JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Calcium Absorption from Three Salts and $CaSO_4$-Fortified Bread in Premenopausal Women

BERDINE R. MARTIN,† CONNIE M. WEAVER,*,† ROBERT P. HEANEY,‡ PATRICIA T. PACKARD,‡ AND DAVID L. SMITH§

Department of Foods and Nutrition, Purdue University, West Lafayette, Indiana 47907-1264, Osteoporosis Research Center, Creighton University, Omaha, Nebraska 68178, and Department of Chemistry, University of Nebraska, Lincoln, Nebraska 68588-0304

Two studies were conducted to measure calcium absorption from calcium sulfate fortified bread and three salts (calcium lactate, calcium carbonate, and calcium sulfate) in healthy premenopausal women using a crossover design. In study I, calcium fractional absorption levels from the three salts labeled with a stable isotope, ^{44}Ca, were not significantly different (0.039−0.47) as determined by the fecal recovery method. In a second study, absorption of ^{45}Ca from $CaSO_4$-fortified labeled bread or labeled milk was measured in serum exactly 5 h postingestion. Fractional calcium absorption was slightly but significantly greater from fortified bread than from milk (mean within subject difference = 0.0675 ± 0.016). Calcium sulfate is a bioavailable fortificant for white bread that compares favorably with milk and two other salts.

KEYWORDS: Calcium; stable isotope; absorption; bread; fortification

INTRODUCTION

Adequate calcium intake is important both for building peak bone mass in the first three decades of life and for attenuating loss of bone in later years. Average calcium intakes in the American diet fall well below calcium requirements (*1*) beginning in early pubertal years. Calcium intakes in adolescents fall below the 75th percentile for boys and the 90th percentile for girls as determined using the CSFII data (*1*). In adult women, calcium intakes fall below the 90th percentile. In adult men, calcium intakes decrease from the 50th percentile to the 95th percentile with age as calcium requirements increase and intakes decrease. Achieving the recommended calcium intake is difficult for those who do not include dairy products with every meal. Although some plant foods are good sources of calcium, it would be difficult on a Western diet to consume sufficient quantities of vegetables such as broccoli, bok choy, and kale to provide the majority of an individual's calcium needs (*2*). Fortified foods and supplements offer alternative sources of calcium to traditional foods. The National Academy of Science panel recommends that individuals who could not meet their calcium needs by traditional food consume these alternative sources. However, such alternative sources need to be evaluated in terms of the bioavailability of their calcium.

Measuring calcium bioavailability in humans using isotopic tracer techniques is considered the ideal approach to estimate calcium bioavailability. Intrinsic labeling of the salts or foods to be studied best represents the true physiological experience unless the appropriateness of an extrinsic label has been validated, as has been done for milk (*3*) and wheat (*4*). The purposes of the studies reported here were to use isotopic tracer methodology with premenopausal women to measure the absorption of calcium from three calcium salts (calcium carbonate, calcium lactate, and calcium sulfate) that are commonly used in supplements and fortified foods (study I) and to evaluate the bioavailability of one of them (calcium sulfate) as a fortificant for bread (study II).

MATERIALS AND METHODS

Preparation and Labeling of Calcium Salts. *Study I.* Calcium carbonate enriched in ^{44}Ca (98.57% enrichment, Oak Ridge National Laboratories, Oak Ridge, TN) was converted to calcium lactate and calcium sulfate by adding a slight molar excess of the respective anions in the form of lactic or sulfuric acid. Salts were precipitated upon cooling. The respective salts were then freeze-dried (Dura-Top, FTS Systems, Inc., Stone Ridge, NY). To ensure accurate doses, the hydration state of the respective compounds was verified by elemental analysis. Actual calcium content was measured by atomic absorption spectrometry (5100 PC, Perkin-Elmer Corp., Norwalk, CT). Stable isotope enrichment was quantified by high-resolution fast atom bombardment mass spectroscopy (*5*). Capsules were prepared containing 135 mg of Ca as $CaCO_3$, $CaSO_4$, or calcium lactate including 36 mg of ^{44}Ca.

