Dietary Xylitol, Sorbitol and D-Mannitol But Not Erythritol Retard Bone Resorption in Rats

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ABSTRACT The aim of the present study was to compare the ability of four dietary polyols to reduce bone resorption. Urinary excretion of 3H radioactivity from [3H]tetracycline-prelabeled rats was used as a marker of bone resorption. After prelabeling, the rats were divided randomly into five groups of 10, and fed for 1 mo a nonpurified diet that was supplemented in four groups with either xylitol, sorbitol, D-mannitol or erythritol, respectively, to give a polyol concentration of 1 mol/kg. Xylitol (42%), sorbitol (44%) and to a lesser degree D-mannitol (23%) decreased the excretion of 3H relative to the basal diet. The erythritol group, however, did not differ from the controls. Sorbitol caused continuous diarrhea, whereas in the other groups, intestinal adaptation took place during the 1st wk of polyol feeding. In conclusion, dietary xylitol, sorbitol and to a lesser degree D-mannitol supplementations in rats retard bone resorption, whereas dietary erythritol has no effect. J. Nutr. 126: 1865-1870, 1996.

INDEXING KEY WORDS:
• rats • polyols • bone resorption

Several natural polyols have been used widely as dietary sugar substitutes. They have also been used in the diets of diabetic subjects and in infusion therapy. Isotonic solutions of xylitol and sucrose have approximately equal sweetness (Moskowitz 1971), whereas erythritol is 75-80% (Kawanabe et al. 1992), D-mannitol 45-57% (Moskowitz 1974) and sorbitol 35-60% (Wright 1974) as sweet as sucrose at equal weight. Xylitol and sorbitol have energy contents similar to that of sucrose. D-Mannitol, when consumed as part of mixed diet, has a reduced energy value (Dills 1989). Erythritol is a very low-energy sweetener, the available energy value being <10% of that of sucrose (Noda and Oku 1992). Xylitol has noncariogenic and even anticariogenic properties (Scheinin and Mäkinen 1975). Sorbitol and D-mannitol can be classified as low-cariogenic polyols, although they are normal substrates of mutans streptococci (Mäkinen 1994). Recently, erythritol was shown to be a promising sugar substitute from a cariologic point of view (Kawanabe et al. 1992).

Polyols occur naturally in many plants. Sorbitol is found in numerous berries and higher plants (Lohmar 1962). D-Mannitol is also widely distributed, but unlike sorbitol, it is present more frequently in plant exudates (Lohmar 1962). Xylitol is present in many vegetables and fruits, although the quantities are quite low (Mäkinen and Söderling 1980, Washüttl et al. 1973). Erythritol exists in fruits (Shindou et al. 1989) and mushrooms (Yoshida et al. 1986), but the amounts are extremely low. Xylitol (Touster 1969), sorbitol (Winegrad et al. 1972) and D-mannitol (Laker and Gunn 1979) are also endogenous metabolites in mammals. Exogenous polyols are absorbed slowly and metabolized mainly by the hepatic enzymes. Xylitol and sorbitol are metabolized completely after moderate administration, whereas D-mannitol is poorly utilized because its low affinity for L-iditol dehydrogenase, causing an increased D-mannitol concentration in the urine (Dills 1989). Exogenous erythritol is very poorly metabolized, being excreted almost completely in urine without degradation (Noda et al. 1994).

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Figure 1 Structural formulas of the polyols studied.

We previously showed that 10 and 20% dietary xylitol supplementations retard bone resorption in rats (Mattila et al. 1995, Svanberg and Knuuttila 1994a). The mechanism causing this effect is obscure, but an increased absorption of calcium (Hämäläinen et al. 1985), associated with its ability to form complexes with sugar alcohols (Angyal 1974), may be involved. Xylitol supplementation increases the bone calcium concentration [Knuuttila et al. 1989], enhances calcification of previously calcium-deficient bone [Hämäläinen et al. 1990] and protects against bone mineral loss after ovariectomy [Svanberg and Knuuttila 1994b] in rats. Furthermore, the serum level of 1,25-dihydroxycholecalciferol, a potent stimulator of bone resorption, is reduced during dietary xylitol supplementation [Hämäläinen et al. 1985].

