Bioavailability of Calcium from Tofu as Compared with Milk in Premenopausal Women

C.M. WEAVER, R.P. HEANEY, L. CONNOR, B.R. MARTIN, D.L. SMITH, AND S. NIELSEN

ABSTRACT: Using a cross-over design, two studies were conducted to measure calcium absorption from calcium-set tofu compared to milk in healthy, premenopausal women. In "study 1," calcium absorption from tofu set with $CaCl_2$ was determined in Caucasian women by fecal recovery of the stable isotope, ^{44}Ca. In "study 2," calcium absorption was determined in Asian women from tofu set with $CaSO_4$ by appearance of ^{45}Ca in serum after 5 h. Analysis of the studies, both separately and pooled, showed that calcium absorption was similar between calcium-set tofu and milk. Calcium-set tofu is a concentrated source of bioavailable calcium.

Keywords: calcium bioavailability, tofu, humans

Introduction

MEDIAN INTAKES OF CALCIUM IN WOMEN ARE LOWER THAN THE recommended intakes in the U.S.A. and many other countries (Institute of Medicine 1997). While over 70% of dietary calcium in the United States comes from dairy products, many individuals do not consume dairy products in sufficient quantities to meet the recommended intakes. Calcium-rich soy products are popular alternative calcium choices for some populations, particularly in Asia. Although soybeans are high in both oxalate and phytate, calcium from whole soybeans is well tolerated by lactase-deficient individuals. They also have a fractional absorption efficiency comparable to that of milk, although inversely related to phytate content (Heaney and others 1991). On the other hand, calcium fractional absorption from fortified soymilk is only 75% of cow's milk (Heaney and others 2000).

Among the few calcium-fortified foods, tofu provides calcium in concentrations comparable to milk in an acceptable serving-size portion. United States Department of Agriculture (USDA) Handbook 8 lists the calcium content of milk as 117 mg/100g and tofu as 128 mg/100g. Poneros and Erdman (1988) found that calcium in tofu was highly bioavailable to rats, 107% compared to 100% for $CaCO_3$. But bioavailability of calcium from tofu has not been studied in humans.

The purpose of this study was to compare in humans the bioavailability of calcium from tofu to that for milk, using 2 different methodologies and populations. In the 1st study, calcium absorption was determined in Caucasian women by fecal recovery of a stable calcium isotope. In the 2nd study, calcium absorption was determined in Asian women using a 5-h post-administration blood sample of ^{45}Ca.

Materials and Methods

THIS STUDY'S PROTOCOLS WERE APPROVED BY THE PURDUE AND Creighton University's "Human Subjects in Research" Committees. Healthy Caucasian women, aged 19 to 24 at Purdue University and healthy Asian women, aged 20 to 38.5, at Creighton University were recruited from those campuses. Exclusion criteria included individuals with predisposing conditions known to influence calcium absorption (thyroid dysfunction, diabetes mellitus, kidney disease, hypertension, gastrointestinal disorders, steroid therapy, pregnancy and lactation, past or current eating disorders, and amenorrhea) as well as women who were taking medications known to impact calcium absorption directly.

Preparation of labeled test foods

Milk. In study 1, each subject received 36 mg of ^{44}Ca as $^{44}CaCl_2$ (prepared from $^{44}CaCO_3$ by dissolving in a molar excess of HCl, filtering with a Buchner funnel, and freeze-drying), added to 67.3 grams of liquid 2% lowfat milk. Extrinsic labeling with $^{44}CaCl_2$ was done the night before ingestion to assure equilibration of the calcium. Extrinsic labeling of milk by this method previously has been shown to give values for calcium absorption not different from intrinsically labeled milk (Nickel and others 1996). Six representative samples (0.5 mL each) of labeled milk were chosen randomly and evaluated for ^{44}Ca enrichment and calcium content.

Tofu. In study 2, whole milk was labeled by the addition of 5μCi $^{45}CaCl_2$ per serving and stored at 4 °C for 14 to 16 h until administration to the subjects.

