Oligofructose stimulates calcium absorption in adolescents

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

ABSTRACT
Background: In rats, nondigestible oligosaccharides stimulate calcium absorption. Recently, this effect was also found in human subjects.

Objective: The objective of the study was to investigate whether consumption of 15 g oligofructose/d stimulates calcium absorption in male adolescents.

Design: Twelve healthy, male adolescents aged 14–16 y received, for 9 d, 15 g oligofructose or sucrose (control treatment) daily over 3 main meals. The treatments were given according to a randomized, double-blind, crossover design, separated by a 19-d washout period. On the 8th day of each treatment period, \(^{44}\text{Ca}\) was given orally with a standard breakfast containing \(\approx 200\) mg Ca. Within half an hour after administration of \(^{44}\text{Ca}\), \(^{46}\text{Ca}\) was administered intravenously. Fractional calcium absorption was computed from the enrichment of \(^{44}\text{Ca}:^{43}\text{Ca}\) and \(^{46}\text{Ca}:^{45}\text{Ca}\) in 36-h urine samples, which was measured by inductively coupled plasma mass spectrometry.

Results: An increase in true fractional calcium absorption (%) was found after consumption of oligofructose (mean difference \(\pm \text{SE of difference}: 10.8 \pm 5.6; P < 0.05\), one sided). The results are discussed in relation to the methods used.


KEY WORDS Oligofructose, true calcium absorption, male adolescents, double stable-isotope technique, nondigestible oligosaccharides

INTRODUCTION
Oligofructose is a mixture of oligosaccharides composed of fructose units linked together by \(\beta(2\rightarrow 1)\) linkages. Part of these molecules are terminated by a glucose. The total number of fructose or glucose units in an oligofructose molecule generally ranges between 2 and 8.

Like other nondigestible oligosaccharides (NDOs), oligofructose resists hydrolysis by human alimentary enzymes (1), but is fermented by colonic microbiota and induces a decrease in the pH of the human culture medium (2). Because of this cecocolonic fermentation, large amounts of short-chain fatty acids (SCFAs) are produced, which may cause a trophic effect on intestinal epithelium as well as on the triacylglycerol- and cholesterol-lowering effects of these NDOs (1). In addition, NDOs have been shown to improve mineral absorption in rats (3–5). In healthy, adult men (mean age: 22 y) a positive effect of 40 g of the NDO inulin daily on apparent calcium absorption was found by using the chemical balance technique (6). The positive effect found in rats (3–5) and humans (6) likely originates predominantly in the colon. Younes et al (7) showed that the large intestine is a major site of calcium absorption when acidic fermentation takes place. Contrary to the results of Coudray et al (6), we did not find an effect of 15 g/d inulin, oligofructose, or galactooligosaccharides on true calcium or iron absorption in adult men (mean age: 23 y) when we used stable-isotope techniques (8). This finding might have resulted because we used a lower NDO concentration than used by Coudray et al. However, 17 g NDO/d also had no effect on apparent calcium absorption in ileostomy subjects (mean age: 54 y) (9).

Therefore, it is also possible that our previous study (8) did not include the colonic component of calcium absorption because calcium absorption was calculated from the enrichment of both isotopes in 24-h urine samples: calcium absorption takes > 24 h after isotope administration to be complete (10).

In addition, the stimulating effect of a more plausible dose of oligofructose on mineral absorption could be more pronounced in younger volunteers, whose calcium requirement is larger. Therefore, the aim of the present study was to investigate whether 15 g oligofructose/d stimulates true absorption of calcium in male adolescents aged 14–16 y. A supplement of 15 g NDO/d to the diet is feasible by using NDO-enriched products and raises substantially the total amount of NDOs in the diet (from \(\approx 4\) to 19 g/d) without bringing about symptoms of intolerance because the first symptoms of intolerance (eg, excessive flatus) are expected at intakes > 30 g NDO/d (11). True fractional absorption was measured by using the double stable-isotope technique. Colonic calcium absorption was included by extending the collection of urine over 36 h instead of 24 h after isotope administration.

