Lactose does not enhance calcium bioavailability in lactose-tolerant, healthy adults\textsuperscript{1–3}

Armin Zittermann, Petra Bock, Christian Drummer, Kerstin Scheld, Martina Heer, and Peter Stehle

ABSTRACT

Background: There is evidence from animal studies that lactose has a beneficial effect on intestinal calcium absorption. However, data concerning the effect of lactose on calcium absorption in lactose-tolerant adults are inconclusive.

Objective: Our objective was to investigate the effect of lactose on calcium bioavailability in humans by the use of a stable-strontium test under controlled metabolic conditions.

Design: Eleven healthy, lactose-tolerant subjects (8 women, 3 men) randomly received a bolus of 2.27 mmol strontium alone (load A), the bolus with 35 g lactose (load B), or the bolus with 17.5 g glucose and 17.5 g galactose (load C). Blood samples were drawn at 0, 15, 30, 60, 90, 180, 240, and 300 min. Urine specimens were collected during the time intervals 2\textsuperscript{2}–2\textsuperscript{2}, 0–2, 2–4, 4–6, and 6–24 h.

Results: Pharmacokinetic parameters of strontium bioavailability were comparable for all 3 loads. In detail, fractional absorption at 240 min for loads A, B, and C was 12.1\textpm 0.7%, 13.0\textpm 1.1%, and 12.2\textpm 0.7%, respectively. Areas under the curve for 0–240 min were 70.8\textpm 6.3, 69.6\textpm 3.5, and 65.8\textpm 5.1 mmol \cdot h/L for loads A, B, and C, respectively (NS). Moreover, fractional strontium excretion values of 5.1\textpm 0.8% (load A), 5.8\textpm 0.4% (load B), and 5.2\textpm 0.8% (load C) were not significantly different.


KEY WORDS Lactose-tolerant adults, calcium absorption, strontium test, bioavailability

INTRODUCTION

Intestinal calcium absorption is mediated by 2 mechanisms (1): a saturable, vitamin D–regulated active transcellular uptake that is confined mainly to the proximal part of the small intestine, and passive transport by a nonsaturable paracellular route that occurs throughout the length of the intestine. The transcellular process is influenced by genetic factors (2), age (3), and sex hormones (3, 4). The paracellular pathway may be stimulated by various nutrients (1, 5).

Animal studies produced strong evidence that the disaccharide lactose has beneficial effects on intestinal calcium absorption (6–10) and on calcium retention in bone (11). Results of human studies, however, are inconsistent: increased calcium absorption (12–16), no effect (17–20), or even impaired calcium absorption (21) has been observed.

Obvious shortcomings of some of these studies include a lack of control groups (12, 13, 17), insufficient sensitivity of the method used (12, 13, 17), immobilization of test subjects (12), and lack of vitamin D status or sex hormone assessment (12–21). Consequently, reliable conclusions are difficult to draw.

In the present randomized, placebo-controlled, crossover trial, the effect of lactose on human calcium absorption was assessed by using a stable-strontium test. In numerous studies the strontium test has been proven to reliably reflect calcium absorption (22–24). Investigations were restricted to free-living, lactose-tolerant adult white subjects because only this group would benefit from a lactose-induced increase in calcium absorption (25, 26).

SUBJECTS AND METHODS

Subjects

Twelve healthy subjects were enrolled in the study (Table 1). According to a questionnaire filled out by the subjects, no medications known to affect calcium or bone metabolism were used (except oral contraceptives; \( n = 6 \)). Pregnant subjects were excluded by use of standard tests performed 1 d before actual examinations. Lactose tolerance was tested by a standard procedure (consumption of 50 g lactose dissolved in 500 mL water) before the study. Lactose tolerance was proven when blood glucose increased by \( > 1.11 \text{mmol/L} (\geq 20 \text{mg/dL}) \) within 90 min (27) and when subjects were free of gastrointestinal symptoms such as flatulence, diarrhea, or cramps within 12 h of lactose ingestion.

