An Inulin-Type Fructan Enhances Calcium Absorption Primarily via an Effect on Colonic Absorption in Humans

Steven A. Abrams, Keli M. Hawthorne, Oluseyi Aliu, Penni D. Hicks, Zhensheng Chen, and Ian J. Griffin

Abstract
Calcium absorption efficiency and bone mineral mass are increased in adolescents who regularly consume inulin-type fructans (ITF). The mechanism of action in increasing absorption is unknown but may be related to increased colonic calcium absorption. We conducted a study in young adults designed to evaluate these mechanisms with a kinetic technique using $^{42}$Ca orally and $^{46}$Ca dosed i.v. Those who responded to 8 wk of supplementation with 8 g of a mixed short and long degree of polymerization ITF by increasing their calcium absorption had kinetic measurements analyzed to evaluate the time course of absorption. The area under the curve of the oral tracer in the blood during the 26 h after dosing was calculated and the time dependence of increased absorption determined. Eight young adults (of 13 studied), with mean calcium intake $\sim$900 mg/d, responded to the ITF with an increased calcium absorption of at least 3%. In responders, absorption increased from 22.7 ± 1.3% to 31.0 ± 15.3%. Colonic absorption, defined as absorption that occurred >7 h after oral dosing, represented 69.6 ± 18.6% of the increase, or 49 ± 28 mg/d. These findings suggest that, in those who respond to ITF, its effects on calcium absorption occur principally in the colon. This benefit to ITF may be especially important when absorption in the small intestine is impaired for anatomic or physiological reasons.

Introduction
Recent studies have demonstrated increased calcium absorption in response to dietary intake of a prebiotic inulin-type fructan (ITF) that contains a mixture of short and long degree of polymerization fructans (1–5). However, there are conflicting data regarding the likely mechanism for this effect. It is possible that a trophic effect on the gut could lead to an effect along the entire gastrointestinal tract, or increased solubility of calcium in the distal gastrointestinal tract could lead to an effect more localized in the colon (6). Although animal studies have pointed toward a primary colonic effect, the mechanisms must ultimately be elucidated in human studies (6–8). Understanding these distinctions would be important in considering the use of ITF to assist in situations including short-gut or various intestinal disease conditions. Human studies localizing calcium absorption are feasible using isotopic tracers to evaluate the time course of calcium absorption (9). To do this requires consideration of the normal time course of calcium absorption and its likely relationship to calcium absorption in different parts of the intestine. Although such data are approximate, they can provide general localizing information regarding in vivo absorption in humans not obtainable by other means.

The time course of calcium absorption in humans has been measured in 1 study performed using radioactive calcium (9). In that study, calcium absorption in the colon began ~7 h after the oral dose and represented ~4% of total calcium absorption. Absorption of the calcium tracer was >99% complete by 23 h following oral tracer ingestion (9).

It is currently feasible to conduct such kinetic studies using stable isotopes and frequent serum collections. We chose to use this approach to evaluate the relative time course of increased calcium absorption associated with ITF use and to infer the approximate proportion of the increase that occurred in the colon (i.e., >7 h after isotope administration). We hypothesized that we would identify a significant increase in the level of an orally administered calcium stable isotope in serum samples collected between 7 and 26 h after oral dosing, reflecting increased colonic-phase calcium absorption.

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2 Author disclosures: S. A. Abrams, K. M. Hawthorne, O. Aliu, P. D. Hicks, Z. Chen, and I. J. Griffin, no conflicts of interest.
6 Abbreviations used: AUC, area under curve; ICP, inductively-coupled plasma; ITF, inulin-type fructan.

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Inulin-type fructan enhances colonic calcium absorption

Methods

Subjects and protocol. Subjects recruited were aged 18–27 y and had a BMI <27.0. They were healthy and were not taking any medications. Subjects discontinued vitamin or mineral supplement use at least 2 wk before beginning the study. Antacid use was not permitted during this time or the study. Written informed consent was obtained from each subject. The Institutional Review Board of Texas Children’s Hospital/Baylor College of Medicine approved this protocol.

