Improved Bone Biomechanical Properties in Xylitol-Fed Aged Rats

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Our previous studies have shown that dietary xylitol protects against weakening of bone biomechanical properties in experimental postmenopausal osteoporosis. To study whether xylitol preserves bone biomechanics also during aging, a long-term experimental study was performed with rats. Twenty-four male Sprague-Dawley rats were divided into 2 groups. The rats in the control group (NON-XYL group) were fed a basal rat and mouse no. 1 maintenance (RM1) diet, while the rats in the experimental group (XYL group) were continuously fed the same diet supplemented with 10% xylitol (wt/wt). The rats were killed after 20 months. Their femurs were prepared for biomechanical analyses and scanning analyses with peripheral quantitative computed tomography (pQCT). In 3-point bending of the femoral diaphysis, maximum load, maximum elastic load, stiffness, energy absorption, elastic energy absorption, ultimate stress, and yield stress were significantly greater in the XYL group than in the NON-XYL group. This indicates a xylitol-induced improvement of both structural and material strength properties of cortical bone. Accordingly, the maximum load of femoral neck was significantly greater in the XYL group. In the pQCT analysis of femoral diaphysis, cortical bone area, cortical thickness (CtTh) periosteal circumference, and cross-sectional moment of inertia were greater in the XYL group. The endosteal circumference was smaller in the XYL group. In the pQCT analysis of the femoral neck cortical area of the midneck was significantly greater in the XYL group. This data indicates that xylitol exerted beneficial effects on the cross-sectional architecture of the bones. In conclusion, continuous moderate dietary xylitol supplementation leads to improved bone biomechanical properties in aged rats concerning both bone structural and material strength properties.

XYLITOL IS A 5-carbon polyalcohol, which is widely distributed in nature. Most fruits, berries, and plants contain xylitol.[1] Considerable amounts of xylitol also occur as an intermediate of the mammalian carbohydrate metabolism. In the human body, 5 to 15 g of xylitol is formed daily.[2] The absorption rate of xylitol is quite slow, which means that high oral doses may induce osmotic diarrhea. Unadapted persons can consume orally 30 to 60 g of xylitol per day without side effects. After adaptation, up to 400 g of xylitol daily have been taken without side effects.[3]

Our previous studies have shown that moderate dietary xylitol supplementation protects against weakening of bone biomechanical properties in experimental postmenopausal osteoporosis.[4] This is related to xylitol-induced protection against loss of bone mineral[5] and against decrease of trabecular bone volume.[6]

Osteoporotic changes are also common during aging, although they appear much slower than in postmenopausal osteoporosis. Aging-related osteoporosis is characterized by impaired bone formation[7] and decreased calcium absorption.[8] The defect in calcium absorption is probably caused by an age-related intestinal resistance to 1.25-dihydroxyvitamin D_3[9] and by a decrease in intestinal vitamin D receptor concentration with aging.[10] Dietary xylitol, on the other hand, is known to increase calcium absorption independently of vitamin D action.[11] Xylitol might also have beneficial effects on bone metabolism despite the apparent vitamin D resistance during aging.

We hypothesize that dietary xylitol can protect against weakening of bone biomechanical properties during aging. To study this hypothesis, a 20-month study was performed with rats.

MATERIAL AND METHODS

Animals

Twenty-four newborn male Sprague-Dawley rats (Laboratory Animal Center, University of Oulu, Finland) were used in the study. The animals were fed a basal powder diet, rat and mouse no. 1 maintenance diet (RM1 diet) (Special Diet Services, Witham, Essex, Great Britain). A total of 1 kg of this diet contains 885 g of cereal products (wheat, barley, and wheatfeed), 60 g of vegetable proteins, 25 g of animal proteins (whey powder), 5 g of soya oil, 7.1 g of calcium, 2.9 g of phosphorus, and 15 μg of cholecalciferol. A list of the other minor components of the diet is shown in the manufacturer's brochure. The rats had free access to tap water. They were housed in a temperature and light-controlled room (21°C to 23°C, 12-hour light-dark cycle).

After weaning, the rats were divided into 2 groups of 12. The rats in the control group were fed the basal RM1 diet, while the rats in the experimental group were fed the same diet supplemented with xylitol (Cultor, Espoo, Finland). The xylitol concentration in the diet was 10% (wt/wt). After 20 months, the rats were killed with CO_2 followed by decapitation. The femurs of the rats were prepared for the analyses. The study protocol was approved by the Ethical Committee on Animal Experiments of the University of Oulu. The experimental procedures were in compliance with the Guiding Principles in the Care and Use of Animals, approved by the Council of the American Physiological Society in 1991.

