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Dietary Silicon Affects Bone Turnover Differently in Ovariectomized and Sham-Operated Growing Rats

Forrest H. Nielsen* and Rhonda Poellot

United States Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, North Dakota

An experiment was performed to test the hypothesis that low dietary silicon affects blood, bone, and urine indices associated with bone formation and breakdown, bone strength and physical characteristics, and the circulating amount of an extracellular matrix protein (osteopontin) involved in bone cell adhesion and activation. A second objective was to ascertain whether ovariectomy (estrogen deficiency) alters the effects of low dietary silicon on bone formation. Female rats weighing about 56 g were assigned to groups of 10 in a factorially arranged experiment. The variables were supplemental dietary silicon at 0 or 35 mg/kg and ovariectomy (estrogen-deficient) or sham operation at the start of the experiment. The basal silicon-low diet contained about 2 mg Si/kg. Low dietary silicon compared with adequate silicon decreased plasma osteopontin concentration, increased plasma sialic acid concentration, and increased urinary helical peptide excretion. Low dietary silicon also affected the response to estrogen deficiency. Ovariectomy increased plasma alkaline phosphatase in the silicon-supplemented, but not in the silicon-low rats. In contrast, ovariectomy decreased liver ornithine aminotransferase in silicon-low but not in silicon-supplemented rats. Ovariectomy increased the urinary excretion of deoxypyridinoline and decreased the femur concentration of sialic acid more markedly in silicon-supplemented than silicon-low rats. Silicon and an interaction between silicon and ovariectomy only mildly changed bone strength and physical measurements and did not affect femur calcium concentration. The findings suggest that silicon has a biochemical function that affects bone growth processes before bone crystal formation by affecting bone collagen turnover and sialic acid-containing extracellular matrix proteins such as osteopontin. J. Trace Elem. Exp. Med. 17:137–149, 2004. Published 2004 Wiley-Liss, Inc.†

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*Correspondence to: Forrest H. Nielsen, USDA, ARS, Grand Forks Human Nutrition Research Center, PO Box 9034, Grand Forks, ND 58202–9034. E-mail: fnielsen@gfhnrc.ars.usda.gov

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INTRODUCTION

More than 25 years ago, it was reported that the dietary restriction of silicon results in undesirable changes in bones and articular cartilage of experimental animals. Carlisle [1–3] described silicon-deficient chicks as having structural abnormalities of the skull associated with depressed collagen content, small, poorly formed joints, and long bones with defective endochondral growth and depressed contents of articular cartilage, water, hexosamine, and collagen. The effects of silicon deficiency on bone organic matrix components and the finding that silicon is present in high concentrations in metabolically active osteogenic cells resulted in the conclusion by Carlisle [4] that silicon has an essential function involved in the formation of the organic matrix rather than with the mineralization of bone. Schwarz [5] also found that silicon deprivation resulted in disturbances in bone formation. Because he found high concentrations of silicon in glycosaminoglycans and polyuronides, Schwarz [6] proposed that silicon is present as silanolate bridges that have role in the structural organization of glycosaminoglycans and polyuronides. The proposed organizational function of silicon has never been rigorously proven. Since the passing of Carlisle and Schwarz, identification of the possible role of silicon in bone formation apparently has received very little research attention.

In 1991, it was suggested that silicon might have a role that affects the interaction between one of the many extracellular matrix proteins being discovered and its cell-surface receptor [7]. Because silicon deficiency has effects beyond that on bone formation, extracellular matrix proteins such as glycoproteins containing sialic acid with physiological roles other than just in bone formation became of interest. Osteopontin, variously known as 44-kDa bone phosphoprotein, sialoprotein I, secreted phosphoprotein I, uropontin, and early T-lymphocyte activation-1 or Eta-1, received attention in the present study because it affects similar processes in vivo as silicon. Osteopontin is a phosphorylated acidic glycoprotein that apparently is important in bone remodeling, wound healing, and certain types of cell-mediated immune responses [8,9]. Recent reports confirm that silicon is involved in bone formation [10], silicon deficiency impairs splenic lymphocyte proliferation in response to an immune challenge [11], and silicon may be important for wound healing [12]. Thus, the following experiment was designed to test the hypothesis that silicon deficiency affects the production or function of osteopontin such that circulating amounts would be modified and this would be associated with changes in indicators of bone turnover, strength, and physical characteristics. Because of reports that osteopontin synthesis is increased in bone cells of ovariectomized animals [13], the effect of ovariectomy on the possible relationship between silicon and circulating osteopontin also was examined. Furthermore, because both ovariectomy [14] and silicon deprivation [4] of growing rats alters organic matrix characteristics, and both affect ornithine aminotransferase activity [12,15], which is involved in

collagen formation, it was hypothesized that ovariectomy would alter changes in bone turnover indices induced by silicon deprivation.

