Biochemical and Molecular Roles of Nutrients

Dietary Fructooligosaccharides Prevent Postgastrectomy Anemia and Osteopenia in Rats

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ABSTRACT Gastrectomized rats develop anemia and osteopenia, and ingestion of fructooligosaccharides leads to an increase in iron absorption and promotes recovery from anemia in iron-deficient rats. Laparotomized (sham-operated control) rats and totally gastrectomized (Billroth II) rats, in groups of 14 each, were fed a control diet without fructooligosaccharides or a diet containing fructooligosaccharides (75 g/kg of diet) for 6 wk. All rats received an intramuscular injection of vitamin B-12 every 2 wk. Tail blood was collected every week for determination of hematocrit and hemoglobin concentration. At the end of the experiment, the rats were killed and the femur and tibia were collected for measurement of bone mineral density (BMD). The hematocrit, hemoglobin concentration, hemoglobin regeneration efficiency, and BMD of both femurs and tibias were significantly lower in gastrectomized rats fed the control diet than in the other three groups. Dietary fructooligosaccharides prevented anemia and osteopenia in totally gastrectomized rats. J. Nutr. 128: 485–490, 1998.

KEY WORDS: • rats • gastrectomy • fructooligosaccharides • anemia • osteopenia

Anemia occurring as a complication of gastrectomy is observed frequently (Adams 1968, Bradley and Isaacs 1976, Kelly et al. 1953, Nishimura and Kaibaram 1991). Gastrectomy anemia may be multicausal (Bradley and Isaacs 1976), but it is clear that this anemia is affected by malabsorption of iron (Brintnall et al. 1956, Fischermann et al. 1967) and vitamin B-12 (Gough et al. 1965). In the early phase after gastrectomy, the leading factor contributing to anemia is malabsorption, because it is necessary for dietary iron to be dissolved by gastric acid for absorption (Monsen and Cook 1976). In the later phase (after several years), the anemia is due to deficiency of vitamin B-12, because vitamin B-12 cannot be absorbed without an intrinsic factor that is secreted from the stomach (Gough et al. 1965). Intravenous administration of these elements usually fails to prevent such an anemia completely (Bradley and Isaacs 1976, Haurani and Wirts 1971), however, a suitable therapy has not been established.

Recently, there have been many reports indicating that indigestible carbohydrates, such as fructooligosaccharides (FOS)1,2 (Baba et al. 1995, Ohta et al. 1997a, 1997b), inulin (Rémesy et al. 1993), guar gum hydrolysate (Hara et al. 1996) and resistant starch (Schultz et al. 1993), stimulate mineral absorption. The effects of FOS with respect to increasing the absorption of minerals such as Ca, Mg and Fe have been especially well examined. Moreover, we have reported previously that FOS-feeding promotes recovery from anemia in iron-deficient rats (Ohta et al. 1995a). We showed that the stimulatory effect of FOS on Ca and Mg absorption mainly takes place in the large intestine (Baba et al. 1996, Ohta et al. 1994b, 1995b, and 1997a). We speculated that FOS might have a similar stimulatory effect on iron absorption in the large intestine because FOS-feeding was found to increase the soluble fraction of iron in the cecal contents of rats (Ohta et al. 1995a). Therefore we examined whether FOS-feeding prevents postgastrectomy anemia.

Postgastrectomy osteopenia also is observed commonly (Morgan et al. 1965, Pääkkönen et al. 1984). Previously, we showed that FOS-feeding reduces the severity of this osteopenia significantly but does not prevent it completely (Ohta et al. 1997b). Therefore in this study, we started feeding the experimental diet earlier after gastrectomy, prolonged the feeding period and measured the bone mineral density (BMD) in the rats.

We previously examined whether the coprophagy, which is a common habit in rats, affects the stimulatory effect of FOS on the mineral absorption and confirmed that the effects of FOS occur in rats irrespective of the prevention of coprophagy (Ohta et al. 1996). Moreover Zang et al. (1992) reported that coprophagy did not affect iron absorption from a ferric compound. Therefore in the present study, we did not prevent coprophagy in rats (Ohta et al. 1997b).

