Increased Intestinal Calcium Absorption from the Ingestion of a Phosphorylated Guar Gum Hydrolysate Independent of Cecal Fermentation in Rats

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The apparent calcium absorption was increased in rats fed on P-GGH and GGH. However, this increase in calcium absorption from GGH feeding was cancelled by a cecectomy, whereas the corresponding increase from P-GGH feeding was not. The change in femoral calcium content was similar to that in calcium absorption. The calcium solubility in the ileum was increased in those rats fed on P-GGH. We conclude that cecal fermentation did not contribute to the increased calcium absorption by the rats fed on P-GGH.

Key words: calcium; rat; absorption; guar gum; cecal fermentation

Such bone disorders as osteoporosis, fracture, and increased backache accompany aging. Insufficient calcium intake,1-5 a decrease of calcium absorptive capacity and hormone imbalance after menopause3-7 cause these disorders. Osteoporosis after menopause results in the balance of ossification and bone resorption not being maintained in the body, and an increase in the calcium supply is necessary to improve this situation. We have prepared phosphorylated water-soluble dietary fiber as a phosphorylated guar gum hydrolysate (P-GGH) that has phosphate residues in its fiber structure, and have already examined the effect of P-GGH on calcium absorption in rats in comparison with that of GGH.8 The results of the previous study suggested that the increase in apparent calcium absorption by rats fed on P-GGH may have been the result of increased calcium solubility in the lower part of the small intestine. We have previously demonstrated that the increased calcium absorption with the ingestion of GGH depended on the cecum.9 Moreover, it has been reported that increased calcium absorption from feeding soluble fiber or oligosaccharides was associated with cecal fermentation.10-13 The aim of this present study is to determine whether cecal fermentation contributes to enhanced calcium absorption when feeding P-GGH by using cecctomized rats (CCX).

GGH is a partial hydrolysate of guar-gum produced by β-1,4-mannanase (EC 3.2.1.78) and has an average molecular weight of 15,000. P-GGH was prepared by chemically modifying GGH which was phosphorylated by a procedure based on the method previously used to phosphorylate soy protein.14 A GGH solution (10% (w/v)) was boiled for about 5 min, and powdered sodium metaphosphate (Kanto Chemical Co., Tokyo, Japan) was then added to the solution. The mixture was continuously stirred at 45℃ for 4 h. During the reaction, the pH value was kept constant at 12.5 by titrating with 16.7 m NaOH by using a pH stabilizer. After the reaction, the solution was dialyzed for about 24 h with a dialysis membrane (M.W. of 8,000), and freeze-dried.

Male Sprague Dawley rats (Japan SLC, Hamamatsu, Japan), weighing about 100 g, were given free access to tap water and a stock diet (Table 1) for one week.

The rats were divided into six groups of six or eight rats based on their body weight.

Three groups of rats (n = 8) were operated on for a cecectomy under anesthesia with sodium pentobarbital (Nembutal; 50 mg/ml of sodium pentobarbital; Abbott, North Chicago, IL, U.S.A). The cecum was excised after ligating the cecal sac. The other three groups of rats (n = 6) were only given an abdominal incision as a sham operation. The body temperature was maintained at 37-40℃ throughout the operations by a warmed surgical plate. All the rats were given free access to tap water and a stock diet for 5 days to recover.

Paired groups (CCX and Sham) were fed on a control diet, or on one of two test diets containing either GGH or P-GGH (50 g/kg of diet) for 10 days (Table 1).

Note

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Abbreviations: GGH, guar gum hydrolysate; P-GGH, phosphorylated guar gum hydrolysate

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Table 1. Diet Composition (g/kg of Diet)

<table>
<thead>
<tr>
<th></th>
<th>Stock diet</th>
<th>Control diet</th>
<th>GGH diet</th>
<th>P-GGH diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>250.0</td>
<td>237.5</td>
<td>225.0</td>
<td>225.0</td>
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<tr>
<td>Sucrose</td>
<td>645.0</td>
<td>612.7</td>
<td>580.5</td>
<td>580.5</td>
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<tr>
<td>Corn oil</td>
<td>50.0</td>
<td>47.5</td>
<td>45.0</td>
<td>45.0</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>40.0</td>
<td>38.0</td>
<td>36.0</td>
<td>36.0</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>11.0</td>
<td>10.4</td>
<td>9.9</td>
<td>9.9</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>4.0</td>
<td>3.8</td>
<td>3.6</td>
<td>3.6</td>
</tr>
<tr>
<td>Cellulose</td>
<td>—</td>
<td>50.1</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>GGH</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>P-GGH</td>
<td>—</td>
<td>—</td>
<td>50.0</td>
<td>50.0</td>
</tr>
</tbody>
</table>

GGH, guar gum hydrolysate; P-GGH, phosphorylated guar gum hydrolysate.

The maximum breaking force of the left femur was measured as the bone strength by a rheometer (RE-3305 RHEONER, Yamaden, Tokyo, Japan) under the following conditions: load cell, 20 kgf; pranger, L-50; pranger speed, 0.5 mm/sec.; method, down stroke.

