True Calcium Absorption in the Intestine Is Enhanced by Fructooligosaccharide Feeding in Rats

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Tomio Morohashi, Tsuneyoshi Sano, Atsutane Ohta and Shoji Yamada

Departments of Pharmacology and *Oral Anatomy, School of Dentistry, Showa University, Shinagawa-ku Tokyo 142–8555, Japan and ^Bioscience Laboratories, Meiji Seika Kaisha, Sakado 350–0289, Japan

ABSTRACT Fructooligosaccharides (FOS) have been shown to stimulate apparent calcium absorption in the intestine. In this study, we examined the effect of FOS on true calcium absorption using the calcium balance in combination with the ^45Ca kinetics method. Sixteen 45-d-old male Wistar rats were randomly divided into two groups, a control group (n = 8) and a FOS group (n = 8). The diet fed to the FOS group contained 5% FOS, at the expense of half of the sucrose in the control diet. After an adaptation period (3 d) and a free-access period (3 d) that were used to estimate the amount of food required for pair-feeding on the basis of calcium, all of the rats were pair-fed throughout the experiment from the age of 51 d. A constant amount of calcium was fed to the rats in each group (95 mg/d). At age 60 d, a 3-d metabolic study was started by the intravenous injection of ^45Ca. Several variables were calculated on the bases of measurements of calcium intake, calcium in feces and serum, and ^45Ca in feces, urine and serum. Both true and apparent calcium absorption in the intestine (Vad and Vna) and urinary calcium were significantly greater in rats that had been fed FOS. There were no differences between the groups in endogenous net calcium excretion into feces (Vf; Vad – Vna). The calcium balance was also enhanced by FOS. Calcium balance in the FOS group was significantly correlated with the absorbed calcium (r^2 = 0.936, P < 0.01), as was that in the control group (r^2 = 0.994, P < 0.01). These results suggest that the increased true calcium absorption and balance by FOS feeding might improve bone calcification. J. Nutr. 128: 1815–1818, 1998.

KEY WORDS: • calcium • kinetics • rats • fructooligosaccharides • intestine

Indigestible carbohydrates have beneficial effects (Hidaka et al. 1986 and 1991, Rombeau et al. 1990). These dietary components such as inulin (Levrat et al. 1991), resistant starch (Schulz et al. 1993) and guar gum hydrolysate (Hara et al. 1996) increased apparent intestinal calcium absorption, calcium balance and bone mineral density in rats. The stimulatory effects of fructooligosaccharides (FOS), which are low-molecular-weight indigestible carbohydrates (Oku et al. 1984, Tokunaga et al. 1986), on intestinal calcium absorption have been particularly well examined (Ohta et al. 1993, 1994a and 1995). FOS also increase apparent intestinal calcium absorption, calcium balance and bone mineral density in growing rats (Ohta et al. 1993) and in models of disease, such as gastrectomized (Ohta et al. 1998a and 1998b) rats. Several studies of such indigestible carbohydrates have attempted to determine the mechanism of their stimulating effects in the intestine.

Recently, it has been demonstrated that the stimulatory effects of FOS and other indigestible carbohydrates on calcium absorption occur in the large intestine (Ohta et al. 1994c, 1995 and 1998a). However, the detailed mechanism has not yet been clarified. This effect may involve the production of short-chain fatty acids (SCFA) in the large intestine, resulting from fermentation in the large intestine because SCFA stimulate the proliferation of epithelial cells in the intestine (Sakata 1987) and reduce luminal pH (Demigné et al. 1989, Ohta et al. 1994a, Schulz et al. 1993, Younes et al. 1996). These findings would lead us to expect an increase in the absorption of dietary calcium (true absorption). However, true calcium absorption in rats fed these indigestible carbohydrates is still unclear because previous studies have relied solely on the simple calcium balance method. A simple calcium balance study can examine only apparent calcium absorption, urinary calcium and calcium balance, and cannot be used to evaluate true intestinal calcium absorption, the excretion of calcium into the intestine or the kinetics of calcium into or from bone. Moreover, apparent calcium absorption cannot explain whether an increase is due to an enhancement of calcium absorption or a reduction of calcium excretion into the intestine. It is very important to clarify the details of the effects of dietary indigestible carbohydrates on calcium metabolism before such effects are applied clinically.

Calcium balance in combination with the ^45Ca kinetics method is a useful technique for analyzing calcium metabolism. By using this approach, it is possible to observe the details of calcium metabolism such as calcium movement in the intestine, kidney and bone (Aubert and Milhaud 1960, Morgan et al. 1975, Sammon et al. 1970, Yamada 1994). The aim of this study was to clarify the effects of dietary FOS on calcium metabolism in growing rats with the use of this method.