Written, informed consent was given by each subject. The study protocol was approved by the ethical committee of the Ärztekammer Nordrhein, Düsseldorf, Germany.

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TABLE 1
Age and anthropometric data of the study group at study entrance

<table>
<thead>
<tr>
<th></th>
<th>Women (n = 9)</th>
<th>Men (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>25.2 ± 0.7</td>
<td>29.7 ± 4.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172 ± 2</td>
<td>175 ± 1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>62.4 ± 2.5</td>
<td>74.1 ± 3.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.1 ± 0.5</td>
<td>24.1 ± 1.2</td>
</tr>
</tbody>
</table>

\[ \text{BMI (kg/m}^2\text{)} = \frac{\text{weight (kg)}}{\text{height (m)}^2} \]

\[ \text{SERM.} \]

Study protocol

Control of confounding factors

The study was performed during the winter at a geographic latitude of 51°N. A vitamin D supplement of 16.6 µg/d was given for 4 d before each investigation to ensure that active, vitamin D–dependent calcium absorption was comparable during the different study periods. According to the mean length of female menstrual cycles and the rhythm of oral contraceptive intake, the tests were performed every 28 d. All participants received a list of calcium-rich foods that they had to include in their daily diet to guarantee a calcium intake >1000 mg/d. Nutrient intake was assessed by a prospective, standardized food record on the 4 d before the actual examination. The food record is described in detail elsewhere (28).

Test procedure

With use of a crossover design, subjects were randomly assigned to 3 groups. After an overnight fast, subjects were institutionalized in a metabolic ward at the German Aerospace Center–Institute of Aerospace Medicine, Cologne, Germany. All participants received a standardized breakfast at 0900 (1 roll, 5 g butter, and 200 mL apple juice) containing 3.5 g protein and 0.63 mmol Ca alone (load A), the breakfast with an additional 35 g lactose (load B), or the breakfast with an additional 17.5 g glucose and 17.5 g galactose (load C), respectively. So that we could assess calcium absorption rates (29), all breakfasts also included 2.27 mmol (200 mg) stable strontium as strontium chloride hexahydrate (Merck, Wiesbaden, Germany) dissolved in the 200 mL apple juice. The subjects were not allowed to eat or drink for 4 h after breakfast. Two hours after breakfast, body height and weight of subjects without shoes and wearing indoor clothing were measured. Moreover, the total body water (TBW) of each subject was assessed by bioimpedance analysis using a single-frequency, 50-kHz, 800-µA device (BIAMED, Cologne, Germany). Four hours after breakfast, lunch (1 roll, 5 g butter, and 200 mL apple juice without strontium) was served. The subjects fasted again for 1 h. No further dietary restrictions were made thereafter. To measure the kinetics of strontium absorption, blood was collected at several time points (before and 15, 30, 60, 120, 180, 240, and 300 min after strontium administration). Urine specimens were collected before (0700–0900) and 2, 4, and 6 h after strontium administration and during the next 18 h. Aliquots of samples were frozen consecutively and stored at −20°C until analyzed.

Analytic procedures

Serum calcium was analyzed after the formation of a complex with o-cresolphthalein by a commercial colorimetric assay at 578 nm (Boehringer, Mannheim, Germany). Intact parathyroid hormone was measured by enzyme-linked immunoassay (DRG Diagnostics, Marburg, Germany) and calcidiol was quantified by using a commercial radioceptor assay (RRA; Immunodiagnostics, Bensheim, Germany). Serum calcitriol was extracted by use of a column technique and was subsequently analyzed by RRA with a calf thymus cytosol binding protein (30). 17β-Estradiol and testosterone concentrations were measured in serum samples by means of an enzyme-linked immunosorbent assay using commercial kits supplied by IBL (Hamburg, Germany). The CVs for all assays described above were <10%. Serum and urine strontium was measured by means of graphite furnace atomic absorption spectrophotometry (model HGA-600; Perkin-Elmer, Überlingen, Germany). The within-day CV was 4.8% and the between-day CV was 3.9%. Blood glucose concentration was determined by using a colorimetric test kit supplied by Merck.

