Transgalactooligosaccharides Stimulate Calcium Absorption in Postmenopausal Women[1]

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ABSTRACT The aim of this study was to investigate whether a product rich in transgalactooligosaccharides (TOS, Elix′or) stimulates true Ca absorption in postmenopausal women. The study was a double-blind, randomized crossover study, consisting of two 9-d treatment periods separated by a 19-d washout period. During the treatment periods, 12 subjects drank 200 mL yogurt drink twice (at breakfast and lunch) containing either TOS (20 g/d) or the reference substance, sucrose. On d 8 of each treatment period, ^{44}Ca and ^{48}Ca were administered orally and intravenously, respectively. Before and during the 36 h after isotope administration, urine was collected and the ratios of isotopes present were measured by inductively coupled plasma mass spectrometry (ICP-MS). From the isotope enrichments, true calcium absorption was calculated. TOS increased true calcium absorption 16%, from (mean ± SD) 20.6 ± 7.0% during the reference treatment to 23.9 ± 6.9% during the TOS treatment ($P = 0.04$, one-sided). In conclusion, in this study in postmenopausal women, greater Ca absorption was observed after consumption of a product rich in TOS (Elix′or) compared with the reference treatment. This increase in Ca absorption was likely due solely to TOS. The increased Ca absorption was not accompanied by increased urinary Ca excretion, meaning that TOS also may indirectly increase the uptake of Ca by bones and/or inhibit bone resorption. J. Nutr. 130: 2938–2942, 2000.

KEY WORDS: • *transgalactooligosaccharides* • *calcium absorption* • *stable isotopes* • *postmenopausal women*

Transgalactooligosaccharides (TOS)[3] are nondigestible carbohydrates produced from lactose by enzymatic transgalactosylation. They consist of chains of galactose molecules ending mainly with a glucose molecule, varying in chain length [degree of polymerization (DP) range 2–8] and type of linkages. Like other nondigestible oligosaccharides (NDO), TOS escape digestion in the human stomach and small intestine and consequently arrive quantitatively in the colon. Colonic fermentation of NDO leads to the formation of high concentrations of short-chain fatty acids (SCFA) and the presence of Ca^{2+} and Mg^{2+} in the colonic lumen. The high concentration of Ca^{2+} may lead to the formation of insoluble bile and fatty acid salts, therefore decreasing their damaging effect on the colonic epithelium (Roberfroid and Delzenne 1998, Wargowich et al. 1984). This hypothesis is underscored by the results of Wijnands et al. (1999) who reported an inhibitory effect of TOS on multiplicity and size of colorectal tumors in rats. In addition, colonic fermentation of TOS can facilitate calcium absorption. This has been shown in rat experiments (Chonan et al. 1995, Chonan and Watanuki 1995 and 1996). Only one human study has reported the effect of TOS on Ca absorption. No effect of 15 g NDO, including TOS, on iron or calcium absorption was found in healthy men (van den Heuvel et al. 1998). As suggested previously (van den Heuvel et al. 1999a), the absence of a positive effect in that study was probably due to the method applied. i.e., dual stable isotope technique, which may not have included the colonic component of Ca absorption because the amount of Ca absorbed after 24 h of isotope administration was not measured. Although data concerning the effect of other NDO on Ca absorption in humans are scarce, they do underscore the positive effect found in rats (Coudray et al. 1997, van den Heuvel et al. 1999a and 1999b). In these studies, complete Ca absorption was measured, including the colonic component.

The aim of this study was to investigate whether a product rich in TOS (Elix′or, Borculo Domo Ingredients, Borculo, The Netherlands) stimulates true Ca absorption in postmenopausal women, who have a decreased efficiency of Ca absorption (Heaney et al. 1989). True Ca absorption was determined with the dual stable isotope technique by measuring the excretion of Ca isotopes in urine collected over 36 h after oral and intravenous isotope administration.