Study II. Calcium sulfate used for bread fortification was labeled by dissolving the salt in the water used for dough-making and adding a sub-microgram quantity of ^{45}Ca as the chloride (Amersham, Arlington Heights, IL). This calcium sulfate solution was then added to a mixture of flour, yeast, and other ingredients, and a loaf of bread was baked in a bread maker (Panasonic, model SD-BT65P, Secaucus, NJ). The resulting bread was sliced, cubed, and dried to constant weight. The

* Address correspondence to this author at 1264 Stone Hall, Purdue University, West Lafayette, IN 47907-1264 [telephone (765) 494-8231; fax (765) 494-0674; e-mail weavercm@cfs.purdue.edu].
† Purdue University.
‡ Creighton University.
§ University of Nebraska.

10.1021/jf020065g CCC: $22.00
Published on Web 05/21/2002

added calcium raised the calcium content of the bread to 300 mg per serving. This amount was the targeted level of fortification to provide the amount of calcium delivered in a cup of milk. Cubes equivalent to one slice of bread (16.8 g) were used as the test substance. The tracer dose was 5.2 μCi per serving.

Subjects. Study I was conducted at Purdue University and study II at Creighton University. Healthy premenopausal women were recruited for both studies, but they were younger in study I (21.2 ± 2.5 years) than in study II (37.0 ± 5.7 years). Both weight (67.0 ± 18.7 kg, study I; and 69.7 ± 13.5 kg, study II) and height (165.6 ± 8.7 cm, study I; and 164.4 ± 5.5 cm, study II) were similar. None of the women smoked or were taking oral contraceptives or medication known to interfere with calcium absorption. Women were selected as a convenience sample but were milk drinkers and had never been diagnosed as lactose intolerant. Study II was conducted at Creighton University on 18 healthy women 22−45 years of age. All participants gave informed consent. Investigative protocols had been approved by the Purdue University and Creighton University Institutional Review Boards, respectively.

Feeding Protocols. *Study I.* For testing of the three salts, subjects were fed a controlled diet containing 1800 kcal, 73 g of protein, 56 g of fat, 823 mg of calcium, and 6−7 g of fiber per day for 4 days during the follicular phase of their menstrual cycles. On the third day subjects ingested the capsules described above with a breakfast consisting of orangeade, an omelet, white bread toast, margarine, and jelly for a total meal content of 250 mg of calcium. Total feces were collected in acid-washed containers for 2 days prior to and 12 days after isotope ingestion. Six grams of polyethylene glycol (PEG E3350, Dow Chemical Co., Midland, MI) was administered with the test meal to monitor completeness of fecal collections. PEG recovery was used to determine subject compliance but was not used to adjust recovery of ^{44}Ca. If PEG recovery had been <70%, the subjects would have been excluded. Each subject repeated the protocol in a randomized order with each salt separated by at least 4 weeks between tests.

Study II. For the study that compared the calcium of fortified bread to milk, test meals, constructed as described previously, were served in the morning after an overnight fast (*6*). In this instance, one of the two pieces of bread, which is a part of a typical test meal with this protocol, was calcium fortified and served, as noted, as croutons. The subjects were studied in a randomized crossover design using milk as the referent. The calcium load for both sources was 300 mg, equivalent to one serving.

Analyses. In study I, calcium absorption from the three salts was estimated from fecal recovery of tracer. All stools were weighed and combined in 3−4 day pools. Pooled feces were then homogenized in a Stomacher Lab Blender (Tekmar Co., Cincinnati, OH) after appropriate quantities of distilled water had been added to form a slurry of pourable consistency. To facilitate final quantitation, accurate weights were taken at each step, and a fecal/slurry weight ratio was calculated. Concentrated HCl (1−4 mL) was added to each pooled sample to keep the calcium in a soluble state. After homogenization, aliquots were taken for total calcium measurement, stable isotope measurement, and PEG quantitation. All aliquots were lyophilized (Dura-Top, FTS Co.). Samples for determination of total calcium and stable isotope ratios were dry-ashed at 600 °C for 3 days. The ash was dissolved in 1 mol/L HCl. Aliquots specified for total Ca determination were diluted appropriately with 0.5 mol/L HCl containing 0.5% lanthanum as $LaCl_3$. Total calcium was measured by atomic absorption spectrophotometry (Perkin-Elmer 5100 PC). Laboratory technique was verified by calcium determination of NBS wheat flour (standard reference material 1567, National Bureau of Standards, Washington, DC). Average calcium values (193.83 ± 8.7 μg/g; CV = 4.49%) were similar to certified values (190 ± 10 μg/g).

