DOI:10.1021/jf904086d

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Novel Fibers Increase Bone Calcium Content and Strength beyond Efficiency of Large Intestine Fermentation

Connie M. Weaver,*,† Berdine R. Martin,† Jon A. Story,† Ingrid Hutchinson,§ and Lisa Sanders§

†Department of Foods and Nutrition, Purdue University, West Lafayette, Indiana 47907, and §Tate & Lyle Health & Nutrition Sciences, Decatur, IL

Dietary fibers are thought to benefit bone health through increasing mineral absorption and retention following fermentation in the lower gut and solubilization of minerals. This study compared eight fibers to cellulose following a 12 week intervention for production of short-chain fatty acids (SCFA), calcium absorption, mineral retention and bone content, and bone density and strength in a weanling rat model. Benefits to bone were poorly to modestly related to SCFA production, calcium absorption, or mineral retention, but some parameters were better predicted by cecal content weight, suggesting other mechanisms may be important. Nevertheless, two resistant starches, a soluble fiber dextrin and Polydextrose, increased bone calcium content. Soluble corn fiber and soluble fiber dextrin had the greatest benefit to bone properties including whole body bone mineral content and density and greater volumetric bone mineral density, cortical thickness and area, and peak breaking strength of the distal femur.

KEYWORDS: Dietary fiber; bone health; mineral absorption; fermentation; short-chain fatty acids

INTRODUCTION

Bone health is a growing issue worldwide. Musculoskeletal disorders are the cause of disability around the world and account for one-fourth of the total cost of illness according to the World Health Organization (WHO) (*1*). A U.S. Surgeon General's report brought attention to the importance of addressing bone health in the United States (*2*). The report highlights the role of diet in bone health with particular emphasis on calcium. Calcium is the largest constituent of bone mineral content and is a nutrient likely to be consumed in amounts well below recommended intakes by most individuals.

Dietary fibers and oligosaccharides are being investigated for their potential to improve bone health largely through their influence on mineral metabolism. These carbohydrate ingredients vary in their composition and structure, but all are considered to be nondigestible because of the lack of appropriate intestinal enzymes to hydrolyze them or structural hindrances that prevent enzyme access (i.e., high crystallinity). Bacteria in the lower gut can ferment these carbohydrates, but the rate and degree of fermentation vary with the polysaccharide. The range of fermentation in the colon for various fibers is broad, from approximately 5% for cellulose to nearly complete for pectin (*3*). The resulting short-chain fatty acids (SCFA) including butyrate and proprionate are thought to solubilize minerals, thereby improving their absorption and subsequent utilization (*4*−*7*). Inulin, a long-chain fructo-oligossacharide (FOS) often obtained from chicory root, and other FOSs have been the most studied (*7*). Fewer data are available for other fibers and oligosaccharides. Results of effects of fibers on mineral utilization are mixed and may be affected by life stage, intervention dose, and level of mineral, especially calcium, in the diet (*7*).

Several novel fibers were tested in an in vitro large intestine model for their effects on microbial stimulation and production of SCFA (*8*). All fibers stimulated growth in the beneficial bifidobacteria and some *Lactobacillus* species as well as increased SCFA production. There was a large range in SCFA production by the fibers, with cellulose generating the lowest levels to a biogum, pullulan, and the soluble fiber dextrin generating over twice the amount of SCFAs as cellulose. Therefore, we hypothesized that fibers with the greatest SCFA production would have the greatest impact on mineral utilization. The aims of our study were to compare a practical level of six novel fibers, inulin, and inulin plus short-chain FOS with cellulose, a negative control, in the same animal model, that is, weanling male rats, at adequate dietary calcium intake, for their effects on true calcium absorption, mineral balance, and cecal SCFA production.

MATERIALS AND METHODS

Test Materials. All fibers were obtained from Tate & Lyle (Decatur, IL) except for the inulin/FOS blend (Synergy 1, Beneo Orafti, Tienen, Belgium). The control fiber was cellulose. The eight test fibers are described in **Table 1**.

Animals. Four-week-old, male Sprague−Dawley rats (n = 150) were purchased from Harlan (Indianapolis, IN). They were maintained on a 12 h light−dark cycle with food and deionized water ad libitum.

Study Design. Rats were assigned to 10 groups of 15 rats each. The groups were two identical control groups and groups fed the eight test

*Address correspondence to this author at the Department of Foods and Nutrition, Purdue University, 700 W. State Str., West Lafayette, IN 47907 [phone (765) 494-8231; fax (765) 494-0674; e-mail weavercm@purdue.edu].