Subjects underwent a baseline stable isotope study including calcium absorption and kinetics (see below). After completion of this study, they began 8 g/d for 8 wk of an ITF that has previously been shown to increase calcium absorption (1–5) (Beneo Synergy1, Orafti). A repeat study was performed at the end of that time period. Subjects continued to receive the ITF until 2 d after this study. Subjects were enrolled until a total of 8 subjects were identified who responded with a 3.0% or greater increase in calcium absorption in response to 8-wk consumption of the ITF. Due to the large number of samples generated, oral tracer kinetics were subsequently analyzed only for those 8 subjects. The 3.0% cut-off had been predetermined a priori as the definition of a responder, based on our previous studies in adolescents (4). We expected to need to perform the study in 11–14 subjects to find 8 responders based on a response rate of 66% in our studies in adolescents (3,4). Ultimately, we studied 13 subjects to identify the 8 responders (62% response rate). No subjects withdrew due to intolerance of the ITF.

Calcium absorption and kinetic methods. A stable isotope of calcium, 42Ca (8 mg), was given orally with a standardized breakfast meal that provided a total of 300 mg of calcium. The 42Ca isotope was mixed into 120 mL of calcium and vitamin D-fortified orange juice. A second isotope, 46Ca (10 μg) was administered intravenously 2 h after the oral isotope. These isotopes were selected due to the speed and relative ease of analyzing large groups of serum samples for 42Ca by ICP.

Serum samples were obtained for enrichment of the 42Ca at 30-min intervals for the first 3 h after dosing, then hourly for the next 5 h, and then every 2 h until the end of the study. The final serum sample was collected 24 h after the i.v. dose (26 h after the oral dose). Isotope enrichment in all serum samples was determined using inductively coupled plasma (ICP) MS. The final serum sample was also analyzed for enrichment of 46Ca (using thermal ionization MS). The 48-h urine pools were collected 24 h after the i.v. dose (26 h after the oral dose). Isotope enrichment in all serum samples was determined using inductively coupled plasma (ICP) MS. The final serum sample was also analyzed for enrichment of 46Ca (using thermal ionization MS). The 48-h urine pools were collected 24 h after the i.v. dose (26 h after the oral dose). Isotope enrichment in all serum samples was determined using inductively coupled plasma (ICP) MS. The final serum sample was also analyzed for enrichment of 46Ca (using thermal ionization MS). The 48-h urine pools were collected 24 h after the i.v. dose (26 h after the oral dose).

Subjects were maintained on diets with ~800–1000 mg/d calcium intake (target mean of 900 mg/d) throughout the study period. Dietary control began 10 d before the first study and continued throughout the study with extensive interaction with the study dietitian. Weighed 3-d meal patterns were consistent with stable isotope studies. The actual total increase in calcium absorption attributable to the ITF was determined from the 48-h urine samples (Eq. 1).

The total area under the curve (AUC) (the product of time and dose-corrected enrichment) was calculated using a rhomboid approximation based on the isotopic enrichment in the blood samples. For each subject, the cumulative increase in the AUC at the last time point of the study (Eq. 2) was set as the value in which 100% of the benefit for that subject had occurred. At each serum sample time point, the proportion of that 100% attributable to the increased AUC was determined.

This information was used to determine the relative proportion of post-ITF increase in calcium absorption that was attributable to increased absorption in the small vs. large intestine by determining the proportion absorbed in the first 7 h (Eq. 3) (9). We assumed that any effect after >7 h occurred in the colon (Eq. 4 and 5).