MATERIALS AND METHODS

Study Design

Female Sprague–Dawley rats that had undergone ovariectomy or sham operation and weighing about 56 g were obtained from Charles River/SASCO (Wilmington, MA) and immediately assigned to four weight-matched groups of 10 in a 2×2 factorially arranged experiment. The variables were supplemental dietary silicon as sodium metasilicate at 0 and 35 mg/kg and ovariectomy or sham operation. Although 35 mg/kg of dietary silicon is about 20 times less than that found in a commercially prepared rodent diet [16], it has been found adequate for rats fed purified diets [10,11]. The rats were housed individually in plastic cages in laminar air-flow racks. The racks were located in a room maintained at 23°C and 50% relative humidity with a 12-h light and dark cycle. The rats had free access to food and deionized drinking water (Super Q; Millipore Corporation, Bedford, MA). Absorbent paper under the false-bottom cages was changed daily. Animals were weighed and provided clean cages weekly.

After 6 weeks on their respective treatments, each rat was placed in a metabolic cage with free access to drinking water, but not to diet, for a 16-h collection of urine in a plastic tube kept on ice. After 8 weeks on their dietary regimens, the rats were anesthetized with ether for the collection of blood in a heparin-coated syringe. After euthanasia by decapitation, the liver and both femurs were excised from each rat. The liver was immediately frozen in liquid nitrogen. Some tissue was left on the femur used for measuring bone physical characteristics; the other femur used for mineral analysis was completely cleared of tissue. Collected urine, plasmas, livers, and femurs were stored at −70°C for later analyses.

The composition of the basal diet is shown in Table I. The silicon supplement providing 35 mg Si/kg replaced ground corn in the diet and was added as a mix of 0.3543 g $Na_2SiO_3 \cdot 9H_2O$ (Analyzed Reagent Grade, J.T. Baker, Phillipsburg, NJ) and 4.6457 g of acid-washed ground corn. The basal diet contained about 2 mg Si/kg as determined by inductively coupled plasma atomic emission spectroscopy after ashing in platinum crucibles by a lithium-boron fusion technique [17]. A standard reference material (National Institute of Standards and Technology, Gaithersburg, MD), no. 1515 apple leaves, was used for quality control purposes in the diet analysis. The diets were not pelleted and were stored at −16°C in tightly capped plastic containers. The study was approved by the Animal Care Committee of the Grand Forks Human Nutrition Research Center, and the lawfully acquired animals were maintained in accordance with the NIH guidelines for the care and use of laboratory animals.

Biochemical Analyses of Plasma, Urine, and Liver

Commercially available assay kits were used to determine urine creatinine (Kit no. 555, Sigma, St. Louis, MO), urine pyridinoline (PYD EIA Kit no. 8010, Quidel,

TABLE I. Composition of Basal Diet[1]

Ingredient	g/kg
Ground corn, acid washed	400.00
Casein, vitamin-free, water washed	120.00
Sucrose	367.00
Corn oil	50.00
DL-α-Tocopherol	0.20
Choline chloride	0.75
L-Methionine	10.00
L-Lysine	5.00
KH_2PO_4	10.00
$CaCO_3$	12.50
Vitamin mix[2]	4.55
Mineral mix[3]	20.00
Total	1,000.00