Calculations

Fractional absorption rates \((F_{c_{240}})\) were calculated as follows considering serum strontium at time 0 \((t_0)\) and \(t_{240}\) (31) and TBW as the distribution volume (29):

\[ F_c(\%) = \left[ (\text{total SrS}) V_s + \left( \frac{\text{UfSrS}}{V_{ef}} \right) D \right] \times 100 \]

where total SrS is the net strontium serum concentration \((\Delta S_{r_{240-0}})\) in µmol/L, \(V_s\) is the serum volume in L, \(\text{UfSrS}\) is ultrafiltrable serum strontium in µmol/L (67% of total serum strontium), \(V_{ef}\) is the volume of the extravasal compartment in L, and \(D\) is the orally administered amount of strontium in mmol. Calculations of \(V_s\) and \(V_{ef}\) are based on TBW measurements, with corrections made to obtain intravasal \((V_s)\) and interstitial \((V_{ef})\) fluid volumes (28).

Area under the time curve (AUC_{0-240}) of serum strontium concentrations (31) was calculated by the trapezoidal method described earlier (32). Results are expressed as µmol·min/L.

Renal strontium output is given as the amount of strontium excreted per minute during a given time interval. Total amount of fractional strontium excretion \((FE)\) within 24 h of strontium administration was determined by using the following equation:

\[ FE(\%) = \left[ \sum (qE_{12h} + qE_{2-4h} + qE_{4-6h} + qE_{6-24h}) \right] \times 100 \]

where \(qE\) is the amount of strontium (above baseline) measured during the time intervals of 0–2 h, 2–4 h, 4–6 h, and 6–24 h, and \(D\) is the orally administered amount of strontium.

Statistics

Statistical analyses were performed by using SPSS/PC+ (SPSS Inc, Chicago). Data were tested for homogeneity of variance by using the Kolmogorov-Smirnov test. A two-factor repeated-measures analysis of variance with time and type of load as the within-subjects factors was used to analyze blood glucose, serum strontium concentrations, and renal strontium excretion. Post hoc analyses were based on the Tukey test. A one-factor analysis of variance was used to analyze the effect of treatment on the pharmacokinetic parameters AUC_{0-240}, \(F_{c_{240}}\), and FE. \(P\) values < 0.05 were considered significant. Considering the observed intra-individual variations in AUC_{0-240}, \(F_{c_{240}}\), and FE, the statistical power \((\alpha = 0.05; \beta = 0.80)\) was sufficient to detect differences of 12%, 14%, and 23.5%, respectively. Data are presented as means ± SEMs.
TABLE 2
Mean daily energy and nutrient intake 4 d before a standardized breakfast (load A), a standardized breakfast + 35 g lactose (load B), and a standardized breakfast + 17.5 g glucose + 17.5 g galactose (load C)

<table>
<thead>
<tr>
<th></th>
<th>Load A</th>
<th>Load B</th>
<th>Load C</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 11)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kJ/d)</td>
<td>8948 ± 1175</td>
<td>8766 ± 537</td>
<td>9838 ± 962</td>
</tr>
<tr>
<td>Carbohydrates (g/d)</td>
<td>224 ± 29</td>
<td>219 ± 17</td>
<td>227 ± 27</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>98 ± 17</td>
<td>84 ± 8</td>
<td>103 ± 12</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>82 ± 9</td>
<td>80 ± 5</td>
<td>85 ± 7</td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>1652 ± 192</td>
<td>1711 ± 173</td>
<td>1624 ± 127</td>
</tr>
<tr>
<td>Phosphorus (mg/d)</td>
<td>1584 ± 183</td>
<td>1697 ± 111</td>
<td>1670 ± 130</td>
</tr>
</tbody>
</table>

1 ± SEM. There were no significant differences between the loads (ANOVA).

