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FRUCTOOLIGOSACCHARIDES STIMULATE THE ABSORPTION OF MAGNESIUM FROM THE HINDGUT IN RATS

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ABSTRACT

We investigated the effects of feeding fructooligosaccharides (FO) on the absorption of Mg from the hindgut in cecal-cannulated rats *in vivo*. Male rats, four wks old male rats, were divided into four groups. Rats in two groups were fed Mg-containing diet and other two groups were fed Mg-free diet with cecal infusion of Mg. Rats of both groups were fed FO-free or FO-containing diet, respectively. The absorptive ratio of Mg administrated into the cecum was similar to that of Mg fed orally in rats fed FO-free and FO-containing diet, respectively. FO-feeding significantly increased the apparent absorption of Mg in both rats fed Mg orally and rats with Mg infused into the cecum. Moreover, the extent of the increase in the absorption of Mg by infusion into the cecum and by oral feeding was the same. In a separate experiment, we observed that only the rats fed Mg-free diet exhibited auricular and facial peripheral hyperemia and hemorrhage. However such symptoms were not observed in the rats with Mg infused into the cecum. In conclusion, these results indicate that FO-feeding stimulates absorption of Mg in the hindgut mainly.

KEY WORDS: Fructooligosaccharides, Hindgut, Magnesium, Rats, Absorption

INTRODUCTION

Previously, we reported that feeding fructooligosaccharides (FO) increased the absorption of calcium (Ca) and magnesium (Mg) in rats (1-3). FO are not digested by human digestive enzymes, but instead are fermented by bifidobacteria in the hindgut (4). Also, there have been reported that feeding of undigestible carbohydrates, such as inulin (5), lactulose (6) and maltitol (7) increases the absorption of minerals, such as Ca and Mg, in rats.

Recently, several reports have demonstrated that the hindgut have the ability to absorb Mg (8-10). Karbach reported that Mg flux from the mucosa to the serosa was higher than that from

the serosa to the mucosa in the colon by using the ussing chamber method, which shows that Mg is absorbed from the colon (8-9). Hardwick et al. reviewed that Ca and Mg are absorbed from both the small and large intestines (10). Also, we reported that the cecectomy decreased the stimulatory effect of FO on the absorption of Mg and Ca (3). However, there is no evidence indicating how much the stimulatory effect of FO on the absorption of Mg occurs in the hindgut.

In the present study, we investigated the effects of FO-feeding on the net absorption of Mg from the hindgut by a cecal cannulation method *in vivo* in unanesthetized and unrestrained rats.

MATERIALS AND METHODS

Diets and animals: Four-week-old male Sprague-Dawley rats (Clea Japan, Tokyo, Japan) were housed in individual stainless-steel wire-mesh cages in a room at 25℃ and 55% relative humidity. Rats were fed a pelleted diet (MF®: Oriental Yeast Co. LTD., Tokyo, Japan) for one week. Then, all rats were operated for cecal cannulation. They were anesthetized by diethyl ether inhalation. A polyethylene tube was implanted into the cecum and sutured. A silicone tube was joined to the polyethylene tube and was fixed on the back of the head with the protector through under the skin. Rats were not allowed any diet for the first 24h after surgery but they were given free access to distilled deionized water. Then they were divided into four experimental subgroups of 7 rats each and fed experimental diet. The composition of each diet is shown in Table 1. The rats in two groups were fed Mg-containing diet and the other two groups were fed Mg-free diet with cecal infusion of Mg (as 0.343mol/L $MgCl_2$) twice per day (900h and 1800h). The amount of Mg infused in the cecum was the average of Mg in rats fed Mg-containing diet. Rats fed Mg-containing diet administrated distilled deionized water in the cecum by the same procedure. Rats of both groups were fed FO-free or FO-containing diet, respectively. All rats were fed these experimental diets for 15 days. Three and 11 days after feeding experimental diets, rats were subjected to the Mg-balance study for 5 days. All feces and urine were collected over a 5-day period in each case. Animals were allowed free access to their respective diets and to deionized water throughout the experimental period. Also, we observed effects of feeding Mg-free diet in a separate experiment in the present study. Cecal-cannulated rats, similar to the above experiments, were fed FO-free or FO-containing diet with administration of distilled deionized water in the cecum for 15 days. On the final day of the experiment, we examined auricular peripheral and facial hyperemia and hemorrhage in detail. The extent of auricular peripheral and facial hyperemia and hemorrhage was graded as follows: −, rats with no inflammation; ±, rats with only auricular hyperemia; +, rats with fewer than 5 sites of auricular and facial hemorrhage; ++, rats with fewer than 10 sites of auricular and facial hemorrhage; +++, rats with more than 10 sites of auricular and facial hemorrhage. FO are a mixture of 42% 1-kestose, 46% nystose and 9% 1F-β-fructofuranosyl nystose (Meioligo-P®, Meiji Seika Kaisha, LTD., Tokyo, Japan) (11). Other dietary components apart from the minerals were from Oriental Yeast Co. (Tokyo). All other reagents were of analytical grade from Wako Pure Chemical Industries, LTD. (Tokyo, Japan).

