Nondigestible oligosaccharides do not interfere with calcium and nonheme-iron absorption in young, healthy men1–3

Ellen GHM van den Heuvel, Gertjan Schaalma, Theo Muys, and Wim van Dokkum

ABSTRACT The effect of inulin, fructooligosaccharides, and galactooligosaccharides on true intestinal absorption of iron and calcium was measured in men by using double stable-isotope techniques (oral 57Fe and 44Ca and intravenous 58Fe and 40Ca). The incorporation of iron isotopes into erythrocytes and the urinary excretion of calcium isotopes was measured with an inductively coupled plasma mass spectrometer. Twelve healthy, nonanemic, male subjects aged 20–30 y received four treatments consisting of a constant basal diet supplemented with 15 g/d inulin, fructooligosaccharide, or galactooligosaccharide, or not supplemented (control treatment). These four treatments were given for 21 d each according to a randomized, crossover design. Iron absorption was measured over the last 7 d of treatment (days 15–21) and calcium absorption was measured on day 21 of each treatment period. Mean (± SEM) iron absorption was 5.5 ± 1.6%, 6.1 ± 1.9%, 5.3 ± 1.9%, and 5.1 ± 1.5%, respectively, during treatment with inulin, fructooligosaccharide, galactooligosaccharide, or the control; mean (± SEM) calcium absorption was 25.8 ± 2.3%, 26.3 ± 1.9%, 26.3 ± 2.6%, and 28.1 ± 4.3%, respectively. None of the differences between treatments was significant. It is concluded that 15 g/d inulin, fructooligosaccharide, or galactooligosaccharide did not have a negative effect on iron and calcium absorption in young healthy men. Am J Clin Nutr 1998;67:445–51.

KEY WORDS Calcium, iron, absorption, fructooligosaccharide, galactooligosaccharide, inulin, stable isotopes, nondigestible oligosaccharides, men

INTRODUCTION

There is increasing interest in so-called functional foods or functional food ingredients, foods with an additional nutritional value that may be beneficial to health. Nondigestible oligosaccharides (NDOs) may fit into this category of functional food ingredients. NDOs are defined as oligosaccharides that escape digestion in the stomach and small intestine of humans and consequently arrive quantitatively in the colon. Examples are inulin, fructooligosaccharides (fructans from the chicory root), and galactooligosaccharides. Favorable effects of the ingestion of NDOs on gastrointestinal function [eg, fecal bulking and production of short-chain fatty acids (SCFAs)] as well as on metabolism (eg, reduction of serum cholesterol and improved tolerance to glucose) have been found (1).

Before NDOs can be considered as favorable for health, their effect on calcium or iron metabolism should be established in humans. An adequate supply and bioavailability of calcium is essential to attain maximum bone mass, on which adult bone mass depends (2). Many studies have also shown a significant relation between iron status and consumption of foods known to influence iron absorption (3). Therefore, a fundamental understanding of dietary factors affecting availability of iron and calcium for absorption is needed to optimize both iron and calcium nutrition.

In rats, positive effects of NDOs on iron and calcium absorption have been found (4–9). The results of a few human studies on other nondigestible and fermentable carbohydrates (gums and pectins) indicate that with the possible exception of iron, pectin and gums do not adversely affect mineral balance (10, 11). The bioavailability of iron has been shown to be decreased by pectin in some, but not in all, human studies (10). Calcium showed the greatest potential for negative balance with longer locust bean gum consumption (12).

Like soluble dietary fiber (gums and some pectins), NDOs are substrates for both hydrolysis and fermentation by cecal and colonic microflora. As a consequence of fermentation, the large intestinal pool of SCFAs and other organic acids increases and the pH may decrease. These environmental changes in the large intestine may improve solubility and absorption of several minerals and trace elements. Moreover, it is possible that NDOs form complexes with minerals in the small intestine, which could either promote or decrease their absorption.

