

## Urinary excretion of magnesium and calcium as an index of absorption is not affected by lactose intake in healthy adults

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The effect of lactose on the urinary excretion of Mg and Ca, as an index of absorption, was studied in a double-blind, crossover study during three 1-week periods. Twenty-four healthy, lactose-tolerant, adult volunteers maintained their habitual diets with the exception that all lactose-containing dairy products in the diet were replaced by 600 g/d of three specially prepared dairy products. These products were based on either lactose-enriched cow's milk or lactose-enriched, lactase (*EC* 3.2.1.23)-treated cow's milk, with or without added Mg, and were given in turn during 1 week. Lactose intake was increased by 127 mmol/d (46 g/d) while taking the lactose-enriched products. While taking the Mg-enriched products, Mg intake was increased by 2.8 mmol/d (69 mg/d) which was equivalent to 17% of the habitual Mg intake. Apart from the lactose and Mg intake, nutrient intake was comparable during the three dietary periods. Urinary excretions of Mg and Ca were used as indicators for their absorption. Mg supplementation significantly increased urinary Mg excretion by 0.97 mmol/d (equivalent to an increase of 18%,  $P < 0.001$ ), indicating that urinary Mg excretion is a valid indicator for intestinal Mg absorption. Hydrolysis of lactose did not affect urinary excretion of Mg and Ca, which implies that lactose intake does not affect the absorption of Mg and Ca in healthy adults.

Lactose: Magnesium: Calcium: Humans

Marginal intakes and impaired intestinal absorption of Mg and Ca may contribute to the pathogenesis of osteoporosis, coronary heart disease and cancer (Shills, 1988; Berner *et al.* 1990). Thus, it is of interest to identify nutrients that either reduce or improve absorption of these minerals. The milk sugar lactose enhances intestinal absorption of Mg in rats (Fournier *et al.* 1971; Andrieux & Sacquet, 1983; Schaafsma *et al.* 1988; Greger *et al.* 1989; Behling & Gregor, 1990; Brink *et al.* 1991, 1992). Such an effect also occurs in infants (Kobayashi *et al.* 1975; Ziegler & Fomon, 1983; Wirth *et al.* 1990), but it is not known whether lactose influences Mg absorption in adults. The effect of lactose on Ca absorption in rats and lactose-tolerant humans is controversial with both an improvement (Fournier, 1954; Fournier & Dupuis, 1960; Vaughan & Filler, 1960; Condon *et al.* 1970; Pansu & Chapuy, 1970; Kocian *et al.* 1973; Pansu *et al.* 1975; Armbrrecht & Wasserman, 1979; Andrieux & Sacquet, 1983; Cochet *et al.* 1983; Schaafsma *et al.* 1988; Greger *et al.* 1989) and no effect (Debognie *et al.* 1979; Greger *et al.* 1987; Sheikh *et al.* 1987; Recker *et al.* 1988) being reported.

The lack of conclusive information about the effect of lactose on the absorption of Mg and Ca in lactose-tolerant adult humans prompted us to perform the present study. We

determined urinary excretion of Mg and Ca in adults given lactose-enriched cow's milk and cow's milk with hydrolysed lactose either without or with added Mg in a crossover design. The latter treatment served as a positive control for the detection of an increased urinary excretion of Mg. Under steady-state conditions and at normal intakes of Mg and Ca, urinary excretion of these minerals is a valid indicator for their absorption (Spencer *et al.* 1984; Morris *et al.* 1988).

## SUBJECTS AND METHODS

### *Subjects*

Twenty-four healthy subjects (fourteen males and ten females), all employees of The Netherlands Institute for Dairy Research, participated in the present study. Subjects were 21–43 years of age, 54–91 kg body weight, did not take any medication and were apparently lactose-tolerant. For this age-group it may be assumed that bone turnover is relatively stable. All participants gave their informed consent, and the study protocol was approved by the Medical Ethical Committee of the Wageningen Agricultural University. All participants completed the study.

### *Experimental protocol*

The subjects were asked to maintain their usual lifestyle for the duration of the study. They were instructed to follow their habitual diet with the exception that all lactose-containing dairy products were replaced by specially prepared (see pp. 864–865) chocolate milk (400 g/d) and vanilla custard (200 g/d). These products were based on either lactose-enriched cow's milk or lactose-enriched, lactase (*EC* 3.2.1.23)-treated cow's milk with or without added Mg. Lactose-enriched instead of normal milk products were used in order to limit the volume of the specially prepared dairy products that had to be consumed; the daily lactose intake from these products corresponded to 1 litre normal milk. The chocolate milk was consumed with breakfast (200 g/d) and lunch (200 g/d) and the vanilla custard with dinner (200 g/d). The calculated daily lactose intake was 135.6 mmol/d from the lactose-enriched products and 8.6 (products without added Mg) or 9.2 mmol/d (products with added Mg) from the lactase-treated products. The calculated extra intake of Mg from the Mg-enriched products was 3.1 mmol/d.