[1]Analyzed concentration of silicon was about 2 mg/kg.
[2]Composition of the vitamin mix (in mg): all-*trans* vitamin A palmitate (500,000 IU/g), 16.0; vitamin D (400,000 IU/g), 3.8; menadione, 1.0; biotin, 1.0; folic acid, 2.0; inositol, 50.0; niacin, 30.0; pantothenic acid, 48.0; riboflavin, 27.0; thiamine-HCl, 10.0; pyridoxine-HCl, 15.0; cyanocobalamin, 0.05; paraminobenzoic acid, 5.0; and glucose, 4,341.15.
[3]Composition of the mineral mix (in g): KCl, 1.5; NaCl, 2.1; $Mg(C_2H_3O_2)_2 \cdot 4H_2O$, 4.4; $Mn(C_2H_3O_2)_2 \cdot 4H_2O$, 0.225. $Zn(C_2H_3O_2)_2 \cdot 4H_2O$, 0.15; $FeSO_4 \cdot 7H_2O$, 0.175; $CuSO_4 \cdot 5H_2O$, 0.02; NaF, 0.002; KI, 0.0002; $Na_2SeO_3 \cdot 5H_2O$, 0.0005; $(NH_4)_2MoO_4$, 0.0005; $Cr(C_2H_3O_2)_3 \cdot 2H_2O$, 0.002; H_3BO_3, 0.006; NH_4VO_3, 0.0003; $NiCl_2 \cdot 6H_2O$, 0.002; $Na_2HAsO_4 \cdot 7H_2O$, 0.005; and acid-washed corn, 11.4115.

Mountain View, CA), urine deoxypyridinoline (DPD EIA kit no. 8007, Quidel, Mountain View, CA), urine helical peptide (EIA kit no. 8022, Quidel, Mountain View, CA), plasma alkaline phosphatase (kit no. 104-105, Sigma, St. Louis, MO), plasma and femur sialic acid (kit no. 784192, Roche, Indianapolis, IN), plasma osteopontin (kit #900089, Assay Designs, Ann Arbor, MI), plasma osteocalcin (EIA kit #BT-490, Biomedical Technologies, Stoughton, MA), plasma tartrate-resistant acid phosphatase isoform 5b (TRAP) (Code TR 102, Suomen Bioanalytiikka Oy, Kiviharjuntie, Finland), and bone extract and liver protein (BCA Protein kit no. 23225, Pierce, Rockford, IL). It should be noted that values obtained by using PYD EIA kit no. 8010 included both pyridinoline and deoxypyridinoline because the assay cross-reacts with both. Liver ornithine amino transaminase activity was determined by the method of Herzfeld and Knox [15].

Biochemical Analyses of Bone

For calcium analysis, femurs were cleaned to the periosteal surface with cheesecloth, lyophilized, then subjected to a wet-ash, low-temperature digestion in Teflon tubes [18]. Calcium was determined by inductively coupled argon plasma atomic emission spectroscopy. A Standard reference material (National Institute of Standards and Technology, Gaithersburg, MD), no. 1515 apple leaves, was used for quality control.

For sialic acid analysis, femurs were cleaned to the periosteal surface with cheesecloth and demarrowed by breaking in half and rinsing the interior with

deionized water introduced via a syringe fitted with a 22-gauge needle. After drying for approximately 45 min, the femurs were weighed, placed in a 15-mL conical screw-cap tube containing 5 mL of 0.1 N HCl, and rocked for 36 h at 4°C. Then the HCl was removed, and 5 mL of 4 M guanidine HCl in 50 mM Tris, pH 7.4, was added for extracting the sialic acid by rocking at 4°C for 72 h. After centrifuging at 2500 rpm for 20 min at 4°C, the supernatant was removed and frozen until analysis. For analysis, 1 mL of the supernatant was placed in a microcentrifuge tube and centrifuged at 4500*g* for 5 min at 22°C, then 0.480 mL of the clarified extract was placed into a 50,000 MWCO Amicon Micron centrifuge filter device (Millipore Corporation) and centrifuged for 12 min at 15,000 g at 22°C. After centrifugation, the filter with the retentate was inverted into a clean microcentrifuge tube and centrifuged at 1000*g* for 3 min at 22°C to obtain the final retentate. Twenty microliters of deionized water was added to the retentate before being analyzed for sialic acid content by using a commercially available kit (kit no. 784192, Roche, Indianapolis, IN).

