

Original Article

Calcium absorption in Australian osteopenic post-menopausal women: an acute comparative study of fortified soymilk to cows' milk

Anne Lise Tang BSc(Hons)¹, Karen Z Walker PhD², Gisela Wilcox MD³, Boyd J Strauss MD³, John F Ashton PhD⁴, Lily Stojanovska PhD¹

¹From the School of Biomedical and Health Sciences, Victoria University, St Albans Campus, Melbourne, Australia

²Nutrition and Dietetics Unit, the Department of Medicine, Monash University, Clayton, Australia

³Monash University Department of Medicine and Clinical Nutrition and Metabolism Unit, Monash Medical Centre, Clayton, Australia

⁴Sanitarium Development and Innovation, Cooranbong, Australia

Calcium loss after menopause increases the risk of osteoporosis in aging women. Soymilk is often consumed to reduce menopausal symptoms, although in its native form, it contains significantly less calcium than cow's milk. Moreover, when calcium is added as a fortificant, it may not be absorbed efficiently. This study compares calcium absorption from soymilk fortified with a proprietary phosphate of calcium versus absorption from cow's milk. Preliminary studies compared methods for labelling the calcium fortificant either before or after its addition to soymilk. It was established that fortificant labelled after it was added to soymilk had a tracer distribution pattern very similar to that shown by fortificant labelled before adding to soymilk, provided a heat treatment (90°C for 30 min) was applied. This method was therefore used for further bioavailability studies. Calcium absorption from fortified soy milk compared to cow's milk was examined using a randomised single-blind acute cross-over design study in 12 osteopenic post-menopausal women aged (mean \pm SD) 56.7 \pm 5.3 years, with a body mass index of 26.5 \pm 5.6 kg/m². Participants consumed 20 mL of test milk labelled after addition of fortificant with 185 kBq of ⁴⁵Ca in 44 mg of calcium carrier, allowing the determination of the hourly fractional calcium absorption rate (α) using a single isotope radiocalcium test. The mean hourly fractional calcium absorption from fortified soymilk was found to be comparable to that of cows' milk: $\alpha = 0.65 \pm 0.19$ and $\alpha = 0.66 \pm 0.22$, $p > 0.05$, respectively.

Key Words: calcium, soymilk, osteopenia, labelling, bioavailability

INTRODUCTION

Calcium is a nutrient essential for maintenance of bone health and mineralisation.¹ In women, the loss of bone mineral greatly increases around the time of menopause as circulating oestrogen declines. This in turn increases the risk of osteoporosis, a reduction in the amount of bone in the bone.² Prevention of osteoporosis in women depends in part on maintenance of a high calcium intake throughout life and particularly after menopause to slow the rate of bone loss.³

In Australia, around 60 percent of dietary calcium comes from dairy foods.⁴ Yet post-menopausal women may choose to consume soymilk rather than cows' milk due to perceived health benefits such as reduced menopausal symptoms, although such effects still require substantiation.⁵ One negative effect from a change to soymilk may be reduced calcium intake as native soymilk contains much less calcium (~20 mg/100mL).⁶ Manufacturers have addressed this issue by fortifying soymilks with calcium, although methods of fortification vary considerably between products.⁷ Soymilk fortification appears to be an

effective way to increase calcium intake and the total amount of absorbed calcium⁸ although calcium bioavailability will depend considerably on the choice of fortificant.⁹ To date, few studies have examined the absorption of calcium from different kinds of fortified soymilk available on the market.^{6,9} To our knowledge, this is the first study examining calcium absorption from an Australian fortified soymilk.

MATERIALS AND METHODS

Calcium-fortified soymilk

The calcium-fortified soymilk (CFSM) used in this study is widely sold throughout Australia (So Good, Sanitarium

Corresponding Author: Prof Lily Stojanovska, School of Biomedical and Health Sciences, Victoria University, St Albans Campus, P.O Box 14428, Melbourne, VIC 8001, Australia.
Tel: 61 3 9919 2737; Fax: 61 3 9919 2465
Email: lily.stojanovska@vu.edu.au

Manuscript received 8 October 2009. Initial review completed 31 December 2009. Revision accepted 22 February 2010.

Health Foods, NSW, Australia). It is made from soy protein (4 percent) and has been fortified to achieve similar calcium content to cows' milk.

Labelling the fortificant with ^{45}Ca before its addition to so milk

The main source of calcium in CFSM is from the added fortificant. Labelled salts of the proprietary phosphate of calcium fortificant used for *So Good* were prepared by dissolving the amount of the proprietary salt required to yield 120 mg of calcium/100 mL in water before adding one microgram of $^{45}\text{CaCl}_2$ (Amersham Biosciences, England). This solution was kept at 90°C for 24 hours to evaporate to dryness. The dry salts were then ground into a fine powder using a mortar and pestle, and added to 100 mL of unfortified soymilk. Fortified soymilk samples were blended at high speed for 5 min, heat treated for 30 min at 90°C and stored at 4°C for 24 h. Each sample had a final total tracer concentration of ≈ 185 kBq/100 mL.

