF P = 0.002

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)

<table>
<thead>
<tr>
<th>Diet</th>
<th>n</th>
<th>Mean ± SE</th>
<th>Mean ± SE</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>7</td>
<td>2.67 ± 0c</td>
<td>3.59 ± 0c</td>
<td>7.26 ± 0c</td>
</tr>
<tr>
<td>GGH</td>
<td>7</td>
<td>4.31 ± 0b</td>
<td>8.25 ± 0b</td>
<td>6.93 ± 0b</td>
</tr>
<tr>
<td>Polydextrose</td>
<td>7</td>
<td>4.48 ± 0b</td>
<td>12.1 ± 0a</td>
<td>6.29 ± 0c</td>
</tr>
<tr>
<td>Gastrectomized</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>7</td>
<td>3.06 ± 0c</td>
<td>3.04 ± 0c</td>
<td>7.07 ± 0b</td>
</tr>
<tr>
<td>GGH</td>
<td>8</td>
<td>4.30 ± 0b</td>
<td>6.93 ± 0b</td>
<td>6.32 ± 0c</td>
</tr>
<tr>
<td>Polydextrose</td>
<td>7</td>
<td>5.17 ± 0a</td>
<td>9.84 ± 0a</td>
<td>6.06 ± 0b</td>
</tr>
</tbody>
</table>

Statistical significance (ANOVA) of effect of:
- Gastrectomy (GX) P = 0.039
- Dietary fibre (DF) P < 0.001
- GX × DF NS

a,b,c,dMean values within a column with unlike superscript letters were significantly different (P < 0.05; NS P ≥ 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.

References

Hara H, Nagata M, Ohta A & Kasai T (1996) Increases in calcium absorption with ingestion of soluble dietary fibre, guar-gum...
hydrolysate, depend on the caecum in partially nephrectomized and normal rats. *British Journal of Nutrition* 76, 773–784.


© Nutrition Society 2000