Dietary Xylitol Supplementation Prevents Osteoporotic Changes in Streptozotocin-Diabetic Rats

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The effects of 10% and 20% dietary xylitol supplementation on the biomechanical properties, trabeculation, and mineral content of long bones were studied in streptozotocin-diabetic rats. Forty 3-month-old male Wistar rats were divided randomly into four groups of 10. Rats in three groups were administered a single injection of streptozotocin (50 mg/kg body weight) to induce type I diabetes, while animals in the fourth group were given a sham injection of physiological saline. The sham-injected group and one of the streptozotocin-diabetic groups were fed the basal diet, while the two diabetic groups were fed the same diet supplemented with 10% and 20% xylitol (wt/wt). After 3 months, the rats were killed and the long bones were prepared for analysis. The 10% and 20% dietary xylitol supplementation significantly prevented the type I diabetes–induced decrease in the mechanical stress resistance of the tibia in the three-point bending test, the shear stress of the femur in the torsion test, and the stress resistance of the femoral neck in the loading test. No statistically significant differences were found between any groups in the values for strain or Young's modulus in the three-point bending test, or in the values for the shear modulus of elasticity in the torsion test. These findings indicate that dietary xylitol protects against the weakening of the bone strength properties of both cortical and trabecular bone without affecting the elastic-plastic properties. Supplementation with 10% and 20% dietary xylitol significantly prevented the type I diabetes–induced decrease of humeral ash weight and tibial density. Histomorphometric data for the secondary spongiosa of the proximal tibia showed that 10% and 20% dietary xylitol supplementation also significantly prevented the type I diabetes–induced loss of trabecular bone volume. In conclusion, dietary xylitol supplementation protects against the weakening of bone biomechanical properties in streptozotocin-diabetic rats. This is related to the preserved bone mineral content and preserved trabecular bone volume.

OSTEOPOROSIS is one of the complications of type I diabetes. Diabetic osteopathy has been reported in many clinical human studies[1-6] and in experimental animal studies.[7-13] Typical findings are an abolished active duodenal calcium absorption,[11] decreased deposition of calcium in bone,[14] decreased bone volume affecting especially the trabecular bone,[1] and reduced bone biomechanical properties. The femurs of alloxan-diabetic rats are more fragile and require less force to break than the femurs of control rats.[7] Torsional strength of the femoral diaphysis is decreased[10] and the structural and material properties of the femoral neck are decreased[12] in streptozotocin-diabetic rats compared with controls. Type I diabetic osteoporosis has been explained by reduced bone formation without a comparable reduction in bone resorption.[11]

Xylitol is a five-carbon polyalcohol present in many vegetables and fruits.[15] It is also an endogenous metabolite of the liver in mammals.[16] Xylitol can be used in the diet of diabetic subjects because it is slowly absorbed, its initial metabolic steps are independent of insulin, and it does not cause rapid changes in blood glucose.[17,18] It is also used as a source of energy in intravenous nutrition, because tissues can use xylitol under postoperative and posttraumatic conditions in which considerable insulin resistance prevents effective glucose utilization. Xylitol effectively converts the catabolic status in such situations toward anabolism.[17,18]

Dietary xylitol supplementation increases the intestinal absorption of calcium in rats.[19] It also increases the bone calcium concentration[20] and retards bone resorption.[21] On the basis of these results, it can be hypothesized that dietary xylitol supplementation may protect against osteoporotic changes in diabetes.

The purpose of the present study was to investigate whether dietary xylitol can protect against the loss of bone mineral, the loss of trabecular bone volume, and the weakening of bone biomechanical properties in streptozotocin-diabetic rats.

MATERIALS AND METHODS

Laboratory Animals

Forty 3-month-old male Wistar rats weighing 398 ± 34 g (mean ± SD) were divided randomly into four groups of 10. All of the animals were fed a basal diet, Lactamin R3 (Labfor, Stockholm, Sweden) consisting of (percent by weight) barley meal 28%, wheat meal 20%, wheat germ 20%, wheat middling 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% Ca, 0.8% P, and 1,500 IE/kg vitamin D_3. The rats had free access to tap water ad libitum.