1From the TNO Nutrition and Food Research Institute, Department of Physiology, Zeist, Netherlands.

2Supported by the European Union and ORAFTI sa, Tienen, Belgium.

3Reprints not available. Address correspondence to EGHM van den Heuvel, TNO Nutrition and Food Research Institute, Department of Physiology, PO Box 360, 3700 AJ Zeist, Netherlands. E-mail: vandenheuvel@Voeding.TNO.NL.

Received January 27, 1998.

Accepted for publication August 25, 1998.
SUBJECTS AND METHODS

Subjects

Subjects were recruited through an advertisement in a local newspaper. Twelve healthy boys were selected for the study. On the basis of our experience with adults, we calculated that with 12 subjects a difference of 5% true calcium absorption could be detected with a power of 90%. At the start of the study, the subjects were aged 14–16 y (x: 15.3 y) and their body mass index (in kg/m²) was between 16.0 and 20.7 (x: 18.4). Normal health was assessed at prestudy screening, which included a medical history, a physical examination, measurements of blood pressure and heart rate, and routine clinical laboratory tests. The study protocol was approved by the TNO external Medical Ethics Committee and all subjects and their parents signed informed consent forms.

Preparation of stable-isotope solutions

The double stable-isotope technique involves the administration of 2 stable isotopes, one orally and one intravenously. Taking the amount administered and the natural abundances of these stable isotopes into account, true fractional calcium absorption can be calculated from the enrichment of both stable isotopes in a urine sample (12). The isotopic labels were obtained from NEDRAY (Bunschoten, Netherlands) in the form of calcium carbonate. The abundances of the different isotopic labels, as determined by inductively coupled plasma mass spectrometry (ICPMS), were as follows: enriched 44Ca (3.39% 40Ca, 0.06% 42Ca, 0.03% 43Ca, 96.5% 44Ca, <0.01% 46Ca, and 0.02% 48Ca) and enriched 48Ca (8.96% 40Ca, 0.09% 42Ca, 0.02% 43Ca, 0.24% 44Ca, <0.01% 46Ca, and 0.03% 48Ca). One subject was given calcium enriched in 44Ca that was leftover from an earlier experiment, which had the following enrichment: 2.80% 40Ca, 0.05% 42Ca, 0.02% 43Ca, 97.1% 44Ca, <0.002% 46Ca, and 0.03% 48Ca. The 42Ca carbonate was converted into chloride salt, diluted with deionized water, and then adjusted to a pH of 5. A similar procedure was followed for 44Ca carbonate, except that saline was used instead of deionized water (13). After filtration, the solution was distributed into 10-mL injection bottles and sterilized for 25 min.

Study design

The subjects were asked to maintain their normal food intake during the study as best as possible, but to restrict consumption of fiber-rich and oligosaccharide-containing food products. During the two 9-d treatment periods, subjects drank 100 mL orange juice containing 5 g oligofructose or the control treatment (fine-powdered sucrose) 3 times daily (at breakfast, lunch, and dinner). Oligofructose is obtained by partial enzymatic hydrolysis of inulin, which is prepared by hot-water extraction of chicory roots. Because the pure oligosaccharide content of the oligofructose-containing product (Raftilose P95; ORAFTI, Tienen, Belgium) was not 100% (Table 1), the weight of oligofructose was adjusted for a constant intake of 5 g pure oligofructose. Aspartame was added to the oligofructose-containing product to obtain the same sweetness as the control treatment. The treatments were given to the subjects according to a randomized, crossover design. This strictly controlled study was double blind.

During the first 7 d of each treatment period, the subjects consumed the study substances at home. On the last 2 d of each treatment period, the subjects were housed in the metabolic unit of the TNO Nutrition and Food Research Institute and calcium absorption was determined. During their stay at the institute, the diet was standardized and contained 1267 mg Ca and 12 MJ energy, of which 12% was from protein, 59% from carbohydrate, and 29% from fat, as estimated from the Dutch Food Composition Table (14).

