Retention of Calcium from Various Xylitol-Calcium Combinations in Rats (43205)

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Abstract. **Previous studies have shown that xylitol, the five-carbon sugar alcohol, increases the intestinal absorption of Ca in the rat. In this study an optimum xylitol:Ca molar ratio for Ca absorption and retention was determined in 10-week-old Long-Evans male rats. Xylitol was intubated with 20 mg of ^{45}Ca (as $^{45}CaCl_2$ or $^{45}CaCO_3$) into the stomach of fasted rats in 1 ml water using xylitol:Ca molar ratios of 0:1, 0.5:1, 1:1, 1.5:1 or 2:1. Radioactivity of urine, feces and humerus was determined from 48-hr samples. The highest retention rate of ^{45}Ca in the whole body or in the humerus was found when 114 mg of xylitol was given with Ca (xylitol:Ca molar ratio 1.5). Xylitol affected humeral retention more significantly than whole body retention. For comparison, the retention of ^{45}Ca was determined from four lactose:$CaCl_2$ combinations and from Ca-lactate and Ca-citrate salts. None of these combinations increased Ca retention significantly, although lactose at 114 mg dose showed slight positive effect. The results favor the use of a xylitol:Ca molar ratio of 1.5 in Ca supplements.**
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Xylitol is a pentacarbon sugar alcohol used globally as a noncariogenic sugar substituent in sweets and chewing gums (1, 2). Xylitol has also been used as an energy source in parenteral nutrition (3). It has been shown that 5–20% dietary xylitol increases intestinal absorption of calcium in the rat (4–6) and 5% dietary xylitol improves rat bone recalcification after a 3-week Ca deficiency period (7). As with other calcium absorption promoting carbohydrates, e.g. lactose and glucose polymers (8), the effect of xylitol was found to be independent of vitamin D action. Thus, the passive, paracellular calcium absorption route was involved. In view of the reduced active (vitamin D dependent) calcium absorption rate found in many elderly people (9–11), a calcium supplement which increases passive Ca absorption would be advantageous in preventing a negative calcium balance and osteoporosis. In previous studies (4–6) xylitol was administered at a 5 to 20% level in rat diet. If a supplement for human use is desired, absorption data of xylitol-containing calcium bolus is needed. To find the optimum ratio of xylitol to calcium, xylitol and radioactive calcium were administered in various molar ratios to rats. Whole body and humeral retention of radioactive calcium were measured.

Materials and Methods

Male Long-Evans rats (inbred strain of the Institute of Dentistry, University of Turku), age 11 weeks and weight 220–245 g were used in this study. The study protocol was approved by the Ethical Committee on Animal Experiments of the University of Turku. Five days before onset of the experiments the normal laboratory diet (1% Ca) of 137 rats was changed for a semisynthetic calcium-free diet[2] supplemented with $CaCO_3$, 10 g/kg (0.4% Ca). The rats were deprived of food 18 hr prior to radioactive calcium administration. Distilled water was freely accessible to the rats. The test load of calcium was either 50 mg of $^{45}CaCO_3$ or 73.5 mg of $^{45}CaCl_2 \cdot 2H_2O$ given in 1 ml of

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[2] The composition of the diet per 100 g serving was as follows: casein, 20 g; corn starch, 62.65 g; cellulose meal, 5 g; soybean oil, 2 g; fat 5 g; NaH_2PO_4, 0.35 g; salt mixture (without Ca), 4 g; vitamin mixture, 1 g; Ca, 0.02%; Mg, 0.04%; P, 0.6%; vitamin D_3, 1.5 IE/g; energy, 3.3 kcal/g (EWOS R414 diet, Södertälje, Sweden).

distilled water. Both doses contained 20 mg of calcium labeled with 1.5 μCi of ^{45}Ca. Pharmacopeia grade (Ph Eur) xylitol (CAS No. 87–99–0, Cultor Ltd, Helsinki, Finland) or lactose was added to the solution in 0–152 mg/ml concentrations to produce xylitol:Ca molar ratios of 0:1 to 2:1 (lactose:Ca molar ratios of 0:1 to 0.89:1). These solutions were administered to the rats that were lightly ether-anesthetized using gastric gavage. Two organic calcium salts, calcium-L-lactate · $4H_2O$ (145.1 mg) or tricalcium-dicitrate · $4H_2O$ (95.1 mg), were administered using the same method to rats. $^{45}CaCl_2$ (specific activity 16.8 mCi/mg, Amersham) was used as tracer for the soluble organic calcium salts. Radioactive $^{45}CaCO_3$ was prepared from $^{45}CaCl_2$ by adding 2 ml of 1 *M* Na_2CO_3 to 1 ml of 1 *M* $CaCl_2 \cdot 2H_2O$ containing 20 μCi of $^{45}CaCl_2$. The precipitate was collected on a cellulose nitrate filter (pore size 0.45 μm), washed twice with 3 ml of water and dried at 60°C for 20 hr. The calcium concentration of this product was 39.1%, which is close to the theoretical 40% calcium of $CaCO_3$.