The animals were housed in cages (Makrolon III; Tecniplast, Buguggiate, Italy), two or three per cage, on a bed of European aspen shavings with a 12-hour light/dark cycle at a room temperature of 21 to 23°C and 40% to 60% humidity. They were weighed weekly, and the food intake was measured.

Under light ether anesthesia, rats in three groups were administered a single injection of streptozotocin (Sigma Chemical, St Louis, MO, 50 mg/kg body weight via the tail vein) dissolved in saline to induce type I diabetes. The diabetic status of the rats was verified by measuring the serum glucose concentration using the glucose-oxidase method and a Sigma kit (Sigma Chemical). Streptozotocin-injected rats were confirmed to be diabetic because serum glucose was higher than 15 mmol/L. Animals in the fourth group were given a sham injection of physiological saline. The diabetic status of the rats was also evaluated by measuring the serum insulin level by a radioimmunoassay using rat insulin (Novo-Nordisk, Bagsvaerd, Denmark) as a standard. Sham-injected animals and one of the streptozotocin-injected groups were fed the basal diet, while animals in the other two streptozotocin groups were

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fed the same diet supplemented with xylitol (Xyrofin, Kotka, Finland) with a final xylitol concentration in the diet of 10% and 20% (wt/wt). After 3 months, the rats were killed with CO_2 followed by decapitation, and the right humerus, tibiae, and femurs were prepared for analysis.

Bone Ash Weight, Bone Density, and Trabecular Bone Volume

The epiphyses and bone marrow of the right humerus were removed, followed by drying of the bone samples at 60°C for 24 hours and pulverization with a micromill Mixer Type III 695 (Retsch, Haan, Germany). The bone ash weight was determined by ashing 25 mg pulverized bone at 900°C for 24 hours.

The whole tibial weight was determined with a weighing machine (Mettler A30; Mettler Instrumente, Zürich, Switzerland), followed by pycnometric measurement of the bone density.

The proximal part of the right tibia was selected for measurement of the trabecular bone volume. The proximal tibiae were cut sagittally into two equal halves with a diamond saw, dehydrated with ethanol (40%), and embedded in methylmethacrylate as described by Baron et al.[22] Undecalcified sections of 5 μm were cut with a Polycut S heavy-duty microtome (Reichert-Jung, Leica Instruments, Nussloch, Germany) and stained according to the method of von Kossa. Sections were taken near the sagittal midline of the tibia at five levels 50 μm apart, and one microscopic field per section was evaluated. Trabecular bone volume was measured in an area of 5 mm^2 at 4X objective magnification using a computer image analyzer (MCID, Model M1; Imaging Research, Brock University, Ontario, Canada). The area situated within 1 mm of the upper surface of the growth plates, as well as all trabeculae in contact with the cortices, were excluded from the measurements.

Mechanical Testing Procedures

The left tibiae and the femurs were stored at -20°C until use. Before testing, the bones were thawed at room temperature and kept moist until the test was completed. The bone length was measured with calipers. The area of the femoral neck, as well as the inner and outer perimeters, roundness factor, and diameters of the tibiae and femurs at the point of fracture, were determined using the Image Measure Computer Program (Microscience, Washington, DC).

The three-point bending of the tibia and the loading of the femoral neck were performed using a material-testing machine as described by Peng et al.[23] Before testing, the machine was calibrated using a standard weight.

In the three-point bending test, a supporter with two loading points, 13 mm apart, was used on the stage of the testing machine. The lateral surface of the tibia at the tibiofibular junction was placed on the first point, and the proximal tibia on the other. The press head compressed the middle of the tibial shaft until fracture occurred, and was rounded to avoid cutting into the bone when loaded. The stress, strain, and Young's modulus were derived from the force-deformation curves obtained using equations described by Turner and Burr.[24] The roundness of the tibial cross-section was shown to be within 0.92 to 0.94 in each group, so the cross-sectional moment of inertia was determined by the equation of round specimens.

The failure load of the femoral neck was determined as described by Peng et al.[23] The femoral head-neck complex was tested until failure by loading the head with a force parallel to the shaft. The diameter of the press head was 2.5 mm. The area of the femoral neck was measured at the point of fracture, and the load to area ratio was calculated.