RESULTS

One female subject was excluded during the first session because of problems related to the blood drawings. Total energy consumption and macronutrient intakes of the remaining 11 subjects did not change significantly during the study period (Table 2). Male subjects had higher energy intakes than female subjects (12,861 ± 2626 compared with 7826 ± 1477 kJ/d). Body weight and TBW were comparable during the different loads for all subjects. Mean calcium and phosphorus intakes were >1000 mg/d in all subjects. Fasting serum concentrations of calcium, parathyroid hormone, and vitamin D metabolites were not significantly different on the 3 test days with the different loads (Table 3). In addition, serum 17β-estradiol concentrations of the male subjects did not change significantly during the study period.

Blood glucose concentrations are given in Figure 1. Baseline concentrations were similar on the 3 test days. The highest blood concentrations were measured 15 min after the breakfast. The increase in blood glucose was identical for the lactose and the glucose + galactose loads. Blood glucose returned to baseline 60 min after breakfast during all 3 sessions. The baseline serum strontium concentrations of the 3 sessions were comparable before each load (Figure 2). There was a rapid increase in serum strontium concentrations during the first 2 h after the bolus. Maximum serum strontium concentrations were observed after 1.8 h.

The calculated pharmacokinetic parameters (Fc, AUC0–240) did not differ significantly between loads (Table 4). Renal strontium excretion reached a plateau 2–4 h after strontium administration. During the next 18 h, mean strontium excretion decreased to a similar extent, independent of the load. In addition, renal FE values were similar (Table 4).

DISCUSSION

The strontium bolus given in our study (2.27 mmol, or 200 mg) is comparable with the amount of oral strontium used in previous studies (22, 33, 34). The amount of lactose given (35 g) is equivalent to the amount of lactose in 0.7 L cow milk and is approximately twice the mean daily lactose ingestion of lactose-tolerant adults in Western countries (26). As shown in Tables 2 and 3, possible confounders of calcium absorption, such as concentrations of serum vitamin D metabolites (28), sex hormones (3), and calcium and protein intakes (3, 35) were not significantly different between subjects before the test loads. Thus, the study was performed under standardized conditions.

Although the absolute absorption rate for the positively charged strontium ion is only half as high as the true fractional calcium absorption, a close correlation between the kinetics of strontium and calcium absorption, with coefficients (r) between 0.86 and 0.98, has been shown in numerous human studies (23, 24, 34, 36). The comparable kinetics of calcium and strontium absorption are because of similarities in kind and locus of intestinal absorption. Perfusion studies showed that strontium is absorbed by a 2-component system consisting of a carrier-mediated process and simple diffusion (37). Pharmacokinetic studies in humans indicate the presence of 2 strontium absorption phases because of 2 dominant intestinal loci. Most probably, the first locus is present in the duodenum. Absorption via this locus is primarily an active process in which the calcium binding protein may be involved (38). In analogy with calcium, the second absorption locus of strontium is located at the distal part of the small intestine (31).

Similar to that of calcium, strontium absorption is a complex interplay of nutritional and hormonal factors. Fractional strontium absorption is influenced by dietary calcium intake (39), calcitriol administration (33), calcidiol status (28), and sex hormone status (39). The strontium test has been used to discriminate calcium malabsorbers from normal absorbers and hyperabsorbers (22) and to study the effect of dietetic measures on calcium absorption in gluten-induced enteropathy (40). Moreover, studies in alcoholic subjects have shown that the strontium test is a valid tool to determine marginal, disease-related alterations in calcium absorption (41). Human studies using both radioactive 45Ca and 85Sr isotopes confirmed the applicability of strontium loads to investigating calcium uptake (17). Strontium measures absorption with precision similar to that of 45Ca (22). The strontium test is, thus, a reliable

TABLE 3
Body weight, total body water, and fasting serum concentrations of sex hormones and of measures related to calcium metabolism just before a standardized breakfast (load A), a standardized breakfast + 35 g lactose (load B), and a standardized breakfast + 17.5 g glucose + 17.5 g galactose (load C)