 Published on Web 08/02/2010 © 2010 American Chemical Society

Table 1. Description of Test Fibers

group	test fibers[a]	solubility	viscosity,[b] cP	description
RS 60	PROMITOR resistant starch	insoluble		type 3 resistant starch; contains amylose and amylopectin; digestive enzyme resistant shell formed by heat−moisture treatment of the starch during processing
RS 75	PROMITOR resistant starch	insoluble		type 3 resistant starch; highly crystalline structure confers digestive resistance
SCF	PROMITOR soluble corn fiber	soluble	1.4	product made from nutritive sugars formed through corn starch hydrolysis (GRAS, U.S. FDA 21CFR184.1865); soluble dietary fiber content is >70% (dsb) by AOAC 2001.03 and contains <20% simple sugars; weight-average degree of polymerization is approximately 10; the predominant glycosidic linkage is α-1,4; however, other α glycosidic linkages are present including α-1,6 and also a small fraction of α-1,2 and α-1,3
SFD	soluble fiber dextrin	soluble	2.1	dextrin product purified after traditional processing of tapioca starch (GRAS, U.S. FDA 21CFR184.1277); soluble dietary fiber is 64% (dsb) by AOAC 2001.03; weight-average degree of polymerization is approximately 40; the predominant glycosidic linkage is α-1,4; however, other α glycosidic linkages are present including α-1,6 and also a small fraction of α-1,2 and α-1,3
Pul	pullulan	soluble	24.3	GRAS fermentation product of *Aureobasidium pullulans* from dextrose (U.S. FDA GRAS Notice GRN 000099); soluble dietary fiber is 85% (dsb) by AOAC 991.43; weight-average degree of polymerization is approximately 3000; the predominant glycosidic linkage is α-1,4; however, other α glycosidic linkages are present (including α-1,6)
PDX	STA-LITE III Polydextrose	soluble	1.5	made by thermal polymerization of glucose; random polymerization results in predominantly α-1,6 linkages with some α-1,4; high degree of branching confers digestive resistance; weight-average degree of polymerization is approximately 12
inulin	inulin	soluble	1.6	low molecular weight fructan oligosaccharide; consists of α-glucopyranosyl unit linked to various numbers of β-fructosyl units
inulin/FOS	Synergy 1	soluble	1.5	blend of short chain fructo-oligosaccharides and high molecular weight inulin 30:70 (w/w)

[a] All fibers were sourced from Tate & Lyle, Decatur, IL, except the inulin/FOS blend, which was obtained from Beneo-Orafti, Tienen, Belgium. [b] Viscosity at 10^{-s}, 10% dry solids. Viscosity cannot be determined on insoluble fibers.

fibers listed in **Table 1**. The intervention was 12 weeks. Initially, all test fibers and the control fiber replaced cornstarch at 10% by weight based on the AIN93 G diet formula (*9*). However, due to loose stools in several groups, that is, all groups except the control and resistant starch groups, the fiber content was decreased to 5% after 2 weeks. A further change to 4% fiber plus 1% cellulose was made for the remainder of the study in groups given SFD, SCF, and PDX after another 3 weeks because of continued loose stools. Food intake was recorded every 3−4 days, and body weights were recorded weekly. Food efficiency ratios (FER) were calculated as change in body weight per gram of food consumed. Weight gain and FER were determined through week 10 before the rats' environments were altered by rotating the rats through the metabolic balance cages.

During the last 2 weeks of the intervention, mineral utilization tests were performed as described below. Also during this period, rats were anesthetized with isoflurane for determination of whole body bone mineral density (BMD) and content (BMC) and fat and lean composition by DXA with a Lunar Prodigy (GE Healthcare).

Rats were euthanized by an overdose of CO_2. Serum was frozen immediately at −80 °C. Cecal contents were extruded, frozen in liquid nitrogen, and stored at −80 °C. Both tibia and femur were extracted, cleaned, and stored wrapped in saline-soaked gauze at −20 °C.

Mineral Utilization. Two days before sacrifice, ^{45}Ca absorption tests were performed. Rats were fasted for 6 h prior to receiving a 5 g test meal of their assigned diet to which 10 μCi of ^{45}Ca had been added. After 2 h, the test meal containers were removed and rinsed to collect any residual radioactivity for adjusting the test dose. Two rats from each group received an intraperitoneal injection containing 5 μCi of ^{45}Ca to mimic 100% absorption. These rats received their assigned test meals without any oral ^{45}Ca. Oral and intraperitoneal ^{45}Ca uptake at 48 h by femurs was determined by liquid scintillation counting. Calcium absorption was calculated as

$$(\text{oral Ca as \% dose in femur}/\text{IP Ca as \% dose in femur}) \times 100$$

For determining mineral retention, three cohorts representing rats from each group were rotated into metabolic cages for 3 days to collect urine and feces in 24 h pools. Mineral content of diet, urine, and feces was determined by ICP-OES (Optima 4300 DV, Perkin-Elmer). Mineral retention for calcium, zinc, iron, magnesium, potassium, and copper was calculated as intake − feces − urine over 3 days.

Bone Mineral Content and Parameters. Femur length and width (anterior−posterior and medial−lateral) were determined at midpoint with digital Vernier calipers. Total femur bone density was determined by water displacement (model AG204, Mettler Toledo) with a density determination kit.

Imaging by peripheral quantitative computed tomography (pQCT) (XCT Research SA, Stratec Medizintechnic) was performed on femurs from eight rats per group. Each scan was acquired with a 0.12 mm voxel size, and the scan line was adjusted using the scout view of the software (Stratec, version 5.50 d; Stratec Medizintech). A constant threshold of 364 mg/cm^3 was used to segment bone from marrow. Total, trabecular, and cortical volumetric bone mineral density (vBMD), BMC and area, cortical thickness, periosteal circumference, and endosteal circumference of distal, midshaft, and proximal (12, 50, and 88% from distal end, respectively) femurs were measured.

