Effect of Lactulose on Calcium and Magnesium Absorption: A Study Using Stable Isotopes in Adult Men

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(Received March 29, 2006)

Summary To evaluate the effect of lactulose on calcium (Ca) and magnesium (Mg) absorption, we performed a clinical trial with a double-blind, randomized, crossover design in 24 healthy adult male volunteers. The absorptions of Ca and Mg were evaluated by a single-labeling method using stable isotopes. The test foods, containing lactulose at a dose of 0 g (placebo), 2 g (low-dose), or 4 g (high-dose) together with 300 mg of Ca containing 20 mg of ⁴⁴Ca, and 150 mg of Mg containing 28 mg of ²⁵Mg, were administered orally. Urine samples were collected for 8 h after the ingestion of the test food. The ratios of stable isotopes in urine (⁴⁴Ca/⁴⁰Ca and ²⁵Mg/²⁴Mg) were measured by ICP-MS (inductively coupled plasma-mass spectrometry). The urinary stable-isotopes ratios (⁴⁴Ca/⁴⁰Ca and ²⁵Mg/²⁴Mg) increased with lactulose dosage. Significant differences were observed in the Ca ratio between placebo and high-dose lactulose ($p < 0.01$), and in the Mg ratio between placebo and low-dose lactulose and between placebo and high-dose lactulose ($p < 0.01$). Lactulose ingestion did not change the levels of bone-resorption markers (type I collagen cross-linked N-telopeptide and deoxypyridinoline) in urine. The test foods did not cause any side effects. This study demonstrates that lactulose enhances the absorptions of Ca and Mg in adult men.

Key Words lactulose, stable isotopes, calcium absorption, magnesium absorption

Calcium (Ca) and magnesium (Mg) are essential elements for normal physiological functioning in humans, and dietary reference intakes (DRIs) have been established in many countries (1). However, a recent nutrition survey has indicated that the DRIs are not always satisfied (1). It is important that the DRIs of these elements are achieved, either by consuming an adequate daily diet or by ingesting food materials that increase their absorption from foods. Ca supplements and Ca-supplemented food products that have been designed to increase Ca intake, such as Ca-enriched milk and food products with a high Ca absorption rate (like milk Ca and Ca citrate malate), are available commercially. Vitamin D, casein phosphopeptide, casein phospho-oligopeptide, and indigestible oligosaccharides are also known to increase Ca absorption.

Mg plays a major role in bone and mineral homeostasis (2) and is a required cofactor in over 300 enzyme systems (1). However, there have been fewer studies on Mg than on Ca, and hence further studies into the nutritional importance of Mg and the enhancement of its absorption are needed.

Lactulose (4-O- β -D-galactopyranosyl-D-fructose), an indigestible oligosaccharide, is a disaccharide that was first synthesized in 1930 (3) and is present in heat-sterilized milk (4). The property of lactulose as a bifidus fac-

tor was confirmed in 1957 by Petuely (5). Many subsequent studies have investigated the importance of lactulose in the growth of intestinal bacteria, and it has also played a pioneering role in the field of prebiotics (6). In clinical applications, lactulose is used widely as a therapeutic drug for hepatic encephalopathy, and as a laxative worldwide (6).

It has been reported that lactulose increases the absorptions of Ca and Mg in dogs (7) and rats (8), and increases the rupture strength of the femur in an ovariectomized rat model of osteoporosis (9). In a clinical trial using double labeling with stable isotopes, Van den Heuvel et al. (10) found that lactulose (at 5 and 10 g) increased the Ca absorption in postmenopausal women. However, there have been no reported clinical trials on the promotion of Mg absorption by lactulose.

The present clinical trial evaluated the effects of lactulose on Ca and Mg absorption, using a double-blind, randomized crossover design. The absorptions of Ca and Mg were evaluated on the basis of ratios of stable isotopes of the two elements in urine after the oral administration of test food containing these isotopes.

