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Effects of Fructooligosaccharides on the Absorption of Iron, Calcium and Magnesium in Iron-deficient Anemic Rats

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Summary We investigated the effects of fructooligosaccharides (FO)-feeding on the absorption of iron (Fe), calcium (Ca) and magnesium (Mg) and on the biochemical parameters in Fe-deficient anemic rats. Fe-deficient anemic rats were made by feeding an Fe-deficient diet for 3 weeks. Then these Fe-deficient rats were fed an experimental diet that contained one of two levels of Fe (15 or 30 mg/kg diet), in the form of ferric pyrophosphate, and one of two levels of FO (0 or 50 g/kg diet) for 2 weeks. After the rats were fed these experimental diets, FO-feeding increased the hematocrit ratio, the concentration of hemoglobin and the hemoglobin regeneration efficiency during the first week. Also, the apparent absorption of Fe was increased by FO-feeding. The levels of Fe in the diet did not affect the absorption of Ca and Mg. However, FO-feeding increased the absorption of Ca and Mg. FO-feeding lowered the pH and raised the solubility of Fe, Ca and Mg in the cecal contents, suggesting that those increasing effects of FO-feeding on absorption of these minerals is correlated with fermentation of FO in the large intestine, namely, the cecum and colon. We concluded that FO-feeding improved recovery from anemia and increased the absorption of Fe, Ca and Mg in Fe-deficient anemic rats.

Key Words iron, iron-deficient, calcium, magnesium, absorption, fructo-oligosaccharides, rat

Iron deficiency, the most common human nutritional deficiency worldwide, is more often the result not only of inadequate intake of iron (Fe) but also of poor bioavailability of Fe in the diet (*1*). The absorption of Fe is not yet clearly understood (*2*), however the effects of many food components on the absorption of Fe have been reported. Phytate, tannin and some types of dietary fiber inhibit Fe-absorption (*3–7*), and it is well known that ascorbate increases Fe-absorption (*7,8*). We reported previously that a diet that contained fructooligosaccharides (FO), which stimulate the growth of bifidobacteria (*9*), increased the absorption

of Ca, Mg and phosphorus (P) in healthy rats (*10,11*). The same effects were observed with other undigestible carbohydrates, such as resistant starch (*12*), lactulose (*13*) and inulin (*14*). The undigestible carbohydrates were fermented by the luminal bacteria in the large intestine, namely, in the cecum and colon. Many reports have suggested an important correlation between increase in the absorption of minerals and fermentation of such undigestible carbohydrates in the large intestine (*10–14*). However, the effects of such undigestible carbohydrates on Fe adsorption have not been reported. Therefore, in anemic rats, if FO-feeding has the same increasing effect on the absorption of Fe as it does on Ca and Mg, recovery from anemia is improved.

Few data have been published on the interactions between anemia and the absorption of minerals such as Ca and Mg (*15–17*), and the absorption of Ca and Mg has been reported to be decreased in rats fed an Fe-deficient diet (*18*). Moreover, in anemic rats, the increasing effects of such undigestible carbohydrates on Ca and Mg adsorption have not yet been confirmed.

In the present study, we investigated the effects of FO-feeding on the absorption of Fe, Ca and Mg in Fe-deficient anemic rats. It is difficult to evaluate the effects of FO-feeding on the recovery from anemia because, in the anemic rats, the absorption of Fe was increased (*19*) and Fe-deficient anemic rats recover from anemia very rapidly by feeding an Fe-containing diet. Therefore, ferric pyrophosphate which is a low-bioavailability iron source (*20*) and is added to many Fe-supplement foods, was used as the iron source in the experimental diet.

MATERIALS AND METHODS

Animals and diets. Three-week-old male Sprague-Dawley rats (Clea Japan, Tokyo) were housed in individual stainless-steel metabolic cages in a temperature- and humidity-controlled room (25°C and 55% relative humidity). All rats were fed an Fe-deficient diet for 3 weeks, then the rats were divided into four experimental groups of seven rats each. Two levels (15 or 30 mg of Fe per kg diet) of ferric pyrophosphate, as a source of Fe, were added to the experimental diets. FO were added to each of the Fe-containing experimental diets at a level of 50 g/kg diet, with FO replacing an eqal amount of sucrose in the diet (Table 1).