Bone breaking strength at the midpoint of the femur was determined by three-point bending using an Alliance RT (MTS Systems, Eden Prairie, MN) at a test speed of 25 mm/min. Bone mineral content was determined following dissolution in HNO_3 by ICP-OES (Optima 4300 DV, Perkin-Elmer).

Cecal Short-Chain Fatty Acids. Cecal contents were frozen in liquid nitrogen and ground with a mortar and pestle to yield powdered cecal contents, and approximately 0.3 g was dissolved in 500 μL of ethanol containing 69 mol of 2-ethylbutyric acid (2-EBA) as an internal standard and stored at 4 °C for 16 h. Supernaturants were mixed with 70% ethanol/heptanoic acid/10% H_3PO_4. Total SCFA, acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate concentrations were determined with a Hewlett-Packard model 6890 gas chromatograph with a flame ionization detector (Agilent Technologies, Palo Alto, CA) equipped with a 30 m, 0.53 mm i.d., capillary column coated with Hewlett-Packard-Free Fatty Acids phase (HP-FFAP). Conditions were described previously (*10*).

Statistical Analysis. Data for the two control groups were averaged. Statistical analysis was performed using SAS version 9.1 for Windows.

Table 2. Effect of Various Fibers on Food Intake, Weight Gain, FER, and Body Composition[a]

	control	RS60	RS75	SCF	SFD	Pul	PDx	inulin	inulin/FOS
food intake									
end wt, g	427.5, 33.9	419.2, 26.7	427.8, 37.8	453.3,* 38.0	443.7, 25.5	412.0, 26.0	425.4, 34.0	423.7, 23.2	427.8, 23.2
wt gain, g	309.3, 28.1	301.9, 20.8	310.1, 35.0	322.3, 36.8	312.8, 21.7	293.3, 28.7	299.7, 31.2	309.8, 22.9	309.0, 32.1
food intake, g/day	20.2, 1.8	20.5, 1.5	20.1, 1.9	19.0, 1.6	20.7, 1.9	20.1, 1.7	20.5, 1.6	20.6, 1.2	19.5, 1.6
FER, g of wt gain/g of food intake	0.21, 0.02	0.21, 0.02	0.22, 0.02	0.22, 0.02	0.22, 0.02	0.24, 0.02	0.21, 0.03	0.23, 0.02	0.22, 0.03
body composition									
body fat, %	20.9, 3.1	21.2, 2.3	20.7, 3.1	19.4, 1.9	18.7, 2.5	19.5, 2.5	18.7, 2.5	19.0, 4.2	17.7, 3.5
BMD, g/cm^2	0.169, 0.005	0.168, 0.005	0.169, 0.005	0.174,* 0.006	0.174,* 0.004	0.165, 0.008	0.172, 0.006	0.169, 0.006	0.169, 0.004
BMC, g	9.57, 0.54	9.37, 0.45	9.69, 0.69	10.3,* 0.8	10.08,* 0.56	9.18, 0.55	9.81, 0.81	9.34, 0.56	9.47, 0.42

[a] See **Table 1** for group codes. Values are expressed as mean, SD. * indicates significantly different ($P < 0.05$) from control group by Dunnett's test.

Table 3. Effect of Various Fibers on Calcium Absorption and Mineral Balance[a]

	control	RS60	RS75	SCF	SFD	Pul	PDx	inulin	inulin/FOS
femur ^{45}Ca uptake, %	31, 8	39, 28	32, 6	36, 5	37, 9	28, 6	32, 4	35, 7	54,* 17
Ca retention, mg/3 days	164.0, 28.0	163.3, 29.2	167.6, 60.4	103, 42.5	168, 34.2	151.8, 29.9	141.6, 35.3	91.7, 29.9	149.6, 23.5
diet Ca concn, mg/g	6.28	5.93	6.15	5.18	6.38	5.91	5.21	4.95	5.79
Zn retention, mg/3 days	0.786, 0.26	1.143,* 0.27	1.215,* 0.49	0.254, 0.25	1.323,* 0.31	0.594, 0.28	0.541, 0.34	0.223, 0.25	1.115,* 0.18
diet Zn concn, mg/g	0.050	0.052	0.054	0.040	0.057	0.046	0.037	0.038	0.051
Fe retention, mg/3 days	0.482, 0.2	0.200, 0.25	0.348, 0.39	−0.205, 0.28	0.572, 0.31	0.277, 0.25	0.107, 0.3	−0.058, 0.23	0.412, 0.18
diet Fe concn, mg/g	0.042	0.037	0.038	0.030	0.043	0.036	0.028	0.031	0.037
Mg retention, mg/3 days	20.943, 3.86	23.799, 2.86	19.460, 7.23	20.499, 4.84	34.01,* 4.21	17.531, 3.89	17.137, 4.68	19.714, 5.0	23.446, 3.5
diet Mg concn, mg/g	0.678	0.690	0.656	0.580	0.873	0.602	0.500	0.613	0.685
Cu retention, mg/3 days	0.409, 0.06	0.575,* 0.08	0.568,* 0.14	0.516,* 0.08	0.526,* 0.07	0.499,* 0.07	0.513,* 0.07	0.351, 0.07	0.542,* 0.06
diet Ca concn, mg/g	0.013	0.015	0.015	0.014	0.014	0.012	0.013	0.011	0.015

[a] See **Table 1** for group codes. Values expressed as mean, SD. * indicates significantly different ($P < 0.05$) from control group by Dunnett's test.