Bone Strength and Physical Characteristics Measurements

Bone strength variables were determined on right femurs after all flesh was removed and length and outside lateral width and ventral depth measurements at mid-shaft as shown in Figure 1 were determined by using an electronic digital caliper with a precision of 0.01 mm. A custom-designed and -built apparatus was used to perform a three-point bending test the same as that performed by commercially available machines to determine bone-breaking variables. The fulcra length and point of force application was determined by femur length. The point of force (crosshead) was centered over the greatest possible distance between the two fulcra below the femur that was placed in a stable position with the ventral side up and the knee joint to the left while facing the instrument. The rate of deformation was a constant 5 mm/min. Validation of the technique and descriptions of the terms used for the assessment of bone strength and elasticity have been described [19]. Briefly, the definitions of the terms are as follows. Maximum force is the force in kilograms needed to break the bone. Stress, an indicator of strength, is the maximum force per unit cross-sectional area at the breaking point. Strain is a ratio of the bending distance before breaking and the original length; modulus of elasticity, an indicator of flexibility, is stress divided by strain. Area moment of inertia is the effect of bone geometry on resistance to bending. Bending moment is force times fulcra length. After breaking, bone thickness and inside lateral width and ventral depth were measured at the breaking point by using a 40X microscope with a 0.02 mm $\times$ 150 ocular scale. See Figure 1 for measurements made.

Statistical Analysis

Data were statistically compared by using two-way analysis of variance (SAS/STAT, Version 8.02, SAS Institute, Inc. Cary, NC) followed by Tukey's contrasts when appropriate. A P value of < 0.05 was considered significant.

D = outside vertical depth of the bone
B = outside lateral width of the bone
b = inside lateral width of the bone
d = inside vertical depth of the bone
WL = thickness width left
WR = thickness width right

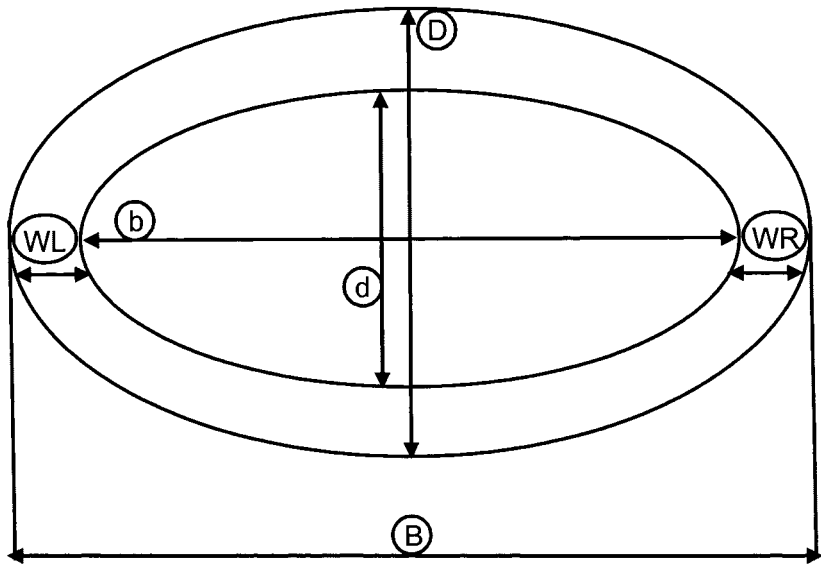

Fig. 1. Exposition of the physical measurements of bone.

RESULTS

Table II shows that ovariectomy increased final body weight. Dietary silicon did not significantly affect weight. Table II also shows that plasma indices associated with bone formation were affected by both dietary silicon and ovariectomy. Plasma osteopontin was decreased by both ovariectomy and low dietary silicon. Plasma sialic acid also was decreased by ovariectomy but increased by low dietary silicon. An interaction between the treatments affected plasma alkaline phosphatase; it was increased by ovariectomy in the silicon-supplemented rats but not affected by ovariectomy in the silicon-low rats. Plasma osteocalcin was increased by ovariectomy; the increase was apparently more marked in the low silicon rats.

As shown in Table III, urinary markers of bone breakdown also were affected by the experimental treatments. Although ovariectomy significantly increased urinary creatinine, on a mmol creatinine basis, ovariectomy still significantly increased the urinary excretion of pyridinoline, deoxypyridinoline, and helical peptide. The increase induced by ovariectomy in all the variables in Table III seemed more marked in the silicon-supplemented rats, but a significant interaction between the two treatments affected only deoxypyridinoline. Feeding low dietary silicon increased the excretion of helical peptide.