There are two methods for measuring mineral absorption using stable isotopes: (i) a double-labeling method in which the stable isotope is ingested orally and another stable isotope is injected intravenously and (ii) a single-labeling method in which the stable isotope alone is administered orally (11). The double-labeling

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Table 1. Ingredients and components of test foods.¹

Ingredient	Placebo	Low-dose lactulose	High-dose lactulose
Lactulose crystals (g)	0.0	2.0	4.0
Milk-based Ca (g)	0.9	0.9	0.9
MgO (g)	0.2	0.2	0.2
Anhydride glucose crystals (g)	4.0	2.0	0.0
CaCO ₃ containing stable isotope ⁴⁴ Ca (g)	0.05	0.05	0.05
MgO containing stable isotope ²⁵ Mg (g)	0.05	0.05	0.05
30% hydrochloric acid (mL)	2.0	2.0	2.0
Distilled water (mL)	73	73	73
Component			
Protein (g)	0.1	0.1	0.1
Fat (g)	0.0	0.0	0.0
Carbohydrate (g)	4.0	4.0	4.0
Ash (g)	1.1	1.1	1.1
Water (g)	74	74	74
Energy (kcal) ²	16.3	12.3	8.3
Ca (mg)	300	300	300
Mg (mg)	150	150	150

¹ Values are given per dose.² Energy value of lactulose=2 kcal/g.

method measures the true absorption of orally ingested minerals, but it requires the subjects to be given an intravenous injection of a stable-isotope solution. In contrast, the single-labeling method cannot be used to evaluate the true absorption of minerals, only the absorption relative to that of a placebo; however, it requires no intravenous injection. Since the objective of the present trial was to evaluate the effect of lactulose on relative mineral absorption, we adopted the single-labeling method.

MATERIALS AND METHODS

Subjects. The subjects were 24 healthy adult males aged 23–42 y (33.5 ± 5.5 y, mean $\pm$ SD). Subjects with diseases considered to affect mineral absorption (i.e., diabetes, renal disorders, galactosemia, osteoporosis, and those requiring hormone treatments) and those for whom the ingestion of milk or lactulose would cause diarrhoea were excluded.

This trial was performed in accordance with the Helsinki Declaration (October 2000, Edinburgh revised version). The institutional review board of the Medical Ethics Committee of Kagawa Nutrition University approved this clinical trial. The trial protocol was explained to each subject, who then provided his written informed consent to participate in the study.

Stable isotopes. The stable isotopes ⁴⁴Ca (in the form of CaCO₃; ⁴⁴Ca, 95.9%) and ²⁵Mg (in the form of MgO; ²⁵Mg, 99.2%) were purchased from Trace Science (Russia).

Test foods. Three types of test food with the following lactulose contents were prepared: 0 g (placebo), 2 g (low-dose), and 4 g (high-dose). The stable isotopes were dissolved in hydrochloric acid (ultrapure 30%, Wako Pure Chemical Industries, Japan) and distributed

in bottles, each containing 20 mg of ⁴⁴Ca and 28 mg of ²⁵Mg. Lactulose crystals (containing more than 98% lactulose; MLC-97, Morinaga Milk Industry, Japan), glucose anhydride, a milk-based Ca (containing about 30% Ca, 1% Mg, 14% phosphate, 7% protein, 5% water; ALAMIN 998, NZMP, New Zealand), Mg oxide, and ten-times-diluted hydrochloric acid (ultrapure 30%, Wako Pure Chemical Industries) (see Table 1) were added to the bottles and mixed. The bottles were then filled to 80 mL with distilled water. The prepared colorless and clear solutions were kept in a refrigerator overnight to allow the ratios of the stable isotopes to equilibrate. The abundance ratios of ⁴⁴Ca to ⁴⁰Ca and of ²⁵Mg to ²⁴Mg in the test foods were about 4.4 and 3.3 times the natural abundance respectively. The test foods contained 300 mg of Ca and 150 mg of Mg.