The Kolmogorov−Smirnov analysis was performed to check for normality. Group means were compared to the control group by Dunnett test. Significance was accepted at $p < 0.05$.

RESULTS

Food intake, weight gain, FER, and body composition are given in **Table 2**. Few significant differences due to group assignment were observed in any of these parameters. The SCF and SFD groups had greater whole body BMD and BMC compared to the control group.

Femur ^{45}Ca uptake, mineral balance, and mineral concentration of diets are given in **Table 3**. Only the inulin/FOS blend significantly enhanced calcium utilization. Copper retention was significantly improved by all of the fibers except inulin as determined by balance studies. Zinc retention was enhanced by RS60, RS75, SFD, and inulin/FOS. Only SFD enhanced Mg retention. Calcium and iron retention were not enhanced as determined by balance studies.

Femur dimensions and total bone BMD by underwater weighing were unaffected by treatment (data not shown). Other femur bone parameters and bone mineral content are given in **Table 4**. Femur calcium and magnesium contents relative to the control group were improved by several fibers, that is, RS60, RS75, SFD, PDX, inulin, and inulin/FOS. Calcium and magnesium concentrations were also improved relative to the control group by those same fibers except the increase with inulin/FOS did not achieve significance. Copper retention was improved by all fibers except inulin. Copper concentration in femurs was too close to the limit of detection by ICP-OE. Zinc content and concentration of femurs were improved by all fibers relative to the control except Pul and SCF. Iron content and concentration were increased by SCF. Many of the measures of proximal femur BMD and bone geometry were improved by SCF, SFD, and PDX, and a few were improved by inulin and inulin/FOS. Of particular importance are the increases in total BMD, due to increases in trabecular rather than cortical BMD, and cortical thickness and area. These differences in bone properties translated into increases in peak breaking force of 8.8% for SCF and 8.4% for SFD (**Figure 1**). Effects of fibers on femur midshaft and distal ends were unremarkable.

Total and individual SCFA contents of the cecum are given in **Table 5**. The fibers that resulted in greater weight of cecal contents than the control group included SFD, SCF, PDX, inulin, and inulin/FOS. All fibers except RS60 and RS75 increased total SCFA and propionate production over the control. Total acetate was increased by these fibers as well (except PDX) compared to the control. Total butyrate was increased by RS60, PDX, inulin, and inulin/FOS.

DISCUSSION

Various fibers have been shown to improve mineral absorption and utilization, but typically at levels of intake that would be difficult to achieve by most individuals. We studied a diverse spectrum of fiber types at more practical levels for their effect on SCFA production and relationship to calcium absorption, bone parameters, and mineral utilization. We hypothesized that fibers which produced the most SCFA upon fermentation in the large bowel would improve mineral utilization through increased solublization at lower pH and, consequently, bone mineral content and mechanical properties. However, there was a much stronger relationship between total SCFA production and cecal weight ($r = 0.59$, $p < 0.0001$) than with mineral utilization. The relationship between total SCFA and mineral content per femur decreased as follows: Zn ($r = 0.27$, $p = 0.0007$) > Mg ($r = 0.26$, $p = 0.0014$) > Ca ($r = 0.19$, $p = 0.02$) > Fe (NS). Cecal weight more strongly related to femoral Ca content ($r = 0.30$, $p = 0.0015$ than did SCFA, but not to other bone minerals.

Bone mineral analysis showed that many fibers increased calcium levels in bone, but the effect was not uniform across minerals. RS60, RS75, SFD, PDx, inulin, and inulin/FOS increased bone calcium content of the femur and, except for inulin/FOS, also calcium concentration compared to the control group.

Table 4. Effect of Various Fibers as Femur Properties and Bone Mineral Content[a]