The rats were placed in Nalgene™ metabolic cages for 48 hr for collection of feces and urine. The semisynthetic diet (without Ca) and water were provided for the rats 6 hr after the Ca dose. After two days, the rats were killed in a CO_2 chamber. The left humerus and the gut were collected. The humeri were epiphysectomized and dried at 120°C for 20 hr and weighed. Radioactive calcium was extracted from the humerus, small intestine, lower gut (including cecum), and feces into 4 *M* HCl, and radioactivity was determined by liquid scintillation counting. Radioactivity of 1 ml urine samples was also measured. It was observed that the stomach and small intestine contained trace amounts of radioactivity. Therefore, these results were excluded from the retention calculations. Retention of ^{45}Ca in the whole body over 48 hr was calculated as follows: 100 × (dose ^{45}Ca − ^{45}Ca in feces and lower gut)/dose ^{45}Ca. Retention of ^{45}Ca in the humerus was also expressed as percentage of dose per gram of dry bone.

Statistical analysis of the effect of xylitol or lactose was done by one-way analysis of variance followed by pairwise comparisons with the Dunnett's method (12). The comparisons were made with the carbohydrate-free control group, and the statistical significance was tested at 1% and 5% risk levels. Statistical comparisons between the retention of calcium chloride, calcium carbonate, calcium lactate, and calcium citrate (without xylitol or lactose) were made by one-way analysis of variance followed by pairwise comparisons with the Tukey's method (12). The statistical significance was tested at 5% risk level.

Results

Two days after administration of the supplements most unabsorbed radioactive calcium was found in the feces. The lower gut contained only 0.1%–3% of

Table I. Retention of ^{45}Ca from Various Xylitol-Calcium and Lactose-Calcium Combinations and from Two Organic Calcium Salts within Two Days in Rats[a,b,c]

Ca-supplement (20 mg Ca)	Organic component:Ca molar ratio	*N*	Whole body retention (% of dose)	Humeral retention (% of dose/g dry bone)	Urinary excretion (% of dose)
$^{45}CaCO_3$	0:1	13	50.1 ± 12.2[d]	2.04 ± 0.45	0.33 ± 0.13[e]
+ xylitol 38 mg	0.5:1	7	51.9 ± 13.9	2.49 ± 0.54	0.69 ± 0.17**
+ xylitol 76 mg	1:1	7	54.6 ± 13.3	2.89 ± 0.33**	1.23 ± 0.30**
+ xylitol 114 mg	1.5:1	7	57.6 ± 5.4	3.03 ± 0.50**	0.88 ± 0.23**
+ xylitol 152 mg	2:1	7	56.4 ± 7.0	2.60 ± 0.42*	0.92 ± 0.21**
$^{45}CaCl_2 \cdot 2H_2O$	0:1	12	62.3 ± 10.4[e]	2.44 ± 0.33[e]	1.17 ± 0.41[d]
+ xylitol 38 mg	0.5:1	6	72.5 ± 9.2	2.57 ± 0.56	0.84 ± 0.23
+ xylitol 76 mg	1:1	6	74.8 ± 12.9	2.84 ± 0.56	0.89 ± 0.34
+ xylitol 114 mg	1.5:1	6	82.3 ± 9.3**	3.47 ± 0.48**	1.19 ± 0.35
+ xylitol 152 mg	2:1	6	72.6 ± 6.5	2.91 ± 0.49*	0.95 ± 0.26
+ lactose 38 mg	0.22:1	6	50.7 ± 12.0	2.76 ± 0.30	0.82 ± 0.36
+ lactose 76 mg	0.44:1	6	56.8 ± 12.5	2.75 ± 0.21	0.83 ± 0.36
+ lactose 114 mg	0.67:1	6	63.7 ± 11.9	2.97 ± 0.25	0.83 ± 0.36
+ lactose 152 mg	0.89:1	6	69.8 ± 16.2	2.43 ± 0.85	0.80 ± 0.26
Ca-lactate · $4H_2O$ 145 mg	1:1	6	57.0 ± 9.0	1.88 ± 0.17[d]	0.65 ± 0.13[e]
Ca-citrate · $4H_2O$ 95 mg	0.67:1	6	61.9 ± 7.4	2.49 ± 0.33[e]	0.69 ± 0.35[e]

[a] The figures shown are mean ± SD values. Statistics: comparison of the groups containing xylitol or lactose with the carbohydrate-free group (analysis of variance and two-tailed Dunnett's test): *$P < 0.05$; **$P < 0.01$. Comparisons between $CaCl_2$, $CaCO_3$, Ca-lactate, and Ca-citrate (without xylitol or lactose) by analysis of variance and Tukey's test: different letters (*d* and *e*) indicate significant differences between the groups.

