The Large Intestine Compensates for Insufficient Calcium Absorption in the Small Intestine in Rats

Kazuki Shiga, Hiroshi Hara* and Takanori Kasai

Laboratory of Nutritional Biochemistry,
Department of Bioscience and Chemistry, Faculty of Agriculture,
Hokkaido University, Sapporo 060-8589, Japan
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Summary We previously demonstrated that the large intestine compensated for decreased calcium (Ca) absorption caused by renal failure in rats fed a highly fermentable dietary fiber. In this study, we examined whether the large intestine compensated for insufficient Ca absorption in the rat small intestine without ingestion of a fermentable dietary fiber. Rats were fed one of four test diets containing either insoluble (carbonate) or soluble (gluconate, lactate, or citrate) Ca sources. The dietary Ca level was 2.0 g/kg, which is lower than the minimum requirement for rats (3.0 g/kg), to conduct the present study under a condition in which rats can maximally absorb Ca. To prevent Ca absorption in the small intestine, we replaced a primary phosphate (KH₂PO₄) with secondary phosphates (K₂HPO₄ and Na₂HPO₄) in diets. The apparent Ca absorption in the small intestine was estimated by adding chromic oxide (Cr₂O₃) as an insoluble and an unabsorbed marker to test diets and by measuring the ratio of Ca:Cr in the cecal content. The apparent Ca absorption in the whole intestine was estimated by the intake and fecal excretion of Ca. The apparent Ca absorption in the small intestine was significantly lower from the Ca carbonate diet than from the Ca gluconate, lactate, or citrate diets. The apparent Ca absorption in the whole intestine was not significantly different among the four groups, and the values were similar to the absorption rates in the small intestines of rats fed diets containing soluble Ca sources. These results show the following: (a) In rats fed 0.2% Ca diets containing soluble Ca salts, Ca is mostly absorbed in the small intestine, even in secondary phosphate intakes; (b) In contrast, in rats fed a 0.2% Ca diet containing an insoluble Ca salt (carbonate), Ca is not sufficiently absorbed in the small intestine. However, the large intestine compensates for the small intestinal Ca absorption decreased by dietary secondary phosphates.

Key Words calcium, calcium absorption, large intestine, rats

*To whom correspondence should be addressed.
Dietary calcium (Ca) is dissolved in the stomach and mainly absorbed in the small intestine by active and passive transports alike. The active transport of Ca in the small intestine depends on vitamin D activated in the kidney (1). Therefore gastrectomy (2), partial small bowel resection (3), and renal failure (4) cause insufficient Ca absorption in the small intestine. When any of these occur, does the large intestine compensate Ca absorption?

Karbach and Feldmeier reported that the large intestine has the highest Ca absorption capacity in rats (5). Some researchers show that it contributes to Ca absorption with the ingestion of a fermentable dietary fiber in case model and/or normal rats (6–9). We have demonstrated that the large intestine in rats fed a highly fermentable dietary fiber compensated Ca absorption decreased by renal failure, but this was not so for rats fed a fiber-free diet (4). These results suggest that the fermentation process is important in large intestinal Ca absorption.

However, fermentation in the large intestine is maintained to some extent by endogenous substrates such as mucin not only in rats fed a fermentable dietary fiber, but also in rats fed a fiber-free diet (10). Therefore under some conditions it is possible that the large intestine in rats fed a fiber-free diet also compensates Ca absorption.

The aim of this study is to examine whether the large intestine compensates insufficient Ca absorption in the small intestine in rats fed an exogenous fermentable substrate-free diet under Ca deficient conditions. To prevent Ca absorption in the small intestine, we used secondary phosphates (K$_2$HPO$_4$ and Na$_2$HPO$_4$). We added chromic oxide (Cr$_2$O$_3$) to all test diets as an insoluble and unabsorbed marker (11) to estimate apparent Ca absorption in the small intestine.