Labelling of fortificant with ^{45}Ca after soymilk manufacture

The fortificant present in CFSM was labelled by adding one microgram of high-specific-activity $^{45}\text{CaCl}_2$ to a known amount of soymilk sample, yielding a tracer concentration of ≈ 185 kBq/100 mL. Labelled milk was vortexed continuously for 1 min and then stored immediately at 4°C or heat treated (90°C for 30 min) before storing at 4°C for 24 h to allow for calcium exchange to occur.

Fractionation of labelled soymilk samples

Calcium-fortified soymilk that had been labelled (as above) either before or after soy milk manufacture were fractionated to determine the ^{45}Ca distribution as described by Heaney and co-workers.⁶ Briefly, CFSM aliquots in 1.5 mL tubes were centrifuged for 30 min at 14,000 x g (Eppendorf centrifuge, model 5415C, Crown Scientific Pty. Ltd., Vic, Australia). Each sample was then separated into four portions: the upper fat layer, an intermediate soluble fluid layer, the remaining supernatant and the pelleted residue. The upper fat layer was discarded and the supernatant portion was analysed for both ^{40}Ca and ^{45}Ca , as detailed below. Samples of unfractionated CFSM were also analysed in a similar way to allow calculation of the ^{40}Ca and ^{45}Ca content of the residual insoluble portion.¹⁰

Calcium analysis

Fractionated and unfractionated samples were heated at 550°C in a muffle furnace and the resulting ashes were dissolved in hydrochloric acid (0.5 mol/L). ^{40}Ca content was determined by diluting the ash sample with 5 mL of 0.5 mol/L HCL containing 0.5 percent lanthanum as LaCl_3 before analysis by atomic spectrophotometry (Varian SpectrAA-300/400, Palo Alto, Ca, USA). ^{45}Ca was measured from ash samples distributed in Ecoscint scintillation fluid (National Diagnostics, UK) using a liquid scintillation counter (Wallac 1410, Perkin Elmer Life Sciences, Massachusetts, USA).

Human study protocol

Participants

Twelve osteopenic, but otherwise healthy, post-menopausal women aged between 50-65 yrs were recruited through poster, email and web advertisements. Potential participants were screened by telephone interview and if inclusion criteria were met, they were asked to complete a questionnaire to determine health and menopausal status. Women were included in the study if they were post-menopausal (at least 12 months amenorrhea), had a diagnosis of osteopenia (i.e. with bone mineral density (BMD) T score between -1 and -2.5 as measured by dual-energy X ray absorptiometry, (Clinical Nutrition and Metabolism Unit, Monash Medical Centre, Clayton, Vic, Australia); otherwise generally healthy (no chronic disease by self-report; including gastrointestinal, kidney, liver, parathyroid or cardiovascular disease); not taking medication or antibiotics that might affect calcium absorption; and not on hormone replacement therapy (HRT) during the preceding 12 months. In addition, participants were required to be lactose tolerant and not allergic to soy or soy products. The body mass index (BMI) of women enrolled in the study ranged between 19-32 kg/m². Each participant completed an eating habit questionnaire (extracted from a food frequency questionnaire developed by Australian Cancer Council, Vic, Australia) to assess dietary calcium intake. This study was conducted according to the guidelines laid down in the Declaration of Helsinki in 1995 (as revised in Edinburgh 2000) and all procedures involving human subjects were approved by the Southern Health Human Research and Ethics Committee. Written informed consent was obtained from all subjects.

Test milk drinks for the acute study

Milk drinks (cows' milk or CFSM) for the acute *in vivo* study were prepared by adding one microgram $^{45}\text{CaCl}_2$ (Amersham Biosciences) in 44 mg calcium carrier to each 20 mL serve of milk giving a tracer concentration of 185 kBq/dose. Milk samples were heat treated (90°C for 30 min) to ensure calcium equilibrium, then cooled and stored at 4°C for 24 h before use.

Study design

Women were asked to come to Monash Medical Centre (MMC) to participate in a randomised crossover design calcium absorption study. Participants were tested on two separate occasions separated by a washout period of at least three weeks. At each test, subjects were randomised (with reference to a set of randomised numbers) to consume a radiolabelled milk sample, either cows' milk or CFSM. Participants arrived at MMC at 8.00 am, after an overnight fast. Bioelectrical impedance was determined (SFB7, Impedimed, Brisbane, Australia). In the Department of Nuclear Medicine, a 22GA catheter was inserted in an antecubital vein to obtain a 10 mL baseline venous blood sample. Participants then consumed a 20 mL test sample of labelled CFSM or cows' milk, immediately followed by 200 mL of distilled water. Blood samples were then collected after 60 min following the method described by Nordin.^{11,12} Blood samples were centrifuged at 3,500 rpm for 10 min and the activity of the ^{45}Ca in the plasma aliquots (1 mL) were measured using a liquid scintillation counter (Wallac 1410) according to the method

of Marshall and Nordin.¹³ The hourly fractional calcium absorption rate (α) was then calculated.¹³

Calculation and statistical analysis

Fortificant labelling before soy milk manufacture was performed once in triplicate, while labelling of fortificant after soy milk manufacture (with and without heat treatment) was performed on two occasions, also in triplicate. Differences in the amount of tracer calcium relative to stable calcium were compared using one way ANOVA. Multivariate analysis of variance was used for multiple comparisons.