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2 To whom correspondence should be addressed.
3 FOS, fructooligosaccharides, is a mixture of 42% 1-kestose, 46% nystose and 9% 1F-glucuronofructofuranosylfructose (Meiloigo-P3, Meiji Seika Kaisha, Tokyo, Japan).
4 Abbreviations used: BMD, bone mineral density; FOS, fructooligosaccharides; Hb-Fe, hemoglobin-iron; HRE, hemoglobin regeneration efficiency; TIBC, total iron binding capacity; UIBC, unsaturated iron binding capacity.


**MATERIALS AND METHODS**

**Animals and surgical procedure.** Thirty-one, 4-wk-old male Sprague-Dawley rats (Clea Japan, Tokyo, Japan) were housed in individual stainless-steel wire-mesh cages in a room at 25°C and 55% relative humidity. The rats were fed a pelleted diet (MF, Oriental Yeast, Tokyo, Japan) for 1 wk before operation as the adaptation period. All rats were anesthetized by fluorothene inhalation (halothan, Takeda Chemical Industries, Osaka, Japan). The rats were assigned randomly to two groups of 14 and 17 rats. In the group of 17 rats, the stomach was removed surgically (Billoth II) (Lambert 1965, Os-

carson et al. 1979). In the other group, the rats were subjected to a sham operation; the abdominal cavity was opened for ~45 min, the same length of time as required for the gastrectomy procedure. Three gastrectomized rats died before the start of the experiment.

**Ethical consideration.** This study was approved by the Animal Committee of Meiji Seika Bioscience Laboratories, and the animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Meiji Seika Bioscience Laboratories.

**Experimental groups and diets.** After the operations, rats were deprived of food for 24 h and then were allowed free access to homog-

enized and pasteurized cow’s milk (Meiji Milk Products, Tokyo, Ja-

pan) for 48 h. Three days after the operations, the rats were divided randomly into four experimental subgroups of seven rats each (sham-

operated vs. gastrectomy, control vs. FOS diet). Rats were fed the assigned experimental diets for 6 wk. All rats received an intramuscular injection of vitamin B-12 (Wako Pure Chemical Industries, Tokyo, Japan) at 0.5 mg/kg every other week, starting on the initial day of the feeding period.

**RESULTS**

Initial and final body weights, body weight gain and total food intake did not vary among the experimental groups (Table 2).

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**TABLE 1**

Composition of experimental diets

<table>
<thead>
<tr>
<th>Ingredient, g/kg</th>
<th>Control diet</th>
<th>FOS diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Dextrin</td>
<td>352</td>
<td>352</td>
</tr>
<tr>
<td>Corn oil</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>25</td>
</tr>
<tr>
<td>FOS</td>
<td>0</td>
<td>75</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Chemical analysis, mmol/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>124</td>
<td>122</td>
</tr>
<tr>
<td>Magnesium</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>87</td>
<td>87</td>
</tr>
</tbody>
</table>

*1* Fructooligosaccharides (Meioglio-P®, Meiji Seika Kaisha, Tokyo, Japan; concentration of oligosaccharides was >95% of total mixture).

*2* Pine-dex #4 (Matsutani Chemical Industry, Hyogo, Japan).

*3* Prepared according to AIN-93 formulation (Reeves et al. 1993).

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**TABLE 2**

<table>
<thead>
<tr>
<th>Treatment diet</th>
<th>Sham</th>
<th>Gastroctomy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>FOS</td>
</tr>
<tr>
<td>Initial body weight, g</td>
<td>243</td>
<td>240</td>
</tr>
<tr>
<td>Final body weight, g</td>
<td>382</td>
<td>385</td>
</tr>
<tr>
<td>Total body weight gain, g/6 wk</td>
<td>138</td>
<td>145</td>
</tr>
<tr>
<td>Food intake, g/day</td>
<td>15.2</td>
<td>15.1</td>
</tr>
</tbody>
</table>

*1* Values are means, *n* = 7.