Interestingly, other polyols share some properties with xylitol regarding their association with calcium. Sorbitol and D-mannitol increase calcium absorption and urinary calcium excretion [Hämäläinen and Mákinen 1986, Knuuttila et al. 1989, Vaughan and Filer 1960]. Dietary sorbitol supplementation increases the concentration of bone calcium, although less than xylitol [Knuuttila et al. 1989]. These similarities indicate that polyols other than xylitol may influence bone resorption. The present experiment was performed to compare the effects of xylitol, sorbitol, D-mannitol and erythritol (Fig. 1) at equimolar levels on bone resorption in male Sprague-Dawley rats. This study thus offered an opportunity to examine whether the effects of these polyols on bone resorption can be regarded as "general polyol effects" [i.e., shared by all dietary polyols], or whether more selective and more specific mechanisms are involved.

Materials and Methods

Labeling procedure. Fifty 4-wk-old male Sprague-Dawley rats [Laboratory Animal Center, University of Oulu, Finland] weighing 119 ± 12 g (mean ± SD), were injected subcutaneously with 1 mL of a solution containing 185 GBq/L of [7-3H(N)]-tetracycline (Du Pont de Nemours GmbH, Dreieich, Germany) dissolved in distilled water. The injections were repeated weekly for 5 wk.

The animals were fed a basal powder diet, RM1 [Special Diet Services, Witham, Essex, United Kingdom]. One kilogram of this diet contains 885 g cereal products (wheat, barley and wheatfeed), 60 g vegetable proteins, 25 g animal proteins (whey powder), 5 g soybean oil, 7.1 g calcium, 2.9 g phosphorus and 1.5 µg cholecalciferol. The rats had free access to the diet and to tap water. The rats were housed in a temperature- and light-controlled room (21–23°C, 12-h light-dark cycle).

The study protocol was approved by the Ethical Committee on Animal Experiments of the University of Oulu.

Urine collection period. One week after the last [3H]tetracycline injection the rats were housed in individual metabolic cages for a 24-h urine collection. After the baseline, the rats were divided randomly into five groups of 10. The animals in the control group continued eating the basal diet (RM1), whereas the other groups were given the same diet supplemented either with xylitol [Xylofin, Kotka, Finland], D-glucitol [generally called sorbitol] [Serestar, Kreseld, Germany], D-mannitol [Sigma Chemical, St. Louis, MO] or mesoerythritol [generally called erythritol] [Fluka Chemie AG, Buchs, Switzerland], respectively, so that the final polyl concentration in the diet was 1 mol/kg. This polyl concentration corresponds to 152 g xylitol, to 182 g sorbitol or D-mannitol and to 122 g erythritol per 1 kg of the diet. The urine was collected on d 1, 3, 7, 10, 14, 21, 28 and 31. Rats were weighed weekly, and their food and water intakes were monitored.

The volume of urine excreted was measured, and the amount of 3H radioactivity present in a 0.001-L aliquot was determined with a scintillation counter 1215 Rachbeta II (Wallac Co., Turku, Finland) using Hydrofluor [Pational Diagnostics, Manville, NJ] as the liquid scintillation counting solution. The total excretion of 3H was calculated as an indicator of the amount of resorbed bone mineral as described by Klein and Jackman [1976]. The continuous monitoring of bone resorption was as described by Mühlbauer and Fleisch [1990].

Bone preparation. After the urine collection period the rats were killed using an overdose of ether, and their tibiae and scapulae were prepared for the determination of the 3H content of bone. The tibial epiphyses and the bone marrow were carefully removed. After drying at 60°C for 24 h the bones were weighed and pulverized with a micromill Mixer Type III 695 (Retsch, Haan, Germany). Thereafter, 25 mg of the pulverized bone was suspended in a 3:1 mixture of concentrated HCl [pro analyse, Riedel-de Haen, Seelze, Germany] and concentrated HNO3 [pro analyse, Merck, Darmstadt, Germany], and the 3H radioactivity was measured as described above. The total amount of 3H left in the tibiae and the scapulae was calculated.


TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Xylitol</th>
<th>Sorbitol</th>
<th>D-Mannitol</th>
<th>Erythritol</th>
<th>Pooled sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total food intake, g/d</td>
<td>23a</td>
<td>23a</td>
<td>23a</td>
<td>22a</td>
<td>25b</td>
<td>2</td>
</tr>
<tr>
<td>Basal diet intake, g/d</td>
<td>23</td>
<td>19</td>
<td>19</td>
<td>18</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Polyol intake, g/d</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Water intake, L/d</td>
<td>0.033a</td>
<td>0.035ab</td>
<td>0.034a</td>
<td>0.041b</td>
<td>0.051c</td>
<td>0.009</td>
</tr>
<tr>
<td>Weight gain, g</td>
<td>96b</td>
<td>77ab</td>
<td>58b</td>
<td>77b</td>
<td>66a</td>
<td>19</td>
</tr>
<tr>
<td>Excretion of urine, L/d</td>
<td>0.011a</td>
<td>0.011a</td>
<td>0.010a</td>
<td>0.020b</td>
<td>0.024b</td>
<td>0.007</td>
</tr>
<tr>
<td>Tibia weight, mg</td>
<td>496</td>
<td>492</td>
<td>482</td>
<td>493</td>
<td>487</td>
<td>39</td>
</tr>
<tr>
<td>Scapula weight, mg</td>
<td>219b</td>
<td>214ab</td>
<td>199a</td>
<td>219b</td>
<td>214ab</td>
<td>22</td>
</tr>
</tbody>
</table>