[b] The supplements were given in a single dose with 1 ml water into rat stomach.

[c] The calcium tracer for Ca-lactate and Ca-citrate was $^{45}CaCl_2$.

tracer. Retention of ^{45}Ca in the whole body and in the humerus, and the urinary ^{45}Ca excretion are shown in Table I. Xylitol increased whole body and humeral retention of calcium from calcium chloride. In both cases the highest retention of ^{45}Ca was found at the 1.5:1 molar ratio of xylitol and calcium. Urinary Ca excretion was not significantly affected by xylitol when $^{45}CaCl_2$ was used as calcium source. When $^{45}CaCO_3$ was used as tracer, whole body radioactivity retention was not significantly increased by xylitol but bone radioactivity was again highest at the 1.5:1 xylitol:Ca molar ratio (Table I). All doses of xylitol increased urinary Ca excretion in the rats when calcium carbonate was used as Ca source. The effect of lactose resembled that of xylitol but was weaker, and the analysis of variance showed no significant differences between the groups. When the four calcium salts (without xylitol or lactose) were compared, it was found that significant differences occurred between the supplements. Retention of ^{45}Ca in the whole body was lower from calcium carbonate than from calcium chloride (Table I). In addition, urinary ^{45}Ca excretion was significantly higher from calcium chloride than from other supplements. However, significant differences in bone calcium retention did not occur between calcium carbonate, calcium chloride, or calcium citrate. By contrast, Ca-lactate showed lower retention of calcium in the bone than calcium chloride and calcium citrate.

Discussion

It is evident from these results that xylitol improves Ca absorption when given as a single dose into the rat stomach. The most effective dose was 114 mg of xylitol with 20 mg of calcium (a molar ratio of 1.5:1). In similar experimental conditions lactose only weakly promoted Ca absorption. The results further suggest that the absorption of calcium is higher from calcium chloride than from calcium carbonate. The calcium carbonate may not have been fully solubilized in the stomach by gastric juice before passing into small intestine. Ionization of a calcium salt is necessary for Ca absorption in the gut, although it is considered that some soluble calcium complexes also undergo absorption (13, 14). However, xylitol increased bone radioactivity from both calcium salts. On the other hand, radioactive calcium retention in the bone from calcium lactate was lower than from calcium chloride. Since Ca-lactate is highly soluble, a similar retention profile was expected. It is concluded that the bioavailability of a calcium supplement cannot be estimated directly from intestinal absorption data. Probably several physiologic and physicochemical factors, such as the influence of the anion on the acid-base balance (15), may affect calcium apposition in bones.

In some cases, the effect of xylitol was clearer on humeral retention of radioactive calcium than on whole body retention. This can be partly explained by more complicated analyses of the whole body experiments with possible cumulative errors from the dosing and the radioactivity determinations together with the large variations in absorption efficiency between individual animals. However, specific effects of xylitol on bone metabolism cannot be excluded. Previous studies have shown higher bone calcium concentrations in rats when xylitol is included in the diet (6, 16). At present, there are no other explanations for increased bone calcification by xylitol than increased absorption via the paracellular pathway due to complexation of calcium with xylitol in the gut (17). Complexes of xylitol and calcium of moderate stability (18) maintain calcium in solution in the intestine but do not prevent absorption. Thus, calcium absorption is permitted for prolonged periods. Concerning the use of xylitol in human nutrition it is not clear that equal xylitol:Ca molar ratios as in the young rats of this study are effective in adult people. The 11-week-old rats still show rapid growth with high Ca absorption, whereas many people in old age suffer from reduced Ca absorption (9–11). Dietary sorbitol increases calcium absorption similarly to xylitol in rat experiments (5). Francis *et al.* (19) studied the effect of 10 g sorbitol (55 mmol) on the absorption of calcium from 0.5 mmol calcium chloride or from 12.5 mmol calcium lactate in osteoporotic women and found a lower absorption rate in the presence of sorbitol. In view of the present results, it might be that the amount of sorbitol was too high. However, bioavailability studies in humans are needed to estimate the value of xylitol in calcium nutrition.

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