**MATERIALS AND METHODS**

*Animals.* Twenty-four 5-week-old (95.8±0.7 g body wt.) male Wistar/ST rats were purchased from Japan SLC (Hamamatsu, Japan). They were housed in individual cages in a room with controlled temperature (22±2°C), relative humidity (40–60%), and lighting (light 8:00–20:00 hours) throughout the experiment. The study was approved by the Hokkaido University Animal Committee, and animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Hokkaido University.

*Diets.* The compositions of test diets are shown in Table 1 (12–15). We used Ca carbonate as the insoluble salt and Ca gluconate, lactate, and citrate as soluble salts. The dietary Ca level was 2.0 g/kg, which is lower than the minimum requirement for rats (3.0 g/kg) (4), to conduct the present study under a condition in which rats can absorb Ca maximally. We replaced the primary phosphate (KH$_2$PO$_4$) in the mineral mixture with secondary phosphates (K$_2$HPO$_4$ and Na$_2$HPO$_4$) to prevent Ca absorption in the small intestine. Also, we added 5.0 g Cr$_2$O$_3$/kg to estimate apparent Ca absorption in the small intestine by the ratio of Ca:Cr in the diet and cecal contents (11).
Table 1. Composition of test diets.

| Dietary component (g/kg diet) | Ca carbonate | Ca gluconate | Ca lactate | Ca citrate |
|------------------------------|--------------|--------------|------------|------------|
| Ca carbonate$^1$             | 5.0          | —            | —          | —          |
| Ca gluconate$^1$             | —            | 21.5         | —          | —          |
| Ca lactate$^1$               | —            | —            | 10.9       | —          |
| Ca citrate$^1$               | —            | —            | —          | 8.3        |
| Casein$^2$                   | 250          | 250          | 250        | 250        |
| Corn oil$^3$                 | 50           | 50           | 50         | 50         |
| Mineral mixture (Ca-free)$^4$| 25.3         | 25.3         | 25.3       | 25.3       |
| Vitamin mixture$^5$          | 10           | 10           | 10         | 10         |
| Vitamin E$^6$                | 1.0          | 1.0          | 1.0        | 1.0        |
| Choline bitartrate           | 4.0          | 4.0          | 4.0        | 4.0        |
| Sucrose                      | —            | —            | —          | 8.3        |
| Chemical analysis$^7$ (g/kg diet) | 2.0          | 2.0          | 1.9        | 2.0        |

1 We used Ca carbonate as an insoluble salt and Ca gluconate, lactate, and citrate as soluble salts. Each Ca salt was added at 0.2% Ca (2.0 g Ca/kg diet).
2 Casein (ALACID; New Zealand Dairy Board, Wellington, New Zealand).
3 Retinyl palmitate (7.66 μmol/kg diet) and ergocalciferol (0.0504 μmol/kg diet) were added to corn oil.
4 The mineral mixture was based on the AIN-76 mixture (12) without Ca salts. However, we replaced a primary phosphate (KH₂PO₄) with secondary phosphates (K₂HPO₄ and Na₂HPO₄). They provided the following (mg/kg diet, calculated values): Na 3,948, K 3,746, Cl 2,892, P 2,856, Mg 375, Fe 100, Zn 34.7, Si 20.0, Cu 6.00, F 2.72, Sn 1.05, Mo 1.00, Cr 0.50, B 0.50, I 0.32, V 0.25, Se 0.20, Co 0.20.
5 The vitamin mixture was prepared in accordance with the AIN-76 mixture (13), except that menadione and L-ascorbic acid were added at levels of 5.81 (14) and 284 (15) μmol/kg diet, respectively.
6 Vitamin E (granulated, Juvela, Eisai, Tokyo, Japan) supplied 423 μmol all-rac-alpha-tocopheryl acetate/kg diet.
7 Chemical analysis was performed with atomic absorption spectrophotometry (Shimadzu AA-6400F, Shimadzu Seisakusyo, Kyoto, Japan).
8 Cr₂O₃ (5.0 g/kg diet) and crystallized cellulose (Avicel; Asahi Chemical Industry, Tokyo, Japan; 50 g/kg diet) were added to all the test diets at the expense of the whole diet.