<table>
<thead>
<tr>
<th></th>
<th>Load A</th>
<th>Load B</th>
<th>Load C</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 11)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>65.2 ± 2.7</td>
<td>65.6 ± 2.6</td>
<td>65.2 ± 2.6</td>
</tr>
<tr>
<td>Total body water (kg)</td>
<td>35.1 ± 1.8</td>
<td>35.0 ± 1.8</td>
<td>35.1 ± 1.7</td>
</tr>
<tr>
<td>17β-Estradiol (pmol/L)</td>
<td>365 ± 102</td>
<td>312 ± 102</td>
<td>297 ± 120</td>
</tr>
<tr>
<td>Testosterone (nmol/L)</td>
<td>13.5 ± 1.7</td>
<td>13.5 ± 1.7</td>
<td>13.5 ± 2.4</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.33 ± 0.03</td>
<td>2.38 ± 0.03</td>
<td>2.31 ± 0.04</td>
</tr>
<tr>
<td>Parathyroid hormone (pmol/L)</td>
<td>1.58 ± 0.22</td>
<td>1.63 ± 0.29</td>
<td>1.30 ± 0.22</td>
</tr>
<tr>
<td>Calcidiol (nmol/L)</td>
<td>59.5 ± 6.6</td>
<td>63.6 ± 7.0</td>
<td>65.4 ± 9.5</td>
</tr>
<tr>
<td>Calcitriol (pmol/L)</td>
<td>87.08 ± 7.8</td>
<td>66.5 ± 10.5</td>
<td>80.8 ± 5.5</td>
</tr>
</tbody>
</table>

1 ± SEM. There were no significant differences between the loads (ANOVA).
procedure with which to assess calcium absorption. Therefore, our results can be interpreted as indicating that lactose has no effect on calcium bioavailability in lactose-tolerant, adult whites.

To measure an effect of lactose on calcium or strontium absorption, the timing of blood sampling may be critical. Under fed conditions, the increase in serum strontium concentrations during the first 2 h after an oral load depends mainly on duodenal receptor-mediated strontium uptake (31). This suggestion is confirmed by the rapid increase in serum strontium concentrations within the first 2 h after strontium administration (Figure 2). However, to accurately determine bioavailability, it is necessary to consider both active and passive absorption, the latter taking place in the lower segments of the small intestine. Absorption of strontium as well as calcium is completed 5–15% lactose to the diet results in an increase in fractional absorption in rat small intestine (43). In contrast with activity in rats, lactose has been shown to enhance both calcium and strontium absorption in a dose-dependent manner (42). Addition of 5–15% lactose to the diet results in an increase in fractional calcium absorption of 5–10% (43). Lactose seems to exhibit its positive effect on calcium absorption predominantly because of its resistance to enzymatic degradation. Allen (44) hypothesized that the presence of nonhydrolyzed lactose in the distal bowel accounts for the increase in calcium absorption. The underlying mechanism might be an increase in the intestinal fluid volume, which might increase the permeability of the intercellular junctions in the jejunum and ileum (1). This hypothesis is confirmed by the fact that other nonabsorbable sugars also promote calcium absorption in rat small intestine (43). In contrast with activity in most white adults, lactase activity in rats declines from a peak value before weaning to ≈10% of the initial value at maturity (45). For that reason, data from experimental rats cannot be compared with results obtained in lactase-tolerant humans. Intestinal perfusion studies in humans have shown that even absorbable monosaccharides enhance calcium absorption via an effect on water movement when they are delivered to parts of the small intestine that are distal to the duodenum (46).

In rats, lactose has been shown to enhance both calcium and strontium absorption in a dose-dependent manner (42). Addition of 5–15% lactose to the diet results in an increase in fractional calcium absorption of 5–10% (43). Lactose seems to exhibit its positive effect on calcium absorption predominantly because of its resistance to enzymatic degradation. Allen (44) hypothesized that the presence of nonhydrolyzed lactose in the distal bowel accounts for the increase in calcium absorption. The underlying mechanism might be an increase in the intestinal fluid volume, which might increase the permeability of the intercellular junctions in the jejunum and ileum (1). This hypothesis is confirmed by the fact that other nonabsorbable sugars also promote calcium absorption in rat small intestine (43). In contrast with activity in most white adults, lactase activity in rats declines from a peak value before weaning to ≈10% of the initial value at maturity (45). For that reason, data from experimental rats cannot be compared with results obtained in lactase-tolerant humans. Intestinal perfusion studies in humans have shown that even absorbable monosaccharides enhance calcium absorption via an effect on water movement when they are delivered to parts of the small intestine that are distal to the duodenum (46).