Protocol. The trial was performed according to a previously described method (12), and was performed in three periods using a three-group, crossover design. The placebo, low-dose lactulose, and high-dose lactulose foods were ingested following a 2-wk washout interval, where this duration was prescribed based on a previous report (12). The order in which the test foods were ingested at each period and in each of the groups is shown in Fig. 1. The trial was performed at the Life-style-Related Diseases Research Center, Kagawa Nutrition University, Saitama, Japan. The subjects were randomly divided into three groups by the permuted-blocks method within strata according to the Ca concentration in their urine before the trial and their age. After random allocation, the Mg concentrations in their urine and weight were compared among the groups, which revealed no differences. This trial was performed in a double-blind manner; the allocation table was sealed and kept strictly confidential until all of the data

Fig. 1. Trial design. The trial was performed in three periods using a three-group, crossover design. The placebo (lactulose: 0 g), low-dose (lactulose: 2 g), and high-dose (lactulose: 4 g) foods were ingested following a 2-wk washout interval.

Fig. 2. Schedule of the ingestion day. After urination at 7:00 on the test day, the subjects drank 150 mL of mineral water every hour (○) until 16:00 (excluding 9:00). The subjects ate two toasted rice balls and the test food with 70 mL of mineral water at 9:00 (⊙) and three toasted rice balls at 12:00 (●). All urine was collected every 2 h from 9:00 to 17:00 (i.e., five times; ▲). Blood was sampled (about 20 mL) before the ingestion of the test food (◇).

were finalized.

The subjects did not consume any foods containing indigestible oligosaccharides (e.g., lactulose, fructooligosaccharide, or difructose anhydrides III or IV) or supplements containing Ca, Mg, vitamin D, or vitamin K from 1 wk before the trial until after the completion of the trial. In addition, the subjects did not consume dairy products or foods containing bifidobacteria or lactic acid bacteria on the day before the trial.

The schedule of the ingestion day is shown in Fig. 2. The subjects ate Japanese wheat noodles (Ca, 20 mg; Mg, 17 mg) and a toasted rice ball (Ca, 3 mg; Mg, 14 mg) at 19:00 on the day before the trial, and drank mineral water thereafter (Ca, 9.7 mg/L; Mg, 1.5 mg/L). After urination at 7:00 on the trial day, the subjects drank 150 mL of mineral water every hour until 16:00 (excluding 9:00). The subjects ingested the test food and two toasted rice balls with 70 mL of mineral water at 9:00, and three toasted rice balls at 12:00. All urine was collected every 2 h from 9:00 to 17:00 (i.e., five times). Blood was sampled before the ingestion of the test food.

Stable-isotope analysis. The ratios of the stable isotopes ($^{44}\text{Ca}/^{40}\text{Ca}$ and $^{25}\text{Mg}/^{24}\text{Mg}$) were measured in the urine collected at 9:00 before the ingestion of the test food and in all of the urine collected between 11:00 and 17:00 (Combined-urine). The samples used for the measurement of stable isotopes were prepared as follows: 1 mL of ultrapure nitric acid (70%, Kanto Chemical, Japan) was added to 1 mL of Combined-urine in a Teflon test tube, which was heated at 120°C for 12 h and then its volume was increased to 10 mL with distilled water. The ratios of the stable isotopes were measured by inductively coupled plasma-mass spectrometry (HP4500, Yokokawa Analytical Systems, Japan) at Mitsui Chemical Analysis and Consulting Service (Japan).

Urinary mineral concentration. The Ca, Mg, and creatinine concentrations were measured in the Com-

bined-urine and urine samples at each time point using the *o*-cresolphthalein complexone, xylydyl-blue methods and Jaffe's method, at SRL (Japan). The amounts of Ca and Mg in the Combined-urine are given as a product of the concentration of mineral and the weight of the urine.

Bone turnover index. Levels of type I collagen cross-linked N-telopeptide (NTx) and deoxypyridinoline in the Combined-urine were measured by ELISA at SRL.

Blood analysis. The following hematological parameters were tested at SRL: GOT, GPT, γ -GTP, total cholesterol, HDL-cholesterol, triglyceride, white-blood-cell count, red-blood-cell count, platelet count, intact parathyroid hormone, osteocalcin, and 25-hydroxy vitamin D.

