Utilization of Calcium in Breads Highly Fortified with Calcium as Calcium Carbonate or as Dairy Calcium

G. S. Ranhotra,[1,2] J. A. Gelroth,[1] and S. D. Leinen[1]

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ABSTRACT

White pan breads were prepared with flour highly fortified with calcium (Ca), using Ca carbonate (Ca, 38.8%) or a high Ca whey powder (Ca, 5.6%) as the Ca source; bread was also prepared using Ca carbonate plus lactose. Ca was added to flour at 924 mg/100 g of flour, a level 4.4 times higher than specified under the U.S. enrichment standards. Breads were dried and finely ground to prepare test diets (Ca, 0.5%) which were then fed to growing rats for four weeks (growth phase) or eight weeks (approaching maturity). At either interval, femur ash content, femur Ca content, femur strength, or Ca absorption values did not differ significantly among groups fed breads fortified either with Ca carbonate, Ca carbonate + lactose, or whey. Thus, breads can be highly fortified with Ca carbonate to be labeled as "high" in Ca, and this Ca may be as well absorbed and utilized as dairy Ca.

It is suggested that women should consume 1,000–1,500 mg of calcium (Ca) daily well before menopause to prevent early onset of osteoporosis (Berner et al 1990, Mortensen and Charles 1996, NAP 1997). Adequate Ca intake is also crucial for peak bone mass during growing years (Ulrich et al 1996, Carter and Whiting 1997). In addition, Ca plays a protective role against essential hypertension, gestational hypertension, and colorectal cancer (Gilliland et al 1987; Hambly et al 1997; Lapre and van der Meer 1992; Ranhotra et al, *in press*). Prompted by these various considerations, the National Research Council recently revised upward the recommended dietary allowances for Ca for many population groups (NAP 1997).

Besides educating consumers to eat more Ca-rich foods, other possible means for ensuring Ca adequacy may include taking Ca supplements and adding Ca-fortified nondairy foods to the diet. As part of the meal, Ca-fortified nondairy foods may be of particular interest to individuals who, for one reason or another, limit their intake of dairy foods, the major source of Ca for many population groups.

A number of foods, including flour, can be fortified (enriched) with Ca. According to the U.S. enrichment standards, flour can be fortified to contain 211 mg of Ca/100 g of flour. This enrichment, however, provides only a modest amount of Ca in finished products such as bread. It was recently shown that flour can be fortified to contain Ca at levels much higher than 211 mg without adversely affecting bread quality, and that this Ca is well absorbed and retained (Ranhotra et al 1999). In that study, Ca carbonate was used as the source of added Ca.

Different Ca sources may be used to fortify foods, but Ca carbonate remains the source of choice as it is reasonably priced, it contains more Ca as compared with most other Ca sources, and it appears to have little or no adverse affect on product quality. No firm conclusion has emerged as to how well the Ca in Ca carbonate is utilized as compared with Ca from dairy foods. Studies done to investigate this question report either no difference (Recker et al 1988, Poneros-Schneier and Erdman 1989, Mortensen and Charles 1996) or show slightly better Ca absorption from a dairy source, perhaps an effect of lactose (Armbrecht and Wasserman 1976, Wong and LaCroix 1980, Ranhotra et al 1981).

This study was not undertaken to arrive at a firm conclusion regarding utilization of Ca from dairy versus nondairy foods but as an extension of our recent study (Ranhotra et al 1999) and with a different objective. Breads were again highly fortified with Ca, and rats were used as the test model. However, the study differed in that it was split into two intervals to allow comparing Ca utilization both during the active growth phase of animals (four weeks after weaning) and at maturity (eight weeks after weaning). More importantly, the utilization efficiency of Ca in Ca carbonate was now compared with that of Ca in a dairy source when both are added to a finished product, namely bread. If differences between the two Ca sources are found to be insignificant, breads highly fortified with Ca carbonate may be viewed as not only a good source of total Ca but also of well-utilized Ca.

MATERIALS AND METHODS

Calcium Sources

Ca carbonate or a high Ca dairy source (whey powder) were used to fortify flour; these were added to provide 924 mg of Ca/100 g of flour, a level also tested in the previous study and which represents 4.4 times the amount of Ca specified under the U.S. enrichment standards. The Ca carbonate and whey powder that were used contained 38.8 and 5.6% Ca, respectively; the source of lactose used contained an insignificant (0.012%) amount of Ca.

