

Increase in Bone Calcification in Young Rats Fed Breads Highly Fortified with Calcium

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ABSTRACT

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Breads were prepared with flour fortified with calcium (Ca) at four levels: 211 (U.S. enrichment standard), 446, 924, and 1,412 mg/100 g of flour. Corresponding diets prepared with these breads and fed to growing rats for four weeks provided 25 (diet A), 50 (diet B), 100 (diet C), and 150% (diet D) of their Ca requirement. After four weeks, the Ca content of the femurs of these rats had increased significantly as Ca in the diet increased up to 100% of requirement but not beyond. The increase in femur strength was significant only between diet A and diet B. Apparent Ca absorption increased

as Ca in the diet increased, but Ca absorbed from diet D was not retained any better than that absorbed from diet C. Overall, the data suggested that Ca in highly fortified breads was well absorbed and retained, and that the rats' Ca status early in life was greatly improved. Flour fortified with Ca up to the 924-mg level (4.4× the mandated enrichment level) had no adverse effect on bread quality. These breads can be labeled a "good source of" or "high in" Ca.

Studies have shown that adequate calcium (Ca) intake during the crucial growing years maximizes peak bone mass and thus delays the onset and severity of osteoporosis, a multifactorial disease, later in life (Berner et al 1990, Renner 1994, Ulrich et al 1996, Nordin 1997, Carter and Whiting 1997). Adequate Ca intake may also reduce the risk of hypertension and colorectal cancer (McCarron et al 1984, Gilliland et al 1987, Lapre and van der Meer 1992, Osborne et al 1996, Hambly et al 1997, McCarron et al 1998). Because of these health benefits, Ca-rich foods may be viewed as functional (nutraceutical) foods.

Milk and derived products are important Ca-rich foods in our diets (Berner et al 1990, Renner 1994). However, milk is not an important item in the diets of many people during childhood and adolescence because of their preference for other beverages or their desire to control fat and caloric intake. Also, people who are lactose intolerant must limit their intake of dairy foods. For these population groups and many others, Ca-fortified nondairy foods can be an alternative and significant source of Ca.

Several food items, including flour, can be fortified with Ca. According to the enrichment standards, flour can be fortified to contain 211 mg of Ca/100 g of flour. However, products made with this flour provide only a modest amount of Ca. For example, a 50-g serving of Ca-fortified bread provides only ≈6–8% of the daily reference value (1,000 mg) of Ca. If Ca is added to flour at appreciably higher levels, bread and related products can contribute a significant amount of Ca to the diet.

While this contribution can easily be calculated, it is uncertain whether this Ca improves the body's Ca status, especially early in life. Adding Ca to nondairy foods may not achieve the intended purpose unless it is well utilized, as is the Ca from dairy products. This study was undertaken to address this question. Flour was fortified with increasingly higher levels of Ca, and its impact on bone calcification was assessed in growing rats.

MATERIALS AND METHODS

Flour Fortification

Calcium carbonate, a Ca source commonly used in fortification of grain-based foods, was used to fortify flour in batch amounts needed for breadmaking. Ca was added at the level mandated (211 mg/100 g)

under the flour-enrichment standards (CFR 1996) and at levels approximately two (446 mg), four (924 mg), and six (1,412 mg) times the enrichment standard (Table I).

Breadmaking

Pan breads were made with Ca-fortified flours according to the standard sponge and dough method routinely used at the American Institute of Baking and reflecting the industry's practice. The sponge contained 70% flour, 2% compressed yeast, and 42% distilled water, while the dough contained the remaining 30% flour, 7% sugar, 3% shortening, 2% salt, and the rest (20%) of the water. After baking, breads were cooled on a rack and then bagged and saved for scoring the next morning (Table I).