Adverse events were assessed by a doctor during the testing according to symptoms of the subjects.

Statistical analysis. Baseline characteristics: Two-tailed ANOVA was used to compare the baseline characteristics of the subjects between the groups.

Urinary ratios of stable isotopes: A random-effect model (mixed model) analysis was used to assess the urinary ratios of the stable isotopes, in which the fixed effects were the treatment, the period, and the urinary ratio of stable isotopes before the test as covariates, and the random effect was the subjects. The degrees of freedom were adjusted using the Satterthwaite method, and the least-square means of the treatments were calculated. Dunnett's multiple comparisons were used to assess differences in the least-square means between placebo and high-dose lactulose and between placebo and low-dose lactulose. The analyses were two-tailed, and a probability of $p < 0.05$ was considered indicative of statistical significance.

Urinary mineral contents: A random-effect model analysis was used to assess the urinary mineral contents, in which the fixed effects were the treatment and the period, and the random effect was the subjects. The

Table 2. Baseline characteristics of the subjects.¹

	Group A	Group B	Group C	Test result ²
Number of subjects	8	8	8	
Age (y)	33.1±5.0	33.5±5.8	33.8±6.2	<i>p</i> =0.9760
Body weight (kg)	69.8±16.6	65.6±6.6	65.9±8.1	<i>p</i> =0.7192
Urinary Ca/CRE ratio (both mg/mL)	0.063±0.039	0.061±0.060	0.068±0.055	<i>p</i> =0.9662
Urinary Mg/CRE ratio (both mg/mL)	0.041±0.012	0.039±0.012	0.041±0.016	<i>p</i> =0.9220
25-OH vitamin D (ng/mL)	25±3	18±5	21±2	<i>p</i> =0.0032**
Intact parathyroid hormone (pg/mL)	31±9	37±12	37±14	<i>p</i> =0.4420
Osteocalcin (ng/mL)	6.4±1.9	7.3±2.0	6.6±1.1	<i>p</i> =0.5757
Blood Ca (μg/mL)	95±5	96±2	96±5	<i>p</i> =0.8370
Blood Mg (μg/mL)	24±1	24±1	24±1	<i>p</i> =0.5141
Red blood cell counts (×10 ⁴ /μL)	501±54	519±19	511±14	<i>p</i> =0.5883
White blood cell counts (/μL)	5,438±1,651	4,900±731	5,588±969	<i>p</i> =0.4859
Platelet count (×10 ⁴ /μL)	24.5±6.6	20.8±3.0	22.3±4.6	<i>p</i> =0.3485
Total cholesterol (μg/mL)	1,941±282	1,863±229	2,096±383	<i>p</i> =0.3157
Triglyceride (μg/mL)	1,453±1,753	1,234±549	1,473±875	<i>p</i> =0.9037
HDL-cholesterol (μg/mL)	595±181	491±82	546±109	<i>p</i> =0.3042
GOT (IU/L/37°C)	23.5±8.4	20.6±5.2	21.8±5.8	<i>p</i> =0.6850
GPT (IU/L/37°C)	24.8±21.0	18.6±6.4	26.5±14.4	<i>p</i> =0.5603
γ-GTP (IU/L/37°C)	30.3±11.4	23.0±6.6	58.1±86.5	<i>p</i> =0.3584
Bone mineral density ³ (g/cm ²)	1.2±0.1	1.2±0.0	1.2±0.0	<i>p</i> =0.3724

¹ Values are means±SDs.² One-way ANOVA.³ Dual-energy X-ray absorptiometry method.***p*<0.01, comparison among the allocated groups.

CRE, creatinine.