Absorption of minerals. Three days and 10 days after the start of feeding the experimental diets, rats were subjected to a mineral-absorption study for 4 days. All feces were collected for a 4-day period. The apparent absorption of minerals was calculated from the following formula:

$$\text{Apparent absorption } (\%) = ((\text{intake} - \text{fecal excretion})/\text{intake}) \times 100$$

Determination of iron (Fe), calcium (Ca) and magnesium (Mg). The amount of Fe, Ca and Mg in the diets, feces and cecal contents was determined with an inductive coupled plasma emission spectrometer (ICPS-5000; Shimadzu, Kyoto). Diets and feces were first dried and then micropulverized after drying (110°C, 10

Table 1. Composition of experimental diets (g/kg) and designations of the four groups of rats.

	L*[1]	LF*[2]	N*[3]	NF*[4]
Casein	250	250	250	250
Corn starch	495	495	495	495
Corn oil	60	60	60	60
Vitamin mix[a]	10	10	10	10
Salt mix[b]	35	35	35	35
Cellulose	50	50	50	50
Sucrose	100	50	100	50
Fructooligosaccharide[c]		50		50
Ferric pyrophosphate (mg/kg)	5.9	5.9	5.9	5.9
Chemical analyses (mg/kg)				
Iron	15	16	29	28
Calcium	5,830	5,740	5,800	5,830
Magnesium	491	476	503	487

[a] Prepared according to AIN-76 formulation. [b] Prepared according to iron-free AIN-76 formulation (Ca, 5.2 g; Mg, 0.5 g; P, 4.0 g/kg of diet). [c] Meioligo-P® (the concentration of oligosaccharides was above 95%, w/w). *[1] Rats fed 15 mg Fe per kg of diet. *[2] Rats fed 15 mg Fe and 50 g FO per kg of diet. *[3] Rats fed 30 mg Fe per kg of diet. *[4] Rats fed 30 mg Fe and 50 g FO per kg of diet.

h). Micropulverized samples (100 mg) were ashed at 600°C for 24 h in a muffle furnace.

The pH of the suspension of cecal contents was measured with a compact pH meter (Twin pH B-112; Horiba, Ltd., Kyoto). The remaining cecal content was weighed and centrifuged (10 min, 10,000 × *g*) in a high-speed centrifuge (HIMAC SCR20B; Hitachi, Ltd., Tokyo) after adding an equal weight of distilled water. The supernatant and pellet were separated and weighed. The supernatant was appropriately diluted with 0.1 N HCl and directly subjected to atomization. Each cecal pellet was dried (110°C, 10 h), weighed and ashed at 600°C for 24 h in the muffle furnace. Each ashed sample, dissolved in 4 ml of 2 N HCl, was diluted appropriately with distilled water for atomization.

The distribution of minerals between the solid and liquid phases of the cecal contents was calculated by the method of Schulz et al. (*12*).

Measurement of biochemical parameters of anemia. Blood was collected by tail vein puncture during the experimental period. Blood samples were analyzed for hematocrit and the concentration of hemoglobin. On the final day of the experiment, rats were anesthetized by exposure to diethyl ether. After laparotomy, the blood was collected by abdominal vein puncture.