1 All values are expressed as means, n = 10.
2 Statistical differences were calculated by ANOVA, further comparisons being made using Fisher's PLSD. Groups with different superscript letters differ significantly (P < 0.05).
3 Average baseline weight was 294 ± 30 g.

**Statistical analysis.** Information from a series of urinary measurements on each rat was summarized as the area under the curve as described by Altman (1990). Statistical significances of the differences between the groups concerning all measured variables were calculated by analysis of variance, further comparison being made using Fisher's Protected Least Significant Difference (Armitage and Berry 1994). The statistical computer program used was StatView II for Macintosh (Abacus Concepts, Berkeley, CA).

**RESULTS**

Compared with the control rats, the weight gains were smaller in rats fed sorbitol or erythritol (Table 1). Food intake was greater in the erythritol group than in the other groups (Table 1). Slight diarrhea was detected during the 1st wk in all polyol supplementation groups. In the sorbitol group, the diarrhetic effect was maintained throughout the experimental period.

D-Mannitol and erythritol caused diuresis due to their incomplete metabolism. Xylitol and sorbitol did not affect the excretion of urine compared with the basal diet alone (Table 1). Consequently, the mean water intake was greatest in the D-mannitol and erythritol groups.

The dry weights of the tibiae and the scapulae in the xylitol, D-mannitol and erythritol groups did not differ from the controls (Table 1). In the sorbitol group, however, significantly smaller weights of the scapulae than in the control or mannitol group were detected.

The urinary $^{3}$H excretion rates are shown in Figure 2. Dietary xylitol, sorbitol and to a lesser degree D-mannitol reduced the $^{3}$H excretion compared with the basal diet alone. This effect was detected as early as 1 d after the beginning of polyol feeding, and the diminished level was maintained throughout the experiment. However, the $^{3}$H excretion was not affected by dietary erythritol. Preserved $^{3}$H radioactivity in the tibiae and the scapulae after the experiment generally was greatest in the xylitol group (Table 2).

**DISCUSSION**

Dietary xylitol, sorbitol and to a lesser degree D-mannitol caused a decrease in the bone resorption relative to the basal diet as measured by urinary $^{3}$H excretion. Dietary erythritol, however, did not differ significantly from the basal diet. The effect of xylitol is in good accordance with our earlier results (Mattila et al. 1995), whereas no data are available concerning the effects of the other three polyols.

Xylitol, D-mannitol and erythritol caused a temporary, slight diarrhea as a result of their slow absorption from the intestine. An intestinal adaptation process occurred during the 1st wk, however, after which no diarrhea was observed. Although these polyols differed in their effects on $^{3}$H excretion, it seems that the excretion of $^{3}$H is not associated with this slight diarrhetic effect. In the case of sorbitol, no adequate adaptation process took place, resulting in continuous diarrhea. This was probably reflected in the reduced weight gain and the associated decreases in the weights of the bones of the rats given sorbitol. These changes may also be regarded as possible confounding factors when the bone resorption status of the rats fed sorbitol is considered. The lower weight gain and the greater food intake in the erythritol group probably resulted from the very low energy available from this polyol.

D-Mannitol is a strong diuretic agent and an effective osmoregulator in mammalian tissues. The present results indicate that erythritol also is strongly diuretic in rats and that enteral administration of erythritol substantially increases water intake.

The method used in measuring the bone resorption has been described by Klein and Jackman (1976) and
further developed by Mühlbauer and Fleisch (1990). Kelly and Buyske (1960) demonstrated that [3H]-tetracycline does not undergo metabolic transformation in rats. The efficiency of renal excretion of [3H]-tetracycline that is removed during bone resorption ensures that the [3H], unlike isotopes of calcium, will be only minimally reused at new sites of bone formation (Klein et al. 1985). The above studies have confirmed the urinary excretion of [3H]tetracycline to be a valid marker of bone resorption.