Preparation of the tofu was the same for both studies except for the calcium salts and coagulants (Shurtleff and Aoyagi 1984). Silken tofu was manufactured in individual portions to prevent expression of whey (Shen and others 1991). In study 1, silken tofu was set with $CaCl_2$ containing 36 mg ^{44}Ca as $^{44}CaCl_2$ and 94.5 mg glucono-delta-lactone (GDL) (Griffith Labs, Scarborough, Ontario, Canada) to maximize stable isotope recovery. In study 2, $^{45}CaSO_4$ was used as the coagulant. Tofu was prepared the evening before ingestion. Washed soybeans (150 g) were soaked overnight, then made into a paste by placing the beans into an Osterizer® for 1.5 min on the highest setting. The paste was filtered under pressure through 2 layers of cheesecloth to produce soymilk. Percent soluble solids, relating to percent protein, were evaluated with a handheld refractometer. A target of 10% soluble solids in the soymilk was set. If the soymilk soluble solids' values were low, the soy paste was filtered until the desired solids level was reached. Soymilk was heated to 85 °C and held for at least 5 min to inactivate trypsin inhibitors. One hundred mL of the heat-treated soymilk, plus coagulants, were added to a preweighed container. Covered tofu samples were tempered in an 85 °C water bath for 1 h to set the curd. Samples were refrigerated until consumption. Containers were weighed following consumption to determine amount of tofu consumed. Subjects were asked to consume the whole portion quantitatively.

Study 1 protocol

At Purdue University, free-living subjects participated in a crossover design consisting of two 14–d periods. Test foods (either tofu or milk labeled with ^{44}Ca) were assigned to each subject in random order. Subjects consumed controlled diets for 4 d during the follicular phase of their menstrual cycle. Breakfast on the 3rd d consisted of either ^{44}Ca labeled tofu or ^{44}Ca labeled milk. The calcium load of the test meal was 250 mg, including 36 mg of ^{44}Ca tracer as $^{44}CaCl_2$ in the test product. Sufficient unlabeled milk was given along with the extrinsically labeled milk to provide a total calcium load of 250 mg. Milk was excluded from the noon meal on the day the tracer was administered.

This controlled diet assured consistency of intestinal contents prior to administration of the isotope. The diet provided an average of 851 ± 277 mg calcium daily, thus reflecting average daily intakes in this subpopulation determined by 8–d diet records. On "d zero" of each trial, fecal samples were collected into labeled, weighed, acid-washed containers for 13 consecutive d, beginning with baseline 1 d before the controlled diet. A washout period of 3 wk to 1 mo (that is, a menstrual cycle) occurred between trials. Each subject served as her own control.

Sample processing

Fecal samples were weighed, pooled, then homogenized with ultrahigh purity water (1:1.5 to 2.0), and with concentrated HCl (1 mL per 100 mL fecal pool weight) in a stomacher (Tekmar Co., Cincinnati, Ohio, U.S.A.). Baseline fecal samples were processed separately. Aliquots were frozen at –10 °C for later calcium analysis.

Analysis

Tofu contained 1.96 ± 0.53% phytate (net weight basis), as determined by the method of Latta and Eskin (1980).

The calcium concentrations of diluted tofu, milk, and fecal samples were measured by flame atomic absorption spectrometry (AAS) (Perkin-Elmer 5100PC; Norwalk, Conn., U.S.A.). Aliquot samples were placed into preweighed, acid-washed, porcelain crucibles and frozen for a minimum of 24 h at –10 °C. Samples were lyophilized for 5 d in a DuraDry Freeze-Dryer (Model PAC-TC-44; FTS Systems, Inc., Stone Ridge, N.Y., U.S.A.). Lyophilized samples then were ashed in a muffle furnace at 600 °C for a minimum of 72 h prior to diluting with 0.5N HCl and 0.5% La as $LaCl_3$ for determination of total calcium.

As determined by AAS, the calcium content of Durham wheat flour (Reference Material Number 8436; National Institute of Standard Technology, Gaithersburg, Md., U.S.A.) did not differ significantly from the certified values (297 ± 7 mg/kg as compared to 278 ± 16 mg/kg, respectively).