	control	RS60	RS75	SCF	SFD	Pul	PDx	inulin	inulin/FOS
wet weight, g	1.19, 0.09	1.12, 0.06	1.15, 01	1.27, 0.09	1.24, 0.08	1.19, 0.07	1.23, 0.01	1.18, 0.07	1.24, 0.09
mineral analysis									
Ca content, mg/bone	51.4, 19.6	180.2,* 43.5	171.5,* 18.2	161.3, 10.5	182.7,* 10.4	161.3, 44.7	186.1,* 15.7	173.0,* 20.4	171.8,* 11.4
Ca concn, mg/g of bone	126.9, 13.2	161.2,* 39.8	149.5,* 11.7	126.4, 5.8	147.3,* 11.0	134.9, 37.0	151.8,* 8.0	147.1,* 15.4	138.6,* 5.9
Mg content, mg/bone	2.7, 0.3	3.1,* 0.7	3.2,* 0.3	3.0, 0.2	3.4,* 0.2	3.0, 0.8	3.4,* 0.3	3.2,* 0.3	3.1,* 0.2
Mg concn, mg/g of bone	2.3, 0.2	2.8,* 0.6	2.8,* 0.2	2.4, 0.1	2.8,* 0.2	2.5, 0.7	2.8,* 0.1	2.7,* 0.3	2.5, 0.1
Fe content, mg/bone	0.05, 0.02	0.06, 0.04	0.06, 0.02	0.07,* 0.04	0.06, 0.02	0.05, 0.02	0.06, 0.02	0.05, 0.01	0.05, 0.02
Fe concn, mg/g ob bone	0.04, 0.01	0.06,* 0.04	0.05, 0.01	0.05,* 0.03	0.05, 0.01	0.04, 0.02	0.05, 0.01	0.04, 0.01	0.04, 0.01
Zn content, mg/bone	0.20, 0.03	0.23, 0.05	0.23, 0.03	0.22, 0.02	0.25, 0.02	0.21, 0.07	0.27, 0.02	0.24, 0.03	0.24, 0.02
Zn concn, mg/g of bone	0.16, 0.02	0.21,* 0.05	0.19,* 0.02	0.17, 0.02	0.20,* 0.02	0.17, 0.05	0.23,* 0.02	0.21,* 0.02	0.19,* 0.01
pQCT analysis of distal femur									
total BMC, mg/mm	15.1, 1.0	15.9, 0.7	15.9, 0.9	16.6,* 1.1	16.2, 0.8	16.4, 1.3	16.5,* 0.9	16.0, 0.9	16.7,* 1.0
total BMD, mg/cm^3	604.4, 39.0	625.6, 32.7	634.1, 29.5	654.5,* 42.7	657.8,* 31.1	642.7, 43.4	666.5,* 23.4	645.4, 45.8	633.4, 30.2
total area, mm^2	25.0, 1.4	24.5, 0.5	25.1, 1.7	25.4, 1.7	24.6, 0.8	25.6, 1.0	24.7, 1.5	24.8, 1.0	26.4, 1.6
trabecular BMC, mg/mm	10.2, 0.7	10.9, 0.7	11.0, 1.0	11.2,* 0.8	10.8, 0.5	11.3,* 0.9	10.9, 0.6	10.8, 0.6	11.5,* 0.8
trabecular BMD, mg/cm^3	520.6, 32.4	544.6, 40.2	557.7, 24.0	573.2,* 36.8	572.5,* 28.1	564.2,* 42.0	579.8,* 25.3	565.2,* 38.5	555.5, 22.6
trabecular area, mm^2	19.7, 1.5	20.0, 0.7	19.7, 2.0	19.6, 2.0	18.9, 0.8	20.0, 1.2	18.9, 1.2	19.2, 1.4	20.7, 1.8
cortical BMC, mg/mm	8.3, 1.1	9.2, 0.8	8.9, 0.8	9.9,* 1.3	9.7,* 0.9	9.5, 1.4	10.1,* 0.8	9.5, 1.3	9.5, 1.0
cortical BMD, mg/cm^3	897.9, 10.1	892.0, 11.8	890.2, 11.5	893.2, 10.1	898.6, 10.2	892.9, 12.7	898.5, 5.3	890.7, 14.1	889.8, 12.4
cortical area, mm^2	9.2, 1.2	10.3, 0.9	10.0, 0.9	11.0,* 1.4	10.8,* 1.1	10.6, 1.5	11.3,* 0.9	10.6,* 1.3	10.7,* 1.1
cortical thickness, mm	0.58, 0.09	0.65, 0.07	0.64, 0.07	0.71,* 0.11	0.70,* 0.08	0.67, 0.11	0.74,* 0.06	0.69,*0.11	0.67, 0.6
periosteal circumf, mm	17.7, 0.5	17.9, 0.2	17.7, 0.6	17.9, 0.6	17.6, 0.3	17.9, 0.3	17.6, 0.5	17.7, 0.4	18.2, 0.6
endosteal circumf, mm	14.0, 0.8	13.8, 0.5	13.7, 0.8	13.4, 1.1	13.2, 0.7	13.7, 0.7	13.0,* 0.6	13.3, 0.9	14.0, 0.9
breaking strength									
peak breaking force, N	174.5, 14.2	165.2, 14	172.3, 18.2	189.9,* 18.4	189.2,* 18.4	170.3, 9.9	181.6, 14.6	170.5, 14.2	176.0, 16.3
stiffness, N/mm	56.8, 5.4	54.3, 3.6	55.0, 4.9	60.6, 5.4	57.8, 4.3	53.7, 5.0	57.6, 4.8	53.7, 5.7	57.4, 3.3

[a] See **Table 1** for group codes. Values are expressed as mean, SD. * indicates significantly different ($P < 0.05$) from control group by Dunnett's test.