Table 3. Effects of lactulose on mineral indices in the Combined-urine.^{1,2}

	Placebo	Low-dose lactulose	High-dose lactulose
Ca (mg)	68.2±5.3	71.7±5.3	75.5±5.3*
Mg (mg)	39.9±1.7	41.4±1.7	41.6±1.7
NTx (nmol BCE/mmol CRE)	22.33±1.3	22.5±1.3	24.4±1.3
Deoxypyridinoline (nmol/mmol CRE)	3.0±0.1	3.1±0.1	3.0±0.1

¹ Combined-urine (all of the urine collected for 8 h after the ingestion of the test food).² Values are least-square means±SEs.**p*<0.05, difference from placebo.

BCE, bone collagen equivalents. CRE, creatinine.

Table 4. Changes in Ca/creatinine ratio in urine over time.¹

Hours after ingestion of test food	Placebo	Low-dose lactulose	High-dose lactulose
0	0.0717±0.0374	0.0738±0.0382	0.0785±0.0432*
2	0.1348±0.0794	0.1365±0.0657	0.1494±0.0715
4	0.1774±0.0892	0.1840±0.0752	0.1856±0.0872
6	0.1380±0.0458	0.1492±0.0539	0.1510±0.0569**
8	0.0553±0.0377	0.0571±0.0281	0.0616±0.0282

¹ Values are means±SDs.Differences from placebo: **p*<0.05; ***p*<0.01.

degrees of freedom were adjusted using the Satterthwaite method, and the least-square means of the treatments were calculated. Dunnett's multiple comparisons were used to assess differences in the least-square means between placebo and high-dose lactulose and

between placebo and low-dose lactulose. The analyses were two-tailed, and a probability of *p*<0.05 was considered indicative of statistical significance.

The ratios of Ca and Mg to creatinine in the urine at each time point were compared between the groups

Table 5. Changes in Mg/creatinine ratio in urine over time.¹

Hours after ingestion of test food	Placebo	Low-dose lactulose	High-dose lactulose
0	0.0523±0.0147	0.0528±0.0148	0.0487±0.0153
2	0.0768±0.0228	0.0770±0.0224	0.0770±0.0249
4	0.0948±0.0234	0.1009±0.0257	0.0971±0.0296
6	0.0802±0.0208	0.0858±0.0288	0.0848±0.0300
8	0.0417±0.0139	0.0452±0.0139	0.0452±0.0162

¹ Values are means±SDs.

Fig. 3. Effect of lactulose on the ratio of Ca stable isotopes ($^{44}\text{Ca}/^{40}\text{Ca}$) in the Combined-urine (all of the urine collected for 8 h after the ingestion of the test food). The test food contained 0 g (placebo), 2 g (low-dose), or 4 g (high-dose) of lactulose together with 300 mg of Ca (^{44}Ca : 20 mg) and 150 mg of Mg (^{25}Mg : 28 mg). Values are least-square means±SEs. Placebo and low-dose lactulose, $n=24$; high-dose lactulose, $n=23$. ** $p<0.01$, different from placebo (Dunnett-Hsu test).

Fig. 4. Effect of lactulose on the ratio of Mg stable isotopes ($^{25}\text{Mg}/^{24}\text{Mg}$) in the Combined-urine. Values are least-square means±SEs. Placebo and low-dose lactulose, $n=24$; high-dose lactulose, $n=23$. ** $p<0.01$, different from placebo (Dunnett-Hsu test).

using the paired t test.

All statistical analyses were performed using SAS software (version 8.02, SAS Institute, Cary, NC, USA).

RESULTS

Subject characteristics.

All 24 subjects completed the trial. One subject experienced food poisoning that was unrelated to the test food, and hence received an antibiotic 2 d before the second period; the data from this period (high-dose lactulose) for this subject were not used. There was no deviation from the protocol in any of the other subjects, and so all the other data were used. The subjects' baseline characteristics are listed in Table 2. The p value was 0.15 or higher for all of the baseline characteristics except 25-OH vitamin D.

Ratios of stable isotopes

The least-square means of the urinary stable-isotopes ratios ($^{44}\text{Ca}/^{40}\text{Ca}$ and $^{25}\text{Mg}/^{24}\text{Mg}$) increased with increasing lactulose intake. Significant differences ($p<0.01$) were observed in the Ca ratio between placebo and high-dose lactulose, and in the Mg ratio between placebo and low-dose lactulose and between placebo and high-dose lactulose (Figs. 3 and 4).