^{44}Ca isotope measurement

Following the method of Liu and others (1989), duplicate food and fecal samples diluted 1:10 with 1N HCl were analyzed for ^{44}Ca enrichment with a Kratos MS-50 Fast Atom Bombardment Mass Spectrometer (FABMS; Kratos Analytical Instruments, Ramsey, N.J., U.S.A.) which was equipped with a fast atom bombardment ion source. Precision (about 0.2%) and accuracy (RSD of natural abundance = 0.3%) of isotope ratios in biological samples by FABMS are given in the method's paper by Jiang and Smith (1987).

The quantity of tracer in the sample (mmole) was determined as follows:

$$\text{Amount of tracer} = \text{IER} \times \text{NA} \times \text{Total Ca}$$

where IER corresponds to the measured isotope enrichment ratio of ^{44}Ca to ^{40}Ca, NA corresponds to natural abundance of ^{44}Ca, and Total Ca is total calcium in the sample (mmole) as obtained by AAS. The percent calcium absorption from the test meals was determined by taking the sum of tracer excreted, deleting the baseline sample contribution, and dividing the total by the amount of tracer in the original test dose.

$$\%\text{Ca absorption} = 100 - \left(\frac{\text{mg } ^{44}\text{Ca excreted in feces - mg } ^{44}\text{Ca baseline}}{\text{mg } ^{44}\text{ Ca test dose}} \times 100 \right)$$

Table 1—Subject characteristics

Subject Characteristics[a]	Study 1 (n = 9)	Study 2 (n = 11)
Age (years)	21 ± 1.15	25 ± 4.96
BMI[b]	21.28 ± 2.15	22.47 ± 4.05
Daily Calcium Intake (mg)	851 ± 277	ND[e]
Daily Calcium Intake (% AI)[c]	85 ± 28	ND
Daily Protein intake (g)	79 ± 27	ND
% RDA Protein[d]	172 ± 58	ND

[a]Mean ± S.D.
[b]BMI = Body Mass Index
[c]Adequate Intakes Ca = 1000 mg/day
[d]RDA Protein = 45 mg/day
[e]ND = not determined

Statistical analysis

Data were analyzed using SAS Version 6.0 (SAS Institute, Cary, N.C., U.S.A.). Student *t* tests were used to compare means of the percent apparent absorption from milk against tofu. Linear regression analysis of calcium and protein intake as compared with percent calcium absorption from tofu and milk was performed with Microsoft Excel® Version 5.0 (Microsoft Corp. 1993).

Study 2

At Creighton University, free-living subjects participated in calcium absorption studies tested on D 1 to 14 of their menstrual cycle (follicular phase), and only after a negative pregnancy test (performed on the same morning as the absorption study). Each test meal consisted of the labeled skim milk or tofu, 2 slices of low-calcium Italian bread, toasted and served with butter, as well as coffee or tea (with artificial sweetener, if desired). Test meals were ingested at breakfast after an overnight fast and were consumed under the supervision of a project nurse. To assure complete ingestion of the tracer, the serving containers were repeatedly rinsed with distilled water following consumption of their contents, and the rinsings consumed as well.

Analytical methods

Absorption was measured from the concentration of the orally administered isotope in the calcium of a blood sample drawn at precisely 5 h after ingestion of the test load, with fractional absorption calculated using methods slightly modified, as described elsewhere (Heaney and Recker 1985, 1988). Briefly, absorption fraction (AbsFx) is given by:

$$\text{AbsFx} = 0.3537 * (SA_5^{\,0.92373}) * (Ht^{0.92373}) * (Wt^{0.37213})$$

in which SA_5 = 5–h serum calcium-specific radioactivity (fraction of oral dose/g calcium); Ht = height (meters); and Wt = weight (kg). This equation adjusts specific activity in the blood for body size,

Table 2—Calcium absorption from tofu and milk in premenopausal women

		Sample Size	Calcium Test Load (mg)	Calcium Absorption from Milk[a] %	Calcium Absorption from Tofu[a] %
Study 1	$\bar{X}$	9	250	54.8	49.3
Caucasian women	S.D.			± 12.1	± 16.7
Study 2	$\bar{X}$	11	200	39.8	39.0
Asian women	S.D.			± 10.1	± 11.5

[a]Mean ± S.D.

which affects the exchangeable pool. This method has been found to correlate well with a double isotopic tracer method.