Equations used to calculate presumed effect of ITF on colonic calcium absorption.

a. Fractional absorption after supplementation with ITF = ITF

b. Fractional absorption prior to supplementation with ITF = NITF
c. Increased absorption after supplementation:

\[ 100 \cdot \frac{\text{ITF} - \text{NITF}}{\text{NITF}} = \alpha(\%) \]  

(Eq. 1)
d. Cumulative area under \( 42^{\text{Ca}} \) serum curve between 0 h and 26 h ITF = D

e. Cumulative area under \( 42^{\text{Ca}} \) serum curve between 0 h and 7 h ITF = E

f. Cumulative area under \( 42^{\text{Ca}} \) serum curve between 0 h and 26 h ITF = F

g. Cumulative area under \( 42^{\text{Ca}} \) serum curve between 0 h and 7 h ITF = G

Total benefit at end of study:

\[ D - F = H \]  

(Eq. 2)

Total benefit from hours 0–7:

\[ E - G = I \]  

(Eq. 3)
h. The proportion of that benefit occurring after 7 h:

\[ 100(1 - I/H) = J(\%) \]  

(Eq. 4)
i. And the amount of calcium absorption after 7 h

as a percentage of the total absorption:

\[ a \cdot J/100 \]  

(Eq. 5)

Note that the 7-h length was arbitrarily assigned based on earlier data (9). The relative incremental benefit could similarly be determined for any time length.

Sample size determination and statistical analysis. Barger-Lux et al. (9) reported that 4.2% of calcium absorption occurs in the colon. We anticipated a SD of that value similar to the population SD for total calcium absorption of ~30% of the mean percent absorption (3,4). Based on intakes of 900 mg/d and absorption of 33%, the total absorption would therefore be 315 mg/d with 4.2% of that or 13 mg in the colon. We estimated a SD of 30% of the mean (i.e. colonic absorption of 13 ± 4 mg/d). We hypothesized a mean increase in calcium absorption of 5% after 8 wk of the ITF to 40% (360 mg/d), of which 80% of the increase (0.845 mg/d = 36 mg/d) would be colonic. Therefore, we hypothesized that colonic absorption would increase by 36 ± 11 mg/d after 8 wk of ITF. A sample size of 8 had a power >0.9 to detect such a difference (P < 0.05) from 0 in total daily calcium absorbed in the colon.

Comparisons of groups were made by paired t tests using SPSS 13.0 for Windows.

Results

A total of 13 subjects participated in the study. Anthropometric and other baseline characteristics of the study subjects are shown in Table 1.

**TABLE 1** Baseline characteristics of the study subjects

<table>
<thead>
<tr>
<th></th>
<th>All subjects</th>
<th>Responders</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>M/F</td>
<td>6/7</td>
<td>3/5</td>
</tr>
<tr>
<td>Age, y</td>
<td>23.8 ± 2.1</td>
<td>23.7 ± 2.3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>65.9 ± 11.9</td>
<td>61.2 ± 12.4</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.2 ± 2.2</td>
<td>21.6 ± 2.4</td>
</tr>
<tr>
<td>Race³</td>
<td>7W/2B/2H/1A/1Bi (C/H)</td>
<td>3W/2B/1A/1Bi (C/H)</td>
</tr>
<tr>
<td>Calcium intake, mg/d</td>
<td>890 ± 181</td>
<td>858 ± 326</td>
</tr>
</tbody>
</table>

1 Data are means ± SD.

2 Two subjects (1 responder, 1 nonresponder) had a BMI = 26; all others had BMI < 25.

3 W, White; B, Black; H, Hispanic; A, Asian (Chinese); Bi, multiethnic.
in Table 1. Mean and SD values are shown for the whole group and for the subgroup of 8 responders to the ITF. There were no significant differences between responders and nonresponders or between responders and the whole group for any of these characteristics.