Measurements of Mg: The amounts of Mg in diets, feces, cecal contents and urine were determined with an inductive-coupled plasma emission spectrometer (ICPS-5000; Shimadzu, Kyoto, Japan). Diets and feces were first dried and then micropulverized. Micropulverized samples (about 100 mg) were ashed at 600℃ for 24 h. The ashed samples, dissolved in 4 ml of 2

TABLE 1
Composition of the Diets and Designations

Group					Separate study	
Mg administration	oral	oral	cecum	cecum	−	−
Fructooligosaccharides in diet	−	+	−	+	−	+
Ingredient (g / kg diet)						
Casein	250	250	250	250	250	250
Corn starch	505	505	505	505	505	505
Corn oil	50	50	50	50	50	50
Vitamin mix. *1	10	10	10	10	10	10
Mg-free mineral mix. *1	35	35	35	35	35	35
MgO	0.42	0.42	0	0	0	0
Cellulose	50	50	50	50	50	50
Sucrose	100	50	100	50	100	50
Fructooligosaccharides *2	0	50	0	50	0	50
Chemical analysis (mmol / kg diet)						
Calcium	127	125	125	127	122	125
Magnesium	9.05	8.64	0.41	0.41	0.41	0.41
Phosphorus	145	142	142	145	145	145

*1 Prepared according to AIN-76 formulation.
*2 Meioligo-P® (concentration of oligosaccharides were greater than 95% of total mixture.).

N HCl, were diluted appropriately with deionized water for atomization. Urine was diluted appropriately with 0.1 N HCl and subjected to atomization.

Quantification of Mg and pH in the cecum: On the final day of the experiment, after administration of Mg into the cecum in rats fed Mg-free diet with cecal infusion of Mg, the rats were anesthetized with diethyl ether inhalation. Blood was obtained by abdominal aortic puncture and the cecum with its contents were removed. The contents in the cecum were collected into preweighed tubes. The quantification of Mg in the cecal contents is based on the method of Schulz et al. (12). A part of the cecal contents added to five volumes of deionized water. The contents were shaken and centrifuged (10 min, 14,000 rpm), and the supernatant fraction and pellet were separated. Pellet and supernatant fractions were analyzed for Mg. The pH of the cecal contents was measured immediately after death by pH electrode (B-112, HORIBA, Kyoto, Japan).

Calculations and statistics: The data reported are the mean values with standard deviation (SD). Data were analyzed by two-way ANOVA (diet and administration). Significant differences between groups were determined by Tukey's test. Significance was recognized at $p<0.05$. The apparent absorption and retention of Mg were calculated from the following formulae;

Apparent absorption
= (administration - fecal excretion)
Apparent absorptive ratio (%)
= (administration - fecal excretion) / (intake)

Retention
= (administration - fecal excretion - urinary excretion)
Apparent retentive ratio (%)
= (administration - fecal excretion - urinary excretion) / (intake)

RESULTS

Body weight and food intake: The initial and final body weight and food intake are shown in Table 2. The final body weight and food intake of the groups with Mg administrated into the cecum was similar to that of the orally fed groups in rats fed both FO-free and FO-containing diets. In a separate experiment, final body weight and food intake were lower in rats fed a Mg-free diet without Mg administration into the cecum than in rats given Mg orally and cecally.

