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The effect of a simultaneous dietary administration of xylitol and ethanol on bone resorption

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Abstract

Our previous studies have shown that dietary xylitol supplementation diminishes bone resorption in rats, as well as protects against ovariectomy-induced increase of bone resorption during experimental osteoporosis. Interestingly, ethanol, when given simultaneously with xylitol, is known to increase blood concentration of xylitol. On the other hand, ethanol, when given alone, has been shown to increase bone resorption. The aim of the present study was to evaluate the effects of a simultaneous dietary administration of 10% xylitol and 10% ethanol on bone resorption. Bone resorption was determined using measurement of urinary excretion of hydrogen 3 (^{3}H) radioactivity in ^{3}H-tetracycline prelabeled rats. Already 4 days after the beginning of dietary supplementations, excretion of ^{3}H was about 15% lower in the xylitol group (diet supplemented with 10% xylitol) and about 25% lower in the xylitol-ethanol group (diet supplemented with 10% xylitol and 10% ethanol) as compared to the controls. The excretion of ^{3}H in these groups remained smaller than that of the controls throughout the entire study period of 40 days. The excretion of ^{3}H in the xylitol-ethanol group remained also smaller than that of the xylitol group. Bone mineral density and bone mineral content were determined with a peripheral quantitative computed tomography (pQCT) system from the rat tibiae at the end of the experiment. Trabecular bone mineral density and trabecular bone mineral content were significantly greater in the xylitol group and in the xylitol-ethanol group compared to the controls. They were also greater in the xylitol-ethanol group as compared to the xylitol group. Cortical bone mineral density and cortical bone mineral content did not differ significantly between the groups. In conclusion, a simultaneous dietary supplementation with 10% xylitol and 10% ethanol seems to diminish bone resorption and to increase trabecular bone mineral density and trabecular bone mineral content in rats. These effects seem to be stronger than the effects induced by 10% xylitol supplementation alone.

1. Introduction

Xylitol is a 5-carbon polyalcohol that is found in many fruits, berries, and plants. The richest sources are plums, strawberries, raspberries, cauliflower, and endives [1]. Xylitol is also an intermediate of mammalian carbohydrate metabolism. In a human body, 5 to 15 g of xylitol is formed daily [2].

Our previous experimental studies have shown that dietary xylitol supplementation diminishes bone resorption in rats [3], as well as protects against an ovariectomy-induced increase of bone resorption during experimental osteoporosis [4]. Dietary xylitol also increases the trabecular bone volume [5] and protects significantly against an ovariectomy-induced decrease of bone trabecular volume [4].

Interestingly, ethanol, when given simultaneously with xylitol, is known to increase the blood concentration of xylitol [6]. On the other hand, ethanol, when given alone, has been shown to increase bone resorption [7] and to decrease trabecular bone volume [8].

The aim of the present study was to evaluate the effects of a simultaneous dietary administration of xylitol and ethanol on bone resorption and bone trabeculation, and to compare these effects with the effects induced by dietary xylitol alone.

2. Material and methods

2.1. Animals

Thirty 4-week-old male Wistar rats were injected subcutaneously on a weekly basis for 5 weeks with 1 mL

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of a solution containing 5 μCi/mL of [7-^{3}H(N)]-tetracycline (Du-Pont de Nemours GmbH, Dreieich, Germany) dissolved in distilled water. One week after the last injection, the rats were housed in individual metabolic cages for a 24-hour urine collection that served as a baseline measurement for their hydrogen 3 (^{3}H) excretion. Thereafter, the rats were divided randomly into 3 groups of 10. Animals in the control group were fed a basal powder diet, Lactamin R3 (Labfor, Stockholm, Sweden), consisting of barley meal 28%, wheat meal 20%, wheat germ 20%, wheat middlings 10%, soya meal 7%, fish meal 7%, fodder yeast 3%, minerals 3%, vitamins and trace elements 1%, and fat 1%. This diet contains 1.1% calcium, 0.8% phosphorus, and 600 IU/kg vitamin D_3. The rats had free access to tap water. Animals in the first study group (the xylitol group) were fed the same diet supplemented with 10% xylitol (Cultor, Espoo, Finland). Animals in the second study group (the xylitol-ethanol group) were fed the same diet as the xylitol group, but their tap water was supplemented with 10% ethanol. The rats were housed in a temperature- and light-controlled room (21°C, 12-hour light-dark cycle). Their urine was collected about twice a week for 40 days. They were weighed weekly, and their food and liquid intake was measured. After the urine collection period, the rats were killed with an overdose of ether followed by decapitation. Their left tibiae were prepared for bone analyses. The study protocol was approved by the Ethical Committee on Animal Experiments of the University of Oulu, Oulu, Finland. The experimental procedures complied with the Guiding Principles in the Care and Use of Animals, approved by the Council of the American Physiological Society in 1991.