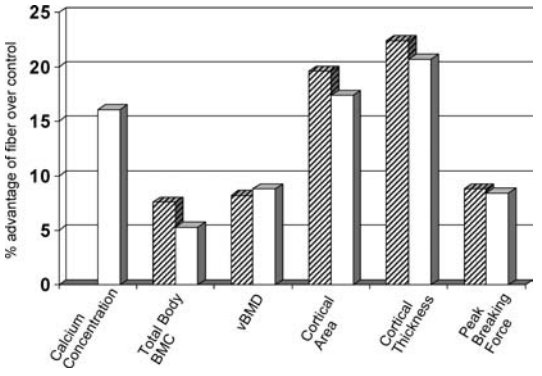

Figure 1. Benefits to femur of soluble fibers over control fed rats; $p < 0.05$ by Dunnett's test (slashed bars are SCF and open bars are SFD).

Yet only inulin/FOS increased femur ^{45}Ca uptake, in agreement with previous studies in children (*11*−*15*), adults (*16*−*18*), and rats (*19*, *20*). When whole body BMC and BMD were assessed, only SCF- and SFD-fed rats showed an improvement in BMC and BMD. None of the fibers improved calcium balance over the control rats fed cellulose. The effect of fibers on utilization of minerals other than calcium was also more evident in bone mineral content than with mineral balance, a less sensitive measure. Femur zinc content and retention were improved by RS60, RS75, SFD, and inulin/FOS, but PDx and inulin also improved bone zinc content. Only SFD improved Mg retention.

Discrepancies among bone mineral content, mineral balance, BMD, and BMC and between calcium absorption and calcium balance may relate to effects of adaptation as well as to different sensitivities of assays. Bone parameters could reflect early diet effects, whereas mineral balance and femur ^{45}Ca uptake reflect end-of-study effects. Measurement of mineral balance and femur ^{45}Ca uptake at an earlier time point in the study may have revealed an early increase in the absorption of calcium and other minerals with some fibers that, due to adaptation, is not detected at a later time point. Adaptation to early improved calcium absorption in the presence of whey proteins has been demonstrated (*21*). Adaptation occurs when the vitamin D-dependent calcium transport protein facilitated diffusion absorption process is down-regulated in response to more calcium being transported over several days across the intestinal epithelium in the presence of calcium absorption enhancing factors. The enhancing effect of an inulin/FOS blend on calcium absorption in adolescents was persistent over 12 months (*11*), in agreement with this study. Not all minerals have been studied for adaptation effects. Zinc and iron are primarily regulated at the site of intestinal absorption, whereas magnesium homeostasis is more closely related to urinary excretion and calcium homeostasis is regulated at the gut, bone, and kidney. Copper absorption is also regulated, but is less studied. Differences in mineral regulation may explain some of the differences observed in mineral retention and bone mineral content such as the greater effect of fibers on bone zinc content than on bone magnesium content.

The ultimate goal of a functional food fiber with respect to bone health is increasing resistance to fracture. Both the novel soluble fibers SFD and SCF significantly increased the force required to break femurs by >8%. This was achieved through increased total BMD, cortical area, and cortical thickness. Although the total BMD increase was associated with increases in trabecular rather than cortical BMD, the increase in cortical area and thickness without altering BMD was more indicative of stronger bone. PDx also improved total and trabecular BMD and cortical area and thickness, but the increase in peak breaking force did not achieve significance.

In this study, significant relationships between total SCFA and bone parameters were few, that is, with total SCFA and distal total and cortical vBMC and cortical thickness ($r = 0.36$, $p < 0.01$ for all). Cecal weight was positively related to BMD ($r = 0.23$, $p < 0.02$) and, more importantly, to peak breaking strength ($r = 0.35$, $p = 0.0002$). Cecal weight reflects water-holding

Table 5. Effect of Various Fibers on SCFA Content[a]

	control	RS60	RS75	SCF	SFD	Pul	PDx	inulin	inulin/FOS
cecal content wt, g	2.70, 0.578	3.25, 0.54	3.27, 0.73	5.58,* 1.26	5.58,* 1.13	3.77,* 1.03	5.73,* 1.51	4.68,* 0.94	4.91,* 0.88
total SCFA content, μmol	539.3, 95.3	568.4, 100.4	617.3, 115.9	765.4,* 190.1	893.0,* 132.3	727.5,* 246.8	685.9,* 73.4	1003.0,* 203.3	851.7,* 150.2
total SCFA concn, μmol/g	76.1, 14.4	74.2, 11.5	80.1, 12.1	77.6, 22.0	89.7,* 12.3	96.0,* 19.9	68.6, 10.4	110.8,* 20.9	92.3,* 16.2
total acetate, μmol	329.4, 70.7	342.8, 88.2	386.7, 73.2	472.6,* 126.7	527.2,* 66.2	434.0,* 156.7	405.9, 44.3	567.8,* 143.7	516.0,* 112.3
acetate concn, μmol/g	45.5, 10.4	44.8, 10.5	50.1, 8.5	47.9, 14.3	53.0, 6.5	57.7,* 15.3	40.7, 6.6	62.7,* 15.0	55.9,* 12.3
total propionate, μmol	97.2, 17.4	102.3, 19.8	117.0, 27.8	186.3,* 45.7	248.8,* 50.3	195.1,* 75.9	160.2,* 20.7	253.7,* 74.5	187.3,* 50.1
propionate concn, μmol/g	13.8, 2.8	13.3, 2.2	15.3, 2.9	18.9,* 5.2	25.0,* 4.7	25.7,* 6.6	16.0,* 2.7	28.2,* 8.6	20.3,* 5.2
total butyrate, μmol	57.6, 13.7	75.7,* 14.4	59.9, 13.5	69.3, 18.0	74.2, 18.2	60.2, 27.2	76.3,* 12.0	140.5,* 44.7	107.4,* 23.7
butyrate concn, μmol/g	8.1, 2.1	9.9, 1.7	7.8, 1.5	7.1, 2.5	7.4, 1.6	7.8, 2.2	7.6, 1.5	15.5,* 4.5	11.7,* 2.8