Peripheral Quantitative Computed Tomography Measurements

The left femur was scanned with a peripheral quantitative computed tomography (pQCT) system, the Stratec XCT 960A (Norland Stratec Medizintechnik GmbH, Birkenfeld, Germany) using a voxel size of $0.148 \times 0.148 \times 1.25$ mm^3. An attenuation threshold of 0.93 cm^{-1} was used to define cortical bone in the analyses. The femoral diaphysis was scanned at midshaft, and the femoral neck was scanned with the neck in an axial direction, adjusting the scan line to the midneck by using the

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scout view, as previously described.[12,13] The obtained scans were used for determinations of geometric parameters and for calculations of bone strength indices.

From the geometric parameters of the femoral diaphysis, cortical thickness (CtTh), as well as periosteal and endosteal circumferences, were measured using a circular approximation for the midfemur cross-sectional shape. The axial cross-sectional moment of inertia (CSMI) was determined in the same direction as the bones were mechanically tested. Furthermore, cross-sectional cortical areas (CtCSA) of the femoral midshaft and of the femoral midneck were measured.

The bone strength index (BSI) was calculated as CSMI × CtBMD, as described by Ferretti et al.[14] The strength strain index (SSI) is a parameter given by the pQCT software and is defined as CSMI weighted by density distribution. The precision and accuracy of the pQCT measurements have been presented previously.[12,15]

Mechanical Testing Procedures

Left femurs were stored at −20°C until used. Before testing, the bones were thawed at room temperature and kept moist until the test was completed. A 3-point bending test of the femoral midshaft and a loading test of the femoral neck were performed using a custom-made materials testing machine, as previously described.[16,17] Prior to testing, the machine was calibrated using a standard weight.

The point of fracture in the 3-point bending test was standardized by always placing the bone similarly in the testing machine. A supporter with 2 loading points, 13 mm apart, was used on the stage of the testing machine. A press head compressed the middle of the femoral shaft at a constant speed of 0.155 mm/s until fracture occurred. The press head was rounded to avoid cutting into the bone during loading. The bone maximum load, maximum elastic load as the load at the yielding point, stiffness as the slope of the linear part of the curve, energy absorption as the area under the curve, and elastic energy absorption as the area under the curve in the elastic region were determined from the load-deformation curves obtained. Ultimate and yield stress, ultimate and yield strain, and Young's modulus were derived from load-deformation curves by using equations described by Turner and Burr.[18] The axial CSMI of the femoral midshaft in the same direction as the bones were mechanically tested was determined using the pQCT system.

The femoral neck was tested as previously described.[16] Briefly, the proximal half of the femur was inserted tightly into a suitable hole on a polymethyl metacrylate plate with a set of holes to fit the size and shape of the bone. The head of the femur was loaded with a force parallel to the shaft of the femur using a concave loading cup at a constant speed of 0.155 mm/s until failure. The diameter of the press head was 2.5 mm. The maximum load was determined from the load-deformation curves.

Statistical Analysis

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

RESULTS

The final body weight of the rats in the aged group that was fed the 10% xylitol-supplemented diet (XYL group) was 584 ± 51 g and in the aged group that was fed the same diet without xylitol supplementation (NON-XYL group) 581 ± 35 g. The difference between the groups was not statistically significant. No adverse effects were observed in the rats during the experimental period of 20 months.

In the 3-point bending test of the femoral diaphysis, all the basic mechanical properties including maximum load, maximum elastic load, stiffness, energy absorption, and elastic energy absorption were greater in the XYL group compared with the NON-XYL group (Table 1). From the derived instrinsic mechanical properties, ultimate stress and yield stress were greater in the XYL group than in the NON-XYL group (Table 1). The values of the other derived parameters, ultimate strain, yield strain, and Young's modulus did not differ significantly between the groups (Table 1). The maximum load of the femoral neck was significantly greater in the XYL group compared with the NON-XYL group (Table 1).