MATERIALS AND METHODS

Male Wistar rats (n = 16) were housed in individual metabolic cages and fed a pelleted diet at the age of 42 d. After 3 d, rats were randomly divided into two groups, a control group (n = 8) and a FOS group (n = 8). The diet in the FOS group contained 5% fructooligosaccharides, at the expense of half of the sucrose in the
control diet. The compositions of the powdered diets are shown in Table 1. The powdered diets were mixed with an equal amount of purified water. The respective wet diets were dried for 1 d (80°C) to calculate the wet/dry ratio, and calcium contents in the dry diets were then measured to determine the amount supplied in the wet diet in each group for pair-feeding on the basis of calcium. The amount of diet was increased every day for 3 d (20, 24 and 32 g wet weight). The rats in each group were given an excessive amount (40 g) of their respective diets to estimate the amount of diet needed for pair-feeding on the basis of calcium without growth retardation. Each rat consumed between 34 and 35 g wet weight of diet. All of the rats were fed a constant amount of calcium (95 mg/d) in their respective diets throughout the experiment beginning when they were 51 d old. Thus, calcium retention in bone depended mainly on absorbed calcium (Fig. 1). 

Table 1 Composition of the experimental diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control diet</th>
<th>FOS1 diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (g/kg dry diet)</td>
<td>5.21</td>
<td>5.18</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>532</td>
<td>532</td>
</tr>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>FOS</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Corn oil</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Mineral mixture1</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mixture2</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Chemical analysis, g/kg dry weight</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Fructooligosaccharides.
2 Prepared according to AIN-93 formulation (Reeves et al. 1993).

The combination of a calcium balance study and a 45Ca kinetics study was performed using the two-compartment model (Aubert and Milhaud 1960; Yamada 1994). This study was performed 4 d before killing (60 d old) over a 3-d period. At the beginning of this study, 1.11 MBq of 45CaCl₂ solution was injected into the tail vein of each rat. Blood samples were obtained from the tail at 2, 4, 6, 25, 49 and 73 h after injection. Urine and feces were harvested on a sheet of filter paper. The filter papers containing feces and urine were ashed (600°C, 3 d) and dissolved in 2.0 mol/L HCl. The samples of feces, urine, and serum prepared from the tail vein before killing were counted for 45Ca by a liquid scintillation counter (Packard Instrument, Meriden, CT) to calculate variables such as endogenous net calcium excretion (Vf), urinary calcium (Vu), bone resorption (Vo−) and bone formation (Vo+). After killing, a blood sample was obtained from each rat from the carotid artery, and the amount of serum was determined. To determine calcium intake (Vi), food consumption was measured. The calcium in feces (Vf) and serum was determined by an atomic absorption spectrophotometer (Perkin Elmer, Norwalk, CT). Apparent intestinal calcium absorption (Vna; Vi – Vf), true intestinal calcium absorption (Vad; Vna + Vi) and calcium balance (Vad / Vna) were calculated.

RESULTS

Body weight in the FOS group increased throughout the experiment in parallel with that in the control group (Fig. 1). No external adverse effects such as weight reduction or diarrhea were observed in the FOS group. Food consumption in the FOS group did not differ from that in the control group (data not shown). There were also no significant differences in the serum calcium concentration (Table 2).

Both true and apparent intestinal calcium absorption (Vad and Vna) were significantly greater in the FOS group. However, there were no significant differences between the groups in endogenous net calcium excretion into feces (Vi; Vad – Vna). Fractional calcium absorption (Vad / Vna) in the FOS group was significantly higher than that in the control group because there was no significant difference in calcium intake (Vi) between the two groups. More than 95% of the absorbed calcium (Vad / Vna, %) was retained in the body, and <5 % of that was excreted into urine in both groups. Thus, the amount of calcium excreted into urine was very small despite the almost twofold greater amount of Vna in rats fed FOS (Table 2).

Calcium balance (VΔ; Vna – Vu or Vo+ – Vo−) was also greater in the FOS group (Table 2). VΔ in rats fed FOS was significantly correlated with Vad (r² = 0.936, P < 0.01), as was that in the control group (r² = 0.994, P < 0.01) (Fig. 2). Thus, the amount of calcium excreted into urine was very small despite the almost twofold greater amount of Vna in rats fed FOS (Table 2).

FIGURE 1 Body weight in rats fed a fructooligosaccharides (FOS) diet or a control diet. The amount of diet was increased every day for 3 d (20, 24 and 32 g wet weight) during the adaptation period. The rats in each group were given an excessive amount (40 g) of their respective diets to estimate the amount of diet needed for pair-feeding on the basis of calcium (free-access period). From the age of 51 d, the respective diets contained 95 mg calcium (pair-feeding period). Values are means ± SD, n = 8.
calcium. Meanwhile, no correlation was observed between $V$,$\Delta$ and variables of bone turnover ($V_0^+$ and $V_0^-$) in either group (data not shown).