[a] See **Table 1** for group codes. Values are expressed as mean, SD. * indicates significantly different ($P < 0.05$) from control group by Dunnett's test. Also measured were isobutyrate, isovalerate, and valerate; no significant ($p < 0.05$) differences compared to control were found.

capacity. The viscosity of these fibers is low compared to fibers such as guar gum as shown in **Table 1** and was unrelated to bone parameters, but the osmotic effect of prebiotics has been suggested as a mechanism for their stimulatory effect on paracellular mineral transport through increased mineral solubilization in a larger fluid volume in the lower bowel (*22*). In support, cecal blood flow increases in rats fed fermentable carbohydrates (*23*, *24*). Another mechanism proposed for the effect of fermentable carbohydrates on mineral absorption by both paracellular and transcellular transport is through increased surface area in response to colonic cell proliferation with SCFA generation (*24*). Perhaps fibers also increase hypertrophy or permeability of gut epithelial cells partially through water-holding capacity, which creates a nutrient reservoir or alters intestinal microflora through which they can also influence mineral absorption (*22*).

Interestingly, both soluble and insoluble fibers were fermentable. A comparison of our results on in vivo production of SCFA with those in the in vitro large intestine model (*8*) showed some interesting differences. Soluble fibers (Pul, SCF, SFD) produced more total SCFA, acetate, and *n*-butyrate than insoluble fibers (RS60, RS75), and all produced more than cellulose in the in vitro model. The authors suggested that resistant starches characterized by their crystalline structure may slow fermentation, which was supported by low lactate production. In that study, production of butyrate and lactate was much higher with Pul than with any other fiber. Our in vivo data showed a general trend of higher total SCFA and acetate production in soluble fibers compared to insoluble fibers, with the lowest production in the control group; however, butyrate production was not related to fiber solubility. When analyzed as a group of soluble fibers (SFD, SCF, PUL PDx, and inulin) and insoluble fibers (RS60 and RS 75), the soluble fibers, but not the insoluble fibers, had greater total SCFA and greater cecal weight compared to the control group. Nevertheless, both soluble and insoluble fibers improved femoral calcium and copper content. Inulin had the highest production of total SCFAs and the greatest femoral ^{45}Ca uptake, but RS 60, with less SCFA generation, had the greatest femoral calcium concentration. Thus, it appears that SCFA generation may correlate to calcium absorption and cecal content, but not necessarily to end effects on bone calcium content.

Most of the fibers had some advantage for mineral utilization. RS60, RS75, SFD, PDx, inulin, and inulin/FOS increased femoral bone calcium content over cellulose. The inulin/FOS blend had the expected benefits to calcium absorption and bone uptake. Bone parameters and mineral absorption weakly correlated with SCFA production, but femur Ca content and breaking strength more strongly correlated with cecal weight, suggesting a role for water-absorbing capacity. The biggest effect of inulin/FOS on calcium metabolism in OVX rats was in suppressing bone resorption (*19*). Kinetic studies would be needed to determine if that mechanism was at work for other fibers. The most effective fibers were the soluble fibers, SCF and SFD, which increased whole body BMC and femoral BMD, cortical area and thickness, and resistance to fracture. PDX also increased femoral BMD and cortical area and thickness. Further study on the effects of SCF, SFD, and PDX on bone health is warranted in humans.

LITERATURE CITED

(1) http://www.boneandjointdecade.org, accessed Nov 1, 2009.

(2) U.S. Department of Health and Human Services. *Bone Health and Osteoporosis: A Report of the Surgeon General*; U.S. Department of Health and Human Services, Office of the Surgeon General: Rockville, MD, 2004.

(3) Cook, S. I.; Sellin, J. H. Review article: short chain fatty acids in health and disease. *Ailment Pharmacol. Ther.* **1998**, *12*, 499–507.

(4) Topping, D. L.; Clifton, P. M. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol. Rev.* **2001**, *81*, 1031–64.

(5) Morohashi, T.; Sano, T.; Ohta, A.; Yamada, S. True calcium absorption in the intestine is enhanced by fructooligosaccharide feeding in rats. *J. Nutr.* **1998**, *128*, 1815–18.

(6) Perrin, P.; Pierre, F.; Patry, Y.; Champ, M.; Berreur, M.; Pradal, G.; Bornet, F.; Meflah, K.; Menanteau, J. Only fibres promoting a stable butyrate producing colonic ecosystem decrease the rate of aberrant crypt foci in rats. *Gut* **2001**, *48*, 53–61.

(7) Weaver, C. M. Inulin, oligofructose and bone health: experimental approaches and mechanisms. *Br. J. Nutr.* **2005**, *93* (1), S99–S103.