In the pQCT analyses of the femoral diaphysis, CtCSA, CtTh, periosteal circumference, and CSMI were significantly greater in the XYL group than in the NON-XYL group, while the endosteal circumference was smaller in the XYL group (Table 2). Furthermore, the BSI and the SSI values of the femoral diaphysis were greater in the XYL group than in the NON-XYL group (Table 2). The cross-sectional cortical area of

Table 1. Results of the Biomechanical Testing Procedures

	Aged Rats Without Dietary Xylitol Supplementation (n = 12)	Aged Rats With 10% Dietary Xylitol Supplementation (n = 12)	Significance of Differences Between Groups
Femoral diaphysis			
Maximum load (N)	348 ± 31	454 ± 44	$P < .001$
Maximum elastic load (N)	172 ± 23	222 ± 28	$P < .001$
Stiffness (N/mm)	801 ± 75	989 ± 118	$P < .001$
Energy absorption ($Nm \times 10^{-3}$)	128 ± 18	159 ± 20	$P < .001$
Elastic energy absorption ($Nm \times 10^{-3}$)	23 ± 4	32 ± 7	$P < .001$
Ultimate stress (N/mm^2)	140.7 ± 13.7	153.3 ± 10.5	$P = .02$
Yield stress (N/mm^2)	66.4 ± 9.6	78.4 ± 15.8	$P = .03$
Ultimate strain	0.121 ± 0.015	0.131 ± 0.028	NS
Yield strain	0.045 ± 0.005	0.050 ± 0.007	NS
Young's modulus (10^3 N/mm^2)	1,481 ± 216	1,591 ± 226	NS
Femoral neck			
Maximum load (N)	129 ± 19	169 ± 26	$P < .001$

NOTE. All values are expressed as mean ± SD. Statistical differences were calculated by the unpaired *t* test.
Abbreviation: NS, nonsignificant.

Table 2. Results of the pQCT Analyses

	Aged Rats Without Dietary Xylitol Supplementation (n = 12)	Aged Rats With 10% Dietary Xylitol Supplementation (n = 12)	Significance of Differences Between Groups
Femoral diaphysis			
CtCSA (mm^2)	12.07 ± 0.87	13.73 ± 1.26	$P < .01$
Cortical thickness (mm)	1.00 ± 0.08	1.14 ± 0.08	$P < .01$
Periosteal circumference (mm)	15.28 ± 0.36	15.79 ± 0.66	$P = .03$
Endosteal circumference (mm)	9.01 ± 0.65	8.49 ± 0.47	$P = .02$
CSMI (mm^4)	19.85 ± 2.28	23.09 ± 4.37	$P = .03$
BSI (mg/mm)	36.17 ± 3.45	42.69 ± 8.12	$P = .02$
SSI (mm^3)	11.1 ± 1.1	12.6 ± 1.9	$P = .03$
Femoral neck			
CtCSA (mm^2)	6.27 ± 1.21	7.32 ± 0.90	$P = .03$

NOTE. All values are expressed as mean ± SD. Statistical differences were calculated by the unpaired *t* test.

Abbreviations: CtCSA, cross-sectional cortical area; CSMI, axial cross-sectional moment of inertia; BSI, bone strength index; SSI, strength strain index.

the femoral midneck was also greater in the XYL group than in the NON-XYL group (Table 2).

DISCUSSION

The final average weights of the rats did not differ between the groups, indicating no major differences in the growth of the animals. This also confirms that the body weight does not act as a confounding factor in the biomechanical tests of the bones.

The results of the 3-point bending test of femoral diaphysis indicate a significant xylitol-induced beneficial effect on bone biomechanics. Bone maximum load, maximum elastic load, stiffness, energy absorption, and energy absorption in the elastic region, which represent mechanical properties of the bone as a structure, were all greater in the XYL group compared with the NON-XYL group. The mineral phase of the bone contributes the major portion of bone strength.[19] The greater load values in the XYL group, representing bone ultimate and elastic strength, might partly be explained by the mineral access in the bones of these animals. Our earlier studies have shown that dietary xylitol increases calcium and phosphorus content of the bones in healthy rats,[20] as well as protects against ovariectomy-induced decreases in calcium and phosphorus content of bone, bone density, and bone ash weight in a rat model of experimental osteoporosis.[5] Bone mineral content is also a significant determinant of bone stiffness,[19] thus partly explaining the greater stiffness values in the xylitol-fed rats. While the energy absorption values are greatly dependent on bone strength and stiffness, it was not surprising that they also were significantly greater in the XYL group.