The torsional test of the femurs was performed using the method reported by Lepola et al.[25] The apparatus used was constructed on the basis of machines introduced by Strömberg and Dalen[26] and Paavolainen.[27] Before testing, the machine was calibrated against a given torque of 0.5 Nm. Testing was performed by twisting the bone inward. The equation for the polar moment of inertia was used to calculate the shear stress and shear modulus of elasticity.

Statistical Analysis

The statistical significance of differences between the groups was calculated using the unpaired *t* test. The statistical program StatView II for Macintosh (Abacus Concepts, Berkeley, CA) was used.

RESULTS

The diabetic status induced by streptozotocin persisted throughout the study period, as evidenced by the serum glucose and insulin concentrations. Glucose in the 20% xylitol group at the end of the experiment was slightly lower compared with the streptozotocin-diabetic rats without xylitol. The weight gain of the diabetic rats (with and without xylitol) was significantly smaller than that of the controls. Also, the weight gain was larger in both diabetic groups fed xylitol versus diabetic rats without xylitol. The mean food intake was significantly greater in diabetic rats (with and without xylitol) versus the controls. The intergroup comparison of food intake of the diabetic groups showed no significant differences (Table 1).

The tibia weight was lower in diabetic rats without xylitol supplementation compared with the controls, whereas the tibial weight of diabetic rats fed 10% and 20% xylitol did not differ significantly from that of the controls (Table 2).

The ash weight of the humerus was significantly decreased in diabetic rats without xylitol administration. The 10% xylitol in the diet of diabetic rats prevented the decrease of ash weight significantly, and 20% xylitol inhibited the decrease completely (Table 2).

The density of the tibia was also significantly lower in

Table 1. Serum Glucose and Insulin, Food Intake, and Weight Gain of the Rats

Parameter	Control (a)	Diabetic (b)	Diabetic + 10% Xylitol (c)	Diabetic + 20% Xylitol (d)
Serum glucose (mmol/L)				
Before streptozotocin	6.9 ± 1.2	7.0 ± 1.4	7.0 ± 1.5	6.9 ± 1.4
1 wk after streptozotocin	8.8 ± 1.1	35.1 ± 2.9	36.4 ± 4.3	32.7 ± 3.5
1 mo after streptozotocin	7.1 ± 1.0	46.3 ± 7.1	37.5 ± 3.5	34.0 ± 5.9
3 mo after streptozotocin	7.5 ± 1.4	45.3 ± 7.7	45.6 ± 4.0	32.8 ± 7.2
Serum insulin (ng/mL)				
Before streptozotocin	4.0 ± 2.7	5.6 ± 2.6	5.5 ± 2.7	3.6 ± 1.9
1 wk after streptozotocin	4.0 ± 3.6	1.8 ± 0.9	1.5 ± 1.0	2.4 ± 1.6
1 mo after streptozotocin	4.8 ± 2.7	1.3 ± 0.5	1.1 ± 0.3	2.3 ± 1.4
3 mo after streptozotocin	7.1 ± 2.1	1.5 ± 0.5	1.8 ± 1.1	2.5 ± 1.7
Weight gain (% of baseline)	28.5 ± 3.3	1.2 ± 15.3	12.5 ± 7.1	11.4 ± 6.1
Food intake (g/d)	19.7 ± 1.4	30.5 ± 2.9	31.9 ± 3.4	30.9 ± 2.2

NOTE. All values are expressed as the mean ± SD; n = 10 per group. Statistical differences were calculated using the unpaired *t* test. Significant differences in weight gain: a *v* b, a *v* c, and a *v* d, $P < .001$; b *v* c, $P = .009$; b *v* d, $P = .030$. Significant differences in food intake: a *v* b, a *v* c, and a *v* d, $P < .001$.