An analysis was performed incorporating baseline 25-OH vitamin D values as a covariate, but this did not change the results.

Urinary mineral excretion

The least-square means of the amounts of Ca and Mg in the Combined-urine increased with lactulose dosage, and a significant difference was observed in the amount of Ca between placebo and high-dose lactulose ($p<0.05$, Table 3).

The ratios of Ca and Mg to creatinine in the urine at each time point increased with lactulose dosage, and a significant difference was observed in the ratio of Ca to creatinine at 6 h between placebo and high-dose lactulose ($p<0.01$; Tables 4 and 5).

Bone turnover index

The least-square means of bone-resorption markers (NTx and deoxypyridinoline) were not associated with the lactulose intake, as indicated by an absence of significant differences among the test-food groups (Table 3).

Correlations between the amounts of minerals and ratios of stable isotopes in the Combined-urine

The amount of Ca was compared with the ratio of stable Ca isotopes ($^{44}\text{Ca}/^{40}\text{Ca}$), and the amount of Mg was compared with the ratio of stable Mg isotopes ($^{25}\text{Mg}/^{24}\text{Mg}$), in the Combined-urine (Fig. 5). The correlation coefficient between the urinary Ca excretion and the ratio of stable Ca isotopes was 0.633 ($p<0.001$), and that of Mg was 0.457 ($p<0.001$).

Fig. 5. Correlations between the ratios of stable isotopes in Combined-urine and the urinary mineral excretion after ingesting the test food ($n=71$). Placebo and low-dose lactulose, $n=24$; high-dose lactulose, $n=23$. The correlation coefficients between the urinary excretions and the ratios of stable isotopes for Ca and Mg were 0.633 ($p<0.001$) and 0.457 ($p<0.001$), respectively.

Safety evaluation

The test foods caused no side effects.

DISCUSSION

In this clinical trial, we adopted the single-labeling method to evaluate the effects of lactulose on mineral absorption. The absorptions of Ca and Mg were evaluated on the basis of ratios of the stable isotopes in urine after the oral administration of test foods containing stable isotopes of Ca and Mg. In the present study, the subjects were administered test foods in which the ratios of ^{44}Ca and ^{25}Mg were about four and three times higher than their natural abundance, respectively. A high ^{44}Ca or ^{25}Mg ratio in the urine after digestion of the test food indicates that the absorption of the mineral was also relatively high. Unlike the double-labeling method, the single-labeling method cannot be used to measure the true absorption, but it has been shown to exhibit a high correlation with the double-labeling method (13, 14), and its accuracy can be improved if the body size is included in the regression equation (15, 16); however, this method has needed ingestion of

radioactive tracer. With the parallel design, it is impossible to measure the absorption of Ca or Mg using a single isotope method without adjusting by body size. With the crossover design, however, the influence of body size can be cancelled out. Therefore, a measurement of the relative absorption of Ca can be achieved using the single-labeling method with a crossover design, as has been described previously (12).

Correlations between the absorptions of Ca and Mg and their amounts in urine have been reported previously (17, 18), and many clinical trials have evaluated the absorptions of Ca and Mg based on urinary excretion (19, 20). In the present study, the amounts of minerals were compared with the ratios of stable isotopes in the Combined urine (Fig. 5). The presence of positive correlations between these variables suggests that the urinary excretions of Ca and Mg reflect Ca and Mg absorption, as reported previously (17, 18).

In the present clinical trial, to evaluate the absorptions of Ca and Mg from the ingested test foods, urine samples collected 2, 4, 6, and 8 h after the ingestion of the test food were combined and compared between the placebo group and the lactulose foods groups. Since the Ca/creatinine and Mg/creatinine ratios in urine had almost returned to the preingestion levels by 8 h after the ingestion of the test food (Tables 4 and 5), measurement of the ratios of the stable isotopes in the Combined-urine should have accurately reflected the absorptions of Ca and Mg.