SUBJECTS AND METHODS

Subjects. The subjects in this study were recruited from a pool of volunteers from TNO Nutrition and Food Research Institute and via

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[3] Abbreviations used: AAS, atomic absorption spectrometry; DNFCS, Dutch National Food Consumption Survey; DP, degree of polymerization; E2, estradiol; FFQ, food-frequency questionnaire; FSH, follicle-stimulating hormone; ICP-MS, inductively coupled plasma mass spectrometry; NDO, nondigestible oligosaccharides; SCFA, short-chain fatty acids; TOS, transgalactooligosaccharides.

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an advertisement in the local newspaper and television news. Fourteen women (including two substitutes) were selected; they reported being postmenopausal for at least 5 y, which was confirmed by a high level of follicle-stimulating hormone (FSH; 64–126 U/L, mean 104 U) and a low level of estradiol (E2; from <20 to 43 ng/L). One woman had an E2 concentration >35 ng/L and five had levels <20 ng/L. At the start of the study, the subjects' age ranged from 55 to 65 y (mean 62 y); their body weights were from 57.2 to 85.8 kg (mean 73.1 kg) and their body mass indices (BMI) from 20.7 to 32.4 kg/m^2 (mean 26.4 kg/m^2). Normal health was assessed at the prestudy screening, which involved physical examination, medical history, measurement of blood pressure and heart rate, and routine clinical laboratory tests. On the basis of the third Dutch National Food Consumption Survey (DNFCS) of 1997 and 1998, which showed that women aged 50–65 y have a mean fiber intake of 21 g/d (Centre of Nutrition 1998), only subjects with a habitual fiber intake between 18 and 26 g/d (mean 22.3) were selected on the basis of a food-frequency questionnaire (FFQ). The FFQ was developed in FOFREX (Food Frequency Expert), a computerized system with data from the second DNFCS of 1992 (National Education Board 1992) and a predefined question matrix.

Before the study, subjects were challenged with a single dose of ~10 g of TOS (Elix′or, Borculo Domo Ingredients) dissolved in a yogurt drink to check for possible intolerance. No signs of intolerance or intestinal discomfort were reported. None of the subjects were taking calcium or fiber supplements, were smokers or were taking drugs known to interfere with calcium metabolism. All reported a typical Dutch food pattern (National Education Board 1992) and all reported consumption of dairy products. All subjects gave informed consent to participate in the study after procedures had been explained to them. The study protocol was approved by the TNO external Medical Ethics Committee.

Study design and execution of the study. The study was a double-blind, randomized crossover study, consisting of two 9-d treatment periods separated by a 19-d washout period. At breakfast and lunch during the treatment periods, the subjects drank 200 mL of a yogurt drink (213 g) containing either TOS (Elix′or) or the reference substance, sucrose. A supplement of 20 g TOS to the diet was achieved by using NDO-enriched products and was expected to be well tolerated. The first symptoms of intolerance (excessive flatulence) seemed to occur after consumption of >30 g/d of fructooligosaccharides (Briet et al. 1995) and after a single dose of short-chain fructans (DP < 10; median: DP = 3) and long-chain fructans (DP > 12) of over 20 g (Rumessen and Gudmand-Høyer 1998). In this study, adaptation to the oligosaccharides was ensured by gradually increasing the daily dose of TOS from 10 g/d (22 g Elix′or) to 20 g/d (44 g Elix′or) over a period of 5 d (10 g TOS on d 1 and 2, 15 g on d 3 and 4, 20 g on d 5–9). Thus, 20 g TOS was administered for only 5 d. **Table 1** shows physicochemical properties of the study substance, Elix′or, and **Table 2** shows the composition of the yogurt drinks. All yogurt drinks had the same taste (peach) and sweetness, as tested by a sensory panel.