Test Diets

After scoring, all loaves were sliced, air-dried, and finely ground, and samples were taken for analyses. Diets were formulated by using the finely ground breads to supply 25, 50, 100, or 150% of the Ca required (0.5%) by the growing rats (NRC 1987). Since flours were fortified with increasingly higher levels of Ca, Ca in the resultant bread-based diets differed significantly, but amounts of breads used were very similar (Table II). All diets were complete in the nutrients (except Ca) required by the rats, including phosphorus (NRC 1987).

Animals

Weanling (21-day-old) Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) were housed individually in suspended mesh-bottom stainless-steel cages in a controlled environment (24°C, 60% rh, and a 12-hr light and dark cycle). After a two-day adaptation period, the animals were weighed and assigned by selective randomization to four groups of 10 animals each or one group of five animals. The five-animal group was sacrificed on day 0 to obtain baseline information on serum and femur Ca. The other groups were then fed test diets daily for four weeks. The rats' food intake increased gradually but differed minimally among groups. This minimal difference was adjusted to equalize the food intake among all groups. Deionized water was offered ad libitum. Animals were weighed weekly. During the entire four-week feeding period, feces from each rat were collected from the tray underneath the cage, air-dried, finely ground, and weighed. Samples were then taken for Ca determination.

Tissue Sampling

After four weeks on the test diets, rats were lightly anesthetized (under ether), and 2 mL of blood was withdrawn by cardiac puncture. The clotted blood was centrifuged (8 min) to obtain serum for

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Ca analysis. Rats were then killed, and both femurs were removed, cleared of adhering tissues, dried, and saved for Ca determination (right femur) or for bone-strength measurements (left femur).

Analytical and Statistical Methods

Finely ground breads were analyzed for protein, fat, dietary fiber, and phosphorus by Approved Methods (AACC 1995). Ca levels in Ca carbonate, finely ground breads, test diets, serum, and femurs (ether-extracted and vacuum-dried) were determined by atomic absorption spectrophotometry (IL model Video 11, Baird Thermo Jarrell Ash Corp., Franklin, MA). Bone strength was determined with a texture analyzer (model TA.XT2, Texture Technologies Corp., Scarsdale, NY). Air-dried femurs were placed in an apparatus that held the bone (supported at the two extremities only) perpendicular to the rounded edge blade of the texture analyzer, and the force (g) required to break the bones was measured. The data were analyzed statistically by analysis of variance by the Tukey test for means separation (SigmaStat Statistical Software, Jandel Scientific Software, San Rafael, CA).

RESULTS AND DISCUSSION

Fortifying Flour

Calcium carbonate and Ca sulfate are the major sources of Ca used to fortify commercial flour. Carbonate is often preferred because it is less expensive and contains more Ca than Ca sulfate (Ranhotra et al 1980). The Ca carbonate used in this study contained 38.6% Ca.

Calcium was added to flour at four levels. The lowest level (211 mg/100 g of flour) is that mandated for enriched flour, a standard-

TABLE I
Quality Characteristics of Calcium-Fortified Breads

Score ^a	Bread ^b			
	A	B	C	D
Dough (27)	20.8	20.3	20.3	20.3
External (18)	14.8	15.5	15.5	14.5
Internal (55)	42.0	41.8	41.5	41.5
Total (100)	77.6	77.6	77.3	76.3

^a Maximum possible points are given in parentheses. Dough scores include sponge out of fermentation, dough out of mixer, and dough at make-up. External bread scores include symmetry, crust character, crust color, and break and shred. Internal bread scores include grain, texture, crumb body, crumb color, taste and aroma, and mouth-feel (bread D tasted chalky).

^b Added Ca/100 g of flour: bread A, 211 mg; bread B, 446 mg; bread C, 924 mg; and bread D, 1,412 mg.

ized product (CFR 1996). In nonstandardized products, Ca, like any other nutrient, may be added at any level consistent with product quality. In this study, Ca was added to flour at three higher levels; a limited assessment of sensory characteristics by four baking technologists suggested no adverse effect on bread quality (Table I), except for the chalky mouth-feel noticed at the highest level of Ca addition (bread D).