To eliminate any bias due to the attitudes of the subjects and investigators and to control for time trends and carry-over effects, a double-blind crossover design was used in which each treatment followed each other the same number of times. The subjects were divided into six groups of four individuals each. Each group underwent one of the six treatment orders. The groups were stratified for body weight, age and male:female ratio. Each dietary treatment was given for 1 week and the entire study was conducted over a period of 3 weeks. During each 1-week period the subjects recorded their actual intake of nutrients on three arbitrary days (two working days and one weekend day) in specially designed diaries. Two samples of 24 h urine were collected; on days 5 and 7 each week. Body weight was measured once weekly.

### *Experimental products*

The experimental dairy products were manufactured by the Technology Department of The Netherlands Institute for Dairy Research. Sterilized, semi-skimmed cow's milk was supplemented with 116 mmol lactose/l (Pharmatose; DMV, Veghel, The Netherlands). Milk with hydrolysed lactose was prepared by adding 400 mg (2000 neutral lactase units) lactase (Maxilact LX 5000; Gist-Brocades, Delft, The Netherlands) to 1 litre of this product. After incubation for 24 h at 10°, about 92% of the lactose was found to be

Table 1. *Analysed concentrations of nitrogen, fat, lactose and minerals\* in the experimental dairy products† (per kg product)*

Component (/kg product)	Chocolate milk			Vanilla custard		
	Control‡	Lactose-enriched	Mg-enriched§	Control‡	Lactose-enriched	Mg-enriched§
N (g)	5.5	5.5	5.4	5.0	5.1	5.0
Fat (g)	17	17	16	15	14	15
Lactose (mmol)	14	229	15	15	220	16
Ca (mmol)	26.3	26.5	26.1	25.0	25.2	25.3
Mg (mmol)	7.2	7.2	12.0	4.0	4.0	9.7
P (mmol)	29.6	29.7	29.5	25.4	25.5	25.6
Water (g)	840	840	840	800	790	800

\* For details of procedures, see p. 865.

† For details of the experimental procedures, see pp. 864-865.

‡ Lactose-enriched, lactase (*EC* 3.2.1.23)-treated.

§ Lactose-enriched, lactase-treated and supplemented with Mg.

hydrolysed. Part of the lactase-treated milk was supplemented with  $\text{MgCO}_3$  (Fluka, Buchs, Switzerland). In order to ensure a double-blind study design, chocolate milk and vanilla custard were prepared from the lactose-enriched milk, the lactose-enriched, lactase-treated milk and the lactose-enriched, lactase-treated, Mg-enriched milk using standard recipes. The analysed lactose and mineral concentrations in the experimental dairy products are given in Table 1.

#### Analyses

Nutrient intakes were calculated from the computerized Dutch food composition table (Stichting NEVO, 1989) with adjustments for the experimental dairy products. After appropriate dilution with a solution containing  $\text{SrCl}_2$  and  $\text{CsCl}$  (final concentrations 48 and 3 mmol/l respectively), the experimental products and urine samples were analysed for Mg and Ca by atomic absorption spectrophotometry (Perkin Elmer 1100; Bodenseewerk Perkin Elmer, Überlingen, Germany). P was determined by the Fiske & Subbarow (1924) method. Lactose in the experimental dairy products was analysed by high performance liquid chromatography (Brons & Olieman, 1983) and N by the macro-Kjeldahl method (International Dairy Federation, 1986). Urine was analysed for Na by atomic emission spectrophotometry (Perkin Elmer 1100). Urine samples were also analysed for creatinine and urea by a colorimetric method (Spayd *et al.* 1978; Goren *et al.* 1986) with the Ektachem 700 XR (Kodak, Rochester, New York, USA). The urinary excretion of minerals was expressed relative to that of creatinine.

#### Statistics

Changes in body weight and dietary and urinary variables were evaluated for statistically significant differences between males and females and between time-intervals by analysis of variance; no such differences ( $P > 0.99$ ) were found. There were no significant differences in urinary mineral excretion between days 5 and 7 of urine collection ( $P > 0.67$ , Student's paired *t* test) within dietary periods. The effects of lactose and Mg were then evaluated for pooled subjects and time-intervals with the use of Student's paired *t* test. In case of urinary variables, average values of days 5 and 7 of each dietary period were used. The level of significance was pre-set at  $P < 0.05$ . On the basis of the observed variation in urinary Mg

Table 2. *Nutrient intake of subjects (n 24) when taking the lactose-enriched, lactase (EC 3.2.1.23)-treated products (control treatment), the lactose-enriched, or the lactose-enriched, lactase-treated, magnesium-enriched products†*