Serum hemoglobin concentration, serum iron (SI) and unsaturated iron binding capacity (UIBC) were analyzed with commercial test kits (Fe C-test, UIBC-test, Wako Pure Chem. Ind., Tokyo). Hemoglobin-Fe (HI) and hemoglobin regeneration efficiency (HRE) were calculated from the following formula by the

method of Miller (*21*):

Hemoglobin-Fe (HI) (mg) = (body weight (g))
× (ml blood/g body weight (assumed to be 0.067 ml))
× (g hemoglobin/ml blood)
× (mg Fe/g hemoglobin (assumed to be 3.35 mg))

Hemoglobin regeneration efficiency (HRE)
= (HI (mg) at the end of each period
− HI (mg) at the begining of each period)/mg Fe consumed

Chemicals. Fructooligosaccharides (FO: mixture of 42% 1-kestose, 46% nystose and 9% 1F-β-fructofuranosyl nystose, with a concentration of oligosaccharides above 95%) were obtained from Meiji Seika Kaisha, Ltd. (Tokyo). Other dietary components apart from minerals were purchased from Oriental Yeast Co. (Tokyo). Ferric pyrophosphate was food additive grade and was purchased from Kokusan Chem. Co. Inc. (Tokyo). Other minerals and all other reagents were analytical grade and were purchased from Wako Pure Chem. Ind. (Tokyo).

Statistics. The data reported are the mean values with SEM. Two-way ANOVA was used to compare means among groups. The level of significance was taken as $p < 0.05$.

RESULTS

Body weight and food intake

Body weight and food intake are shown in Table 2. Increasing Fe concentration in the diet increased the final body weight and food intake. FO-feeding did not affect the body weight and food intake.

Table 2. Body weight and food intake of the rats fed experimental diets for 14 days.

Group*1	Body weight (g)		Food intake (g)
	Initial	Final	
L	172±4	240±16	237±6
LF	173±4	257±4	223±13
N	173±3	265±7	253±9
NF	174±3	269±6	240±7
Statistical significance of effects of:			
(Fe)*2	ns	ns	*
(FO)*3	ns	ns	ns
(Fe) × (FO)*4	ns	ns	ns

M±SEM ($n=7$). *1 See legend to Table 1. *2 Effect of Fe in diets. *3 Effect of FO in diets. *4 Interaction between (Fe) and (FO). ns = No significant difference. Significant difference at $p < 0.05$ (*).

Table 3. Changes in hematocrit ratio (%) and hemoglobin concentration (g/dl).

Group*1	day 0	day 3	day 7	day 10	day 14
Ht (%)					
L	15.9±3.3	15.5±1.6	17.9±1.7	19.4±2.4	22.2±2.5
LF	16.4±3.4	17.1±2.0	20.4±3.4	21.6±2.6	24.3±2.4
N	17.5±4.8	18.2±2.2	26.4±4.1	27.9±3.8	32.1±5.0
NF	17.9±3.9	20.4±1.8	24.7±2.8	30.5±3.3	33.9±2.3
Statistical significance of effects of:					
(Fe)*2	ns	***	***	***	ns
(FO)*3	ns	*	ns	*	ns
(Fe)×(FO)*4	ns	ns	ns	ns	ns
Hb (g/dl)					
L	6.03±1.24	5.03±0.94	5.28±0.35	5.71±0.87	5.10±0.47
LF	6.40±1.93	6.31±0.72	6.65±1.00	6.33±0.82	6.21±0.55
N	6.20±0.63	6.60±0.68	7.56±1.21	8.60±0.88	8.82±1.66
NF	5.71±0.97	6.87±0.97	7.74±1.24	9.64±0.69	9.45±0.57
Statistical significance of effects of:					
(Fe)*2	ns	***	***	***	***
(FO)*3	ns	*	ns	*	*
(Fe)×(FO)*4	ns	ns	ns	ns	ns

M±SEM ($n=7$). *1 See legend to Table 1. *2 Effect of Fe in diets. *3 Effect of FO in diets. *4 Interaction between (Fe) and (FO). ns=No significant difference. Significant difference at $p<0.05$ (*) and $p<0.001$ (***).

Table 4. Biochemical parameters of anemia in the various groups of rats.