For the calcium absorption test, the orange juice containing the study substance was extrinsically labeled with 44Ca and given to the subjects with a standard breakfast (with ñ200 mg Ca) on the 8th day of each treatment period after a 12-h overnight fast. No food or drinks were allowed, except for water, for 4 h after 44Ca administration. 48Ca was administered intravenously within 30 min after the oral administration of 44Ca. Before and after the bolus injection, blood pressure and heart rate were recorded for safety reasons.

The exact quantity of isotopes given by each route, as calculated by weighing the bottles or syringes before and after administration, was 14.0 mg (range: 13.0–15.5 mg) true 44Ca and 1.15 mg (range: 1.09–1.16 mg) true 48Ca. 44Ca:43Ca and 48Ca:43Ca values in urine collected before isotope administration (basal urine sample) and over the 36 h after administration were used to compute fractional calcium absorption according to the formula reported by van Dokkum et al (12).

Stable-isotope analysis

44Ca:43Ca and 48Ca:43Ca in basal and 36-h urine samples were measured by ICPMS (Elan 500; Perkin-Elmer Sciex, Norwalk, CT). The accuracy of this method was evaluated by analyzing enriched urine samples with our inductively coupled plasma mass spectrometer and a thermal ionization mass spectrometer. Comparable results were found with both methods, as described by Luten et al (15).

All measurements were carried out in isotope-ratio peak hopping mode. ICPMS was operated 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, and total measuring time 270 s.

Trichloroacetic acid (3.5%) was added to the urine samples for deproteinization. The calcium was concentrated by precipitation of calcium with saturated ammonium oxalate and dissolution of the formed calcium oxalate into 1.2 mol HCl/L (15). The calcium concentration in the HCl solution was measured by atomic absorption spectrometry and, if necessary, adjusted by dilution to ñ10 mg Ca/L.

The prepared urine samples taken during each treatment, before and after isotope administration for the same subject, were tested within 1 d. Between each 4 urine samples, one standard solution of 10 mg Ca/L and one blank solution were measured. Mean 44Ca:43Ca values of the standard solutions, measured within 1 d, ranged between 15.560% and 15.858% (CV:

| TABLE 1 Composition of the oligofructose-containing product |
|-----------------|-----------------|
| Oligofructose (% of dry matter) | 95 |
| Glucose:fructose:sucrose (% of dry matter) | 5 |
| Aspartame (%) | 0.35 |
| Dry matter (%) | ≥95 |
| Ash (% of dry matter) | <0.2 |
| Degree of polymerization (%) | 2–8 |

Downloaded from https://academic.oup.com/ajcn/article-abstract/69/3/544/4694202 on 11 June 2018 by guest
of small amounts of calcium in some basal urine samples, no enrichment (\%) shown in the mean percentage enrichment of these ratios by treatment are 44 Ca: 43 Ca and 48 Ca: 43 Ca and the percentage enrichment by treatment.

**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>Control treatment ((n = 12))</th>
<th>Oligofructose ((n = 11))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(44\text{Ca}^{41}\text{Ca})</td>
<td>15.47 ± 0.03 [9]</td>
<td>15.45 ± 0.04 [10]</td>
</tr>
<tr>
<td>Basal</td>
<td>16.13 ± 0.18</td>
<td>16.28 ± 0.18</td>
</tr>
<tr>
<td>(44\text{Ca}^{43}\text{Ca})</td>
<td>1.40 ± 0.02 [9]</td>
<td>1.41 ± 0.03 [10]</td>
</tr>
<tr>
<td>Basal</td>
<td>1.53 ± 0.03</td>
<td>1.54 ± 0.04</td>
</tr>
<tr>
<td>Enriched</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(44\text{Ca}^{41}\text{Ca})</td>
<td>4.3 ± 1.2</td>
<td>5.4 ± 1.1</td>
</tr>
<tr>
<td>Enrichment (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(44\text{Ca}^{43}\text{Ca})</td>
<td>8.9 ± 1.8</td>
<td>8.8 ± 2.1</td>
</tr>
</tbody>
</table>

\(1^2\) ± SD; \(n\) in brackets. 

\(2\) Significantly different from control treatment, \(P < 0.01\) (one sided).

