Lactose does not enhance calcium bioavailability in lactose-tolerant, healthy adults

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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 2–0, 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 ± 0.7%, 13.0 ± 1.1%, and 12.2 ± 0.7%, respectively. Areas under the curve for 0–240 min were 70.8 ± 6.3, 69.6 ± 3.5, and 65.8 ± 5.1 mmol · h/L for loads A, B, and C, respectively (NS). Moreover, fractional strontium excretion values of 5.1 ± 0.8% (load A), 5.8 ± 0.4% (load B), and 5.2 ± 0.8% (load C) were not significantly different.

Conclusions: Lactose does not have a beneficial effect on calcium bioavailability in lactose-tolerant adults.

Subejcts 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 mmol/L (> 20 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.

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).
**TABLE 1**

| Age and anthropometric data of the study group at study entrance$^\dagger$ |
|-----------------|-----------------|
|                 | Women ($n = 9$) | Men ($n = 3$) |
| Age (y)         | 25.2 ± 0.7      | 29.7 ± 4.2    |
| Height (cm)     | 172 ± 2         | 175 ± 1       |
| Weight (kg)     | 62.4 ± 2.5      | 74.1 ± 3.4    |
| BMI (kg/m$^2$)  | 21.1 ± 0.5      | 24.1 ± 1.2    |

$^\dagger\bar{x} \pm$ SEM.

**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 determined by using a commercial diet kit 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, Uberlingen, 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 ($F_{c240}$) were calculated as follows considering serum strontium at time 0 ($t_0$) and $t_{240}$ (31) and TBW as the distribution volume (29):

$$F_{c1} (%) = \frac{([\text{total SrS}]_S + (\text{UfSrS})_V_{ex} / D) \times 100}{(1)}$$

where total SrS is the net serum strontium concentration (ΔSr$^{240-0}$) in μmol/L, $V_S$ is the serum volume in L, UfSrS is ultrafiltrable serum strontium in μmol/L (67% of total serum strontium), $V_{ex}$ 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 $V_{ex}$ are based on TBW measurements, with corrections made to obtain intravasal ($V_S$) and interstitial ($V_{ex}$) 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:

$$FE (%) = \frac{[\text{qE}_{2-4h} + \text{qE}_{2-4h} + \text{qE}_{6-24h} + \text{qE}_{2-24h}] / D)}{\times 100}{(2)}$$

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$^{0-240}$, $F_{c240}$, and FE. $P$ values < 0.05 were considered significant. Considering the observed intraindividual variations in AUC$^{0-240}$, $F_{c240}$, 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.

**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 radioimmunoassay (RRA; Immundiagnostica, Bensheim, Germany). Serum calcidiol 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, Uberlingen, 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.
Calcium (mg/d) 1652
Protein (g/d) 82
Fat (g/d) 98

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 89Sr 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 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>Nutrient</th>
<th>Load A (n = 11)</th>
<th>Load B (n = 11)</th>
<th>Load C (n = 11)</th>
</tr>
</thead>
<tbody>
<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>

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

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>Parameter</th>
<th>Load A (n = 11)</th>
<th>Load B (n = 11)</th>
<th>Load C (n = 11)</th>
</tr>
</thead>
<tbody>
<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>

1X ± SEM. There were no significant differences between the loads (ANOVA).
procedures 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 h after oral administration with or without coingestion of a standardized meal (31) or lactose (15). Absolute strontium bioavailability is thus assessed best by fractional absorption at 240 min and by AUCs at 240 min (31, 34). Comparable AUCs together with similar renal FE (Table 4) during all loads clearly indicate that the lactose load had no specific effect on strontium or calcium metabolism.

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 ≈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>Fractional absorption</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_{0-240} (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>

* × SEM. AUC_{0-240} 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).
REFERENCES