Table 2. Weight and Density of the Tibia and Ash Weight of the Humerus

Parameter	Control (a)	Diabetic (b)	Diabetic + 10% Xylitol (c)	Diabetic + 20% Xylitol (d)
Weight of tibia (g)	0.68 ± 0.09	0.60 ± 0.09	0.62 ± 0.08	0.65 ± 0.05
Density of tibia (kg/dm^3)	1.60 ± 0.02	1.53 ± 0.04	1.58 ± 0.05	1.57 ± 0.04
Ash weight of humerus (%)	62.2 ± 1.0	60.2 ± 1.3	61.8 ± 1.0	62.4 ± 0.8

NOTE. All values are expressed as the mean ± SD; n = 10 per group. Statistical differences were calculated using the unpaired *t* test. Significant difference in weight of tibia: a *v* b, $P = .03$. Significant difference in density of tibia: a *v* b, $P = .001$; b *v* c, $P = .007$; b *v* d, $P = .04$. Significant difference in ash weight of humerus: a *v* b, $P = .002$; b *v* c, $P = .005$; b *v* d, $P < .001$.

diabetic rats without xylitol versus the controls. However, 10% and 20% dietary xylitol supplementation prevented the diabetes-induced decrease in tibial density (Table 2).

Histomorphometric data for the secondary spongiosa of the proximal tibia revealed that the trabecular bone volume was significantly decreased by about 47% in diabetic rats without xylitol compared with the controls. In diabetic rats with 10% and 20% dietary xylitol supplementation, the loss of trabecular bone volume was only 13% and 20%, respectively (Fig 1).

The results for bone biomechanical properties assessed from the three-point bending test of the tibia showed that dietary xylitol supplementation prevented the decreased resistance to mechanical stress caused by experimental diabetes, while no statistically significant differences were found between any groups in the values for strain or Young's modulus (Fig 2).

The torsion test of the femur yielded a result parallel to the tibial bending. The shear stress of the femur was significantly lower in diabetic rats without xylitol compared with the controls, while both xylitol supplementations caused a significant preventive effect (Fig 2). Values for the modulus of elasticity did not differ significantly between any of the groups.

In the loading test of the femoral neck, a significant preventive effect against the type I diabetes–induced decreased resistance to mechanical stress was obtained by both 10% and 20% xylitol administration (Fig 2).

DISCUSSION

The dosage of streptozotocin used in this study (single injection of 50 mg/kg body weight) maintained the diabetic status throughout the study period. This is in correspondence with previous studies, where injection of streptozotocin 50 to 70 mg/kg induced a stable chronic diabetes in rats.[28] This type of animal model is used to induce an insulin-dependent type I diabetes.[29] Xylitol administration did not significantly change serum glucose and insulin levels, indicating that the observed xylitol-induced effects in diabetic rats were not caused by altered diabetic status.

Food intake was increased in diabetic rats. This is also in agreement with prior studies,[30,31] and it most likely occurs to compensate for the decreased nutritional availability and the reduced body weight.[30] Food intake was similar in diabetic rats fed xylitol versus the basal diet.

As in previous studies,[28,30] the weight gain of the diabetic rats was significantly decreased as compared with that of the control animals. This is caused by a decreased availability of glucose and amino acids to cells, creating a shortage of substrates for cellular biosynthesis and affecting related cellular metabolism.[30] Dietary supplementation with 10% and 20% xylitol in the diabetic rats led to increased body weight as compared with the diabetic rats without xylitol. This is most likely a consequence of the ability of diabetic animals to use xylitol as a substrate in energy metabolism. Bässler and Prellwitz[32] have shown that the turnover rate and utilization of xylitol are unimpaired in diabetes.

The long bones are lighter and the density and mineral content are markedly decreased in streptozotocin-diabetic rats compared with healthy controls. In parallel to our previous study with healthy rats,[20] dietary xylitol supplementation significantly increased the tibial density and humeral ash weight of