**DISCUSSION**

Calcium absorption in the small intestine is systematically regulated by endogenous factors such as parathyroid hormone and 1,25-dihydroxycholecalciferol (Bronner 1987). On the other hand, it has recently been shown that luminal fermentation of indigestible carbohydrates such as FOS plays an important role in calcium absorption in the large intestine in rats (Ohta et al. 1994c and 1995). In the FOS group in this study, both true and apparent calcium absorptions were stimulated without changes in calcium excretion into the intestine. The stimulating effect of indigestible carbohydrates in the intestine has been explained as follows: 1) indigestible carbohydrates reduce luminal pH by microbial fermentation in the large intestine and dissolve otherwise insoluble calcium salts (Demigné et al. 1989, Ohta et al. 1994c, Schulz et al. 1993, Younes et al. 1996); 2) fermentation results from the production of SCFA, which have been reported to stimulate calcium absorption in humans (Trinidad et al. 1996); 3) SCFA induce the proliferation of epithelium cells (Sakata 1987). FOS is also known to enhance the production of SCFA (Oku et al. 1984, Tokunaga et al. 1986). Thus, the increase in true calcium absorption in the FOS group might be due in part to the SCFA produced from FOS in the large intestine (Table 2). There was no significant difference between the control and FOS groups in endogenous net calcium excretion into feces ($V_i$). On the other hand, true calcium absorption ($V_d$) and its fraction ($V_d/V_i$) in the FOS group were both greater than those in the control group (Table 2). Assuming that endogenous actual calcium excretion is counteracted by reabsorption throughout the whole intestine, where its fractional absorption rate is defined as $V_i(1 - V_d/V_i)$, the amount of calcium in the intestine may be higher in rats fed FOS. Unfortunately, this experiment is not suitable for clarifying this point.

The intake of FOS by rats enhances calcium balance, bone mineral density and calcium contents in bone (Ohta et al. 1993, 1998a and 1998b). In this study, feeding FOS increased both true intestinal calcium absorption ($V_d$) and calcium balance ($V_\Delta$), compared with those in the control group (Table 2). However, there were no significant differences in bone formation ($V_0^+$) or resorption ($V_0^-$) between the two groups (Table 2). There are several possible explanations for this result. Sammon et al. (1970) examined the relationship between calcium intake and apparent absorption using diets with various doses of calcium (4–200 mg/d). They found that the increase in apparent absorption was correlated with calcium intake. Moreover, apparent calcium absorption generally changes in direct proportion to calcium balance, but is inversely proportional to bone resorption and is relatively unassociated with bone formation (Morgan et al. 1975). Thus, if $V_d$ is drastically stimulated by FOS feeding, $V_0^-$ would be suppressed. However, in this experiment, rats were fed adequate calcium, but not an excess, so that the increase in $V_n$ in the FOS group was significant, but still <9 mg/d. In addition, because both $V_0^+$ and $V_0^-$ are calculated from other variables, they may vary more than other variables. Thus, changes in calcium flow in bone might be undetected.

Urinary calcium excretion ($V_u$) in the FOS group was significantly greater than that in the control group (Table 2). Calcitriol may induce the accumulation of calcium in the kidney. In fact, although excessive phosphorus and calcium intake induce the accumulation of calcium in the kidney, the addition of FOS to the diet reduces such accumulation (Ohta et al. 1994b). However, the effects of FOS on calcium excretion
and reabsorption in the kidney are unclear, and further studies are required to evaluate this point.

Peak bone mass in humans is achieved after sexual maturity and is then maintained for two decades. Thereafter, the mass of virtually all bones declines until death. In fact, we would expect that high calcium deposition into bone in a period of growth would increase the peak bone mass and would prevent bone disorders with aging. Meanwhile, only an excess supply of calcium reduces magnesium absorption (Ohta et al. 1994a). Some experimental data indicate that a decrease in magnesium has an adverse effect on osteogenesis (S-Lindberg et al. 1993). However, FOS stimulates both calcium and magnesium absorption in rats (Ohta et al. 1993, 1994a and 1995). In this study, the intake of FOS in rats enhanced calcium balance, and absorbed calcium was significantly correlated with calcium balance, which might improve bone calcification.

**LITERATURE CITED**


Younes, H., Demigné C. & Rémésy C. (1996) Acidic fermentation in the cecum and is then maintained for two decades. Thereafter, the mass of virtually all bones declines until death. In fact, we would expect that high calcium deposition into bone in a period of growth would increase the peak bone mass and would prevent bone disorders with aging. Meanwhile, only an excess supply of calcium reduces magnesium absorption (Ohta et al. 1994a). Some experimental data indicate that a decrease in magnesium has an adverse effect on osteogenesis (S-Lindberg et al. 1993). However, FOS stimulates both calcium and magnesium absorption in rats (Ohta et al. 1993, 1994a and 1995). In this study, the intake of FOS in rats enhanced calcium balance, and absorbed calcium was significantly correlated with calcium balance, which might improve bone calcification.