Calcium absorption fraction for each study subject is shown in Table 2. Values shown are based on the urinary analysis of calcium absorption from the 48-h urine pool collected after dosing for each subject. Statistical analysis of responders vs. nonresponders was not conducted due to the small samples size and the fact that this study was not designed for this comparison. Furthermore, neither the study intervention nor the sample analysis was blinded or placebo controlled. A single large negative responder (Nonresp4) was almost certainly a technical error in the study or an unidentified change in diet, because such a result is nonphysiological.

The overall mean absorption increase for the 13 subjects of 3.2% was similar to the change of 5.1% seen in a group of 15 elderly adults with very similar mean absorptive efficiency (5).

When the single nonphysiological responder (Nonresp4) was excluded, the increase for the remaining 12 averaged 5.1%, essentially identical to the previous study of adults (5). We did not evaluate the time course of absorption or the urinary excretion of calcium in nonresponders.

Results for the mean AUC of the 8 subjects before and after ITF are shown in Figure 1. The difference in AUC is shown in Figure 2 and demonstrates a difference \( P < 0.05 \) by 120 min. Of importance is to consider the time course of the AUC. The relative amount of the total AUC achieved by 7 h, for example, was essentially identical at 32% in the pre-ITF group and 33% in the post-ITF group. This demonstrates there was no change in relative rate of absorption by ITF during that time span. In an evaluation of the completion of calcium absorption in responders performed by comparing the absorption at 24 h (from the blood) and that at 48 h (from urine), the blood value at 24 h represented \( \sim 98\% \) of the final absorption.

The 50% benefit level was achieved at \( \sim 750 \) min (12.5 h) after the oral dose was given (time-dependent mean proportion of benefit to ITF is shown in Fig. 3). Using the approximation that absorption becomes colonic after 7 h of the oral dose, we calculated the relative increase associated with the colon (results in Table 3) for each responder. Overall, from this model, 70% of the benefit was associated with colonic phase absorption.

**Discussion**

At the onset of the study, we predicted that a significant increase in calcium absorption would occur in the colon, its magnitude would be at least 36 mg/d, and at least 80% of the increase in calcium absorption attributable to ITF would occur in the colon.

Our results are in agreement with the first 2 predictions and are very close to the last prediction. That is, a significant increase in calcium absorption from the colon occurred and the proportion of overall ITF benefit that could be attributed to the colon was \( \sim 70\% \). This study was the first to our knowledge to evaluate these issues in humans. The net increased colonic absorption of 49 mg/d was higher than our prestudy estimate of 36 mg/d (Table 3). The use of the 7-h time period to determine colonic absorption is based on a previous kinetic study in adults.

We found no evidence for a time shift in calcium absorption in responders. No single time point is an absolute for all subjects in determining colonic phase absorption, but the fundamental results of this study are not substantially affected by the use of
Calcium intake is the mean of the first and second study and therefore differs slightly after 4 h, then it would have ~80% and if after 10 h, it would have ~60%.

As outlined by Cashman (11) and Scholz-Ahrens et al. (6), mechanisms of increased absorption include but are not limited to the following: 1) increased passive absorption in the colon due to increased solubility of calcium associated with pH and microbial changes in the colon; 2) direct effect of short-chain fatty acids in increasing transcellular calcium absorption; and 3) increased cell growth and adaptive surface area in the small and large intestine. Similar hypotheses were generated based on studies in rats by Raschka and Daniel (12), who considered the possibility that ITF could affect calbindin or other transcellular absorption-related proteins. In Caco-2 human intestinal cells, a 300–400% increase in paracellular calcium absorption was associated with ITF (13).

Our findings indicate that although the hypothesized mechanisms for increased colonic absorption, such as increased colonic solubility of calcium, are the predominant mechanisms involved in the ITF effect in responders, whole gut mechanisms must also be involved. In this regard, it is important to remember that in humans, calcium absorption primarily occurs in the upper portion of the small intestine compared with the large intestine in rats.