The aim of this study was to investigate the effect of 15 g/d inulin, fructooligosaccharides, and galactooligosaccharides on the absorption of iron and calcium in humans. A supplement of 15 g NDO/d to the diet is feasible by using NDO-enriched products and raises substantially the total amount of NDO in the diet (from ~4 to 19 g/d) without bringing about symptoms of intolerance because the first symptoms of intolerance (excessive flatus) are expected at an intake > 30 g NDO/d (13). True absorption of iron and calcium was determined by using double stable-isotope techniques to measure the incorporation of iron isotopes into hemoglobin or the excretion of calcium isotopes in urine collected over the 24 h after isotope administration. Also,
fetal pH and the fecal output of SCFAs were measured because these indexes could be involved in the mechanism by which NDOs influence iron or calcium absorption.

**SUBJECTS AND METHODS**

**Subjects**

This study was part of a larger study in which the effect of NDOs on blood glucose, blood lipids, breath-hydrogen excretion, and some fecal indexes was measured. To avoid inhomogeneity of these variables, 12 healthy, male subjects aged 20–30 y were selected for the study (x ± SD: body weight, 79.8 ± 9.2 kg; height, 186 ± 6.1 cm). Normal health was assessed in a prestudy screening and included a medical history, a physical examination, measurement of blood pressure and heart rate, electrocardiography, and routine clinical laboratory tests. Only subjects reporting a typical Dutch food pattern (14) and normal bowel habits were selected. None of the subjects were taking dietary supplements and all were nonsmokers. The study protocol was approved by the TNO External Medical Ethics Committee and all subjects gave signed, informed consent.

**Isotope methods**

The double stable-isotope technique involved the administration of two isotopes of one element, one orally (57Fe and 44Ca) and one intravenously (58Fe and 48Ca). The incorporation of iron isotopes into erythrocytes and the excretion of calcium isotopes into urine collected after isotope administration was then measured. Blood was analyzed for both 57Fe:56Fe and 58Fe:56Fe and urine was analyzed for both 44Ca:43Ca and 48Ca:43Ca to correct for isotope fractionation. The true fractional absorption of iron (15) and calcium (16) can be calculated by taking into account the amount administered and the natural abundances of these isotopes.

**Preparation of stable-isotope solutions**

Stable iron and calcium isotopes were obtained from Eurisotope (Saint Aubin, France) in the form of iron oxide and calcium carbonate. The abundances of the different iron isotopes according to inductively coupled plasma–mass spectrometry (ICP-MS) were as follows: enriched 57Fe (0% 54Fe, 3.6% 56Fe, 95.5% 57Fe, and 0.9% 58Fe) and enriched 58Fe (0% 54Fe, 0.45% 56Fe, 6.5% 57Fe, and 93.05% 58Fe). The natural abundances of iron isotopes are 5.8% 54Fe, 91.72% 56Fe, 2.2% 57Fe, and 0.28% 58Fe (17).

Under sterile conditions, iron trioxide was dissolved in 10.2 mol H2SO4/L and 14.5 mol HNO3/L, followed by the addition of 0.5 mol H2SO4/L. The solution was then heated for 30 min at both 230 and 250°C followed by cooling. The white powder was redissolved in 0.2 mol H2SO4/L and filtered through a 0.5-μm polytetrafluoroethylene filter (18). Ascorbic acid, to reduce ferric into ferrous iron, and deaerated, deionized water (final concentration 3 g ascorbic acid/L) were added (15). The final concentration of iron was 0.924 g enriched 57Fe/L and 0.112 g enriched 58Fe/L. The final pH of the intravenous 58Fe solution was 1.7.

The abundances of the different calcium isotopes according to ICP-MS were as follows: enriched 44Ca (2.80% 40Ca, 0.05% 42Ca, 0.02% 43Ca, 97.10 ± 0.2% 44Ca, < 0.002% 46Ca, and 0.03% 48Ca) and enriched 48Ca (8.96% 40Ca, 0.09% 42Ca, 0.02% 43Ca, 0.24% 44Ca, < 0.01% 46Ca, and 90.69% 48Ca). The natural abundances of calcium are 96.941% 40Ca, 0.647% 42Ca, 0.135% 43Ca, 2.086% 44Ca, 0.004% 46Ca, and 0.187% 48Ca (17). 44Ca carbonate was converted into chloride salt, diluted with deionized water, then adjusted to a pH of 5. A similar procedure was followed for 48Ca carbonate, except that saline was used instead of deionized water. After filtration the solution was distributed into 10-mL sterilized bottles and autoclaved for 20 min (16). The final concentration was 1.2 g enriched 44Ca/L for the oral 44Ca solution and 0.12 g enriched 48Ca/L for the intravenous 48Ca solution.