TABLE II. Effect of Dietary Silicon, Estrogen Status, and Their Interaction at 8 Weeks on Final Body Weight, Plasma Osteopontin, Sialic Acid, and Osteocalcin Concentrations and Alkaline Phosphatase Activity

Treatment[1]	Body weight (g)	Osteopontin, (ng/mL)	Sialic acid, (mg/100 mL)	Alkaline phosphatase, (U/mL × 10)[2]	Osteocalcin, (ng/mL)
0 Si Sham	204 ± 5	55 ± 4[3]	75 ± 3	0.542 ± 0.020[4,5]	207 ± 9
35 Si Sham	215 ± 5	65 ± 4	64 ± 3	0.462 ± 0.025[4]	214 ± 8
0 Si OVRx	293 ± 8	32 ± 2	52 ± 4	0.532 ± 0.028[4,5]	239 ± 7
35 Si OVRx	300 ± 7	44 ± 6	44 ± 3	0.564 ± 0.031[5]	219 ± 6
Analysis of Variance *P* Values					
Silicon	0.19	0.02	0.005	0.37	0.41
OVRx	0.0001	0.0001	0.0001	0.09	0.02
Silicon × OVRx	0.74	0.84	0.80	0.05	0.09

[1]Treatments were dietary silicon supplements of 0 and 35 mg/kg, and ovariectomy (OVRx) or sham operation. The basal diet contained about 2 mg Si/kg.
[2]Units were μmoles of *p*-nitrophenyl phosphate hydrolyzed/min.
[3]Mean ± SEM.
[4,5]Values not followed by the same superscript are significantly different according to Tukey's contrasts.

TABLE III. Effect of Dietary Silicon, Estrogen Status, and Their Interaction at 6 Weeks on the Urinary Excretion of Creatinine, Pyridinoline, Deoxypyridinoline, and Helical Peptide

Treatment[1]	Creatinine, μmole/16 h	Pyridinoline, mmole/mmole creatinine	Deoxypyridinoline, mmol/mmole creatinine	Helical peptide μg/mmole creatinine
0 Si Sham	30 ± 2[2]	215 ± 10	264 ± 12[3]	227 ± 18
35 Si Sham	40 ± 4	181 ± 8	196 ± 14[3]	162 ± 13
0 Si OVRx	48 ± 3	239 ± 10	383 ± 28[4]	240 ± 18
35 Si OVRx	47 ± 2	244 ± 14	425 ± 15[4]	218 ± 4
Analysis of variance *P* values				
Silicon	0.16	0.19	0.51	0.006
OVRx	0.0002	0.0002	0.0001	0.03
Silicon × OVRx	0.06	0.07	0.007	0.15

[1]Treatments were dietary silicon supplements of 0 and 35 mg/kg and ovariectomy (OVRx) or sham operation. The basal diet contained about 2 mg Si/kg.
[2]Mean ± SEM.
[3,4]Values not followed by the same superscript are significantly different according to Tukey's contrasts.

Other indices associated with bone that were affected by ovariectomy and/or dietary silicon are shown in Table IV. Femur sialic acid was significantly decreased by ovariectomy in the silicon-supplemented rats. Plasma TRAP and femur calcium concentration were both decreased by ovariectomy, but not significantly affected by dietary silicon. Liver ornithine aminotransferase was affected by a significant interaction between the treatments; it was significantly increased by low dietary silicon in the sham-operated but not in the ovariectomized rats.