Group*1	SI*2 (μg/dl)	UIBC*3 (μg/dl)	TIBC*4 (μg/dl)	HRE*5	
				1st period (%)	2nd period (%)
L	57.2±11.0	772±23	829±22	24.1±9.4	23.6±5.9
LF	39.7±5.2	775±11	815±15	62.6±10.1	22.4±10.6
N	80.5±10.3	699±21	779±16	44.3±5.3	50.8±11.7
NF	95.4±12.2	627±20	722±15	61.2±11.7	68.0±10.9
Statistical significance of effects of:					
(Fe)*6	***	***	***	ns	***
(FO)*7	ns	ns	ns	***	ns
(Fe)×(FO)*8	ns	ns	ns	ns	ns

M±SEM ($n=7$). *1 See legend to Table 1. *2 SI: serum iron concentration, *3 UIBC: unsaturated iron-binding capacity, *4 TIBC: total iron-binding capacity, *5 HER: hemoglobin regeneration efficiency. *6 Effect of Fe in diets. *7 Effect of FO in diets. *8 Interaction between (Fe) and (FO). ns=No significant difference. Significant difference at $p<0.001$ (***).

Table 5. Absorption of iron, calcium and magnesium.

	L*1	LF*1	N*1	NF*1	Statistical significance of effects of: (Fe)*4	(FO)*5	(Fe)×(FO)*6
Iron (3–6 days)							
Intake (mg/day)	0.259±0.005	0.250±0.010	0.519±0.017	0.485±0.009	***	*	ns
Fecal excretion (mg/day)	0.100±0.007	0.084±0.005	0.209±0.009	0.160±0.005	***	***	*
Absorption (mg/day)*2	0.159±0.006	0.166±0.004	0.310±0.017	0.325±0.006	***	ns	ns
Absorption ratio (%)*3	61.5±2.4	66.5±1.7	59.6±4.8	67.0±0.6	ns	***	ns
(10–13 days)							
Intake (mg/day)	0.299±0.009	0.279±0.006	0.568±0.021	0.554±0.016	***	ns	ns
Fecal excretion (mg/day)	0.206±0.014	0.149±0.011	0.294±0.019	0.250±0.014	***	***	ns
Absorption (mg/day)*2	0.093±0.010	0.130±0.014	0.274±0.028	0.304±0.024	***	ns	ns
Absorption ratio (%)*3	31.2±3.3	46.3±4.5	47.9±3.8	54.4±2.9	***	***	ns
Calcium (3–6 days)							
Intake (mg/day)	96.9±1.8	89.1±1.4	99.3±3.3	91.7±1.7	ns	***	ns
Fecal excretion (mg/day)	32.2±2.2	22.1±0.7	36.5±2.1	23.0±1.4	ns	***	ns
Absorption (mg/day)*2	64.6±1.7	67.0±1.7	62.7±2.5	68.8±1.3	ns	*	ns
Absorption ratio (%)*3	66.8±1.9	75.2±0.9	63.2±1.6	75.0±1.3	ns	***	ns
(10–13 days)							
Intake (mg/day)	111.7±3.4	99.7±2.3	112.9±4.3	114.1±3.3	*	ns	ns
Fecal excretion (mg/day)	47.0±2.5	34.7±1.9	47.6±2.8	34.7±1.7	ns	***	ns
Absorption (mg/day)*2	64.8±5.3	65.0±3.4	65.3±2.1	79.5±3.8	*	*	*
Absorption ratio (%)*3	58.0±1.3	65.0±5.8	57.9±1.3	69.5±1.7	ns	***	ns
Magnesium (3–6 days)							
Intake (mg/day)	8.16±0.15	7.39±0.11	8.95±0.79	8.35±0.15	***	***	ns
Fecal excretion (mg/day)	3.35±0.20	1.77±0.09	4.07±0.22	1.71±1.20	ns	***	*
Absorption (mg/day)*2	4.80±0.19	5.62±0.13	4.87±0.25	6.64±0.13	***	***	*
Absorption ratio (%)*3	58.9±2.3	76.1±1.2	54.5±2.0	79.5±1.2	ns	***	*
(10–13 days)							
Intake (mg/day)	9.41±0.29	8.27±0.19	9.78±0.37	9.52±0.28	***	*	ns
Fecal excretion (mg/day)	5.30±0.25	2.05±0.13	4.94±0.27				