Virtually all the Ca in the breads originated from Ca added to the flour because other Ca sources (e.g., yeast food, milk replacers, Ca propionate) were excluded from the bread formula and only deionized water was used. Flour itself contributed an insignificant amount of Ca.

Assessing Calcium Status

More than 99% of the body's Ca is present in the skeletal mass. The presumption was made that the Ca level of the femur exemplifies the Ca status of the entire skeletal mass, and thus it was used as the parameter to assess the body's Ca status. It was also presumed that Ca from the Ca carbonate used in the test diets was as well absorbed as Ca from other potential sources, including dairy products. Several studies indicate this to be the case (Ranhotra et al 1981; Shiekh et al 1987; Lewis et al 1989; Garcia-Lopez and Miller 1991; Ranhotra et al 1997a,b). Serum Ca levels and Ca absorption

TABLE II
Composition of Bread-Based Test Diets

	Diet ^a			
	A	B	C	D
Dietary Ca, %	0.125	0.25	0.50	0.75
Diet composition, %				
Bread ^b	55.6	56.8	57.0	56.9
Casein ^c	10.2	10.0	10.0	10.2
Soybean oil	4.1	4.1	4.3	4.2
Constants ^d	7.1	7.1	7.1	7.1
Cornstarch	23.0	22.0	21.6	21.6
Dietary protein, %	15	15	15	15
Dietary phosphorus, %	0.4	0.4	0.4	0.4
Dietary fiber, %	3	3	3	3
Dietary fat, %	5	5	5	5

^a Flours used in breads for diets A, B, C, and D contained 211, 446, 924, and 1,412 mg of Ca/100 g, respectively.

^b Finely ground.

^c Virtually free of calcium.

^d Included (% in diet) cellulose, 0.3; vitamin mix, 2.2; mineral mix (Ca free, P free), 3.5; and NaH₂PO₄·H₂O, 1.1. Vitamin and mineral mixes (AIN no. 76) were obtained from ICN Biochemicals, Inc., Aurora, OH.

TABLE III
Tissue Calcium Concentration, Calcium Absorption, and Femur Strength^a

	Initial ^b	After Four Weeks ^c			
		Diet A (0.125% Ca)	Diet B (0.25% Ca)	Diet C (0.50% Ca)	Diet D (0.75% Ca)
Diet intake, g	...	376 ± 0a	376 ± 1a	376 ± 1a	375 ± 1a
Body weight gain, ^d g	...	150 ± 4a	150 ± 6a	151 ± 6a	148 ± 7a
Tissue concentrations ^e					
Serum Ca, mg/dL	10.1 ± 0.2a	10.3 ± 0.4a	10.2 ± 0.3a	10.3 ± 0.4a	10.4 ± 0.4a
Femur weight, mg	57 ± 10d	167 ± 21c	204 ± 21b	240 ± 14	245 ± 8a
Femur ash, %	42.6 ± 1.2c	42.3 ± 3.6c	54.1 ± 3.6b	57.9 ± 2.0a	57.4 ± 1.7a,b
Femur Ca (total), mg	7.1 ± 1.8d	22.3 ± 1.8c	32.9 ± 2.6b	44.8 ± 2.1a	44.8 ± 2.2a
Femur Ca, %	12.5 ± 1.4c	13.5 ± 1.4c	16.3 ± 1.5b	18.7 ± 1.2a	18.3 ± 1.0a
Femur strength, ^f g	...	2,994 ± 712b	6,347 ± 1,224a	6,243 ± 1,386a	6,799 ± 1,289a
Calcium absorption					
Ca intake, mg	...	470 ± 1d	939 ± 2c	1,878 ± 3b	2,814 ± 7a
Fecal Ca loss, mg	...	16 ± 3c	45 ± 12c	481 ± 56b	900 ± 128a
Ca absorbed, mg	...	454 ± 3d	894 ± 12c	1,398 ± 55b	1,914 ± 130a

^a Four-week study. Values are averages of 10 rats per diet ± standard deviation. Values within a row not sharing a common letter are different ($P < 0.05$).