We did not evaluate how variations of calcium intake affected the ITF effect. In previous studies, we did not find a relationship between intake and ITF benefit, but we have found that those with lower absorption efficiency have a greater ITF benefit (1). It is likely that at very low calcium intakes, absorption efficiency is likely high and less calcium is presented to the colon to be absorbed. However, in the elderly or those with lower absorptive efficiency, a benefit even at very low intakes might be present. This possibility should be subject to experimental confirmation.

This study was specifically not designed to evaluate the issue of overall response or responders vs. nonresponders. We have shown in our previous studies that about two-thirds of individuals are responders. The reasons that some respond and others do not may be related to genetic differences (4) or unidentified dietary factors (5). Larger studies in adults, preferably using DEXA as an outcome to look at specific bone sites, should be conducted to evaluate these effects.

This study provided confirmation that the animal studies, which had identified a benefit of ITF for calcium absorption, accurately identified the principal mechanisms as well. However, given the multiple methods by which ITF acts, it is not surprising that some human subjects have a much greater response than others. Our results demonstrate that in those individuals who respond to ITF, its effects primarily occur in the colon. Thus, a benefit of ITF may be especially important when absorption in the small intestine is impaired due to anatomic or physiological abnormalities. Furthermore, understanding the site of action may be helpful in considering the effects of ITF and related products on the absorption of other minerals or on the design of other ITF products.

**Acknowledgments**
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**Literature Cited**

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**TABLE 3** Colonic calcium absorption using a model that assumes that calcium absorption occurs in the colon after 7 h and is complete 26 h after oral isotope dosing

<table>
<thead>
<tr>
<th></th>
<th>First 7 h</th>
<th>Next 19 h</th>
<th>Calcium intake</th>
<th>Colonic total</th>
<th>Total absorption in colon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>mg/d</td>
<td>percentage</td>
<td>mg/d</td>
</tr>
<tr>
<td>Resp1</td>
<td>6.03</td>
<td>3.24</td>
<td>996</td>
<td>34.9</td>
<td>32.2</td>
</tr>
<tr>
<td>Resp2</td>
<td>5.35</td>
<td>14.85</td>
<td>755</td>
<td>73.5</td>
<td>112.1</td>
</tr>
<tr>
<td>Resp3</td>
<td>2.36</td>
<td>5.14</td>
<td>1158</td>
<td>68.5</td>
<td>59.5</td>
</tr>
<tr>
<td>Resp4</td>
<td>0.00</td>
<td>2.80</td>
<td>1210</td>
<td>100.0</td>
<td>46.0</td>
</tr>
<tr>
<td>Resp5</td>
<td>2.19</td>
<td>5.41</td>
<td>830</td>
<td>71.2</td>
<td>44.9</td>
</tr>
<tr>
<td>Resp6</td>
<td>1.82</td>
<td>6.98</td>
<td>635</td>
<td>79.3</td>
<td>44.3</td>
</tr>
<tr>
<td>Resp7</td>
<td>2.37</td>
<td>3.13</td>
<td>650</td>
<td>56.8</td>
<td>20.3</td>
</tr>
<tr>
<td>Resp8</td>
<td>1.10</td>
<td>2.90</td>
<td>1050</td>
<td>72.4</td>
<td>30.4</td>
</tr>
<tr>
<td>Mean</td>
<td>2.65</td>
<td>5.68</td>
<td>910</td>
<td>69.6</td>
<td>48.7</td>
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<tr>
<td>SD</td>
<td>2.0</td>
<td>4.0</td>
<td>224</td>
<td>18.6</td>
<td>28.3</td>
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<tr>
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</tr>
<tr>
<td></td>
<td>t = 3.7;</td>
<td>t = 5.1;</td>
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</tr>
<tr>
<td></td>
<td>P = 0.008</td>
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<td></td>
<td>P = 0.002</td>
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</table>

*Data are means ± SD. *Different from 0.
1 Calcium intake is the mean of the first and second study and therefore differs slightly from Table 1.