During the treatment periods, the subjects were asked to keep their habitual food consumption as constant as possible but to exclude consumption of products containing pre- or probiotics. During the first 7 d of each treatment period, the subjects consumed the yogurt drinks supplied at home. On the last 2 d of each treatment period, the subjects stayed in the Institute's metabolic unit, where the yogurt drinks were consumed, the Ca absorption test was carried out and the diet was standardized. For the Ca absorption test, after a 12-h overnight fast, a yogurt drink enriched with ^{44}Ca was consumed just at the start of a standard breakfast. The amount of carrier Ca present in the yogurt drink and standard breakfast was 250 mg. Within 0.5 h after ingestion of the oral dose of stable isotopes, the ^{48}Ca solution was administered intravenously. The exact quantity of isotopes given by each route, as calculated by weighing the syringes in which the isotope solutions were taken up before and after isotope administration, ranged between 13.6 and 13.8 mg ^{44}Ca and 0.97 and 0.99 mg ^{48}Ca. Before and after the bolus injection, blood pressure and heart rate were recorded for safety reasons.

TABLE 1

Physical/chemical properties of Elix'or (Borculo Domo Ingredients, Borculo, Netherlands)

	Average
Dry matter	75 g/100 g
Transgalactooligosaccharides	60 g/100 g dry matter
Lactose anhydrate	20 g/100 g dry matter
Glucose anhydrate	19 g/100 g dry matter
Galactose	1 g/100 g dry matter

TABLE 2

Composition of yogurt drink

Ingredient	Reference treatment, d 1–9	TOS treatment d 1–2	TOS treatment d 3–4	TOS treatment d 5–9
Low-fat yogurt, *g/100 g* (3.5% protein)	86.72	85.76	86.02	84.34
Calcium, *mg/L*	1050	1050	1050	1050
		g/100 g		
Water	5.0	2.0	—	—
Sucrose	7.66	6.42	5.55	4.63
Pectin	0.25	0.25	0.25	0.25
Guar gum	0.05	0.05	0.05	0.05
Peach concentrate	0.30	0.30	0.30	0.30
Pigment Annatto	0.0125	0.0125	0.0125	0.0125
Elix'or[2]	—	5.21	7.82	10.42

[1] Computed from the Dutch Food Composition Table.
[2] Checked by analysis with HPAEC-PAD (Dionex, Sunnyvale, CA).
TOS, transgalactooligosaccharides.

Preparation of stable isotope solutions. The stable isotopes were obtained from Chemgas (Boulogne, France) in the form of $CaCO_3$. The abundances of the different Ca isotopes according to analysis by inductively coupled plasma mass spectrometry (ICP-MS) were as follows: 3.39% ^{40}Ca, 0.06% ^{42}Ca, 0.03% ^{43}Ca, 96.5% ^{44}Ca, <0.01% ^{46}Ca, 0.02% ^{48}Ca for Ca enriched in ^{44}Ca, and 2.82% ^{40}Ca, <0.001% ^{42}Ca, 0.007% ^{43}Ca, 0.10% ^{44}Ca, <0.001% ^{46}Ca, 97.07% ^{48}Ca for Ca enriched in ^{48}Ca. The $^{44}CaCO_3$ was converted into chloride salt, diluted with deionized water, followed by pH adjustment to 5. A similar procedure was followed for $^{48}CaCO_3$, except that saline was used instead of deionized water. After filtration, the solution was distributed over 10-mL injection bottles and sterilized for 20 min at 121°C. The $^{44}CaCl_2$ was added to the yogurt drink in the morning of the test days ~1 h before oral administration.

Stable isotope analysis. The concentration of Ca in the urine samples was measured by atomic absorption spectrometry (AAS). ^{44}Ca/^{43}Ca and ^{48}Ca/^{43}Ca isotope ratios in urine were measured by ICP-MS (VG PlasmaQuad, Fisons Instruments, UK) after protein precipitation with 3.5% trichloroacetic acid, precipitation of Ca with saturated ammonium oxalate and dissolution of the formed calcium oxalate in 1.2 mol/L HCl. The Ca concentration in the HCl solution was measured by AAS and diluted if necessary to adjust to ~1 mg/L Ca. Urine spot samples and 36-h urine samples taken during each treatment, before and after isotope administration to the same subject, were measured by ICP-MS within 1 d, together with a blank and a standard of 1 mg/L Ca. All values were adjusted for minor deviations from standard Ca solutions with accepted natural ratios. All samples were measured in duplicate.