(10–13 days)							
Intake (mg/day)	9.41±0.29	8.27±0.19	9.78±0.37	9.52±0.28	***	*	ns
Fecal excretion (mg/day)	5.30±0.25	2.05±0.13	4.94±0.27	2.24±0.18	ns	***	ns
Absorption (mg/day)*2	4.11±0.30	6.21±0.31	4.84±0.33	7.28±0.40	*	***	ns
Absorption ratio (%)*3	43.5±2.6	74.9±2.0	49.3±2.4	76.1±2.2	ns	***	ns

Mean±SEM ($n=7$). *1 See legend to Table 1. *2 Intake-fecal excretion. *3 (Absorption value/intake)×100. *4 Effect of Fe levels in diets. *5 Effect of FO in diets. *6 Interaction between (Fe) and (FO). ns=No significant difference. Significant difference at $p<0.05$ (*) and $p<0.001$ (***).

Table 6. Distribution of iron, calcium and magnesium between the liquid and solid phases in cecal lumen of rats fed experimental diet.

	L*1	LF*1	N*1	NF*1	Statistical significance of effects of: (Fe)*3	(FO)*4	(Fe)×(FO)*5
Weight of cecal contents (g)	2.96±0.38	8.37±0.38	3.82±0.36	7.92±0.49	ns	***	ns
pH of cecal contents	7.0±0.1	5.9±0.1	7.0±0.1	6.3±0.2	ns	***	ns
Iron							
Solid phase (μg/g content)	75.0±7.7	38.6±3.9	152.6±3.7	129.8±6.8	***	***	ns
Liquid phase (μg/g content)	4.0±0.5	27.4±5.9	3.4±0.2	34.3±8.8	ns	***	ns
Ratio of liquid to solid phase (%)*2	5.6±1.0	77.2±11.0	2.2±0.1	28.0±8.0	***	***	***
Calcium							
Solid phase (mg/g content)	27.1±0.6	19.5±0.9	22.9±3.9	17.8±0.7	ns	***	ns
Liquid phase (mg/g content)	2.0±0.4	2.4±0.4	1.5±0.2	3.8±0.3	ns	***	*
Ratio of liquid to solid phase (%)*2	7.6±1.8	12.7±2.4	5.3±0.6	21.3±1.8	ns	***	***
Magnesium							
Solid phase (mg/g content)	2.58±0.13	0.53±0.06	2.49±0.12	0.32±0.05	ns	***	ns
Liquid phase (mg/g content)	0.56±0.06	0.48±0.04	0.40±0.06	0.70±0.07	ns	ns	***
Ratio of liquid to solid phase (%)*2	22.6±4.0	102.2±18.8	16.4±2.6	246.0±33.4	**	***	***

M±SEM ($n=7$). *1 See legend to Table 1. *2 (Solid phase/Liquid phase)×100. *3 Effect of Fe in diets. *4 Effect of FO in diets. *5 Interaction between (Fe) and (FO). ns=No significant difference. Significant difference at $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***).

Biochemical parameters of Anemia

Changes in the hematocrit ratio and hemoglobin concentration during the experiment are shown in Table 3. An increase in the concentration of Fe in the diet increased the hematocrit ratio at experimental days 3, 7, 10 and 14. FO-feeding increased the hematocrit ratio at experimental days 3 and 10. An increase in the concentration of Fe in the diet increased the hemoglobin concentration at experimental days 3, 7, 10 and 14. FO-feeding increased the hemoglobin concentration at experimental days 3, 10 and 14. SI, UIBC, TIBC and HRE are shown in Table 4. During the first week of the experimental period, FO-feeding increased HRE. During the second week of the experimental period, an increase in the concentration of Fe in the diet increased HRE. SI, UIBC and TIBC were improved by increasing the Fe concentration in the diet. However FO-feeding did not affect SI, UIBC or TIBC.