^b Based on the group sacrificed on day 0.

^c Breads for diets A–D contained 211, 446, 924, and 1,412 mg of Ca/100 g of flour, respectively.

^d Initial body weight, 50 ± 3 g.

^e Values for femurs are expressed on a moisture-free, fat-free basis.

^f Force (g) required to break the bone in a texture analyzer unit.

(Ca balance) were also measured, but they are not viewed as sufficiently sensitive parameters to be reflective of the body's Ca status.

Weight Gains

Body weight gains of the four groups of rats were nearly identical after four weeks (Table III). Ca represents <2% of the body weight. Thus, although diet A provided submarginal Ca and diet D excessive Ca, the resultant differences in skeletal mass were likely inconsequential in affecting the overall body weight gains of the rats in any significant way.

Serum Calcium

Serum Ca levels of rats on all diets were in the normal range and nearly identical (Table III). Apparently, the very efficient homeostatic mechanism that keeps serum Ca levels, a critical parameter, within the normal range (Clark 1969, Miller 1989) was operative even in rats fed a submarginal (diet A) level of Ca.

Femur Weight and Ash

Expressed on a moisture-free, fat-free basis, femur weights increased significantly ($P < 0.05$) with increases in dietary Ca levels, except at the highest Ca level (Table III). Increases in femur ash content followed the same trend. Femur ash increased from 42.3% on diet A to 54.1% on diet B and then to 57.9% on diet C but not beyond (diet D). On a moisture-free, fat-free basis, ash represents about two-thirds of the mature bone mass. Apparently, the skeletal mass did not reach full maturity in this short-term study, and this ash level was not reached.

Femur Ca Content

Since nearly all of a body's Ca is present in the bones, bone Ca content has been used as an index of the body's Ca status. Femur Ca content, taken as indicative of the entire body's Ca status, increased significantly ($P < 0.05$) as dietary Ca levels increased, except at the highest dietary Ca level (Table III). Total femur Ca increased from 22.3 mg with diet A to 32.9 mg with diet B and to 44.8 mg with diet C; no further increase occurred with diet D. The same trend was observed in femur Ca increases expressed on a percentage basis. This strongly suggests that as Ca in the bread increased, it became increasingly more available (net amount) and improved the Ca status of the growing rats. However, this occurred only to the point at which Ca adequately met the body's need toward achieving maximum bone density (diet C) but not beyond (diet D).

Femur Strength

Bone strength was measured as a function of the weight required to break air-dried femurs. Bone strength doubled as the dietary Ca level increased from 0.125 to 0.25% (diet A vs. diet B) but not beyond. Thus, while Ca may be a critical factor in improving bone strength when intake is extremely low, it may be less of a determining factor at levels still considered low but not extremely low.

Calcium Absorption and Retention

As dietary Ca levels increased, fecal losses of Ca also increased (Table III). Although this resulted in a gradual decrease in the percentage of Ca absorbed, the amount of Ca absorbed increased significantly ($P < 0.05$). The amount absorbed was highest in rats on the highest dietary Ca level (diet D). However, this did not result in increased bone strength compared with that of rats on diet C, which adequately met the rats' Ca requirement. This suggests that substantially higher Ca intake does not necessarily result in better utilization of absorbed Ca, perhaps because more Ca is excreted in the urine.

CONCLUSIONS

Breads made with flour fortified with Ca at the U.S. enrichment standard provide only a modest amount in our diet, ≈ 60 mg per 50-g

serving. However, if flour is fortified with Ca at levels that exceed the enrichment standard, the resultant breads and related products can be a significant source of Ca. Thus, these products could play a significant role in improving the body's Ca status early in life. High levels of added Ca do not appear to adversely affect bread quality.

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