Taking into account the amount administered and the natural abundance of these isotopes, true absorption was calculated from the isotope ratios in urine collected before dose administration and over the 36 h after dose administration, as reported by Van Dokkum et al. (1996).

Statistics. The data were analyzed by regression analysis for a Latin-square design (SAS V6.12/V8.0, SAS Institute, Cary, NC).

Statistically, the null hypothesis was that there is no positive effect of TOS consumption on Ca absorption. Because previous human studies (Coudray et al. 1997, van den Heuvel et al. 1999a and 1999b) indicated a positive effect of NDO on Ca absorption, the alternative hypothesis was a positive effect of 20 g TOS/d on Ca absorption. The null hypothesis was to be rejected at $P < 0.05$ (one-sided). Regression analysis was used to assess associations between variables.

RESULTS

Twelve subjects completed the study. One subject was replaced by a substitute because of illness. Because the substitute with the same treatment sequence also was ill, seven subjects received the reference treatment during the first period and six received the treatment with TOS. In the second period, this sequence was reversed. This resulted in an unbalanced design. In addition, the data of another subject collected during the first treatment period were not included because factors other than treatment (i.e., sickness, no meal with the isotopes) may have influenced her outcome.

Compliance as checked by returned yogurt drinks and questionnaires was 100%. No adverse side effects (gastrointestinal complaints or changes in stools) were seen after intake of 20 g TOS compared with the control treatment.

All samples were measured in duplicate. Within-duplicate variation (CV) was 0.72% for the $^{44}Ca/^{43}Ca$ ratio and 0.91% for the $^{48}Ca/^{43}Ca$ ratio.

It was not possible to measure the basal urine sample of one subject for each treatment period due to a small amount of Ca in the urine. The basal ratios were replaced in these cases by the average of basal $^{44}Ca/^{43}Ca$ and $^{48}Ca/^{43}Ca$ ratios per treatment period. The average basal $^{44}Ca/^{43}Ca$ ratio was 15.431 (CV 0.98%) and the $^{48}Ca/^{43}Ca$ ratio was 1.380 (CV 1.05%). The average enrichment value (mean ± SEM; $n = 23$) was 3.30 ± 0.21% for the urinary $^{44}Ca/^{43}Ca$ ratio and 12.58 ± 0.42% for the urinary $^{48}Ca/^{43}Ca$ ratio.

Table 3 shows the mean basal and enriched $^{44}Ca/^{43}Ca$ and $^{48}Ca/^{43}Ca$ ratios and the enrichment percentages of these ratios per treatment. No significant differences were found between treatments. Ca absorption (mean ± SEM) was 23.9 ± 6.9% during the treatment with TOS ($n = 11$) and 20.6 ± 7.0% during the reference treatment ($n = 12$) ($P = 0.04$, one-sided). A relative increase in Ca absorption by 16% was found [standard error of the difference (SED) = 5.76]. **Figure 1** shows the mean and individual differences in Ca absorption. Even excluding the woman who showed the highest TOS-induced increase in Ca absorption (see Fig. 1), the difference was significant (SED = 3.01; $P = 0.02$, one- sided).

Total Ca excretion in the 36-h urine was 210 ± 112 and 204 ± 85 mg for the reference and the TOS condition, respectively ($P = 0.45$, one-sided). There was a significant correlation between the mean Ca absorption and the mean amount of Ca excreted in 36-h urine ($y = 0.03x + 15.80$, $r = 0.56$, $P < 0.1$). No correlation was found between the TOS-enhanced effect on Ca absorption and the excretion of Ca in 36-h urine (mean of total Ca excretion) ($y = 0.01x + 1.70$, $r = 0.15$, $P \geq 0.05$).