**Study design and execution of the study**

The subjects consumed a controlled diet consisting of a basal diet supplemented with 15 g/d inulin, fructooligosaccharide, or galactooligosaccharide, or a diet with no added NDO (5 g during each meal). Because the pure oligosaccharide content of the supplements was not 100% (Table 1), the weight of the individual NDO was adjusted for a constant intake of 15 g pure oligosaccharides. These four 21-d treatments were given to the subjects according to a randomized, crossover design. This strictly controlled study was executed in a double-blind fashion. The composition of the basal diet is shown in Table 2. The analyses were performed on freeze-dried samples of the diet. The protein content was determined according to Kjeldahl analysis. The percentage of crude fat was analyzed according to Weibull-Stoldt (19). Total carbohydrates were determined according to Van de Kamer (20). Total dietary fiber was analyzed according to Proskey et al (21). Iron was measured by flame atomic-absorption spectrometry (AAS) at a wavelength of 248.3 nm with deuterium background compensation, and calcium at a wavelength of 422.7 nm.

During the first 2 wk of each treatment period the subjects lived in their own environment and consumed at home the diet supplied, except for dinner, which was consumed in the institute’s metabolic unit. In the last week (days 15–21) of each treat-

---

**TABLE 1**

Composition of the nondigestible oligosaccharide supplements

<table>
<thead>
<tr>
<th>Inulin</th>
<th>Fructooligosaccharide</th>
<th>Galactooligosaccharide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligofructose</td>
<td>92</td>
<td>95</td>
</tr>
<tr>
<td>Oligogalactose</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Glucose:fructose:sucrose</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Galactose</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lactose</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ash</td>
<td>&lt; 0.2</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>DP range</td>
<td>2–60</td>
<td>2–8</td>
</tr>
</tbody>
</table>

1 DP, degree of polymerization.
ment period the subjects were housed in the metabolic unit. The NDOs (5 g) were added to 100 mL orange juice, which was consumed at the start of breakfast, lunch, and dinner during the whole study. Body weight loss was corrected for by supplying extra soft drinks, cake, or both if necessary.

After an overnight fast, the subjects attended the research unit on day 15 of each treatment period. A 20-mL blood sample was taken and 10 mL was put in a tube containing lithium and heparin for the measurement of basal iron isotope ratios. The remaining 10 mL was divided into sample tubes for measurement of the subjects’ hemoglobin concentration, serum ferritin concentration, and transferrin saturation [ie, serum iron/total-iron-binding capacity (TIBC) × 100]. Hemoglobin was determined with a Sysmex K-1000 Hematology Analyzer (Toa Medical Electronics Corp, Kobe, Japan). Serum ferritin was measured with FerroZine (Boehringer, Mannheim, Germany) and the concentration of SCFAs was measured by HPLC with an ion-exclusion column (22).

In addition to the 5 g NDO, 2 mL FeSO4 and 100 mg vitamin C were added to 100 mL orange juice, which increased the total amount of vitamin C to 136 mg. This isotope-enriched orange juice was consumed at the start of breakfast, lunch, and dinner during the last 7 d (days 15–21) of each treatment period. Vitamin C was added to improve absorption so as to obtain the extra amount of iron, the iron content of the basal diet in subsequent treatment period another batch of iron isotopes was incorporated into erythrocytes, in addition to the iron isotopes incorporated during an earlier treatment period because erythrocytes live ~110 d. Therefore, the enrichment ratio of the earlier iron absorption test also represented the basal ratio of the next iron absorption test performed during the following treatment period. From the enrichment of 57Fe:56Fe and 58Fe:56Fe, true fractional absorption was calculated according to the formula of Barrett et al (15), in which absorption was adjusted for the small amount of 57Fe present in the intravenous injection and conversely the small amount of 58Fe in the oral preparation.

In the morning on day 19 of each treatment period the subjects consumed the NDO and FeSO4, and vitamin C–containing orange juice, to which 15 mL CaCl2 was added. After breakfast, which contained 513 mg Ca, 10 mL CaCl2 was given intravenously. Before and after the bolus injection, blood pressure and heart rate were recorded for safety reasons. The exact quantity of isotopes given orally and intravenously, which was calculated by weighing the bottles or syringes before and after administration, varied between 16.8 and 18.8 mg Ca and between 1.00 and 1.14 mg Ca, respectively. From the measurement of 44Ca:43Ca and 48Ca:43Ca in urine collected before and after administration and over the 24 h after dose administration, fractional calcium absorption was computed according to the formula reported by Eastell et al (16).