Absorption of Fe, Ca and Mg

The absorption of Fe, Ca and Mg is shown in Table 5. FO-feeding increased the apparent absorption ratio of Fe, Ca and Mg during both periods (experimental days 3–6 and 10–13). An increase in the concentration of Fe in the diet increased the apparent absorption of Fe during the second period (experimental days 10–13), but did not affect the apparent absorption ratios of Ca and Mg.

Weight and pH of the cecal contents and the distribution of Fe, Ca and Mg in the cecal contents

The weight and pH of the cecal contents and the distribution of Fe, Ca and Mg are shown in Table 6. The weight of the cecal contents were increased and the pH of the cecal contents were reduced by FO-feeding. FO-feeding increased the liquid phase and decreased the solid phase of Fe, Ca and Mg in the cecal contents. Therefore, FO-feeding increased the ratios of the liquid phase to the solid phase of Fe, Ca and Mg. An increase in the concentration of Fe in the diet increased the solid phase and decreased the ratio of the liquid phase to the solid phase of Fe in the cecal contents.

DISCUSSION

FO-feeding improved recovery from anemia based on the hematocrit ratio, hemoglobin concentration and hemoglobin regeneration efficiency (HRE) in Fe-deficient rats. FO-feeding also increased the apparent absorption of Fe. This is one of the reasons why FO-feeding improved recovery from anemia.

Some type of pectin which is an undigestible and fermentable carbohydrate, increased HRE in Fe-deficient anemic rats and increased the apparent absorption of Fe in healthy rats (*22*,*23*). The mechanism of Fe-absorption is not clearly understood yet. However, solubilization of Fe is essential before it can be absorbed in the lumen (*15*). The increasing effect of these pectins on Fe-absorption was

suggested to be caused by inhibiting the insoluble complex formation with Ca, phosphate or other nutrients in the lumen (*22,23*). However, FO are neutral carbohydrates, and do not chelate Fe directly. Therefore, FO-feeding stimulates Fe-absorption by a mechanism different from that of pectin. There are three hypotheses which may explain the stimulating effect of FO-feeding on the absorption of Fe. Organic acids such as lactate and short-chain fatty acids were produced by luminal bacteria from FO. First, organic acids lowered the pH of the luminal contents and raised the solubility of Fe. It is well known that ascorbate stimulates the absorption of Fe (*7,8*). Ascorbate acts in two ways: by reducing ferric iron to ferrous iron, and by forming an absorbable small molecular complex with ferric iron (*1*). Lactate has also been reported to form an absorbable small molecular complex with ferric iron (*24*). Second, lactate which was produced by luminal fermentation of FO formed an absorbable small molecular complex with Fe. It has been reported that high levels of dietary Ca supplementation decreased the absorption of Fe (*16,17*). Because Ca supplementation stimulates the formation of an insoluble complex of Fe with Ca in the lumen. Third, increased Fe absorption follows an increase in Ca absorption by FO-feeding.

In rats, the main site in the lumen where the undigestible carbohydrates are fermented by luminal bacteria is the large intestine, namely, the cecum and colon. There is relatively little fermentation of undigestable carbohydrates in the small intestine. Although the role of the large intestine on the absorption of Fe is not known entirely, our data suggested that the large intestine is able to absorb Fe.

The absorption of Ca and Mg were reportedly reduced in Fe-deficient anemic rats (*18*). In our present study, an increase of Fe in the experimental diet improved the recovery from anemia, but did not affect the absorption of Ca or Mg. Moreover, also in Fe-deficient anemic rats, as in healthy rats, FO-feeding increased the absorption of Ca and Mg. These results suggested that anemia did not affect Ca and Mg absorption directly, at least in the short-term period.

We concluded that FO-feeding improved the recovery from anemia in the Fe-deficient anemic rats. This effect might be due to increased absorption of Fe. Also, FO-feeding increased the absorption of Ca and Mg in anemic rats.

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