**DISCUSSION**

An increase in true fractional calcium absorption was found in male adolescents after consumption of oligofructose. In addition, the study in humans by Coudray et al (6) and experiments in rats (3, 4) showed a positive effect of NDOs on apparent calcium absorption. Contrary to these results, we did not find an effect of NDOs on true calcium absorption in an earlier study (8). This might have been because colonic calcium absorption was not considered in that study. Coudray et al’s study (6) and most experiments in rats used the chemical balance technique to measure apparent calcium absorption. The advantage of the chemical balance technique is that it measures complete calcium absorption, including that from the colon. Absorption from the colon should be considered when investigating the effect of NDOs on calcium absorption, mainly because the large intestine may represent a major site of calcium absorption when acidic fermentation takes place (7), but also because it is hypothesized that SCFAs, produced during fermentation of NDOs, improve calcium absorption through an exchange of intracellular H\(^+\) for Ca\(^{2+}\) in the distal colon (17). Experiments in rats have shown that fermentable oligosaccharides facilitate colorectal (18) and cecal absorption of calcium (3, 19). A disadvantage of the chemical balance technique, however, is that it does not distinguish...
between unabsorbed and endogenously secreted minerals, so true absorption cannot be determined. This was the main reason for using the double stable-isotope technique in the present study. In addition, we compared the results of the present study with those of our earlier study in humans (8). The measurement of colonic calcium absorption was included by extending the collection of urine from 24 h to 36 h after isotope administration.

One of the oldest approaches developed for the measurement of true fractional calcium absorption is determination of the ratio of isotopes excreted in complete urine collections. This method was used in our work with stable isotopes administered orally in a single meal (8, 12). Use of complete urine collections over a period of time instead of a single urine or blood sample gives better approximations of fractional calcium absorption (20, 21) because intravenously injected isotopes and absorbed orally administered isotopes do not necessarily arrive in the bloodstream at the same time or are metabolized in parallel. Measurement of isotopes in a complete 24-h urine sample largely reflects the ratio of the areas under the plasma disappearance curves for the 2 isotopes (20) and is therefore much less dependent on equal metabolic kinetics of the orally and intravenously administered isotopes. Measurement of calcium absorption, based on 24-h urinary excretion of isotopes, is a proven, valid method; therefore, there is no reason to doubt the validity of the method when urine collection is extended to 36 h to include colonic calcium absorption.

Nevertheless, we cannot exclude the possibility that the metabolic kinetics of calcium absorbed in the colon are different from those of calcium absorbed earlier in the duodenum or ileum or from that of the injected isotope because of the lag time between the entrance of these isotopes in the blood and the existence of a diurnal rhythm in the metabolism of calcium (22, 23). Such a bias would, however, not change the outcome of the present study because such a bias would exist with both treatments.

If the only difference between the earlier and the present study in humans had been the duration of urine collection, we have speculated that most of the enhancement of calcium absorption due to oligofructose takes place in the large intestine. However, there were other differences between the 2 studies: age, the duration of adaptation to oligofructose consumption, and the carrier dose of calcium given at breakfast with the stable isotopes.

Calcium absorption was not correlated with urinary calcium excretion, which is consistent with the results found in white girls and boys (24, 25). Apparently, during the period of bone development, absorbed calcium is largely taken up by the bone tissue so that no relation between absorption and urinary excretion becomes apparent. The oligofructose-induced enhancement of calcium absorption was not reflected by an increase in urinary calcium excretion. Moreover, the enrichment of \(^{46}\text{Ca}:^{43}\text{Ca}\), and hence the excretion of injected calcium, was not affected by oligofructose. In rats, a positive effect of nondigestible carbohydrates on bone development was found (26–28). Therefore, oligofructose may help to maximize the peak bone mass in boys.

In conclusion, 15 g oligofructose/d increases calcium absorption in adolescent boys. More research is warranted to explore in which part of the intestine the oligofructose-induced enhancement takes place and whether it can improve calcium balance in humans.

We are grateful to P van Aken-Schneyder, J van Assum-van den Ziel, FW Sieling, C Kistemaker, and EJ Brink for technical assistance.

REFERENCES