(Mean values with their standard errors)

Dietary treatment...	Control‡		Lactose-enriched		Mg-enriched§	
	Mean	SE	Mean	SE	Mean	SE
Energy (MJ/d)	11.9	0.4	11.5	0.5	11.5	0.5
Protein (% of energy)	13.9	0.4	14.0	0.4	14.2	0.3
Fat (% of energy)	34.1	0.9	32.9	0.9	32.6	1.3
Carbohydrates (% of energy)	49.7	0.9	51.3	1.2	50.4	1.4
Lactose (mmol/d)	8.6	0	135.6*	0	9.2	0
Ethanol (% of energy)	2.3	0.6	2.7	0.8	2.8	0.8
Fibre (g/d)	35.8	1.6	36.0	2.0	35.2	2.2
Mg (mmol/d)	16.8	0.7	17.5	0.8	19.7*	0.6
Ca (mmol/d)	37.0	1.6	36.4	1.7	36.8	1.9
P (mmol/d)	62.7	2.3	62.7	2.5	62.6	2.5
K (mmol/d)	114	3	117	4	112	3
Na (mmol/d)	167	10	155	8	174	12

Mean value was significantly different from those of other groups (one-sided, one-sample *t* test): \**P* < 0.001.

† For details of products and procedures, see pp. 864–865.

‡ Lactose-enriched, lactase-treated.

§ Lactose-enriched, lactase-treated and supplemented with Mg.

and Ca excretion it could be calculated that an increase in urinary Mg excretion of only 0.3 mmol/d, corresponding to a lactose-induced increase in percentage apparent Mg absorption from the complete diet of 1.8, would reach statistical significance. For Ca these values were 0.5 mmol/d and 1.3% respectively.

## RESULTS

### *Body weight*

Body weight was not influenced by dietary treatments and remained stable throughout the experiment. Initial and final mean (*n* 24) body weights (kg) were 71.8 (SEM 1.9) and 71.7 (SEM 1.9) respectively.

### *Nutrient intake*

Table 2 shows that the actual lactose intake was increased by 127 mmol/d after consumption of the lactose-enriched products. The actual Mg intake was significantly increased by 2.8 mmol/d during the period that the lactose-enriched, lactase-treated, Mg-enriched products were consumed. This was associated with a negligible increase in lactose intake when compared with the lactose-enriched, lactase-treated period (control treatment). Otherwise, there were no significant differences in nutrient intake between the dietary periods.

### *Urinary variables*

Urinary excretions of creatinine, urea, phosphate and Na were not significantly affected by increased lactose or increased Mg intake (Table 3). Likewise, extra lactose did not significantly influence the urinary excretion of Mg and Ca. However, urinary Mg excretion

Table 3. *Urinary excretion of minerals by subjects (n 24) when taking the lactose-enriched, lactase (EC 3.2.1.23)-treated products (control treatment), the lactose-enriched, or the lactose-enriched, lactase-treated, magnesium-enriched products†*

(Mean values with their standard errors)

	Control‡		Lactose-enriched		Mg-enriched§	
	Mean	SE	Mean	SE	Mean	SE
Volume (l/d)	1.39	0.09	1.40	0.11	1.41	0.08
Creatinine (mmol/d)	14.1	0.5	14.0	0.5	13.8	0.6
Urea (mmol/d)	402	14	400	16	403	18
Mg:creatinine (mmol/mmol)	0.38	0.02	0.37	0.01	0.46*	0.02
Ca:creatinine (mmol/mmol)	0.32	0.03	0.31	0.03	0.32	0.02
P:creatinine (mmol/mmol)	2.20	0.07	2.22	0.07	2.19	0.08
Na:creatinine (mmol/mmol)	11.2	0.5	11.1	0.7	11.8	0.9

Mean value was significantly different from those of other groups (one-sided, one-sample *t* test): \**P* < 0.001.

† For details of products and procedures, see pp. 864–865.

‡ Lactose-enriched, lactase-treated.

§ Lactose-enriched, lactase-treated and supplemented with Mg.

was significantly increased when the lactose-enriched, lactase-treated, Mg-enriched products were consumed.