Each data value is expressed as the mean ± SE. The results were analyzed by two-way ANOVA, and the significance of differences between groups was evaluated by Duncan's multiple-range test (P<0.05).

The final body weight, body weight gain and food intake were not significantly different among the six groups (data not shown). The apparent calcium absorption (%) during the first and second periods after administering the experimental diets is shown in Fig. 1. The calcium absorption was higher in both the sham and CCX rats fed on P-GGH and in the sham rats fed on GGH than in the sham and CCX rats fed on the control diet and in the CCX rats fed on GGH during the first period. These results show that the increased calcium absorption with GGH feeding in this experiment depended on cecal fermentation; however, the increase in calcium absorption with P-GGH feeding did not depend on the cecal fermentation. The calcium absorption was not significantly different among the six groups during the second period. This might have resulted from downregulation of the absorptive system during the second period. The calcium solubility in the ileum was significantly higher in the rats fed on P-GGH than in the rats fed on the control or GGH diet (Table 2). This suggests that calcium absorption from P-GGH depended on the increased calcium solubility of the ileal contents (pH: control, 6.8±0.1; GGH, 6.7±0.1; P-GGH, 6.4±0.1; P<0.001). The calcium content in the femur was significantly higher in the sham and CCX rats fed on P-GGH and in the sham rats fed on GGH than in the other groups (Fig. 2-A). These results are similar to those for the apparent calcium absorption during the
Ca Absorption Independent of Cecal Fermentation

**Fig. 1.** Apparent Calcium Absorption by a Balance Test in Rats.
Graph A shows the result for the first period (d3-d5), and graph B shows the result for the second period (d8-d10). The rats were fed on the control diet, GGH diet or P-GGH diet. Each value is the mean for eight (cecectomized, □) or six (sham, ■) rats with SE shown as vertical bars. Values not sharing a common superscript are significantly different at $P<0.05$.

**Fig. 2.** Calcium Content and Maximum Breaking Force of the Femur.
Graph A shows the result for the calcium content of the femur. The right femur was used for determining the calcium content. Graph B shows the result for the maximum breaking force of the femur. The left femur was used for determining the bone strength. The rats were fed on the control diet, GGH diet or P-GGH diet. Each value is the mean for eight (cecectomized, □) or six (sham, ■) rats with SE shown as vertical bars. Values not sharing a common superscript are significantly different at $P<0.05$.

In conclusion, cecal fermentation did not contribute to the increase in apparent calcium absorption with P-GGH feeding. We presume that the increase in calcium absorption was the result of increased calcium solubility in the lower part of the small intestine.

first period. The higher level of calcium may have been stored as calcium phosphate in the femur in the case of the P-GGH groups and in the sham rats fed on GGH. The femur strength tended to be higher in the P-GGH group than in the GGH and control groups (Fig. 2-B).
Table 2. Concentrations of Soluble Calcium and Total Calcium and Calcium Solubility and pH of the Ileal Content of Rats

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Soluble Ca (μmol/g of contents)</th>
<th>Total Ca (μmol/g of contents)</th>
<th>Ca Solubility (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>238.1 ± 49.0^a</td>
<td>941.3 ± 79.3</td>
<td>25.7 ± 4.7^a</td>
<td>6.8 ± 0.1^a</td>
</tr>
<tr>
<td>Sham</td>
<td>234.3 ± 34.9^a</td>
<td>933.6 ± 74.3</td>
<td>24.5 ± 2.1^a</td>
<td>6.8 ± 0.1^a</td>
</tr>
<tr>
<td>GGH</td>
<td>258.5 ± 21.7^b</td>
<td>960.0 ± 78.1</td>
<td>27.3 ± 0.8^a</td>
<td>6.7 ± 0.1^ab</td>
</tr>
<tr>
<td>Sham</td>
<td>252.3 ± 46.8^a</td>
<td>984.9 ± 150.1</td>
<td>26.5 ± 3.5^a</td>
<td>6.7 ± 0.1^ab</td>
</tr>
<tr>
<td>P-GGH</td>
<td>327.0 ± 35.7^ab</td>
<td>951.8 ± 123.7</td>
<td>35.4 ± 2.6^a</td>
<td>6.5 ± 0.0^bc</td>
</tr>
<tr>
<td>Sham</td>
<td>395.1 ± 19.5^c</td>
<td>1111.0 ± 57.3</td>
<td>35.9 ± 2.2^a</td>
<td>6.3 ± 0.1^c</td>
</tr>
</tbody>
</table>

P values by ANOVA

- Diet (A): $P = 0.003$
- Operation (B): $P = 0.524$
- A × B: $P = 0.520$

GGH, guar gum hydrolysate; P-GGH, phosphorylated GGH.

Each value is the mean ± SE. Values in the same row not sharing a common superscript are significant different at $P < 0.05$ by two-way ANOVA.

References