Isotopic abundance was determined by high-resolution fast atom bombardment mass spectrometry (*5*). Fractional absorption was determined as the difference between the administered isotopic dose and the quantity of ^{44}Ca excreted in the feces, divided by the respective dose. PEG was analyzed by a modification of the turbidimetric method of Malawer and Powell (*7*).

In study II, absorption from the ^{45}Ca-labeled fortified bread was measured from the level of ^{45}Ca in serum calcium at exactly 5 h after oral ingestion, as described previously (*8*, *9*).

Table 1. Calcium Absorption in Premenopausal Women (n = 6) from Salts (Study I)

salt	fractional absorption[a] (mean ± SD)
calcium carbonate	0.39 ± 0.07
calcium sulfate	0.41 ± 0.07
calcium lactate	0.47 ± 0.08

[a] Means were not significantly different (p < 0.05) as determined by ANOVA.

Table 2. Calcium Absorption in Premenopausal Women (n = 18) from Milk and $CaSO_4$-Fortified Bread (Study II)

	fractional absorption (mean ± SEM)
milk	0.3627 ± 0.022
$CaSO_4$-fortified bread	0.4302 ± 0.025
within-subject difference (bread minus milk)	0.0675 ± 0.016[a]

[a] Significantly different from 0 (p < 0.05), paired t test.

Differences in fractional absorption from intrinsically labeled salts were considered to be significant at $p < 0.05$ using analysis of variance. The single pairwise comparison, within subject, of the difference in fractional absorption from milk and calcium sulfate enriched bread was analyzed with a standard paired t test (*10*).

RESULTS

Average fractional absorption values of the intrinsically labeled calcium carbonate, calcium sulfate, and calcium lactate are given in **Table 1**. Analysis of variance indicated that there were no differences in fractional calcium absorption among the salts. In the crossover study comparing milk and calcium sulfate enriched bread, the mean within-subject difference (bread minus milk) was significantly different from zero, the value predicted under the null hypothesis (**Table 2**). This indicates superior absorbability for the calcium in the fortified bread; however, the difference was relatively small and may have little nutritional significance.

DISCUSSION

Calcium fractional absorptions from $CaSO_4$ as a salt or incorporated into bread were nearly identical in both studies despite the different vehicles, the different methods employed in the two protocols, and the different study populations. Thus, calcium ingested as one of these three salts as a fortificant in food was at least as good as milk calcium.

Calcium carbonate, calcium lactate, and calcium sulfate are three of the most commonly used salts in supplements and fortification of foods. Calcium absorption values from the three salts were not significantly different from one another despite a range of aqueous solubility spanning 3 orders of magnitude (0.14 mM/L for $CaCO_3$ vs 15.4 mM/L for $CaSO_4$ and 103.4 mM/L for calcium lactate). Previous studies have also shown that solubility in water has little impact on calcium absorption in humans (*11*). Similar results were also seen when the lavage method of measuring calcium absorption was used. Shiekh et al. (*12*) concluded that several calcium salts including carbonate and lactate and calcium from milk were similarly absorbed in young men when ingested in a fasting state.

Calcium retention in premenopausal women has been previously reported and found to be equivalent for lactate, sulfate, and carbonate salts using balance techniques (*13*, *14*). We felt that calcium absorption from common salts should be reevaluated using newer isotopic tracer techniques. Balance studies have

much higher variability than isotopic techniques and often fail to show even large differences. For example, calcium absorption from milk was found to be significantly greater than that from spinach (27.6 ± 8.8 vs 5.1 ± 2.6%) using isotopic tracer techniques (*6*), whereas balance studies failed to show a significant difference for spinach versus dairy products (*15*). Furthermore, in healthy adults with a fully formed skeleton, retention is a poor measure of absorbability because calcium balance under such conditions would be expected to be zero.