TABLE 3

The basal and enriched $^{44}Ca/^{43}Ca$ and $^{48}Ca/^{43}Ca$ ratios and isotope enrichments in urine of postmenopausal women after a treatment without (reference) or with 20 g of transgalactooligosaccharides (TOS) per day[1]

	Reference, $n = 12$	20 g TOS, $n = 11$[2]
$^{44}Ca/^{43}Ca$ ratio		
Basal	15.433 ± 0.16	15.429 ± 0.14
Enriched	15.915 ± 0.18	15.966 ± 0.13
$^{48}Ca/^{43}Ca$ ratio		
Basal	1.376 ± 0.01	1.383 ± 0.02
Enriched	1.552 ± 0.04	1.555 ± 0.03
Enrichment, %		
$^{44}Ca/^{43}Ca$ ratio	3.1 ± 1.1	3.5 ± 0.9
$^{48}Ca/^{43}Ca$ ratio	12.8 ± 2.7	12.4 ± 1.9

[1] Values are means ± SD.
[2] Without the data obtained from Subject 6.

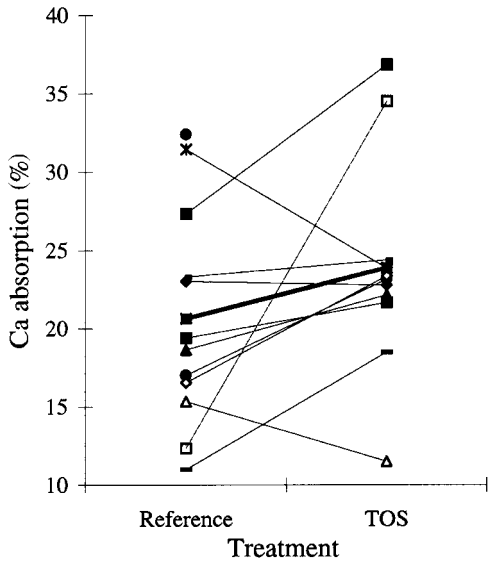

FIGURE 1 Individual changes of Ca absorption, expressed as percentage of intake, in postmenopausal women after a treatment without (reference) or with 20 g of transgalacto-oligosaccharides per day. The bold line represents the mean change of Ca absorption (mean difference ± SED = 3.3 ± 5.76, $P = 0.04$, one-sided). Without the women with the highest increase, mean difference ± SED = 1.4 ± 3.01, $P = 0.02$, one-sided).

DISCUSSION

The main finding in this study was that consumption of a product rich in transgalactooligosaccharides (Elix′or; 20 g/d of TOS) increases calcium absorption by an average of 16% in postmenopausal women. Although some human studies on NDO have been performed, differences in design and subjects make it difficult to compare them. Only one study (van den Heuvel et al. 1999b) exists that is nearly comparable to the present study. In that study, in postmenopausal women with an unknown fiber intake, relative increases in Ca absorption of 8 and 16% were found as a result of a daily intake of a single dose of 5 or 10 g, respectively, of lactulose during breakfast. This breakfast provided a smaller amount of calcium (162 mg) than the breakfast given in the present study (250 mg). In the present study, the dose of TOS was divided between breakfast and lunch, and a 16% increase in Ca absorption was found. We assumed that TOS given during lunch would still exert an effect on the absorption of Ca from breakfast, which was enriched with stable isotopes. However, there are indications that the increase in Ca absorption was due solely to the 10 g of TOS taken during breakfast. In rats, the direct and indirect effects on Ca absorption of another nondigestible carbohy-

drate were studied by feeding lactulose either before or after the test meal containing ^{47}Ca. From the results, it was concluded that lactulose must be present in the meal in order to observe its stimulant effect (Brommage et al. 1993). This may suggest that the increase in Ca absorption found in the present study was due to the 10 g TOS present in the breakfast containing the stable isotope. The acute positive effect of lactulose was nonspecific and shared, at least in rats, by other NDO (Brommage et al. 1993). Whether this is also the case in humans should be confirmed by further research because the studies on lactulose and TOS involved two different human studies.