Breadmaking

Pan breads (pound loaves) were made according to the standard sponge and dough method as described earlier (Ranhotra et al 1999). Flour was fortified (924 mg of Ca/100 g) in batch amounts with the test ingredients, and three sets of breads were prepared (Table I). These sets differed only in the source of Ca: Ca carbonate (bread A), Ca carbonate plus lactose (bread B), or a high Ca whey (bread C). Breads B and C contained the same level of lactose added either as a separate ingredient (bread B) or as part of the whey used (bread C). After baking, breads were cooled on a rack, sliced, dried, and finely ground. They were not scored because no adverse effect of Ca carbonate on bread quality was observed in the previous study.

Test Diets

Finely ground breads were used to formulate test diets (Table II). These diets contained the same level (0.5%) of Ca each which originated either from Ca-carbonate-fortified bread (diet A), Ca carbonate plus lactose-fortified bread (diet B), or whey-fortified bread (diet C). All diets were complete in nutrients required by the rats including Ca and phosphorus (NRC 1987).

Animals

Sixty-five weanling, Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) were housed individually in suspended mesh-bottom stainless steel cages in a controlled environment (24°C,

[1] Nutrition Research Program, American Institute of Baking, 1213 Bakers Way, Manhattan, KS 66505-3999.
[2] Corresponding author. Phone: 785-537-4750. Fax: 785-537-1493. E-mail: granhotra@aibonline.org

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TABLE I
Bread Formula (based on baker's %)

Ingredient	Bread A ($CaCO_3$)	Bread B ($CaCO_3$ + Lactose)	Bread C (Whey)
Sponge			
Bread flour	70	70	70
Yeast (compressed)	2	2	2
Water (deionized)	42	42	42
Dough			
Bread flour	30	30	30
Sugar (granulated)	7	7	7
Salt	2	2	2
Shortening	3	3	3
Calcium carbonate	2.38	2.38	. . .
Lactose	. . .	8.76	. . .
Whey (high Ca)[a]	. . .	. . .	16.59
Water (deionized)	20	22	24

[a] Level added (bread C) provides the same amount of Ca as Ca carbonate in breads A and B.

TABLE II
Composition of Bread-Based Diets

	Diet[a]		
	A	B	C
Diet composition, %			
Bread (finely ground)	59.7	62.6	63.0
Casein	10.5	10.6	9.8
Soybean oil	3.9	4.0	4.0
Cellulose	0.2	0.1	0.0
Vitamin mix (AIN 76)	2.2	2.2	2.2
Mineral mix (AIN 76)[b]	3.5	3.5	3.5
NaH_2PO_4	1.2	0.9	1.0
Corn starch	18.8	16.1	16.5
Dietary Ca, %	0.5	0.5	0.5
Dietary phosphorus, %	0.4	0.4	0.4
Dietary protein, %	15	15	15
Dietary fat, %	5	5	5
Dietary fiber, %	3.3	3.3	3.3

[a] Diets A–C correspond to breads A–C in Table I.
[b] Calcium and phosphorus free.

60% rh, and a 12-hr light and dark cycle). After a three-day adaptation period, these animals were weighed and assigned by selective randomization to six groups of 10 rats each and one group of five animals. The five-animal group was sacrificed at the start of the experiment (day 0) to obtain baseline information. Other groups were then fed bread-based diets daily: two groups were fed diet A, two diet B, and two diet C. One group from each set was sacrificed after four weeks and the other group after eight weeks.

Diets were fed to the animals daily, and records of diet intake were maintained throughout the study. Food intake of the rats increased gradually over time, but all animals were pair-fed to ensure that food intake, and thus Ca intake, was identical among all groups during each test period (Table III). Deionized water was offered ad libitum. Animals were weighed weekly. During both test periods, feces from each rat were collected from the trays underneath the cages (throughout 4 or 8 weeks). To minimize contamination from urine and spilled diet, feces were collected daily and were cleaned of adhering diet. They were then air-dried, weighed, finely ground, and stored frozen for Ca determination.

Tissue Sampling

After four or eight weeks on test diets, rats were lightly anesthetized (under ether), and 2 mL of blood was withdrawn by cardiac puncture. The clotted blood was centrifuged, and the serum obtained was analyzed for Ca. Rats were sacrificed under ether, and both femurs were removed, cleaned of adhering tissues, dried and saved for Ca determination (left femur) or for bone-strength measurement (right femur).

Analytical

AACC Approved Methods (AACC 2000) were used to analyze finely ground breads for protein, fat, and dietary fiber; phosphorus was determined by the method of Fiske and Subbarow (1925). Ca in Ca sources, finely ground breads, test diets, serum, and femur (ether-extracted and vacuum-dried) was determined by atomic absorption spectrophotometry (IL model Video 11, Baird Thermo Jarrell Ash Corporation, 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 ends only) perpendicular to the rounded edge blade of the texture analyzer, and the force (g) required to break the bones was measured.