2.2. Measurement of ^{3}H radioactivity

The volume of urine excreted was measured and the amount of ^{3}H-radioactivity present in a 1-mL aliquot was determined with a scintillation counter 1215 Rachbeta II (Wallac, Turku, Finland) using Hydrofluor (Pational Diagnostics, Manville, NJ) as the liquid scintillation counting solution. The total excretion of ^{3}H was calculated as an indicator of the amount of resorbed bone mineral, as described by Klein and Jackman [9]. The continuous monitoring method was that described by Mühlbauer and Fleisch [10].

2.3. Peripheral quantitative computed tomography measurements

The left tibiae of the rats were scanned with a peripheral quantitative computed tomography (pQCT) system, the Stratec XCT 960A (Norland Stratec Medizintechnik GmbH, Birkenfeld, Germany), using a voxel size of 0.148 × 0.148 × 1.25 mm^3. The diaphysis was scanned at midshaft. The distal metaphysis was scanned adjusting the scan line to 5 mm proximal to the distal end of the tibia using the scout view property of the pQCT software. Cortical bone mineral density and cortical bone mineral content were determined from the scans of the tibial diaphysis. The tibial distal metaphysis scans were used for the determinations of trabecular bone mineral density and trabecular bone mineral content. The concentrical peeling method (peel mode 1) with a 45% inner region corresponding to a pure trabecular area was used, as described by Tuukkanen et al [11].

2.4. Statistical analysis

Information from a series of urinary measurements on each individual rat was summarized as the area under the curve, as described by Altman [12]. The statistical significance of the differences between the groups concerning all measured variables were calculated by analysis of variance (ANOVA), further comparison being made using Fisher's protected least significant difference (PLSD). The statistical computer program used was Stat View II for Macintosh (Abacus Concepts, Berkeley, Calif).

3. Results

The weight gains of the rats did not differ significantly between the groups (data not shown) indicating no major differences in the growth of the animals. The average diet and liquid intakes were also similar in the groups (data not shown) indicating that the dietary supplementations did not change the dietary habits of the rats. There was some difference in urine volume outputs between the groups (data not shown). However, the possible confusing effect caused by different urine volumes was eliminated by calculating the amount of ^{3}H radioactivity in proportion to the whole daily volume of urine.

The amounts of urinary ^{3}H excreted during the experimental period are seen in Fig. 1. A reduction of the excreted radioactivity was observed in every group because of the decreasing amount of ^{3}H in bone. Already in the first mea-

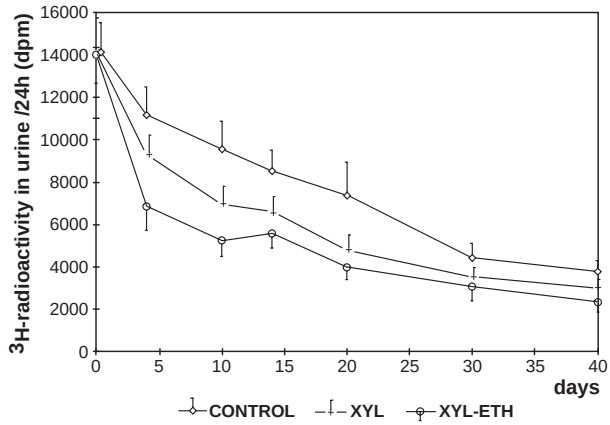

Fig. 1. Urinary ^{3}H excretions of the rats during the experimental period. Values are presented as mean ± standard error of mean; n = 10 per group. Between groups, comparison was measured as the area under the curve. Statistical differences were calculated by the ANOVA, further comparison being made using Fisher's PLSD. Significant differences: control group vs xylitol group (XYL), $P < .002$; control group vs xylitol-ethanol group (XYL-ETH), $P < .001$; xylitol group vs xylitol-ethanol group, $P = .02$.