(8) Maathuis, A.; Hoffman, A.; Evans, A.; Sanders, L.; Venema, K. The effect of the undigested fraction of maize products on the activity and composition of the microbiota determined in a dynamic *in vitro* model of the human proximal large intestine. *J. Am. Coll. Nutr.* **2009**, *28*, 657–666.

(9) Reeves, P. G.; Nielsen, F. H.; Fakey, G. C., Jr. AIN-93 purified diets for laboratory rodents final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **1993**, *123*, 1939–1951.

(10) Lupton, J. R.; Kurtz, P. P. Relationship of colonic luminal short-chain fatty acids and pH to in vivo cell proliferation in rats. *J. Nutr.* **1993**, *123*, 1522–1530.

(11) Abrams, S. A.; Griffin, I. J.; Hawthorne, K. M.; Liang, L.; Gunn, S. K.; Darlington, G.; Ellis, K. J. A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adults. *Am. J. Clin. Nutr.* **2005**, *82*, 471–476.

(12) Van den Heuvel, E. G. H.; Muys, T.; van Dokkum, W.; Schaafsma, G. Oligofructose stimulates calcium absorption in adolescents. *Am. J. Clin. Nutr.* **1999**, *69*, 544–548.

(13) Griffin, I. J.; Davila, P. M.; Abrams, S. A. The effect of adaptation for moderate dietary intakes of oligosaccharides on calcium absorption in children. *Br. J. Nutr.* **2002**, *87*, S187–S191.

(14) Griffin, I. S.; Hicks, P. M. D.; Heaney, R. P.; Abrams, S. A. Enriched chicory inulin increases calcium absorption mainly in girls with lower calcium absorption. *Nutr. Res.* (*N.Y.*) **2003**, *23*, 901–909.

(15) Abrams, S. A.; Hawthorne, K. M.; Aliu, O.; Hicks, P. D.; Chen, Z.; Griffin, I. J. An inulin-type fructan enhances calcium absorption

primarily via an effect on colonic absorption in humans. *J. Nutr.* **2007**, *137*, 2208–2212.
(16) Coudray, C.; Bellanger, J.; Castiglia-Delavaud, C.; Remesy, C.; Vermorel, M.; Rayssignuier, Y. Effect of soluble or partly soluble dietary fibres supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men. *Eur. J. Clin. Nutr.* **1997**, *51*, 375–380.
(17) Tahiri, M.; Tressol, J. C.; Amaud, J.; Bornet, F. R.; Bouteloup-Demange, C.; Feillet-Coudray, C.; Brandolini, M.; Ducros, V.; Pepin, D.; Brouns, F.; Roussel, A. M.; Rayssiguier, Y.; Coudray, C. Effect of short-chain fructooligosaccharides on intestinal calcium absorption and calcium status in postmenopausal women: a stable-isotope study. *Am. J. Clin. Nutr.* **2003**, *77*, 449-457, 2003.
(18) López-Huertas, E.; Teucher, B.; Boza, J. J.; Martinez-Férez, A.; Mojsak-Newman, G.; Baró, L.; Carrero, J. J.; González-Santiago, M.; Fonaliá, J.; Fairweather-Tate, S. Absorption of calcium from milk enriched with fructo-oligosaccharides, caseinophosphopeptides, tricalcium phosphate, and milk solids. *Am. J. Clin. Nutr.* **2006**, *88*, 310–316.
(19) Zafar, T. A.; Weaver, C. M.; Zhao, Y.; Martin, B. R.; Wastney, M. E. Nondigestible oligosaccharides increase calcium absorption and suppress bone resorption in ovariectomized rats. *J. Nutr.* **2004**, *123*, 399–402.
(20) Coudray, C.; Tressol, J. C.; Gueux, E.; Rayssiguier, Y. Effects of inulin-type fructans of different chain length and type of branching on intestinal absorption and balance of calcium and magnesium in rats. *Eur. J. Nutr.* **2003**, *42*, 91–98.
(21) Zhao, Y.; Martin, B. R.; Wastney, M. E.; Schollum, L.; Weaver, C. M. Acute versus chronic effects of whey proteins on calcium absorption in growing rats. *Exp. Biol. Med.* **2005**, *230*, 536–542.
(22) Bongers, A.; van den Heuval, E. G. H. M. Prebiotics and the bioavailability of minerals and trace elements. *Food Rev. Int.* **2003**, *19*, 397–422.
(23) Démigné, C.; Levrat, M. A.; Rémésy, C. Effects of feeding fermetable carbohydrates on the cecal concentrations of mineral sand their fluxes between the cecum and blood plasma in the rate. *J. Nutr.* **1989**, *119*, 1625–1630.
(24) Younces, H.; Coudray, C.; Bellanger, J.; Demigné, C.; Rayssiguier, Y.; Rémésy, C. Effects of two fermentable carbohydrates (inulin and restant starch) and their combination on calcium and magnesium balance in rats. *Br. J. Nutr.* **2001**, *86*, 479–485.

Received for review February 16, 2010. Revised manuscript received July 9, 2010. Accepted July 21, 2010. This study was funded by Tate & Lyle Health & Nutrition Sciences, Decatur, IL.