Statistical

The data in Table III were analyzed statistically by analysis of variance using the Tukey test for means separation (SigmaStat Statistical Software, Jandel Scientific, San Rafael, CA).

RESULTS AND DISCUSSION

Calcium Sources

Because Ca carbonate is widely used in Ca supplements and fortified foods, it was chosen as the preferred source for fortification of the bread. Whey was used as a source of dairy Ca, with the high Ca whey allowing fortification of the bread at the high Ca level required (Table I). In breads and, thus, in bread-based diets (Table II), virtually all of the Ca originated from the two Ca sources used; flour contributed an insignificant amount.

Sensitive Parameters

Nearly all (>99%) of the body's Ca is present in the skeletal mass. Slight differences in the rate of calcification of bones at different sites in the body may occur. For the purpose of this study, however, it was presumed that the level of Ca in the femur exemplifies the Ca status of the entire body. Thus, this parameter was used to assess the body's Ca status. Serum Ca was also measured, but it is not viewed as a sensitive parameter reflective of the body's Ca status. Ca absorption values, perhaps more sensitive than serum Ca, may also be less revealing of the body's Ca status than femur Ca.

Weight Gain

Because <2% of the body weight is represented by Ca, differences in weight gain, even if observed, may not suggest differences in Ca status. Differences in weight gain were, in fact, not observed; weight gains among the three groups of rats were nearly identical within each experimental period (Table III). This may be, in part, due to Ca in all diets being provided at the required (NRC 1987) level.

Serum Calcium

Compared to the initial level of 9.7 mg/dL, serum Ca levels increased slightly but significantly ($P < 0.05$) by week four and then declined slightly by week eight (Table III). At both intervals, serum Ca levels did not differ significantly among diets. They also tended to be in the normal range throughout, apparently because a very efficient homeostatic mechanism keeps serum Ca levels, a critical parameter, in the normal range (Clark 1969, Miller 1989).

Femur Weight and Ash

Femur weights increased fourfold by week four and eightfold by week eight (initial weights vs. four-week and eight-week weights), but no significant differences were noted among the three diets at either interval (Table III). The accompanying increases in femur ash content, however, were modest, apparently because the increase in femur weights resulted mainly from an increase in the organic matrix and not the mineral content.

TABLE III
Femur Calcium Content, Femur Strength, and Apparent Calcium Absorption

		After Four Weeks			After Eight Weeks		
	Initial[a]	Diet A ($CaCO_3$)	Diet B ($CaCO_3$+Lactose)	Diet C (Whey)	Diet A ($CaCO_3$)	Diet B ($CaCO_3$+Lactose)	Diet C (Whey)
Diet intake, g		396 ± 1b[b]	396 ± 1b	396 ± 0b	846 ± 1a	846 ± 0a	846 ± 1a
Body weight gain,[c] g		151 ± 7b	154 ± 9b	155 ± 5b	261 ± 9a	264 ± 5a	266 ± 9a
Tissue concentration							
Serum Ca, mg/dL	9.7 ± 0.5d	10.5 ± 0.3a	10.5 ± 0.3a	10.4 ± 0.3a,b	10.2 ± 0.2a–c	10.0 ± 0.3b–d	9.8 ± 0.3c,d
Femur weight,[d] mg	51 ± 4c	234 ± 11b	235 ± 13b	242 ± 14b	402 ± 26a	404 ± 12a	409 ± 9a
Femur ash, %	39.8 ± 2.7 e	59.5 ± 1.7c,d	60.1 ± 2.1b,c	57.4 ± 2.9 d	62.0 ± 1.6a–c	63.2 ± 1.2a	62.6 ± 0.9a,b
Femur Ca (total), mg	6.2 ± 0.8c	46.3 ± 2.3b	46.4 ± 4.7b	45.7 ± 3.7b	86.2 ± 5.7a	86.3 ± 5.4a	82.2 ± 4.3a
Femur Ca, %	12.2 ± 1.5c	19.8 ± 1.3a,b	19.8 ± 1.9a,b	18.9 ± 1.6b	21.4 ± 0.6a	21.4 ± 1.1a	20.1 ± 1.0a,b
Femur strength,[e] g		9,225 ± 1,644b	9,384 ± 3,272b	9,993 ± 1,855a,b	10,319 ± 1,947a,b	12,682 ± 2,862a	12,063 ± 2,365a,b
Calcium absorption							
Ca intake, mg		1,978 ± 6b	1,978 ± 6b	1,980 ± 0b	4230 ± 5a	4,230 ± 0a	4,231 ± 4a
Fecal Ca loss, mg		426 ± 58b	411 ± 41b	450 ± 33b	1,418 ± 120a	1,385 ± 212a	1,298 ± 195a
Ca absorbed, %		78.5 ± 2.9a	79.2 ± 2.1a	77.3 ± 1.7a	66.5 ± 2.8b	67.3 ± 5.0b	69.3 ± 4.6b