TABLE IV. Effect of Dietary Silicon, Estrogen Status, and Their Interaction on Liver Ornithine Aminotransferase Activity and Plasma Tartrate-Resistant Acid Phosphatase (TRAP), Femur Sialic Acid, and Femur Calcium Concentrations

Treatment[1]	Liver ornithine amino transferase, U/mg protein[2]	Femur sialic acid, μg/mg protein	Plasma TRAP, U/L	Femur calcium, mg/g
0 Si Sham	0.773 ± 0.081[3,4]	301 ± 18[4,5]	1.97 ± 0.19	205 ± 5
35 Si Sham	0.390 ± 0.033[5]	332 ± 8[4]	1.77 ± 0.20	198 ± 6
0 Si OVRx	0.529 ± 0.065[5]	265 ± 12[5,6]	0.96 ± 0.06	178 ± 8
35 Si OVRx	0.417 ± 0.044[5]	233 ± 14[6]	1.37 ± 0.20	176 ± 6
Analysis of variance *P* values				
Silicon	0.0003	0.95	0.55	0.41
OVRx	0.08	0.0001	0.0003	0.0004
Silicon × OVRx	0.03	0.03	0.09	0.70

[1]Treatments were dietary silicon supplements of 0 and 35 mg/kg and ovariectomy (OVRx) or sham operation. The basal diet contained about 2 mg Si/kg.
[2]Units were μmoles of pyrroline-5-carboxylate formed/hour.
[3]Mean ± SEM.
[4–6]Values not followed by the same superscript are significantly different according to Tukey's contrasts.

TABLE V. Effect of Dietary Silicon, Estrogen Status, and Their Interaction on Femur-Breaking Characteristics

Treatment[1]	Maximum force, kg	Bending moment, kg/mm	Moment of inertia, mm^4	Modulus of elasticity, kg/mm^2	Stress, kg/mm^2
0 Si Sham	10.23 ± 0.27[2]	24.6 ± 0.9	3.85 ± 0.15	508 ± 14[3,4]	9.85 ± 0.28
35 Si Sham	11.19 ± 0.29	26.5 ± 1.2	3.88 ± 0.15	560 ± 19[3]	10.22 ± 0.48
0 Si OVRx	12.77 ± 0.33	30.9 ± 0.9	5.47 ± 0.27	480 ± 12[4]	9.11 ± 0.34
35 Si OVRx	12.92 ± 0.45	29.6 ± 1.2	5.97 ± 0.24	415 ± 20[5]	8.22 ± 0.26
Analysis of Variance *P* values					
Silicon	0.12	0.81	0.20	0.71	0.46
OVRx	0.0001	0.0001	0.0001	0.0001	0.0003
Silicon × OVRx	0.25	0.14	0.27	0.002	0.08

[1]Treatments were dietary silicon supplements of 0 and 35 mg/kg and ovariectomy (OVRx) or sham operation. The basal diet contained about 2 mg Si/kg.
[2]Mean ± SEM.
[3–5]Values not followed by the same superscripts are not significantly different according to Tukey's contrasts.

Table V shows that ovariectomy affected femur-breaking characteristics more than dietary silicon. Silicon through an interaction with ovariectomy affected only the modulus of elasticity. As a result of this interaction, the modulus of elasticity of the femur was highest in the silicon-supplemented, sham-operated rats, and the lowest in the silicon-supplemented ovariectomized rats. Ovariectomy significantly increased maximum force, bending moment and moment of inertia, but decreased stress measurements of the femurs.

Femur dimensions shown in Table VI were significantly affected by both treatments. Silicon-supplemented rats had femurs of greater length, thicker lateral bone, and greater outside diameter width than silicon-low rats. Ovariectomy increased all physical measurements shown in Table VI. However, the increase in the thickness of medial bone induced by ovariectomy was modified by dietary silicon; the increase was significant only in the silicon-low rats.

DISCUSSION

The hypothesis that silicon has a function that affects the action of extracellular matrix proteins with physiological roles other than just in bone formation is supported by the osteopontin and sialic acid findings in the present study. The sialoprotein osteopontin is a multifunctional cytokine and adhesion protein that is synthesized by several cell types other than bone cells, including hypertrophic chondrocytes, kidney proximal tubule epithelial cells, and arterial smooth muscle cells [8]. In bone, osteopontin is involved in bone cell attachment to the bone matrix and generates intracellular signals that affect osteoclast motility [9]. The unique significance of sialic acid is that this molecule is usually found at the periphery of the oligosaccharide chains of glycoproteins and glycolipids of cell membranes. Thus, sialic acid has an important role in cellular adhesion, signaling and interaction [20].