Intestinal calcium absorption proceeds by two mechanisms, an active transcellular process and a passive paracellular process (Bronner 1998). Both transport mechanisms may be affected by NDO in several ways, i.e., hypertrophy of the cecal wall and a greater surface area, an increase in soluble calcium due to fermentation and an accelerated blood flow (Chonan and Watanuki 1995, Younes et al. 1996). In addition to these causal factors, it has been hypothesized that NDO stimulate transcellular Ca absorption by a direct effect of the SCFA produced. The direct effect of SCFA probably involves an exchange of intracellular H^+ for Ca^{2+} present in the distal colon (Lutz et al. 1991, Trinidad et al. 1996). The rate-limiting step in transcellular calcium movement is, however, the diffusion of Ca^{2+} across the cytoplasm. Calbindin plays an important role in the intracellular diffusion rate of the calcium ion (Bronner 1998). In gastrectomized rats fed fructooligosaccharides, the amount of calbindin was increased in both cecal and colorectal segments and decreased in the proximal and distal small intestine. The overall effect, however, was an improved Ca absorption due to NDO (Ohta et al. 1998). Future work should address the mechanism by which ingestion of NDO improves mineral absorption.

In addition to TOS, the study substance contained lactose, which is another nutrient that may increase Ca absorption under certain conditions. In this study, the increase in Ca absorption was probably due solely to TOS. In rats, lactose vs. glucose caused an increment in mean Ca absorption, but this effect failed to reach significance (Heijnen et al. 1993). Others have also found that lactose does not induce increases in Ca absorption (Armbrecht and Wasserman 1976, Chonan et al. 1995, Favus and Angeid-Backmann 1984). Rats become lactase deficient after weaning (de Groot et al. 1957) and hence are not capable of hydrolyzing lactose in the intestine. Possibly, fermentation of lactose in the intestine may increase the solubility of intestinal Ca, leading to increased absorption. Although lactose seems to increase Ca absorption in lactase-deficient infants (Saarela et al. 1995), no effect of lactose on Ca absorption has been found in premature children (Stathos et al. 1996), premenopausal women (Nickel et al. 1996) or adult volunteers (Brink et al. 1993). Taking into account the data from the literature, no effect of lactose on Ca absorption in healthy, lactose-tolerant people is to be expected. This is underscored by the results of Zitterman et al. (2000). In addition, Chonan et al. (1995) found in 4-wk old male rats a dose-dependent increase of calcium absorption due to TOS, but not due to lactose. Together with the higher percentage of TOS in the study substance, this may indicate that the increase of Ca absorption in the present study is likely due solely to the TOS present in the study substance.

According to Bronner and Pansu (1999), probably no $>$ 10% of total calcium absorption takes place in the large intestine. However, when acidic fermentation of TOS takes place, the large intestine may represent a major site of Ca and Mg absorption (Younes et al. 1996). This may be of particular importance when the overall process of digestive absorption is inefficient, such as in elderly subjects (Andon et al. 1993). Over a 20-y period of observation, aging and estrogen decline produce a 20–25% deterioration in absorptive performance from age 40 to 60 y (Heaney et al. 1989). The results of the present study demonstrate that TOS may help correct for this decrease in absorption capacity, without increasing urinary Ca excretion. Animal studies support this finding (Chonan et al. 1995, Chonan and Watanuki 1995 and 1996). In two rat studies, calcium balance was examined together with bone mineralization (Chonan et al. 1995, Chonan and Watanuki 1996). The results indicated that the increased calcium absorption produced by TOS coincided with an elevation of the Ca content of bone.

In conclusion, in this study in postmenopausal women, an increased Ca absorption was observed after consumption of a product rich in TOS (Elix′or) compared with the reference treatment. This increase in Ca absorption was probably due solely to the TOS. The increased Ca absorption was not accompanied by increased urinary Ca excretion, implying that TOS also may increase the uptake of Ca by the bones and/or inhibit bone resorption. More research is required to explore whether TOS is able to improve Ca balance in humans and/or reduce the prevalence of osteoporosis.

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