*2* For details of diet, see Table 1.

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Table 1 shows the composition of the two experimental diets (control and FOS). FOS is a mixture of 42% 1-kestose, 46% nystose and 9% 1F-β-bructofuranosylnystose (Meioglio-P®, Meiji Seika Kaisha, Tokyo, Japan) (Hidaka et al. 1988 and 1991). Other dietary components, apart from minerals, were obtained from Oriental Yeast. All other reagents were of analytical grade from Wako Pure Chemical Industries.

One-half of the gastrectomized and sham-operated rats were fed the control diet, and the remaining rats were fed the FOS diet. During the first 2 wk, the rats were fed 15 g of diet per day, and thereafter they were fed 20 g of diet per day for 4 wk.

The rats were allowed free access to deionized water throughout the experimental period. On the final day of the experiment, all rats were anesthetized by exposure to diethyl ether. After laparotomy, whole blood was collected by abdominal vein puncture, and the animals were killed. The femur and tibia of both sides were sampled immediately.

**Measurement of biochemical parameters of anemia.** Blood was collected by tail venous puncture every week during the experimental period. Blood samples were analyzed to determine the hematocrit and hemoglobin concentration. Using the serum samples obtained on the final day of the experiment, serum iron and unsaturated iron binding capacity (UIBC) were determined by means of commercial assay kits (Fe c-test, UIBC-test, Wako Pure Chemical Industries). Hemoglobin-iron (Hb-Fe) and hemoglobin regeneration efficiency (HRE) were calculated from the following formula by the method of Miller (1982).

\[
\text{Hb-Fe (mol)} = \frac{[\text{body weight (g)}]}{[\text{mol Fe/g hemoglobin (assumed to be 0.06 mol)}]} \times \left( \frac{\text{mol Fe consumed}}{\text{Hb-Fe (mol) at the beginning of each period}} \right)
\]

**RESULTS**

Initial and final body weights, body weight gain and total food intake did not vary among the experimental groups (Table 2).
FRUCTOOLIGOSACCHARIDES AND GASTRECTOMY

FIGURE 1 Hematocrits and hemoglobin concentrations in sham operated or gastrectomized rats fed diets with (FOS) or without (control) fructooligosaccharides. Each point represents the mean ± SEM, n = 7. All ANOVA main effects (treatment, diet and time) and interactions except treatment × time were significant,¹ significantly lower than in sham operated rats fed the FOS diet, ¹ significantly lower than in sham operated rats fed either control or FOS diet. §, significantly less than in other three groups, P < 0.05.

The initial hematocrit and hemoglobin concentration in the sham-operated rats were higher than those in the gastrectomized rats (Fig. 1). At every sampling time after the 1st wk after the start of feeding the experimental diets, there was no difference in hematocrit or hemoglobin concentration between the gastrectomized rats fed the FOS diet and the sham-operated rats fed either the control diet or the FOS-containing diet. However, the values in these three groups were significantly higher than those in gastrectomized rats fed the control diet.

The concentration of iron in serum of sham-operated rats fed the control diet did not differ from that in sham-operated rats fed the FOS diet but was significantly lower than those in gastrectomized rats fed either diet (Table 3). Serum iron in gastrectomized rats fed the FOS diet was significantly higher than that in gastrectomized rats fed the control diet. UIBCs in the two sham-operated groups did not differ and were significantly lower than those in gastrectomized rats fed either the control or the FOS diet. UIBC in the gastrectomized rats fed the FOS diet was significantly lower than that in the gastrectomized rats fed the control diet.

HRE in the gastrectomized rats fed the control diet was significantly lower than that in the other three groups (Fig. 2).