Serum calcium was directly measured by atomic absorption spectrophotometry on a Perkin-Elmer Model 2380 (Perkin-Elmer, Norwalk, Conn., U.S.A.). The tofu calcium was analyzed initially by ashing, then by atomic absorption of the dissolved ash. ^{45}Ca was measured by liquid scintillation counting in a Beckman LS-3150T counter (Beckman Instruments, Inc., Irvine, Calif., U.S.A.). Samples were counted with the counting rate error as ≤ 2%.

Statistical Methods

Differences between mean absorption values for the 2 sources were tested by paired *t* tests. An orthogonal regression test was performed to test the null hypothesis that milk calcium absorption was not different from tofu calcium absorption (that is, slope = 1.0 at $p > 0.05$) (Goldberger 1968).

Results and Discussion

SUBJECT CHARACTERISTICS FOR STUDY 1 ARE PRESENTED IN TABLE 1. Subjects in study 1 were slightly younger than those in study 2, but had similar body mass index (BMI).

Calcium absorption within study from milk and tofu was statistically similar for both the Caucasian women of study 1 and the Asian women of Study 2 (Table 2). The paired comparisons within subjects are shown graphically in Figure 1. Although both calcium sources were absorbed similarly by each group, the Caucasian women absorbed calcium more efficiently than the Asian women. An orthogonal regression analysis of the derived slope of tofu calcium absorption on milk calcium absorption resulted in a slope of 1.22 ± 0.55. The slope was not different from the null hypothesis of a slope of 1, $p = 0.37$. A least squares regression analysis of ^{44}Ca absorption from tofu and milk against average pre-study calcium intakes in study 1 is shown in Figure 2. Approximately 21% of the variance was accounted for by usual calcium intake.

True calcium absorption efficiency was effectively identical ($p > 0.05$) between tofu and milk in healthy women. Soybean products would not be expected to provide calcium in a form that was as bioavailable as milk because of their high phytate and oxalate content, known inhibitors of calcium absorption. We reported previously that although phytate significantly affected calcium absorption, calcium bioavailability from intrinsically labeled soybeans of vary-

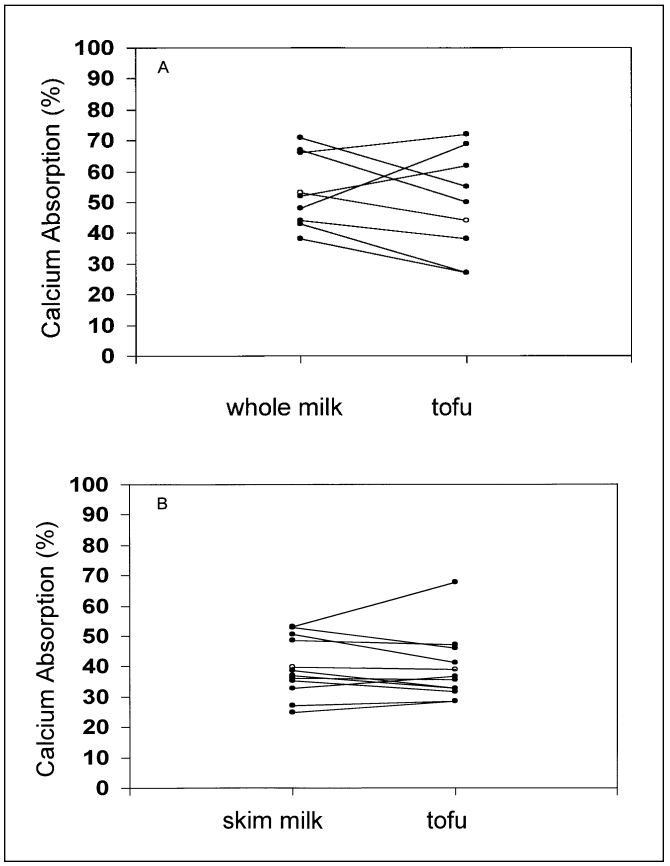

Figure 1—Calcium absorption (%) from milk and tofu in premenopausal (a) Caucasian women (Study 1); and (b) Asian women (Study 2). Each line represents 1 subject. ○ = absorption values