Mg balance: The Mg balance is shown in Figs. 1 and 2 and Table 3. In the first (3~7days) and second (11~15days) periods, the absorptive ratio of Mg in rats fed a Mg- and FO-free diet with cecal infusion of Mg was similar to that in rats fed Mg-containing and FO-free diet. FO-feeding significantly increased the apparent absorption of Mg in both the orally and cecally administrated rats, and the increase in Mg absorption in the cecal group was to the same extent with that in the oral group.

Mg and pH in the cecum: The Mg content and pH in the cecum are shown in Table 4. The pH of the cecal contents was lower in the FO-feeding groups than in the FO-free diet groups. The liquid phase of Mg in rats in the FO-feeding groups were higher than those in the FO-free diet groups. The proportion of soluble Mg to insoluble Mg in the cecum was also increased by FO-feeding, but the values were not different between the oral and cecal groups with or without FO feeding.

Auricular and facial hyperemia and hemorrhage: In a separate experiment, all rats fed Mg-free diet exhibited auricular and facial peripheral hyperemia and hemorrhage, but no such symptoms were observed in the rats given Mg (Table 5).

TABLE 2
Body Weight and Food Intake of the Rats Fed Experimental Diets for 15days

Groups*					ANOVA			Separate study	
Mg administration	oral	oral	cecum	cecum	D**	A***	DxA	−	−
Fructooligo-saccharides in diet	−	+	−	+				−	+
Initial weight (g)	106± 4	107± 4	107± 2	107± 1	NS	NS	NS	107±5	108± 3
Final weight (g)	242±16	244±17	237±12	232±13	NS	NS	NS	169±7	173± 7
Food intake (g)	274±25	265±18	273±23	252±28	NS	NS	NS	182±9	185±11

Values are mean ± SD (n=7).
NS=No significant difference.
*For details of diets and procedures, see TABLE1.
**D: diet, ***A: administration.

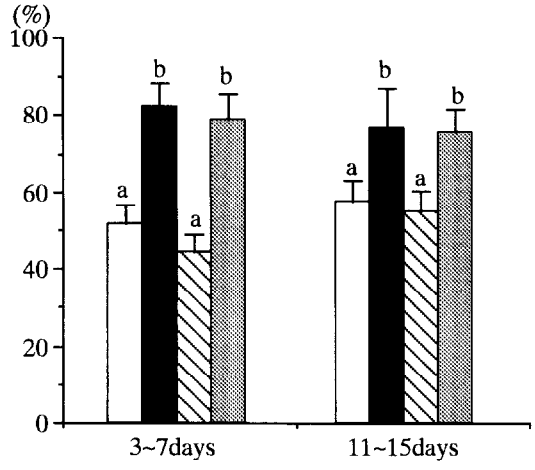

FIG.1 Magnesium Absorptive Ratio of Rats Fed Experimental Diets. Values are mean ± SD (n=7) in each group. Differences among groups were evaluated for significance by Tukey's test after preliminary analysis of variance (two-way ANOVA).

□ Group fed the Mg-containing and FO-free diet.

■ Group fed the Mg- and FO-containing diet.

▨ Group fed the Mg- and FO-free diet with cecal administration of Mg

▩ Group fed the Mg-free and FO-containing diet with cecal administration of Mg

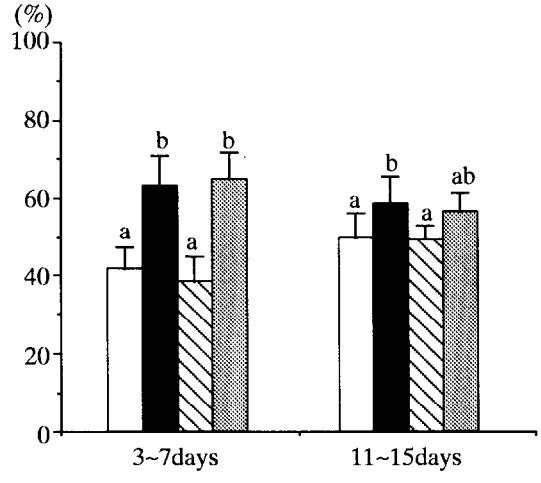

FIG.2 Magnesium Retentive Ratio of Rats Fed Experimental Diets. Values are mean ± SD (n=7) in each group. Differences among groups were evaluated for significance by Tukey's test after preliminary analysis of variance (two-way ANOVA).