Experimental protocol. After a week of acclimatization, the rats were divided into four groups (n=6) by body weight. They were freely given deionized water and one of the four test diets for 13 d. Body weight and food intake were measured every day. Feces were collected from day 11 to day 13 and freeze-dried to evaluate apparent Ca absorption, which reflects whole intestinal absorption. Rats were deprived of food during the dark period on day 13, though they had access to...
deionized water. On day 14, animals were given 2.0 g of each test diet containing 0.5% Cr₂O₃. After 2 h, the cecum was removed with its contents after withdrawal of blood from the aorta under anesthesia (Nembutal: sodium pentobarbital, 50 mg/kg body weight, Abbott Laboratories, North Chicago, IL, USA). The cecal contents were collected and stored at −30°C to evaluate Ca and Cr contents.

Analyses. Ca contents in the diet, cecal contents, and feces were determined by atomic absorption spectrophotometry (Shimadzu AA-6400F, Shimadzu Seisakusyo, Kyoto, Japan) after wet-ashing with an acid mixture (16 N HNO₃: 6 N HClO₄ = 3:1). Cr contents in the diet and cecal contents were found to similarly estimate apparent Ca absorption in the small intestine.

Calculations and statistics. Each value was calculated as follows (16, 17):

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\text{Apparent Ca absorption in the small intestine (\%)} = 100 \times \left( \frac{\text{Ca intake}}{\text{Cr intake}} - \frac{\text{Ca in the cecal contents}}{\text{Cr in the cecal contents}} \right) / \left( \frac{\text{Ca intake}}{\text{Cr intake}} \right)
\]

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\text{Apparent Ca absorption in the whole intestine (\%)} = 100 \times \left( \frac{\text{Ca intake} - \text{Ca in the feces}}{\text{Ca intake}} \right)
\]

We used Smirnoff's test (18) to reject aberrant values. The effects of Ca salt were analyzed by one-way ANOVA. Duncan's multiple-range test (19) was used to determine whether mean values were significantly different between groups (p < 0.05). These statistical analyses were done by SAS Version 6.07 (SAS Institute, Cary, NC, USA).

RESULTS

In our preliminary study, Ca solubility in the stomach and apparent Ca absorption in the small intestine in rats fed a diet containing secondary phosphates (51.7 ± 5.1, 60.0 ± 6.8%, n = 6) were lower than in rats fed a diet containing primary phosphates (66.5 ± 3.7, 79.6 ± 5.5%, n = 6, p < 0.05) at the AIN-76 Ca level. These results indicate that dietary secondary phosphates impaired Ca absorption in the small intestine by reducing Ca solubility in the rats.

Final body weight, body weight gain, and food intake were not significantly different among the four groups (Table 2).

Apparent Ca absorption in the small intestine was significantly lower in the rats fed a diet containing an insoluble Ca salt (carbonate) than in those fed diets containing soluble Ca salts (gluconate, lactate, or citrate). Apparent Ca absorption in the whole intestine was not significantly different among the four groups, and the values were similar to the absorption rates in the small intestines of rats fed diets containing soluble Ca sources (Table 3).
Table 2. Growth parameters in rats fed insoluble Ca carbonate and soluble Ca gluconate, lactate, and citrate.

| Diet group | Initial body weight (g) | Final body weight (g) | Body weight gain (g) | Food intake (g/d) |
|------------|-------------------------|-----------------------|----------------------|------------------|
| Ca carbonate | 149 ± 2 | 236 ± 3 | 87 ± 3 | 15.4 ± 0.3 |
| Ca gluconate | 151 ± 2 | 235 ± 6 | 85 ± 4 | 15.8 ± 0.7 |
| Ca lactate | 148 ± 2 | 228 ± 5 | 80 ± 3 | 14.8 ± 0.2 |
| Ca citrate | 150 ± 2 | 231 ± 3 | 81 ± 2 | 15.0 ± 0.3 |
| ANOVA Ca salt: | p = 0.854 | p = 0.535 | p = 0.383 | p = 0.476 |

All values are means ± SE (n = 5 in the Ca lactate group; n = 6 in the Ca carbonate, gluconate, and citrate group).