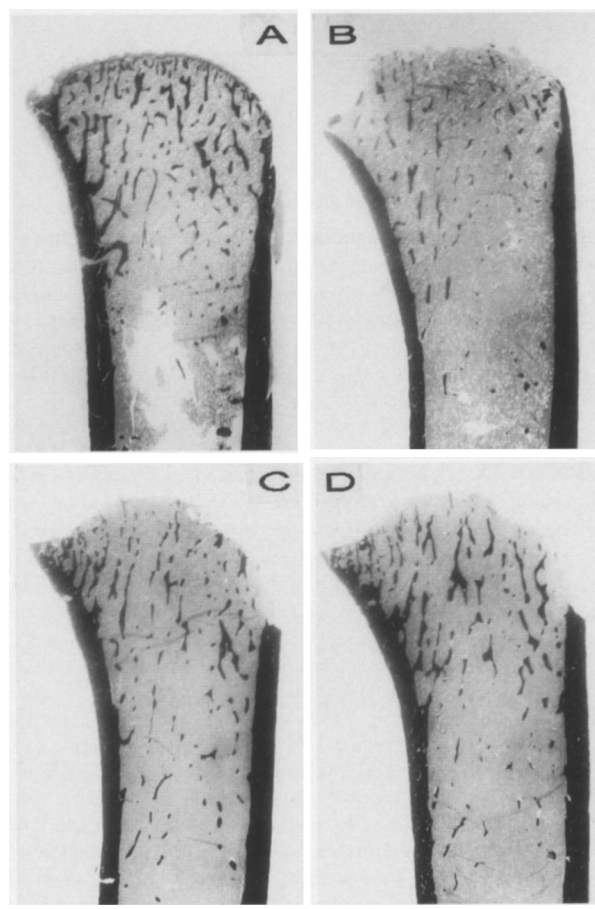

Fig 1. Proximal tibia of control (A), steptozotocin-diabetic (B), 10% xylitol–fed streptozotocin-diabetic (C), and 20% xylitol–fed streptozotocin-diabetic (D) rats (Von Kossa stain, original magnification ×10). Trabecular bone volume (mean ± SD) was 6.75% ± 2.41%, 12.63% ± 3.63%, 10.97% ± 2.89% and 10.14% ± 2.44%, respectively (n = 10 per group). Statistical differences were calculated using the unpaired *t* test: A *v.* B, $P < .001$; B *v.* C, $P = .004$; B *v.* D, $P = .009$.

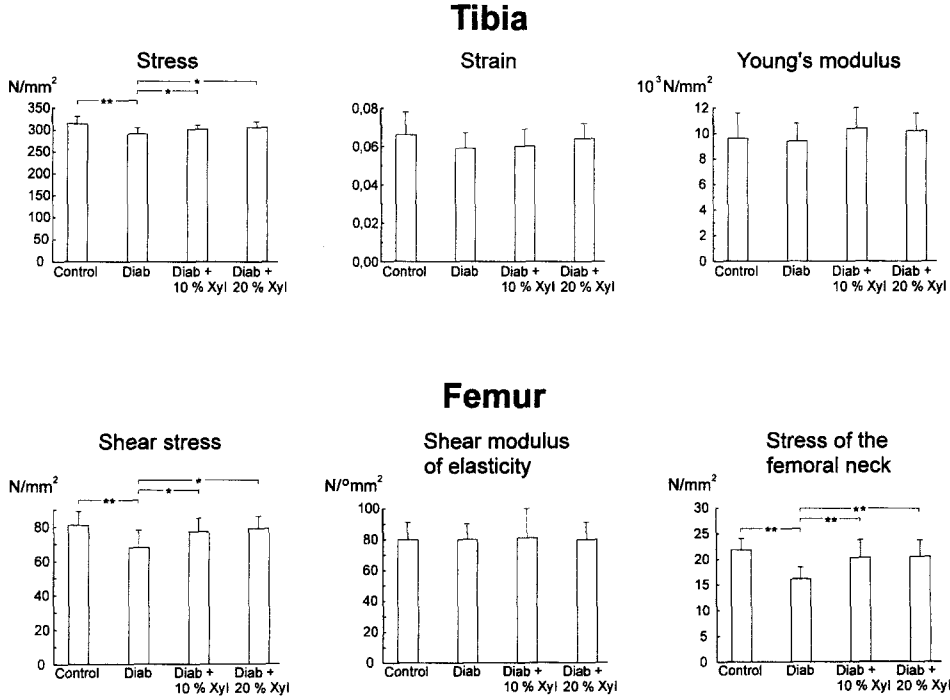

Fig 2. Biomechanical results of the 3-point bending test of the tibia, torsion test of the femur, and loading test of the femoral neck (n = 10 per group). Statistical differences were calculated using the unpaired *t* test: *$P <$ .05, **$P <$ .01.