Bone cross-sectional architecture is an important determinant of bending strength and stiffness.[12,21] The axial CSMI, a geometric indicator of the architectural efficiency of femoral cross-sectional design,[22,23] was significantly greater in the XYL group than in the NON-XYL group. Cross-sectional cortical area and CtTh of the femoral diaphysis were also greater in the XYL group, explaining for their own part the greater bone strength and stiffness of these animals. Interestingly, the periosteal circumference of the femoral diaphysis was greater and the endosteal circumference smaller in the XYL group. This indicates that dietary xylitol supplementation in these rats resulted in both reduced bone resorption in the inner region and increased bone formation in the outer region of the femoral diaphysis. Xylitol-induced diminished bone resorption has been detected in our previous studies with young adult healthy[24] and ovariectomized rats.[6]

During aging, the cortical bone of the diaphysis is subjected to increased endocortical resorption and increased periosteal bone formation, the net result being cortical thinning.[25] In contrast, the age-related cortical thinning of the femoral neck, due to its unique anatomy, is not associated with the periosteal response required for appositional bone growth to occur.[25,26] Hence, the cortex thins without an increase in the outer cross-sectional diameter. However, dietary xylitol in the present study was able to induce about 17% greater cortical bone area in the femoral neck of the aged animals. The maximum strength of the femoral neck was about 31% greater in the XYL group than in the NON-XYL group. Besides the improved architectural properties, this can be explained by increased bone mineral content of the neck in the xylitol-fed rats.

The mechanical properties of bone result from a combination of bone instrinsic material properties and geometric architectural properties.[27,28] From the derived instrinsic material properties, ultimate stress and yield stress, representing tissue strength, were significantly greater in the XYL group than in the NON-XYL group. Previously, stress values have been shown to decrease during aging in male rats.[29] Our findings indicate that this age-dependent decrease in the material strength of the bone was at least partly prevented by dietary xylitol. The values of the other derived parameters, strain, and Young's modulus, describing the elastic and plastic behavior of the bone,[19] did not differ significantly between the groups. This indicates that xylitol causes an increase in bone strength without affecting bone elasticity. This is in accordance with our earlier findings with young adult rats.[30]

The pQCT system has proven to be an effective tool in evaluating geometric properties of bone in experimental studies.[12,15,27] It has also been used to estimate parameters related to the biomechanical behavior of bone. The BSI and SSI indices, which have been shown to closely predict the actually measured biomechanical data,[12,14,27] also confirmed the results of the biomechanical tests in the present study.

The precise mechanism of action of xylitol is not known.

Aging is characterized by decreased calcium absorption, which is related to decreased intestinal responsiveness to vitamin D.[9,10] Dietary xylitol, on the other hand, is known to increase calcium absorption independently of vitamin D action.[11] Xylitol may thus be able to maintain sufficient calcium absorption despite the apparent vitamin D resistance during aging.

During the metabolism of xylitol, a marked reduction of the redox state has been confirmed.[31] The elevations of the NADH/NAD and NADPH/NADP ratios are due to rapid production of the reduced forms of these coenzymes in the polyol dehydrogenase and in the xylitol dehydrogenase reactions. The reduced redox state has been associated with active calcification,[32] increased collagen synthesis, and decreased collagenase activity.[33] The anticatabolic effect of xylitol has been detected in our previous studies, in which dietary xylitol diminished bone resorption in young adult rats,[24] and protected against increased bone resorption in ovariectomized rats.[6] The anabolic effect of xylitol has been detected in many studies in which intravenous or parenteral administration of xylitol has been used.[34,35] An anabolic effect was also suggested in our previous study, in which the xylitol-induced increase in cortical bone mineral content was greater in the newly synthetized bone than in the formerly formed bone compared with the controls.[36] However, whether the beneficial effects of xylitol are induced by affecting bone growth or bone loss cannot be determined on the grounds of the present study.

The absence of adverse effects indicates the safety of moderate long-term dietary xylitol use. The xylitol concentration of 10% (wt/wt) corresponds to the daily intake of approximately 2 g of xylitol. This is about 7% of the total daily caloric intake of the rats. This might suggest about a 40 g daily intake of xylitol in humans, an amount that has been proven to be well tolerated even in nonadapted persons.[37] However, it should be pointed out that no direct decisions regarding human metabolism can be drawn before excluding possible species-specific effects.

In conclusion, continuous moderate dietary xylitol supplementation leads to improved bone biomechanical properties in aged, male rats concerning both bone structural and material strength properties.

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