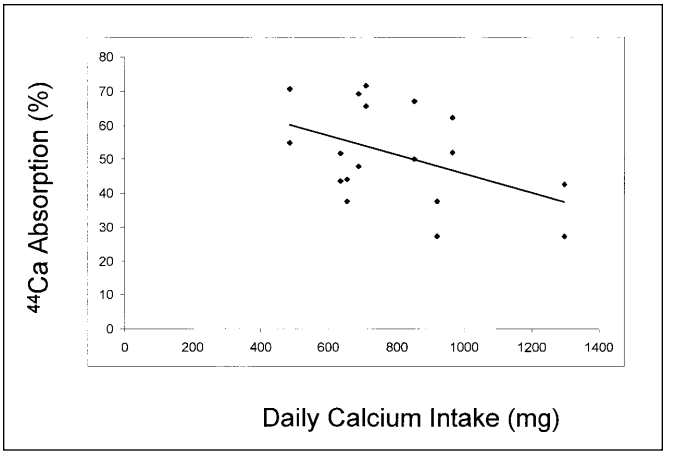

Figure 2—Least squares regression line comparing daily recorded calcium intake to percent ^{44}Ca absorption from tofu and milk ($R^2 = 0.21$, $p = 0.05$) (study 1). ◆ = milk and ◇ = soy

ing phytate levels was nearly equal to milk (Heaney and others 1991). However, that study used whole soybeans whereas the present study used a soy food. Soybeans are typically processed prior to consumption into food ingredients or into more easily digested forms, such as tofu.

Calcium absorption values from milk in the studies reported here were somewhat higher than values for calcium from milk on similar loads reported previously by Heaney and others (1988). The 53% calcium absorption from milk in the Caucasian women in study 1 is similar to that reported previously in young women at Purdue University (44% from a calcium load of 250 mg), using complete kinetic analysis and the same calcium load given with milk (Wastney and others 1996) and to the values (45.5% on a calcium load of 150 mg) reported by Fairweather-Tait and others (1989).

It is tempting to conclude that Caucasian women (study 1) absorbed calcium from milk and tofu more efficiently than did Asian women (study 2). However, because different methods were used in these studies, it is premature to make conclusions based on race, especially given that the Caucasian women on average were slightly younger than the Asian women. We have previously shown that women over age 21 were not in positive calcium balance in contrast to those below age 21 (Weaver and others 1995). Differences did not relate to adaptation to tofu, because neither group regularly consumed tofu.

Evaluation of calcium intakes from diet records in study 1 allowed analysis of the effect of this surrogate marker of calcium status on calcium absorption efficiency. Mean calcium intakes were only 85% of calcium requirements for this age group. As determined by regression analysis of daily calcium intake as compared with percent ^{44}Ca absorption, 21% of the variability in calcium absorption efficiency was explained by calcium intake. Subjects who typically consumed lower amounts of calcium in their diets had higher calcium absorption efficiencies. O'Brien and others (1996) utilized stable isotope methodology to investigate the ability of girls to respond to acute periods of inadequate dietary calcium intake. In 11 girls aged 11.6 ± 2.4 years, fractional calcium absorption was significantly greater (0.582 ± 0.087 as compared with 0.260 ± 0.068, $p < 0.0001$) from a low-calcium diet (7.05 ± 2.03 mmol/d) than a high-calcium diet (35.3 ± 2.28 mmol/d). Additionally, urinary calcium losses were decreased as calcium intake was lowered (3.08 ± 1.98 against 1.30 ± 0.83 mmol/day, $p < 0.004$). These results suggest that during periods of inadequate calcium intake, girls are able to significantly increase the efficiency of calcium absorption and decrease urinary calcium losses to conserve calcium required for bone mineral acquisition. Although the young women in study 1 were beyond the rapid growth period of adolescence, they were still acquiring bone mass through consolidation.