Table 1
Results of the pQCT analyses

	Control rats	Rats with 10% dietary xylitol supplementation	Rats with 10% dietary xylitol and 10% ethanol supplementation
	(a)	(b)	(c)
Tibial metaphysis			
Trabecular bone mineral density (mg/cm^3)	189.3 ± 23.1	254.5 ± 46.6	303.5 ± 73.1
Trabecular bone mineral content (mg/cm)	1.65 ± 0.20	2.02 ± 0.26	2.28 ± 0.35
Tibial diaphysis			
Cortical bone mineral density (mg/cm^3)	1323.0 ± 16.1	1323.6 ± 10.3	1318.3 ± 6.8
Cortical bone mineral content (mg/cm)	7.89 ± 0.54	7.88 ± 0.44	7.70 ± 0.55

All values are expressed as mean ± SD; n = 10 per group. Statistical differences were calculated by the ANOVA, further comparison being made using Fisher's PLSD. Significant differences in trabecular bone mineral density: a vs b, and a vs c, $P < .01$; b vs c, $P < .05$. Significant differences in trabecular bone mineral content: a vs b, and a vs c, $P < .01$; b vs c, $P < .05$.

surement, 4 days after the beginning of dietary supplementations, the excretion of ^{3}H was about 15% lower in the xylitol group and 25% lower in the xylitol-ethanol group, as compared to the controls. The excretion of ^{3}H in both the xylitol and xylitol-ethanol group remained smaller than that of the control group throughout the entire study period of 40 days. When calculating excretions of ^{3}H during the whole experimental period, the xylitol ($P < .002$) and the xylitol-ethanol ($P < .001$) groups differed significantly from the control group. The excretion of ^{3}H in the xylitol-ethanol group was also significantly smaller than that of the xylitol group ($P = .02$).

The results of the pQCT analyses are seen in Table 1. The trabecular bone mineral density at the end of the experimental period was significantly greater in the xylitol group ($P < .01$) and in the xylitol-ethanol group ($P < .01$) compared to the control rats. The xylitol-ethanol group differed also significantly from the xylitol group ($P < .05$). Accordingly, trabecular bone mineral content was significantly greater in the xylitol ($P < .01$) and xylitol-ethanol ($P < .01$) groups compared to the controls. The xylitol-ethanol group differed significantly also from the xylitol group ($P < .05$). Cortical bone mineral density and cortical bone mineral content did not differ significantly between the groups.

4. Discussion

Several studies have confirmed that the urinary excretion of ^{3}H radioactivity in ^{3}H-tetracycline prelabeled rats is a valid marker of bone resorption [9,10]. Multiple prelabeling of rapidly growing rats permits homogenous distribution of ^{3}H-tetracycline throughout the bones [9], and the elimination of ^{3}H directly reflects bone resorption [13]. This is possible because tetracycline incorporated to the bone is removed only during resorption [14], and because very little of the removed tetracycline is reused at new sites of bone formation [15].

Dietary xylitol supplementation in rats induced a significant and rapid reduction of bone resorption that was maintained over the whole experimental period of 40 days. This is well in accordance with our previous findings [3]. A simultaneous ethanol supplementation seemed to further strengthen the xylitol-induced reduction of bone resorption. This is quite interesting, because ethanol, when given alone, has been shown to increase bone resorption [7]. On the other hand, ethanol, when given simultaneously with xylitol, is known to increase the blood concentration of xylitol [6].

Dietary xylitol supplementation also induced a significant increase in the trabecular bone mineral density and in the trabecular bone mineral content of the distal metaphysis of tibia. This is in accordance with our previous findings [5], as well as with the diminished bone resorption detected in the present study. Accordingly, with the bone resorption findings, ethanol, when given simultaneously with xylitol, seemed to induce an even greater increase in the values of the trabecular bone markers compared to the supplementation of the diet with xylitol alone. Interestingly again, ethanol, when given alone, has been shown to decrease the trabecular bone volume [8]. The cortical bone mineral density and the cortical bone mineral content did not differ significantly between the groups. This may indicate that trabecular bone is more sensitive than cortical bone to these kinds of effects induced by xylitol. On the other hand, the experimental period in the present study was quite short, about 1 month, and possible changes in the cortical bone may need a longer time to become detectable.

Although ethanol, when given alone, has been shown to increase bone resorption and decrease bone trabeculation, it can be said that a simultaneous dietary xylitol administration is most probably very effective in protecting against such ethanol-induced changes.

In conclusion, a simultaneous dietary supplementation with 10% xylitol and 10% ethanol seems to diminish bone resorption and increase trabecular bone mineral density and trabecular bone mineral content in rats. These effects seem to be stronger than the effects induced by 10% xylitol supplementation alone.

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