Table 3. Apparent Ca absorption in the small intestine and whole intestine in rats fed insoluble Ca carbonate and soluble Ca gluconate, lactate, and citrate.

| Diet group | Apparent Ca absorption in the small intestine (%) | Apparent Ca absorption in the whole intestine (%) |
|------------|-----------------------------------------------|-----------------------------------------------|
| Ca carbonate | 82.5 ± 7.0 | 94.3 ± 2.4 |
| Ca gluconate | 97.7 ± 0.9 | 98.4 ± 0.7 |
| Ca lactate | 98.8 ± 0.1 | 99.2 ± 0.5 |
| Ca citrate | 98.0 ± 0.5 | 98.4 ± 1.3 |
| ANOVA Ca salt: | p = 0.019 | p = 0.132 |

All values are means ± SE (n = 4 in the Ca lactate group; n = 5 in the Ca carbonate, gluconate, and citrate group). In the same column, values not sharing a common letter are significantly different (Duncan's multiple-range test; p < 0.05). The p value enclosed with a square shows a significant effect of Ca salt in the parameter (one-way ANOVA; p < 0.05).

DISCUSSION

In this study, apparent Ca absorption in the whole intestine was not significantly different among the rats fed different diets (Ca carbonate diet, Ca gluconate diet, Ca lactate diet, and Ca citrate diet) (Table 3). This result shows that Ca absorption in rats fed an insoluble Ca salt (carbonate) is similar to that in rats fed soluble Ca gluconate, lactate, or citrate. Until now, under various conditions there have been many experiments regarding the bioavailability of different Ca salts: Weaver et al showed that Ca absorption in rats fed Ca oxalate was one-tenth that in rats fed Ca carbonate or chloride (20); on the other hand, most researchers reported that Ca bioavailability was similar among rats fed different Ca salts (21–25), which
agrees with our results. Therefore it is generally accepted that the difference of dietary Ca salt does not influence Ca bioavailability except for Ca oxalate. Our study supports this view.

This study revealed that apparent Ca absorption in the small intestine was significantly lower in rats fed a modified mineral diet containing Ca carbonate than in rats fed the diet containing Ca gluconate, lactate, or citrate, even in those with low Ca intakes. The lower small intestinal absorption in the carbonate group was increased in whole intestinal absorption, but not in the soluble Ca groups. Whole intestinal absorption was similar among the four Ca salts groups (Table 3). These results show the following: (a) In rats fed 0.2% Ca diets containing soluble Ca salts, Ca is mostly absorbed in the small intestine even in secondary phosphate intakes; (b) In contrast, in rats fed a 0.2% Ca diet containing an insoluble Ca salt (carbonate), Ca is not sufficiently absorbed in the small intestine. However, large intestinal absorption compensates insufficient Ca availability in the small intestine. We previously reported that the cecum in rats fed a fermentable dietary fiber, guar-gum hydrolysate (GGH), compensated low Ca absorption in partially nephrectomized rats (4). Moreover, it was reported that the large intestine had contributed to Ca absorption through the ingestion of fermentable carbohydrates such as oligosaccharides (8,9,26) and resistant starch (27) in case model and/or normal rats. These findings suggest that the fermentation process is concerned with Ca absorption in the large intestine. Several researchers propose that organic acids produced by fermentation decrease luminal pH and this may dissolve insoluble Ca salts (6,27). However, this study performed in rats fed a fermentable fiber-free diet. Our results show that the large intestine compensates insufficient Ca absorption in the small intestine without the ingestion of a fermentable dietary fiber, and they also may suggest that the same availability in different Ca sources is due to increasing Ca absorption in the large intestine of rats. Possibly under our experimental conditions, fermentation in the large intestine might have been maintained by endogenous substrates such as mucin (10).

In conclusion, the large intestine compensates small intestinal Ca absorption decreased by dietary secondary phosphates without the ingestion of a fermentable dietary fiber in rats fed a 0.2% Ca diet.

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