Conclusions

REGARDLESS OF THE METHODOLOGICAL AND SUBJECT DIFFERENCES between each study, and the higher calcium absorption efficiencies in study 1, both studies showed that calcium absorption from tofu was equivalent to that of milk. Calcium-set tofu is a concentrated source of bioavailable calcium for individuals who may not ingest enough dairy products to meet minimum daily requirements. In addition, both milk and tofu provide good quality protein as well.

References

Fairweather-Tait SJ, Johnson A, Eagles J, Ganatra S, Kennedy H, Gun MI. 1989. Studies on calcium absorption from milk using a double-label stable isotope technique. Brit J Nutr 62:379-388.
Goldberger AS. 1968. Topics in regression analysis. New York: Macmillan. P 69.
Heaney RP, Dowell MS, Rafferty K, Bierman J. 2000. Bioavailability of the calcium in fortified imitation milk with some observations on method. Am J Clin Nutr 71:1166-1169.
Heaney RP, Recker RR. 1988. Estimating true fractional calcium absorption. Ann Int Med 108:905-906.
Heaney RP, Recker RR. 1985. Estimation of true calcium absorption. Am Int Med 103:516-521.
Heaney RP, Weaver CM, Fitzsimmons ML. 1991. Soybean phytate content: effect on calcium absorption. Am J Clin Nutr 53:745-747.
Institute of Medicine. 1997. Dietary Reference Intake. Food and Nutrition Board. Washington, D.C.: National Academy Press. P 71-145
Jiang X, Smith DL. 1987. Quantitation of stable isotopic tracers of calcium by fast atom bombardment mass spectrometry. Analyt Chem 59:2570-2574.
Latta M and Eskin M. 1980. A simple and rapid colorimetric method for phytate determination. J Agric Food Chem 28:1313-1315.
Liu YM, Neal P, Ernst J, Weaver C, Rickard K, Smith D, Lemons J. 1989. Absorption of calcium and magnesium from fortified human milk by very low birthweight infants. Ped Res 25:496-502.
Nickel KP, Martin BR, Smith DL, Smith JB, Miller GD, Weaver CM. 1996. Calcium bioavailability from bovine milk and dairy products in premenopausal women using intrinsic and extrinsic labeling techniques. J Nutr 126:1406-1411.
O'Brien KO, Abrams SA, Liang LK, Ellis KJ, Gagel RF. 1996. Increased efficiency of calcium absorption during short periods of inadequate calcium intake in girls. Am J Clin Nutr 65:1570.
Poneros AG, Erdman JW Jr. 1988. Bioavailability of calcium from tofu, tortillas, nonfat dry milk, and mozarella cheese in rats: effect of supplemental ascorbic acid. J Food Sci 53:208-210.
Shen CF, de Man L, Buzzell RI, de Man JM. 1991. Yield and quantity of tofu as affected by soybean and soymilk characteristics: glucono-delta-lactone coagulant. J Food Sci 56(1):109-112.
Shurtleff W, Aoyagi A. 1984. Soymilk. In: Tofu and Soymilk Production. 2nd ed. Lafayette, Calif.: Soyfoods Center. P 195-234.
Wastney ME, Ng J, Smith D, Martin BR, Peacock M, Weaver CM. 1996. Differences in calcium kinetics between adolescent girls and young women. Am J Physiol 271(R):208-216.
Weaver CM, Martin BR, Plawecki KL, Peacock M, Wood OB, Smith DL, Wastney ME. 1995. Differences in calcium metabolism between adolescent and adult females. Am J Clin Nutr 61:577-581.
MS 20010604 Submitted 10/29/01, Revised 5/24/02, Accepted 5/31/02, Received 6/5/02

This work was funded by Indiana Value Added.

Authors Weaver and Martin are affiliated with the Dept. of Foods and Nutrition at Purdue University, West Lafayette, IN 47907. Author Nielsen is with the Purdue Dept. of Food Science. Author Heaney is with Creighton University, Omaha, NE 68178. Author Connor is affiliated with Amway Corporation, Ada, MI 49355. Author Smith is with the University of Nebraska, Lincoln, NE 68588-0304. Direct inquiries to author Weaver (E-mail: weavercm@cfs.purdue.edu).