The ratio of stable Mg isotopes ($^{25}\text{Mg}/^{24}\text{Mg}$) in urine before the ingestion of the test food increased as the clinical trial progressed. To avoid bias due to the baseline values differing between the three study periods, the ratio of the stable isotope before ingestion of the test food was added as a covariate factor to the analysis model.

No abnormal baseline values of the physiological indices related to Ca and Mg metabolism (25-OH vitamin D, intact parathyroid hormone, osteocalcin, blood Ca, blood Mg, or bone mineral density) were detected in any of the subjects (Table 2). 25-OH vitamin D was the only index that differed among the groups. To assess whether the intergroup difference in 25-OH vitamin D affected the results, analyses were performed with and without 25-OH vitamin D included as a covariate; this did not change the results. Furthermore, this clinical trial was performed using a crossover design, which inherently cancels out any intergroup differences.

Several possible mechanisms underlying the enhancement of indigestible-oligosaccharide-induced mineral absorption have been proposed. The everted-sac and Ussing-chamber techniques have shown that indigestible disaccharides (e.g., maltitol and difructose anhydride (DFA)) directly increase the intestinal permeability of Ca in the absence of fermentation (21–23). This increase depends on the types of indigestible saccharides used in the Ussing-chamber technique (22, 24). DFA also shows a direct effect on the Ca permeability in vivo when the luminal perfusion method is applied (25). These effects may be due to indigestible

oligosaccharides activating the paracellular transport pathway through tight junctions. Intragastric administration of CaCl_2 solution with maltitol reduces gastrointestinal Ca transit and increases luminal fluid content (26). These effects promote the solubility of Ca and enable a larger area of the small intestine to absorb Ca over a longer period. Whether lactulose has the same effects as DFA and maltitol is not known because there are no reports on the relevant mechanisms of lactulose.

The intestinal bacteria ferment indigestible oligosaccharides in the large intestine and thereby produce organic acids (e.g., short-chain fatty acids, lactic acid, and succinic acid). The acidification of the large intestine makes the Ca and Mg salts soluble (they are insoluble at neutral pH), and the resulting increase in the ion concentrations of these minerals increases their absorption in the large intestine (8). It has been reported that acidification in the ileal lumen with lactulose intake increases the apparent Ca and Mg absorptions (8). It has also been reported, in an experiment using the Ussing-chamber technique, that short-chain fatty acids directly increase the Ca permeability of the intestinal mucosa (27). Fermentative degradation of indigestible oligosaccharides such as lactulose by intestinal bacteria produces hydrogen gas, which is absorbed in the intestine and excreted via expiration. It has been reported that when 10 g of lactulose is ingested orally as a single dose, hydrogen is excreted via expiration for 8 h, reaching a peak at 3 h (23). In addition, the total acetate production rate and acetate concentration in plasma are significantly increased from 3 to 6 h after the intake of a single dose of lactulose (28). These results suggest that lactulose is catabolized and converted to organic acids by intestinal bacteria within several hours. In the present study, the Ca/creatinine and Mg/creatinine ratios were compared between lactulose consumption and placebo at each time point (Tables 4 and 5). The biggest difference was found in urine at 6 h after ingestion, at which time assimilation by intestinal bacteria may have progressed, but not in urine at 3 h, at which time the lactulose concentration in the large intestine may have been the highest. This suggests that the enhancement of Ca and Mg absorptions by lactulose is due mainly to organic acids produced by the catabolization of lactulose in the large intestine.

The enhancement of mineral absorption by indigestible oligosaccharides may be attributable not to multiple mechanisms but rather to a single process. Furthermore, the relative contribution of each process to the increasing mineral absorption may depend on the type of indigestible oligosaccharide and intestinal bacterial flora in individual hosts.

In summary this clinical trial has confirmed, using stable isotopes in adult men, that lactulose enhances the absorptions of Ca and Mg.

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