#### DISCUSSION

Total mineral absorption equals the sum of mineral retention in the body, faecal endogenous excretion, urinary excretion and loss of the mineral via the skin. Therefore, three assumptions underlie the use of urinary mineral excretion as an index of absorption. First, it is assumed that mineral retention in the body is zero. This relates to the assumption that the adults participating in the present study were, in view of their age, in a steady state. Further, it is assumed that faecal endogenous excretion and the loss via the skin were constant. Thus, differences in urinary Mg excretion should reflect differences in Mg absorption. Indeed, a positive relationship between absorption and urinary excretion of Mg has been demonstrated (Morris *et al.* 1988). The moderately increased Mg intake (2.8 mmol/d; Table 2) while consuming the lactose-enriched, lactase-treated, Mg-enriched products caused an increase in urinary Mg excretion of 0.97 mmol/d (calculated from Table 3). Thus, urinary Mg excretion as a percentage of intake from the Mg-enriched experimental dairy products was 35. Reported percentages of Mg absorption range from 30 to 40 (Nordin, 1976; Shills, 1984) at a common intake of 10–20 mmol/d. Apparently, a treatment period of 1 week was long enough to demonstrate effects of a diet change.

The lack of an effect of lactose on Mg absorption, as assessed by urinary Mg excretion, in our study is at variance with findings of similar studies with infants (Kobayashi *et al.* 1975; Ziegler & Fomon, 1983; Wirth *et al.* 1990). Kobayashi *et al.* (1975) reported that lactase-treated lactose increased Mg absorption compared with untreated lactose. This is an unexpected finding. However, Mg intake was not reported and differences in Mg intake between experimental groups cannot be excluded. Wirth *et al.* (1990) reported that lactose increases Mg absorption, but lactose ingestion was associated with an increased Mg intake. As shown in our study and in others (Hodgkinson & Heaton, 1965; Schwartz *et al.* 1973), a higher Mg intake by itself results in increased absolute absorption and urinary excretion of Mg so that an independent effect of lactose cannot be determined.

As mentioned previously (p. 863), dietary lactose increases intestinal absorption of Mg in rats. Rats become lactase deficient after weaning (De Groot & Hoogendoorn, 1957) and, thus, are not capable of hydrolysing lactose in the intestine. Possibly, microbial fermentation of lactose in the intestine lowers lumen pH. This would result in increasing solubility of intestinal Mg (Shiga *et al.* 1987), leading to enhanced Mg absorption (Hardwick *et al.* 1991).

There is controversy about the influence of lactose on Ca absorption in humans (Fournier & Dupuis, 1960; Condon *et al.* 1970; Pansu & Chapuy, 1970; Kocian *et al.* 1973; Cochet *et al.* 1983; Sheikh *et al.* 1987; Recker *et al.* 1988). In our lactose-tolerant subjects, hydrolysing lactose did not affect Ca absorption as assessed by urinary Ca excretion. It is possible that the 1-week treatment period was too short to observe an effect of lactose on urinary Ca excretion. However, it has been demonstrated that a change in dietary Ca or protein affects urinary Ca excretion within 1 week (Adams *et al.* 1979; van Beresteijn *et al.* 1990).

It has been shown that lactose increases Ca absorption in lactose-tolerant subjects, whereas it causes a decrease in lactose-intolerant subjects (Condon *et al.* 1970; Pansu & Chapuy, 1970; Cochet *et al.* 1983). Others (Kobayashi *et al.* 1975; Debognie *et al.* 1979) demonstrated that hydrolysed lactose produces higher rates of Ca absorption than intact lactose. These findings indicate that hydrolysis of lactose in the intestine is a prerequisite for increased Ca absorption. In keeping with this, Birlouez-Aragon (1988) showed that after milk consumption lactose-intolerant subjects absorb less Ca than lactose-tolerant subjects. Furthermore, compared with normal milk, lactase-treated milk enhanced Ca absorption in lactose-intolerant subjects but had no effect in lactose-tolerant subjects (Birlouez-Aragon, 1988). The present study supports the latter observation. In lactose-tolerant young adults with a high Ca intake, intact lactose had no specific effect on Ca absorption, as assessed by urinary excretion, when compared with its galactose and glucose components.

Urinary Ca excretion as a percentage of intake from the whole diet was on average 12. Reported percentages of Ca absorption range from 20 to 30 (Nordin, 1976) at a common intake of 20–25 mmol/d for Ca. The low value observed in our experiment might be largely explained by the high Ca intake of 37 mmol/d. Heaney *et al.* (1990) showed that an increase in Ca intake markedly decreased the absorption efficiency for Ca. On the other hand, Ca absorption might have been underestimated in this study as Ca retention and Ca losses via the endogenous route and the skin were assumed to be negligible. However, Ca intake was equal for each dietary period (Table 2) and, thus, the low urinary Ca excretion relative to intake is not likely to have affected the treatment comparisons.

Our study cannot exclude long-term effects of lactose on mineral absorption, neither can it exclude an effect in other age-groups. These aspects need further investigation. However, our findings indicate that hydrolysing lactose does not affect urinary excretion of Mg and Ca, which implies that lactose intake does not affect the absorption of Mg and Ca in healthy, lactose-tolerant adults.

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