Circulating osteopontin was decreased by both silicon deprivation and ovariectomy. Because they had a similar effect, it was surmised that these treatments would similarly affect bone mineral content and breaking characteristics. However, this was not found. For example, ovariectomy decreased femur calcium concentration, but low dietary silicon did not. The effect of ovariectomy was consistent with previous reports showing that ovariectomy of young growing rats results in long bones that are less mineralized, or contain more organic matrix in the metaphysis [14,21]. Further evidence that low dietary silicon was not having the same effect as ovariectomy on variables associated with bone turnover in young rats were the circulating and femur sialic acid findings. The circulating concentration of sialic acid was decreased by ovariectomy but increased by low dietary silicon. In the femur, ovariectomy significantly decreased sialic acid in silicon-supplemented but not in silicon-low rats. The finding of different effects in plasma and femur sialic acid also suggests that the effect of dietary silicon on action of some extracellular matrix proteins is different in bone than in other sites in the body. Nonetheless, because silicon alters the concentrations of circulating sialic acid and osteopontin in blood and bone, and sialic acid in bone, it probably has a function that influences the action or function of some sialoproteins.

Although dietary silicon did not significantly affect femur calcium concentration, it significantly affected several variables associated with bone formation and breakdown; most of these variables were associated with the collagen component of the bone organic matrix. The lowest values for the collagen breakdown products, deoxypyridinoline, pyridinoline, and helical peptide, in urine were in the sham-operated rats fed supplemental silicon. Also, ornithine

TABLE VI. Effect of Silicon, Estrogen Status, and Their Interaction on Femur Dimensions in mm

	Outside diameter		Inside diameter		Bone thickness depth		Bone thickness width		
Treatment[1]	Depth	Width	Depth	Width	Ventral	Dorsal	Lateral	Medial	Femur length
0 Si Sham	3.03 ± 0.03[2]	3.35 ± 0.08	1.97 ± 0.03	2.29 ± 0.06	0.54 ± 0.03	0.52 ± 0.01	0.58 ± 0.01	0.51 ± 0.01[3]	31.6 ± 0.20
35 Si Sham	2.99 ± 0.04	3.52 ± 0.04	1.89 ± 0.04	2.28 ± 0.02	0.56 ± 0.01	0.53 ± 0.01	0.67 ± 0.03	0.54 ± 0.01[3,4]	31.8 ± 0.24
0 Si OVRx	3.26 ± 0.04	3.81 ± 0.08	2.01 ± 0.04	2.53 ± 0.08	0.64 ± 0.01	0.60 ± 0.03	0.69 ± 0.03	0.59 ± 0.02[4]	33.4 ± 0.13
35 Si OVRx	3.30 ± 0.03	3.99 ± 0.04	2.05 ± 0.03	2.70 ± 0.04	0.66 ± 0.03	0.59 ± 0.02	0.73 ± 0.04	0.56 ± 0.01[3,4]	33.9 ± 0.15
Analysis of variance *P* values									
Silicon	0.97	0.01	0.51	0.14	0.34	0.91	0.05	0.98	0.05
OVRx	0.0001	0.0001	0.009	0.0001	0.0001	0.0001	0.008	0.001	0.0001
Silicon × OVRx	0.31	0.89	0.10	0.11	1.00	0.33	0.37	0.04	0.38

[1]Treatments were dietary silicon supplements of 0 and 35 mg/kg and ovariectomy (OVRx) or sham operation. The basal diet contained about 2 mg Si/kg.
[2]Mean ± SEM.
[3,4]Values not followed by the same superscript are significantly different according to Tukey's contrasts.

amino transferase activity was less in silicon-supplemented than silicon-low sham-operated rats. Ornithine amino transferase is an enzyme that forms glutamic semialdehyde that is used in the synthesis of proline, which is hydroxylated in collagen. The finding that silicon influences collagen metabolism is consistent with those reported by others. Carlisle [2,3] found that silicon deprivation reduced the collagen content in skull and long bone. Carlisle also reported that silicon stimulated the activity of prolyl hydroxylase in frontal bones of chick embryos in vitro [4]. Reffitt et al. [22] found that orthosilicic acid stimulated collagen type I synthesis by human osteoblast-like cells in vitro.