Stable-isotopes analysis

The blood samples were prepared for the ICP-MS of the stable-isotope ratios 57Fe:56Fe and 58Fe:56Fe (25). After destruction of the samples in a polytetrafluoroethylene tube with concentrated nitric acid, the pH was adjusted to 3.0 ± 0.5 with ammonia. Iron was isolated by selective extraction with 1% sodium diethylthiocarbamate (trihydrate) into carbon tetrachloride. The water layer was discarded. The carbon tetrachloride layer was then left overnight with 10–20 mL 1.4 mol HNO3/L. The nitric acid layer was used for ICP-MS (Elan 500; Perkin-Elmer Sciex, Norwalk, CT). All measurements were carried out in isotope ratio peak hoppers in the high-resolution mode to provide maximal accuracy. Typical conditions for operations were as follows: plasma power 1.2 kW, reflected power < 5 W, coolant argon-flow rate 18 L/min, dwell time 20 ms, 1 measurement per peak, 10 repeats per integration, total measuring time 270 s.

The iron concentration in the nitric acid solution was measured by AAS. If necessary, the solution was diluted to adjust the iron concentration to ~1.0 mg/L to avoid saturation of the detection system. ICP-MS plasma was optimized to reduce the possible molecular interference at m/z 56 of ArO+ (26).

During each ICP-MS run, solutions of an iron standard (1.0 mg/L), blank, nitric acid, and a blood sample were analyzed for the isotope ratios 57Fe:56Fe and 58Fe:56Fe. The results of the samples were corrected for the small interference of ArO+ at 56Fe.

TABLE 2

Composition of the daily basal diet without nondigestible oligosaccharide supplementation

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Basal diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total energy (MJ)</td>
<td>12.1</td>
</tr>
<tr>
<td>From protein (%)</td>
<td>11.3</td>
</tr>
<tr>
<td>From fat (%)</td>
<td>34.4</td>
</tr>
<tr>
<td>From carbohydrates (%)</td>
<td>54.3</td>
</tr>
<tr>
<td>Dietary fiber (g)</td>
<td>25.0</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>955</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>11.8</td>
</tr>
</tbody>
</table>
Minor adjustments in bias were made for unknown factors by comparing standards with accepted natural abundances. All samples were measured in duplicate. Both the basal and the enriched ratio of one treatment were measured within 1 d.

The calcium isotope ratios $^{44}\text{Ca}:^{43}\text{Ca}$ and $^{48}\text{Ca}:^{43}\text{Ca}$ in urine were measured by ICP-MS after protein precipitation with 3.5% trichloroacetic acid, precipitation of calcium with saturated ammonium oxalate, and dissolution of the formed calcium oxalate into 1.2 mol HCl/L (25).

The calcium concentration in the hydrochloric acid solution was measured by AAS and, if necessary, diluted to $\approx$10 mg Ca/L. Urine spot samples and 24-h urine samples taken before and after isotope administration of the same subject were tested within 1 d, together with a blank and standard of 10 mg Ca/L. All values were adjusted for minor deviations from standard calcium solutions with accepted natural ratios. All samples were measured in duplicate.

Statistics

The differences among treatments for iron and calcium metabolism were tested by analysis of variance (ANOVA) with block structure (27). In addition, all indexes were corrected for body weight, and iron absorption and the other iron isotope values were corrected for serum ferritin by analyses of covariance. The mean values of serum ferritin, SCFAs, fecal pH, enrichment of iron and calcium isotope, and absorption of iron and calcium over the four treatments for each subject were calculated. These overall means were used in correlation analyses (27). Because some indexes (iron and calcium absorption, basal and enriched $^{57}\text{Fe}:^{56}\text{Fe}$, enrichment of $^{57}\text{Fe}$, basal $^{44}\text{Ca}:^{43}\text{Ca}$, hemoglobin, serum iron, and pH) did not have a symmetric distribution, natural logarithms of these data were used in the statistical tests.