diabetic rats, indicating a preventive effect against the loss of bone minerals. The trabecular bone volume of streptozotocin-diabetic animals was only about half that of the controls. Differences of the same magnitude were also seen in the studies by Amir et al[33] and Verhaeghe et al.[11] However, 10% and 20% xylitol administration clearly protected against the loss of bone trabeculae. This indicates that dietary xylitol administration protects against streptozotocin-induced osteoporotic changes in both the organic and inorganic fractions of the bone. This suggests that the mechanism of action of dietary xylitol is most likely multifactorial.

The preventive effect against the loss of bone minerals is most likely partly a consequence of an increased intestinal absorption of calcium caused by xylitol, thus providing more calcium to the bone. Active duodenal calcium absorption is abolished in type I diabetes.[11] This has been linked with decreased 1,25-$(OH)_2D_3$ concentrations, suggesting vitamin D resistance at the duodenal level.[34] Dietary xylitol, on the other hand, is known to increase passive calcium absorption independently of vitamin D action,[19] and xylitol is thus probably able to fulfill its action despite the apparent vitamin D resistance. This supports the idea that the favorable effects of xylitol on the bones of healthy rats also act during the stage of low bone turnover in type I diabetes. Xylitol has also displayed an anticatabolic capacity[17] independently of insulin.[35] In type I diabetes, bone collagen production is diminished.[36] A reduced redox state, on the other hand, is linked with increased collagen synthesis and decreased collagenase activity.[37] The first steps in the metabolism of xylitol produce a reduced redox state (increased NADPH/NADP and NADH/NAD ratios), thus enabling a positive metabolic balance of collagen. Collagen degradation has also been shown to be increased in type I diabetes.[38] On the basis of our previous study,[21] this might be prevented by dietary xylitol because of its effect to diminish bone resorption.

The bending and torsional strength of long bones is used as an indicator of cortical bone strength.[26,27,39,40] Type I diabetes induced a significant reduction in the strength properties of bone without affecting the elastic-plastic properties. This is in agreement with the study by Verhaeghe et al,[41] wherein the torsional strength of the femur was decreased while stiffness remained normal. The 10% and 20% xylitol supplementation in the present study increased tibial stress and femoral shear stress significantly compared with values in the streptozotocin-diabetic rats without xylitol. This is in accordance with the observed xylitol-induced prevention against the loss of bone minerals. None of the measured biomechanical variables were weakened after dietary xylitol administration, negating the possibility that dietary xylitol, despite the increased bone mineral content, could cause such qualitative changes in bone that might compromise its mechanical properties.

Loading of the femoral neck gives an indication of trabecular bone strength.[12,23] The femoral neck is one of the most sensitive regions of trabecular bone remodeling.[42] Thus, as in the study by Hou et al,[13] the diminished trabecular bone volume in the present study led to weakened material properties of the femoral neck of streptozotocin-diabetic rats. However, the 10% and 20% xylitol supplementation increased the stress resistance of the femoral neck significantly as compared with that of the diabetic rats without xylitol. This is most likely greatly due to the increased trabecular bone volume of these rats.

The 10% and 20% xylitol concentrations were chosen because they had the most favorable effects on bone metabolism in our previous dose-response study with healthy rats.[43] These concentrations correspond to a daily intake of approximately 2 and 4 g xylitol, respectively, and are about 7% and 14% of the

total daily caloric intake of the rats. This might suggest a daily intake of about 40 and 80 g xylitol in middle-size adults, amounts that have been proven to be well tolerated.[44] However, it should be noted that no direct conclusions regarding human metabolism can be drawn from the present study.

In conclusion, dietary xylitol supplementation protects against the weakening of bone biomechanical properties in streptozotocin-diabetic rats. This is related to the preserved bone mineral content and preserved trabecular bone volume.

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