[a] Based on a group of rats sacrificed at the start (day 0) of the experiment.
[b] Values are averages ± standard deviations of 10 rats per diet. Values followed by the same letter in the same row are not significantly different ($P < 0.05$).
[c] Initial body weight: 39 ± 2 g
[d] Values are expressed on fat-free, moisture-free basis.
[e] Force (g) required to break the right femur (air-dried) in a texture analyzer unit.

Compared with the initial level of 39.8%, femur ash increased rapidly and significantly ($P < 0.05$) by week four (Table III). In the next four weeks, the increase in percent femur ash was modest but still significant ($P < 0.05$). At maturity, ash represents about two-thirds of the femur weight (fat-free, moisture-free basis). This level was nearly reached by week eight, and thus compares Ca utilization both during the active growing phase (week four) and as animals near maturity (week eight).

Femur Calcium Content

Femur Ca content, taken as indicative of the entire body's Ca status, increased significantly ($P < 0.05$) at the end of week four and again at the end of week eight (Table III); total femur Ca, in fact, doubled between week four and week eight. As percent of femur, Ca also increased significantly ($P < 0.05$) by week four but, unlike total Ca, further increases in percent Ca were only modest and not significant ($P > 0.05$). This is typical of the bone calcification process.

At both intervals, lactose did not appear to promote a higher Ca retention as femur Ca on diet B (Ca carbonate + lactose) or diet C (whey) was no higher than that on diet A (Ca carbonate). A few studies done with animals have shown lactose as promoting Ca absorption (Armbrecht and Wasserman 1976, Wong and LaCroix 1980, Ranhotra et 1981) but convincing evidence has not emerged. In studies with human subjects, Recker et al (1988) concluded that none of the Ca sources tested (milk products, imitation milk or Ca carbonate) were significantly superior or inferior to the others. Studies by Mortensen and Charles (1996) arrived at a similar conclusion: that a Ca carbonate regimen in humans is at least as good a Ca supplement as milk. Even under conditions of decreased gastric acidity, as in the elderly, Ca carbonate, an insoluble Ca salt, is well absorbed if administered with a meal (Wood and Serfaty-Lacrosniere 1992); this may be true when Ca carbonate is added to a food like bread.

In this study, the effect of lactose was examined both as a component of whey (diet C) and as a separate component (diet B). As a separate component, lactose did not appear to promote the retention of Ca from Ca carbonate. It is possible that under experimental conditions different than this study, some positive effect of lactose can still be demonstrated, but such an effect is not likely to be profound.

Femur Strength

During the four-week and eight-week test periods, bone modeling and mineralization processes, including calcification, likely proceeded normally. Under such conditions, bone strength among groups is not likely to differ much. Results in Table III confirm this. After four weeks and, with one exception (diet B, week eight), after eight weeks, femur strength did not differ significantly among groups as well as between the two test periods ($P > 0.05$). Rats on diet B showed slightly higher strength at week eight, but this appears to carry limited physiological significance when the other data on diet B are examined.

Calcium Absorption

Ca intakes among groups of rats were identical within both test periods (Table III). Fecal loses of Ca during the four or eight week periods also did not differ significantly ($P > 0.05$). Consequently, the absorption of Ca did not differ significantly ($P > 0.05$) either. Ca absorption approached 80% during the first four weeks but dropped, as expected, significantly ($P < 0.05$) during the next four weeks. A further drop in Ca absorption would likely have occurred had the experiment been continued beyond week eight. That Ca absorption did not differ significantly among groups strongly supports the observations made for Ca status based on femur Ca content.

Overall Findings

Collectively, these results suggest that bread fortified with Ca carbonate may be as good a source of useable Ca as breads fortified with a dairy source of Ca. A study recently conducted comparing bread-based diet (Ca-enriched bread) and milk-based diet arrived at a similar conclusion (Juma et al 1999).

CONCLUSIONS

In groups of young rats fed breads fortified with Ca carbonate, Ca carbonate plus lactose, or a high Ca whey, femur ash content, femur Ca content, femur strength and Ca absorption values did not differ significantly. This strongly suggested that Ca from Ca carbonate is as well absorbed and retained as Ca from a dairy source. Ca carbonate can be added to bread at sufficiently high levels without adversely affecting bread quality.

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