It is generally thought that the major action of estrogen in enhancing and supporting bone mass is that it suppresses bone resorption [23]. This thought is supported by the finding in the present study that ovariectomy increased urinary deoxypyridinoline, pyridinoline and helical peptide. The plasma TRAP findings suggest, however, that ovariectomy was having an effect on more than just osteoclastic bone resorption to affect these three products of collagen breakdown in young growing rats. Plasma TRAP isoform 5b, which is considered a good marker of osteoclastic activity and bone resorption [24,25], was decreased, not increased, by ovariectomy. Perhaps estrogen deficiency was having more of an effect on chondroclast formation and activity [14] and thus both articular cartilage and bone organic matrix were sources of the increased urinary collagen breakdown products induced by ovariectomy in young growing rats.

As was found with the circulating and femur sialic acid, low dietary silicon affected the outside diameter width, lateral thickness, and femur length differently than ovariectomy. The changes in bone shape in ovariectomized rats were consistent with the report that ovariectomy of young rats increases the longitudinal growth rate of long bones throughout the growth period [14]. Additionally, ovariectomy increased radial bone growth, which results in bones with a larger cross-sectional area. These changes in bone physical characteristics have been characterized by increased chondroclast number and increased resorption of mineralized cartilage in the zone of vascular invasion [14]. Turner et al. [21] interpreted these changes as evidence that estrogen impairs chondroclast differentiation. Perhaps silicon has a role in chondrocyte, not chondroclast differentiation which estrogen inhibits, and thus ovariectomy would have a different effect than silicon deprivation on the shape of the femur.

In addition to liver ornithine amino transferase, plasma alkaline phosphatase, an enzyme also associated with bone formation early in the process, was affected by dietary silicon. Alkaline phosphatase has different isoforms produced through sialylation and one form provides phosphorus for bone mineralization. The activity of this enzyme was less in silicon-supplemented than silicon-low sham-operated rats. Like ornithine amino transferase, alkaline phosphatase activity was not significantly affected by dietary silicon in ovariectomized rats. The lack of an effect of silicon on these two enzymes in ovariectomized rats, however, was the result of different responses to ovariectomy. It decreased ornithine amino-transferase in silicon-low but not in silicon-supplemented rats; whereas it increased plasma alkaline phosphatase in silicon-supplemented but not low-silicon rats.

In the present study, ovariectomy had the expected effect of decreasing liver ornithine amino transferase activity in rats fed low dietary silicon but not in the rats fed supplemental silicon. This enzyme accumulates in the liver and kidney to a greater extent in female than male rats during the first two months of life; ovariectomy prevents this difference [15]. The finding that the effect of silicon was enhanced in estrogen-normal rats, or reduced in estrogen-low rats as the result of ovariectomy, is similar to the findings with urinary collagen-breakdown products and plasma alkaline phosphatase activity. All these findings suggest that estrogen status needs to be normal to see a marked effect of silicon deficiency on bone formation or turnover indices in growing female rats. That a relationship exists between ovariectomy and silicon metabolism and utilization is supported by a report by Charnot and Peres [26]. They found that the concentrations' of silicon in plasma, intestinal tissue and ungual tissue (nails) were markedly reduced by ovariectomy. Castration of males did not affect the silicon concentrations of these tissues. Thus, silicon apparently has a biochemical function that can be modified by estrogen deficiency caused by ovariectomy.

CONCLUSION

The findings in the present study suggest that silicon has a biochemical function that can affect circulating and bone concentrations of some extracellular matrix proteins, the activity of some enzymes associated with bone formation, the excretion of collagen breakdown products, and the response to ovariectomy. Surprisingly, these effects of silicon did not markedly affect bone calcium content and bone strength, but it did enhance the bone elasticity. This indicates that silicon does not have a major effect on bone crystal formation or function once mineralization has been initiated, but has an effect on bone growth processes (most likely through affecting collagen turnover) prior to this process. The urinary excretion of cross-links lends substantial support to the view that silicon is an essential element needed for normal bone cartilage formation and function. Evidence that that silicon exerts its effect through altering the action of an extracellular matrix protein such as the cytokine osteopontin was not obtained. Nonetheless, the findings show that silicon influences the presence of cytokines in tissues and fluids other than bone, which could be the basis for silicon being beneficial to immune function [11] and wound healing [12].

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