$CaCO_3$ and $CaSO_4$ are preferred sources of calcium for the fortification of cereal-based foods as they minimally affect product quality. In young male rats, calcium bioavailability from bread fortified with 1 of 10 different sources including carbonate, sulfate, and lactate was compared (*16*). All sources resulted in equal values for growth, apparent calcium absorption, and femoral calcium content.

This study supports the use of $CaSO_4$ as a highly available source of calcium for premenopausal women when used as a fortificant in white bread. The absorption of the calcium salt from the bread compares favorably with that of milk and does not differ when compared to calcium lactate and calcium carbonate.

LITERATURE CITED

(1) Institute of Medicine. *Dietary Reference Intakes: Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*; National Academy Press: Washington, DC, 1997.

(2) Weaver, C. M.; Plawecki, K. L. Dietary Calcium Adequacy of a vegetarian diet. *Am. J. Clin. Nutr*. **1994**, *59*, 1238−1241.

(3) Nickel, K. P.; Martin, B. R.; Smith, D. L.; Smith, J. B.; Miller, G. D.; Weaver, C. M. Calcium bioavailability from bovine milk and dairy products in premenopausal women using intrinsic and extrinsic labeling techniques. *J. Nutr.* **1996**, *126*, 1406−1411.

(4) Weaver, C. M.; Heaney, R. P.; Martin, B. R.; Fitzsimmons, M. L. Extrinsic vs. intrinsic labeling of the calcium in whole-wheat flour. *Am. J. Clin. Nutr.* **1992**, *55*, 452−454.

(5) Jiang, X.; Smith, D. L. Quantitation of stable isotopes of calcium by fast atom bombardment mass spectrometry. *Anal. Chem.* **1987**, *59*, 2570−2574.

(6) Heaney, R. P.; Weaver, C. M.; Recker, R. R. Calcium absorbability from spinach. *Am. J. Clin. Nutr.* **1988**, *47*, 707−709.

(7) Malawer, S. J.; Powell, D. W. An improved turbidimetric analysis of polyethylene glycol using an emulsifier. *Gastroenterology* **1967**, *53*, 250−256.

(8) Heaney, R. P.; Recker, R. R. Estimation of true calcium absorption. *Ann. Intern. Med.* **1985**, *103*, 516−521.

(9) Heaney, R. P.; Recker, R. R. Estimating true fractional calcium absorption. *Ann. Intern. Med.* **1988**, *108*, 905−906.

(10) Snedecor, G. W.; Cochran, W. G. *Statistical Methods*, 6th ed.; Iowa State University Press: Ames, IA, 1973.

(11) Heaney, R. P.; Recker, R. R.; Weaver, C. M. Absorbability of calcium sources: the limited role of solubility. *Calcif. Tissue Int.* **1990**, *46*, 300−304.

(12) Sheikh, M. S.; Santa Ana, C. A.; Nicar, M. J.; Schiller, L. R.; Fordtran, J. S. Gastrointestinal absorption of calcium from milk and calcium salts. *N. Engl. J. Med.* **1987**, *317*, 532−536.

(13) Patton, M. B.; Sutton, T. S. The utilization of calcium from lactate, gluconate, sulfate, and carbonate salts by young college women. *J. Nutr*. **1952**, *48*, 443−452.

(14) Spencer, H.; Scheck, J.; Lewin, I.; Samachson, J. Comparative absorption of calcium from calcium gluconate and calcium lactate in man. *J. Nutr*. **1996**, *89*, 283−292.

(15) Johnston, F. A.; McMillan, T. J.; Falconer, G. D. Calcium retained by young women before and after adding spinach to the diet. *J. Am. Diet. Assoc.* **1952**, *28*, 933−938.

(16) Ranhotra, G. S.; Lee, C.; Gelroth, J. A. Expanded cereal fortification: bioavailability and functionality (bread making) of various calcium sources. *Nutr. Rep. Int.* **1980**, *22*, 169−175.

Received for review January 22, 2002. Revised manuscript received April 17, 2002. Accepted April 17, 2002. This work was funded by U.S. Department of Agriculture Grant 88-37200-3695 and the Continental Baking Co., St. Louis, MO.

JF020065G