A decline in the amount of excreted radioactivity during the test period was observed in all groups because of the decreasing amount of [3H] in bone. This led to a decrease in numerical, but not in relative differences between the groups. The higher amount of radioactivity preserved in the bones of rats fed xylitol further confirmed the retarded bone resorption detected by the decreased urinary excretion of [3H].

The detailed chemical mechanism of decreased bone resorption caused by the polyols is obscure, but an increased calcium absorption may be involved. The enhanced calcium absorption may be related to the complex formation between calcium and the polyols. It has been suggested that the complex calcium remains soluble in the gut lumen for prolonged periods of time promoting its absorption (Hämäläinen and Mäkinen 1989). Briggs et al. (1981) showed that the relative complexation coefficients (measuring relative stabilities of complexes) of xylitol, sorbitol, D-mannitol and erythritol with calcium are 0.30, 0.40, 0.20 and 0.19, respectively. Although these values were not measured in biologic environments, their magnitudes, nevertheless, provide clues of the relative complexation capacities of these polyols. Accordingly, the polyols (xylitol and sorbitol) that were more effective in retarding bone resorption, have higher complexation coefficients (and stronger complexes) than those polyols (D-mannitol and erythritol), which were less effective.

Xylitol and sorbitol, which are mostly metabolized, seem to retard bone resorption more effectively than the poorly utilized D-mannitol and the unmetabolized erythritol. The first step of the metabolism of absorbed polyols is their oxidation by L-iditol dehydrogenase to the corresponding 2-ketoses with concomitant production of NADH. Ingested xylitol and sorbitol will thus increase the cellular NADH/NAD ratio, whereas the low oxidation rate of D-mannitol should not elevate the cellular NADH level significantly. The high cellular NADH/NAD ratio leads to the suppression of the citric acid cycle, NADH being used for energy production.

**FIGURE 2** Urinary [3H] excretion of the rats fed different polyols (1 mol/kg dry diet) for 1 mo. Values are means, n = 10.

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**TABLE 2**

Average urinary [3H] excretion and [3H] radioactivity preserved in the tibiae and scapulae of the rats fed different polyols for 1 mol

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Xylitol</th>
<th>Sorbitol</th>
<th>D-Mannitol</th>
<th>Erythritol</th>
<th>Pooled SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary [3H] excretion, AUC/d</td>
<td>20S^c</td>
<td>114^a</td>
<td>112^a</td>
<td>159^b</td>
<td>201^c</td>
<td>45</td>
</tr>
<tr>
<td>[3H] radioactivity in the tibia, Bq</td>
<td>997^a</td>
<td>1218^b</td>
<td>1183^ab</td>
<td>1062^ab</td>
<td>1057^ab</td>
<td>253</td>
</tr>
<tr>
<td>[3H] radioactivity in the scapula, Bq</td>
<td>345^ab</td>
<td>430^c</td>
<td>370^b</td>
<td>355^ab</td>
<td>310^a</td>
<td>60</td>
</tr>
</tbody>
</table>

1 All values are expressed as means, n = 10.
2 Statistical differences were calculated by ANOVA, further comparisons being made using Fisher's PLSD. Groups with different superscript letters differ significantly (P < 0.05) from each other.
3 Data presented in Fig. 2. AUC = area under the curve.
through the respiratory chain [Jakob et al. 1971, Williamson et al. 1971]. Coenzymes regulate numerous metabolic reactions and hormonal effects, and it is conceivable that some coenzymes in their reduced stage elicit such reaction conditions that preserve the higher calcium levels in bone structures. Alterations in the cellular NADH/NAD ratio are closely connected with the calcification process. Increased NADH concentrations occur simultaneously with active calcifications of the cartilage [Shapiro et al. 1982]. An increased NADH concentration is also known to promote Ca^{2+} transport across the cell surface membrane and the sarcoplasmic reticulum [Lehninger et al. 1978].

In conclusion, dietary xylitol, sorbitol and to a lesser extent D-mannitol, retard bone resorption in Sprague-Dawley male rats, whereas dietary erythritol has no effect. This polyol-dependent variation in bone resorption may result from differences in calcium absorption (which can be stimulated by some dietary polyols) and from the metabolic fate of those polyols in rat tissues in which a "kinetic pressure" (elicited as the polyols must deposit their "extra" hydrogen atoms to suitable acceptors, such as NAD) will directly or indirectly affect the mechanisms that regulate bone calcium levels. Future research should provide a more detailed chemical explanation to these effects, as well as clues to the possible application of the present findings in human usage of dietary polyols, and whether similar effects can be observed using polyol levels smaller than the 1 mol/kg level used in this study.

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LITERATURE CITED