□ Group fed the Mg-containing and FO-free diet.

■ Group fed the Mg- and FO-containing diet.

▨ Group fed the Mg- and FO-free diet with cecal administration of Mg

▩ Group fed the Mg-free and FO-containing diet with cecal administration of Mg

TABLE 3
Magnesium Balances of the Rats Fed Experimental Diets

Groups*					ANOVA		
Mg administration	oral	oral	cecum	cecum	D**	A***	DxA
Fructooligo-saccharides in diet	−	+	−	+			
3-7days							
Administration (μmol/d)	158±13	154±13	159	159	NS	NS	NS
Fecal excretion (μmol/day)	76.2± 9.5^{b}	27.7±10.8^{a}	88.3± 7.4^{b}	33.4±10.8^{a}	p<0.001	p=0.022	NS
Urinary excretion (μmol/day)	16.1± 8.3^{ab}	29.4±10.8^{c}	9.0± 4.1^{a}	22.4± 6.4^{bc}	p<0.001	p=0.022	NS
Absorption (μmol/day)	81.8±10.0^{a}	126±11.0^{b}	70.3± 7.4^{a}	125±11^{b}	p<0.001	NS	NS
Retention (μmol/day)	65.7± 8.0^{a}	96.8±11.9^{b}	61.4±10.1^{a}	103±11^{b}	p<0.001	NS	NS
11-15days							
Administration (μmol/d)	171±22	174±16	171	171	NS	NS	NS
Fecal excretion (μmol/day)	73.1±16.0^{b}	40.6±18.0^{a}	77.6±9.5^{b}	41.3±17.8^{a}	p<0.001	NS	NS
Urinary excretion (μmol/day)	12.8± 2.6^{a}	31.2±10.2^{b}	9.4±4.1^{a}	32.7± 9.3^{b}	p<0.001	NS	NS
Absorption (μmol/day)	97.9±12.7^{a}	133±22^{b}	93.8±9.6^{a}	130±10^{b}	p<0.001	NS	NS
Retention (μmol/day)	85.1±12.5^{a}	102±15^{b}	84.4±6.9^{a}	97.3± 7.4^{ab}	p<0.002	NS	NS

Values are mean ± SD (n=7).
NS=No significant difference
Values not sharing a common superscript letter are significantly different (p<0.05).
*For details of diets and procedures, see TABLE1.
**D: diet, ***A: administration.

DISCUSSION

As shown in Fig.1, the absorptive ratio of cecally infused Mg was similar to that of orally fed Mg in the FO-free groups. This suggests that the hindgut of rats is able to absorb the same amount of Mg which is ingested orally from the diet. In a separate experiment, all rats fed Mg-free diet without cecal supplementation of Mg exhibited the auricular and facial peripheral hyperemia and hemorrhage which were typical symptoms of Mg deficiency (13-15). These symptoms of Mg deficiency were completely prevented with supplementation of Mg into the

TABLE 4
pH and Distribution of Magnesium between the Liquid Phase and Solid Phase in the Cecum in Rats

Groups*					ANOVA		
Mg administration	oral	oral	cecum	cecum	D**	A***	DxA
Fructooligo-saccharides in diet	−	+	−	+			
pH	7.05±0.50^{bc}	6.30±0.50^{a}	7.37±0.29^{c}	6.47±0.42^{ab}	p=0.007	NS	NS
Cecal weight (g)	2.14±0.37^{a}	4.47±0.74^{bc}	3.13±0.35^{ab}	5.78±1.03^{c}	p<0.001	p=0.016	NS
Amount in the liquid phase (μmol/cecal content)	5.44±2.89^{a}	17.9±0.6^{ab}	16.1±6.4^{a}	35.5±13.7^{b}	p=0.013	p=0.025	NS
Amount in the solid phase (μmol/cecal content)	23.9±2.5^{ab}	7.88±4.70^{a}	36.7±6.9^{c}	23.2±13.8^{ab}	p=0.023	p=0.030	NS
Liquid phase/Solid phase	0.18±0.08^{a}	1.56±0.63^{b}	0.35±0.13^{a}	1.26±0.71^{b}	p=0.001	NS	NS