RESULTS

All subjects completed the study. During the fructooligosaccharide treatment the calcium absorption values of two subjects were excluded because one subject did not collect a basal urine spot and another subject did not show enrichment of the intravenously administered $^{48}\text{Ca}$ in his urine.

The mean iron status during each treatment is shown in Table 3. On the basis of the criteria of Fairweather-Tait (3), none of the subjects were considered to be iron-deficient. When corrected for body weight, TIBC was less when fructooligosaccharide was given than during the control treatment ($P < 0.05$). No other significant differences in iron status were found among treatments.

In blood taken on day 15 of the first treatment period ($n = 12$), basal $^{57}\text{Fe}:^{56}\text{Fe}$ was 0.0237 (CV: 0.6%) and $^{58}\text{Fe}:^{56}\text{Fe}$ was 0.00304 (CV: 1.5%). The mean ($\pm$SEM) enrichment value found in blood taken 2 wk after each isotope administration was 2.82 $\pm$ 0.42% for $^{57}\text{Fe}:^{56}\text{Fe}$ ($n = 48$) and amounted to 10.57 $\pm$ 0.45% for $^{58}\text{Fe}:^{56}\text{Fe}$ ($n = 24$). About half (48%) of the enrichment values of $^{57}\text{Fe}:^{56}\text{Fe}$ were less than the detection limit of 1.9% (three times the SD of the basal ratio). This indicated low iron absorption in these healthy, young men. All enrichment values of $^{58}\text{Fe}:^{56}\text{Fe}$ were above the detection limit. On the basis of two values per subject instead of four, the mean ($\pm$SEM) $^{58}\text{Fe}$ utilization, which indicates the amount of absorbed $^{57}\text{Fe}$ incorporated into erythrocytes, was 88.0 $\pm$ 1.7%.

In urine collected before administration of each isotope ($n = 47$), average basal $^{44}\text{Ca}:^{43}\text{Ca}$ was 15.5 (CV: 1.1%) and $^{48}\text{Ca}:^{43}\text{Ca}$ was 1.45 (CV: 2.5%). The mean ($\pm$SEM) enrichment value ($n = 47$) found in urine collected over 24 h after administration of each isotope was 4.1 $\pm$ 0.2% for $^{44}\text{Ca}:^{43}\text{Ca}$ and 10.7 $\pm$ 0.4% for $^{48}\text{Ca}:^{43}\text{Ca}$. All enrichment values were above the detection limit.

Because iron and calcium absorption values were not symmetrically distributed, the natural logarithms of these values were used. No significant differences in iron or calcium absorption were found among treatments after log transformation ($P = 0.503$ and $P = 0.933$, respectively). The residual SDs of ln (iron absorption) and ln (calcium absorption) were 0.97 and 0.32, respectively. Iron absorption corrected for serum ferritin also did not differ among treatments ($P = 0.490$). Individual iron and calcium absorption values for each treatment are shown in Tables 4 and 5, respectively. The correlation between mean overall iron absorption per subject over the four treatments and mean overall serum ferritin concentrations after log transformation ($r = -0.868$, $P < 0.001$) are shown in Figure 1.

No significant differences in fecal pH and most of the SCFAs were found among treatments, except for the concentration of acetic acid, which increased when the diet was supplemented with 15 g/d galactooligosaccharide or inulin ($P < 0.05$). In an attempt to identify determinants of iron or calcium metabolism, correlations with fecal pH and SCFAs were calculated; none were significant ($P > 0.10$) except for the correlation between $^{57}\text{Fe}$ enrichment and the concentration of butyric acid ($r = 0.588$, $P < 0.05$).