Femur BMD in the sham-operated rats fed the FOS diet was significantly higher than that in rats of the other three groups (Fig. 3). Tibia BMD did not differ between the two sham-operated groups. BMD of both femur and tibia in the gastrectomized rats fed the FOS diet did not differ from that in the sham-operated rats fed the control diet. BMD of both femur and tibia in the gastrectomized rats fed the control diet was lower than that in rats of the other three groups.

DISCUSSION

To our knowledge, there is only one published paper that has described postgastrectomy anemia in rats, but it contains no detailed description of the development of this anemia (Rieber et al. 1967). In the present study, we clarified the process of the postgastrectomy anemia. The primary cause of this anemia was considered to be bleeding during the gastrectomy operation, because only 3 d after the operation (at the start of the experimental feeding period), anemia was already evident in the gastrectomized rats. A secondary cause was iron malnutrition as indicated by the observation that, in gastrectomized rats fed the FOS diet was significantly lower than that in the gastrectomized rats fed the control diet.

| TABLE 3 | Serum iron, unsaturated and total iron binding capacity in sham-operated and gastrectomized rats fed control or FOS-containing diets¹ |
|----------|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|          | Sham                                      | Gastrectomy    | Statistical significance of effects |
|          | Control                                  | FOS            | Control         | FOS            | Pooled SEM      | D̂⁵        | T                  | D̂ × T⁶        |
| Serum iron, μmol/L | 34.5±a  | 32.5ab  | 10.0c  | 26.2b  | 2.1  | 0.003       | <0.001          | <0.001           |
| UIBC³, μmol/L      | 52.8c  | 59.3c  | 109.2a  | 72.9b  | 4.5  | <0.001     | <0.001          | <0.001           |
| TIBC⁴, μmol/L      | 87.3c  | 91.8c  | 119.2a  | 99.1b  | 2.5  | <0.001     | <0.001          | <0.001           |

¹ Values are means, n = 7. UIBC: unsaturated iron-binding capacity; TIBC: total iron-binding capacity. a,b,c Mean values with no superscript letters in common are significantly different, P < 0.05.
² For details of diet, see Table 1.
³ Diet (control or FOS), treatment (with or without gastrectomy).
While in this study, the iron source in the experimental diets was Fe-citrate, which is a water-soluble compound. In this case, the main reason for the iron malabsorption cannot be that gastric acid secretion was lacking or that Fe-citrate was changed into an insoluble form in the lumen.

FOS-feeding prevented the development of anemia. The final hemoglobin concentration and hematocrit in the gastrectomized rats fed the FOS diet did not differ from those in the sham-operated rats fed either of the diets. Serum iron and HRE also significantly increased after FOS-feeding in gastrectomized rats. It is reported that there is a high positive correlation between serum iron concentration and iron absorption (Kim and Atallah 1993). Buchowski et al. (1989) also reported that there is a high positive correlation between HRE and apparent absorption of iron. Moreover, we have confirmed that FOS stimulated iron absorption in iron-deficient anemic rats (Ohta et al. 1995a), suggesting that dietary FOS prevent anemia by increasing iron absorption. In gastrectomized rats fed a FOS-containing diet, increases in unsaturated and total iron binding capacity were observed. It seems that the effect of ingested FOS was not enough to stimulate iron absorption in gastrectomized rats to the same degree as that in the case of healthy rats.

Previously, we reported that FOS-feeding increased iron absorption and improved recovery from anemia in iron-deficient rats (Ohta et al. 1995a). In that study, we used ferric pyrophosphate, which is a water-insoluble compound, as the iron source in the experimental diets. In this case, FOS-feeding led to a decrease in pH of the cecal contents and an increase in iron concentration in the soluble fraction of the cecal contents. We have demonstrated previously that ingested FOS promotes an increase in Ca and Mg absorption from the large intestine (Baba et al. 1996, Ohta et al. 1994b, 1995b, and 1997b). The mechanism of iron absorption via not only the small intestine, but also via the large intestine has not yet been clarified.