Values are mean ± SD (n=7).
NS=No significant difference
Values not sharing a common superscript letter are significantly different ($p<0.05$).
*For details of diets and procedures, see TABLE1.
**D: diet, ***A: administration.

cecum (Table 5). These results suggested that hindgut is able to absorb Mg at nutritionally-sufficient levels, and that Mg which was absorbed from the hindgut was utilized effectively in rats.

The main site (i.e. small intestine versus hindgut) of the stimulation of Mg absorption by feeding of undigestible carbohydrates remains unclear. Fig.1 shows that FO-feeding also significantly increased the absorption of Mg as compared with rats fed FO-free diet. Moreover, the increase in the amount of Mg absorbed by FO-feeding in rats administrated Mg into the cecum was similar to that in rats fed Mg orally. These results suggest that almost of all the stimulatory effect of FO-feeding on the absorption of Mg takes place in the hindgut. Rémésy et al. determined that Mg flux from the cecum was about 200 to 700% higher in rats fed several undigestible carbohydrates such as pectin, lactulose, inulin and wheat bran (16). The extent of the increase in the Mg absorption in their study was larger than that by FO-feeding in our study. In their study, Mg absorption was estimated at just after the dark period. We speculated that Mg absorption shows diurnal fluctuation, and that they observed the maximal level of the increase on Mg absorption from the cecum caused by feeding of undigestible carbohydrates.

A possible mechanism of stimulation of the absorption of Ca and Mg by feeding undigestible carbohydrates is that lowered luminal pH in the hindgut caused by fermentation of these carbohydrates increases the soluble fraction and the absorption of these minerals in the hindgut. Brink et al. speculated that the formation of an insoluble Ca-Mg-P complex in the lumen decreases the absorption of Mg (17). Also, we reported previously that increasing the concentration of Ca and P in the diets decreased the absorption of Mg in rats (2). Schulz et al. reported that the levels of Mg^{2+} ions were increased by lowering the luminal pH in the intestine

TABLE 5
Auricular and Facial Hyperemia and Hemorrhage in Rats Fed Experimental Diets

Groups*					Separate study	
Mg administration	oral	oral	cecum	cecum	−	−
Fructooligosaccharides in diet	−	+	−	+	−	+
−	7	7	7	7	0	0
±	0	0	0	0	1	2
+	0	0	0	0	3	2
++	0	0	0	0	1	1
+++	0	0	0	0	2	2

* See legend to TABLE 1.
− : no inflammation.
± : only auricular hyperemia.
+ : fewer than 5 sites of auricular and facial hemorrhage.
++ : fewer than 10 sites of auricular and facial hemorrhage.
+++ : more than 10 sites of auricular and facial hemorrhage.

(12). Scharrer et al., using an *in situ* perfusion method, reported that short chain fatty acids (SCFA) stimulate Mg absorption from the colon in rats (18). SCFA are mainly produced by luminal fermentation of undigestible carbohydrates in the hindgut. Also, in the present study, FO-feeding lowered the pH of the cecal contents and increased the soluble fraction of Mg. These results suggest that a decrease in luminal pH by fermentation of FO in the cecum raises the solubility of Mg in the cecal contents and stimulates the absorption of Mg (Table 4).

Laboratory rats ingest their feces, and this phenomenon is known as coprophagy (19-21). A few authors suggested that coprophagy is an important behavior in the recycling of nutrients (20). Tadayyon et al. reported that Mg absorption was decreased about 10% by the prevention of coprophagy (22). Therefore, it appears that not all amount of the Mg administered into the cecum is absorbed from the hindgut. However, we reported that the prevention of coprophagy did not alter the absorption of Mg in rats fed FO-free diet, and that FO-feeding increased the absorption of Mg in rats both with or without coprophagy (23). So it seems that coprophagy influences to a lesser extent the absorption of Mg.

In conclusion, our results suggest that FO-feeding stimulates the absorption of Mg mainly from the hindgut.

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