In adult whites, mean lactase activity is ≈40% lower in the duodenum than in the jejunum (47). As discussed above, higher concentrations of sugars in the distal small intestine might enhance calcium absorption in lactose-tolerant subjects (48). The identical time courses of blood glucose after the lactose and the glucose + galactose loads in our study (Figure 1) suggest rapid and quantitative lactose digestion and subsequent absorption of the monosaccharides. Consequently, we assume that only small amounts of intact lactose reached the distal part of the small intestine. A specific osmotic effect can thus be excluded. The different metabolic fate of lactose in lactose-tolerant subjects and in rats might explain the obvious differences in calcium absorption seen in experimental and human studies.

In summary, our results with a stable-strontium test strongly suggest that lactose has no effect on calcium bioavailability in lactose-tolerant adult subjects. Moreover, the data indicate that results from rat studies cannot be used to predict an effect of lactose on calcium absorption in lactose-tolerant adult whites.

### Table 4

Pharmacokinetic parameters of strontium bioavailability and renal strontium excretion after a standardized breakfast (load A), a standardized breakfast + 35 g lactose (load B), and a standardized breakfast + 17.5 g glucose + 17.5 g galactose (load C)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Load A</th>
<th>Load B</th>
<th>Load C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractional absorption 240 (%)</td>
<td>12.1 ± 0.7</td>
<td>13.0 ± 1.1</td>
<td>12.2 ± 0.7</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0–240&lt;/sub&gt; (μmol·h/L)</td>
<td>70.8 ± 6.3</td>
<td>69.6 ± 3.5</td>
<td>65.8 ± 5.1</td>
</tr>
<tr>
<td>Renal excretion (nmol/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−2 to 0 h</td>
<td>1.2 ± 0.3</td>
<td>1.5 ± 0.3</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>0–2 h</td>
<td>83.9 ± 11.7</td>
<td>97.0 ± 15.0</td>
<td>92.6 ± 13.9</td>
</tr>
<tr>
<td>2–4 h</td>
<td>137.1 ± 17.2</td>
<td>167.9 ± 21.5</td>
<td>152.3 ± 25.7</td>
</tr>
<tr>
<td>4–6 h</td>
<td>137.2 ± 13.3</td>
<td>158.9 ± 23.1</td>
<td>149.5 ± 20.2</td>
</tr>
<tr>
<td>6–24 h</td>
<td>79.3 ± 15.3</td>
<td>85.2 ± 6.0</td>
<td>76.7 ± 14.3</td>
</tr>
<tr>
<td>Fractional excretion (% of dose)</td>
<td>5.1 ± 0.8</td>
<td>5.8 ± 0.4</td>
<td>5.2 ± 0.8</td>
</tr>
</tbody>
</table>

<sup>7</sup>x ± SEM. AUC<sub>0–240</sub>, area under the time curve over the interval of 0–240 min. There were no significant treatment effects or treatment × time interactions (one- and two-factor ANOVA).

### Figure 1

Mean (±SEM) blood glucose concentrations after a standardized breakfast (load A; n = 11), a standardized breakfast + 35 g lactose (load B; n = 11), and a standardized breakfast + 17.5 g glucose + 17.5 g galactose (load C; n = 11). Main effects of time and time × treatment were observed (P = 0.0001 and P = 0.045, respectively). Significantly different between treatments, P < 0.05 (post hoc Tukey test).

### Figure 2

Mean (±SEM) changes in serum strontium concentrations after a standardized breakfast (load A; n = 11), a standardized breakfast + 35 g lactose (load B; n = 11), and a standardized breakfast + 17.5 g glucose + 17.5 g galactose (load C; n = 11). No significant interactions between time and treatment were observed.
REFERENCES