DISCUSSION

The method of Barrett et al (15) was chosen to measure iron absorption, which so far has been executed only in women, who generally have a smaller blood volume (28) and absorb more iron

---

**TABLE 3**

Iron status during the different treatments

<table>
<thead>
<tr>
<th>Iron status</th>
<th>Inulin</th>
<th>Fructooligosaccharide</th>
<th>Galactooligosaccharide</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (mmol/L)</td>
<td>9.5 ± 0.5</td>
<td>9.5 ± 0.9</td>
<td>9.4 ± 0.5</td>
<td>9.5 ± 0.5</td>
</tr>
<tr>
<td>(8.8–10.3)</td>
<td>(8.5–11.7)</td>
<td>(8.5–10.1)</td>
<td>(8.6–10.2)</td>
<td></td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td>84 ± 58</td>
<td>86 ± 53</td>
<td>82 ± 49</td>
<td>84 ± 48</td>
</tr>
<tr>
<td>(18–187)</td>
<td>(23–185)</td>
<td>(15–171)</td>
<td>(18–174)</td>
<td></td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>33 ± 11</td>
<td>32 ± 5</td>
<td>32 ± 9</td>
<td>31 ± 5</td>
</tr>
<tr>
<td>TIBC (µmol/L)</td>
<td>60 ± 5</td>
<td>58 ± 5</td>
<td>58 ± 5</td>
<td>60 ± 6</td>
</tr>
<tr>
<td>(53–71)</td>
<td>(46–67)</td>
<td>(53–71)</td>
<td>(50–69)</td>
<td></td>
</tr>
</tbody>
</table>

$^1 \bar{x} \pm SD; n = 12$. Range in parentheses. TIBC, total-iron-binding capacity.

$^2$Significantly different from control when corrected for body weight, $P < 0.05$. 

---

Downloaded from https://academic.oup.com/ajcn/article-abstract/67/3/445/4666130 by guest on 11 April 2019
than men (29). Therefore, a higher dose of isotopes is needed by men to achieve a significant enrichment in the isotope ratio of erythrocytes sampled within 15 d after dose administration. The required dose of 57Fe and 58Fe was calculated assuming an absorption of 6% and an incorporation of the absorbed iron into erythrocytes of 80%. The limits of detection were set at three times the SD above the basal ratio (30), which corresponds to an enrichment of 1.9% for 57Fe:56Fe and of 4.4% for 58Fe:56Fe in the present study. To avoid an excessive and nonphysiologic iron intake, the oral dose of 57Fe was spread over 1 wk and the iron content of the basal diet was adapted somewhat. Extra vitamin C was added to the orange juice with or without NDOs to increase iron absorption to 6%. Despite the addition of vitamin C, mean (± SEM) iron absorption was 5.5 ± 0.8%. About half of the enrichments of 57Fe:56Fe were below the detection limit, indicating low iron absorption in these healthy, young men. The low absorption agrees with the high iron status and the high negative correlation between serum ferritin and iron absorption.

In men with saturated iron stores, we should consider not only the absorption function of the intestine but also the barrier function of the intestine because iron overload can have negative effects on health (31). Total iron losses in males amount to ≈0.9–1 mg/d (32). To compensate for these daily losses, in the present study the average iron absorption should have been 5.4–6.5%, which was indeed the case.

In other studies (33–35) with male subjects, mean iron absorption varied between 2.5% and 5% when the oral isotope was given without added vitamin C. It is possible that the effect of vitamin C on iron absorption is less than has been predicted from tests with single meals, as proposed by Hunt et al (36). Besides, Cook et al (37), using the multiple meal technique, found a geometric mean nonheme-iron absorption of only 6.6% with a diet designed to be highly bioavailable in both men and women. This is similar to the geometric mean of 3.1% found in the present study, in which the high calcium content of the breakfast could have led to a reduction in iron absorption (38). Therefore, the iron absorption values found in the present study correspond to values reported in the literature.

The measurement of fractional absorption of calcium from a 24-h urine collection after oral and intravenous administration of