Several authors have reported that iron malnutrition is the cause of iron malabsorption in postgastrectomy patients (Brinnall et al. 1956, Fischermann et al. 1967) because dietary iron is not dissolved under conditions in which gastric acid secretion is lacking, and thereby iron absorption is decreased (Bjorn-Rasmussen 1973). However, in this study, the iron source in the experimental diets was Fe-citrate, which is a water-soluble compound. In this case, the main reason for the iron malabsorption cannot be that gastric acid secretion was lacking or that Fe-citrate was changed into an insoluble form in the lumen.

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However, sufficient iron is absorbed via the large intestine for recovery from iron-deficiency anemia in rats (Ebihara et al. 1994, Ebihara and Okano 1995). Therefore, we speculate that the effect of FOS in increasing the absorption of iron takes place in the large intestine in gastrectomized rats.
rats. In the present study, we used soluble Fe-citrate as the iron source in the experimental diets and it seems likely that this Fe-citrate was changed immediately into another insoluble chemical form, such as a phosphate salt, in the intestine. There are a few other possible explanations for the stimulatory effect of FOS on iron absorption in gastrectomized rats. FOS-feeding may lead to a change in mucin composition and thereby increase iron absorption in the small intestine. Conrad et al. (1993) proposed that the pathway of iron transport in the intestine consists of several iron-binding proteins including mucin, integrin, mobilferrin and ferritin. Once the iron-mucin complex is formed, it keeps the iron in a soluble form after alkalization in the lumen and thereby facilitates iron absorption (Conrad and Umbreit 1993). Dietary inulin, which is an indigestible carbohydrate with longer chains of fructose residues than those in FOS, modifies the composition of mucin (leading to an increase in sulphomucin in the cecal mucosa) (Fontaine et al. 1996). Second, the increase in iron absorption may be due to the increase in Ca absorption that results from FOS-feeding. Several authors have reported that Ca supplementation decreases iron absorption in rats and humans (Barton et al. 1983, Cook et al. 1991, Monsen and Cook 1976) by an unknown mechanism. However, Hallberg et al. (1991) speculated that Ca and iron may competitively bind to one or more substances that are important in the transcellular absorptive pathway, resulting in the inhibitory effect of Ca on iron absorption. Actually in rats, some duodenal proteins such as mobilferrin and calreticulin have affinity for both Ca and iron (Conrad et al. 1993). Calcium binding proteins exist also in the large intestine of rats (Petith et al. 1979). In our previous studies, we observed that FOS-feeding markedly increased Ca absorption in gastrectomized rats (Ohta et al. 1997b). If FOS stimulates Ca absorption via a route independent of iron absorption, such as the paracellular route, FOS-feeding may reduce the inhibitory effect of Ca on iron absorption. Third, propionate, which is produced by intestinal fermentation of FOS, may stimulate heme production. It has been proposed that propionate could promote δ-aminolevulinic synthesis, hence affecting heme production (Imazumi et al. 1992).

We have not examined the effect of FOS in animals fed other dietary forms of iron, except for ferric citrate and ferric phosphate, such as heme iron. This will be examined in our future studies.

Previously, we reported that FOS-feeding significantly suppressed the decrease in BMD of the femur and tibia that occurs in gastrectomized rats but did not prevent it completely (Ohta et al. 1997b). In that study, we observed a higher significant correlation between BMD and apparent iron absorption in the later balance periods and the extent of Ca absorption during the final balance period in the gastrectomized rats fed FOS diet was similar to that in sham-operated rats (Ohta et al. 1997b). Therefore, we expected that feeding the FOS diet sooner after gastrectomy and prolonging the feeding period would completely prevent the decrease in BMD. In this study, FOS-feeding did prevent completely the decrease in BMD of both femur and tibia. These findings suggest that gastric hormones, such as gastrin or calcium, may not affect femur BMD (Axelson et al. 1991, Håkanson et al. 1990).

In conclusion, FOS-feeding completely prevented postgastrectomy anemia and osteopenia in rats.

LITERATURE CITED