<table>
<thead>
<tr>
<th>Subject</th>
<th>Inulin</th>
<th>Fructooligosaccharide</th>
<th>Galactooligosaccharide</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29.1</td>
<td>25.3</td>
<td>26.0</td>
<td>68.3</td>
</tr>
<tr>
<td>2</td>
<td>23.5</td>
<td>25.0</td>
<td>25.5</td>
<td>32.7</td>
</tr>
<tr>
<td>3</td>
<td>36.8</td>
<td></td>
<td>40.8</td>
<td>27.8</td>
</tr>
<tr>
<td>4</td>
<td>26.0</td>
<td>24.2</td>
<td>18.8</td>
<td>39.1</td>
</tr>
<tr>
<td>5</td>
<td>28.1</td>
<td>34.1</td>
<td>33.7</td>
<td>29.2</td>
</tr>
<tr>
<td>6</td>
<td>18.7</td>
<td></td>
<td>31.0</td>
<td>14.5</td>
</tr>
<tr>
<td>7</td>
<td>34.8</td>
<td>37.9</td>
<td>33.3</td>
<td>30.3</td>
</tr>
<tr>
<td>8</td>
<td>13.8</td>
<td>21.4</td>
<td>25.0</td>
<td>25.2</td>
</tr>
<tr>
<td>9</td>
<td>38.6</td>
<td>24.7</td>
<td>19.6</td>
<td>16.0</td>
</tr>
<tr>
<td>10</td>
<td>19.9</td>
<td>27.3</td>
<td>7.2</td>
<td>18.5</td>
</tr>
<tr>
<td>11</td>
<td>18.9</td>
<td>16.9</td>
<td>21.7</td>
<td>18.5</td>
</tr>
<tr>
<td>12</td>
<td>22.0</td>
<td>26.4</td>
<td>33.0</td>
<td>17.6</td>
</tr>
<tr>
<td>X ± SEM</td>
<td>25.8 ± 2.3</td>
<td>26.3 ± 1.9</td>
<td>26.3 ± 2.6</td>
<td>28.1 ± 4.3</td>
</tr>
</tbody>
</table>
two different isotopes of calcium has been shown to be an accurate and reliable technique (39, 40). Fractional calcium absorption has been found to be inversely correlated with the logarithm of the size of the ingested calcium load (41). Therefore, in the present study mean (±SEM) calcium absorption from a breakfast containing 513 mg Ca was 26.6 ± 1.4% and hence lower than values found by Fairweather-Tait et al. (42), who added 30 mg 44Ca to watercress soup or skim or calcium-enriched milk, which contained ~150 mg Ca (27.4 ± 1.9%, 45.5 ± 1.9%, or 35.7 ± 4.7%, respectively). In fasting men, calcium absorption from orange juice containing 0.5 mg 44Ca/kg was 51.9 ± 3.8% (43). Therefore, the lower calcium absorption found in our study was attributable to the fact that the oral dose was given during a breakfast containing a relatively large amount of calcium.

Like other fermentable, nondigestible carbohydrates, NDOs do not seem to inhibit iron absorption in humans. Contrary to our results, a positive effect of fructooligosaccharide on iron absorption was found in rats (7, 9). In the study by Ohta et al. (7), 8-wk-old, iron-deficient rats were used whereas in our study subjects with normal iron stores were selected. With increasing iron stores, true differences in bioavailability are rare and more difficult to show (44). However, increased iron absorption has also been observed in growing rats with normal iron stores that were given 10% fructooligosaccharide or inulin for 24 d (9). According to Galibois et al. (45), 5% fiber in a rat diet corresponds to roughly 20 g fiber/d in a typical Western diet and 10% fiber to ~40 g/d. If this conversion also holds true for NDOs, Delzenne et al. (9) gave twice the amount of NDO we gave.

Calcium absorption takes place by two routes. The nonsaturable paracellular route of calcium occurs throughout the small intestine and is thought to be driven by passive diffusion. The saturable active transcellular transport is the major absorptive pathway in the proximal intestine (46). The large intestine may be an important absorptive site for calcium, and is thought to be driven by passive diffusion. The saturable paracellular route of calcium occurs throughout the small intestine and is thought to be driven by passive diffusion. The saturable paracellular route of calcium occurs throughout the small intestine and is thought to be driven by passive diffusion. The saturable paracellular route of calcium occurs throughout the small intestine and is thought to be driven by passive diffusion. The saturable paracellular route of calcium occurs throughout the small intestine and is thought to be driven by passive diffusion. The saturable paracellular route of calcium occurs throughout the small intestine and is thought to be driven by passive diffusion. The saturable paracellular route of calcium occurs throughout the small intestine and is thought to be driven by passive diffusion. The saturable paracellular route of calcium occurs throughout the small intestine and is thought to be driven by passive diffusion.

We gratefully acknowledge the cooperation of the subjects. Our special appreciation goes to P van Aken-Schneijder for dietetic advice, J Catsburg and W Sieling for technical assistance, and C Kistemaker for statistical help.

REFERENCES

16. Eastell R, Vieira NE, Yergey AL, Riggs BL. One-day test using sta-