The sample size of 12 women recruited into this cross-over design study gives a probability of 90 percent that this human study can detect a treatment difference at a 5 percent level of significance (two-sided), if the true difference between the treatments is 15 percent. This is based on the assumption that the within-patient SD of the response variable is 10 percent. Data from this study were compared by paired t-tests. SPSS for Windows (Version 11.5, SPSS Australasia Ltd, Melbourne) was used for all statistical analyses. A p value of <0.05 was taken as significant.

RESULTS

Source labelling

Atomic spectroscopy established that only 21–22 percent of the stable calcium (^{40}Ca) present in samples of CFMS samples could be detected in the supernatant fraction, even following 24 h storage at 4°C (Table 1). When the fortificant was labelled with ^{45}Ca before it was added to the soy milk, the relative abundance of ^{45}Ca in the supernatant was found to be only slightly (4–5 percent) but not significantly ($p>0.05$) lower than that of the stable isotope at each time point measured. In contrast, when the fortificant was labelled after it had been added to the soy milk, the relative abundance of calcium tracer in the supernatant was significantly higher than that of stable calcium (^{40}Ca), even following 24 h storage ($p<0.05$). When additional heat treatment was applied however, the abundance of ^{45}Ca in the supernatant was now reduced to a level very similar to that of stable calcium. Tracer abundance continued to remain at this level after 24 h at 4°C.

The percentage of radioactive tracer in the supernatant and in the residual portion of CFMS samples after the three different labelling methods is given in Figure 1. This indicates that provided a heat treatment was applied, the labelling of fortificant after its addition to soy milk resulted in a tracer abundance very similar to that found when fortificant labelling was carried out prior to soy milk manufacture. Given the relative ease of labelling CFMS after manufacture, this method with heat treatment was used for our *in vivo* study.

Calcium absorption comparison from CFMS to cows' milk

Twelve mildly overweight (BMI (mean \pm SD): 26.5 \pm 5.6 kg/m²) post-menopausal women aged 56.7 \pm 5.3 years were recruited for the calcium absorption study. Table 2 shows the individual hourly fractional calcium absorption rates when these women consumed labelled CFMS or cow's milk. The mean hourly fractional calcium absorp-

tion rate (α) for each milk differed by only 1.5 percent (Table 2), a difference that was not of statistical significance.

DISCUSSION

Bioavailability studies require appropriate distribution of label in the test food. Tracer methods to measure calcium absorption are not commonly used on marketed products because they often cannot be easily labelled.¹⁴ This study has shown that, provided that a heat treatment is applied, a calcium fortificant can be labelled for tracer studies after it has been added to soy milk. This method will then generate a tracer abundance very similar to that obtained when the fortificant has been labelled prior to its addition to soymilk. This is a valuable observation indicating a practical method that can be usefully applied to compare calcium absorption across the range of commercially available CFMSs when access to the initial fortificant is not necessarily available.

Earlier studies on cows' milk and other dairy products as well as from studies on a calcium-containing wheat flour product have indicated that for these foods, labelling of fortificant after its addition to the food can generate a similar tracer abundance to that obtained when the fortificant is labelled before it is added to the food.^{15,16} For foods such as cow's milk, a calcium isotope appears to be able to exchange readily with stable calcium unheeded by the physical and chemical nature of the food. In contrast, when heat treatment is not used, fortificant labelling after addition to foods such as green leafy vegetables or soymilk^{6,17} gives rise to non-uniform tracer distribution, and for CFMS a 50 percent overestimation of true absorbability.⁶ Our study indicates that this problem can be avoided by heat treatment. Heat treatment may promote an exchange between soluble calcium and insoluble calcium. Insoluble calcium is the predominant form of calcium in both the proprietary fortificant added to *So Good*, and in CFMS and labelled after manufacture (>80 percent). This heat treatment method was therefore used in our further *in vivo* bioavailability studies.

Our *in vivo* study indicates that, in osteopenic post-menopausal women who are at risk of developing osteoporosis, the bioavailability of calcium in CFMS is similar to that of cows' milk. Many factors can affect calcium absorption including protein content and type and individual absorptive patterns influenced by factors such as vitamin D status.¹⁸ In previous studies, calcium absorption has been measured by a variety of methods,^{10,15,19–23} the most common being tracer methods employing stable or radioactive calcium isotopes.^{10,24,25} In the present study, a single radioisotope test was selected as it provides fast and accurate results after the test dose is taken.²⁶ This method is adopted as a valid basis for calculating calcium absorption.¹² The rate of calcium absorption calculated using this method correlated significantly with the rate of dietary calcium absorption measured in simultaneous calcium balances and very highly with calcium absorption calculated by the double isotope method.^{12,27} The single isotope test produces a relatively sharp peak of radioactivity after 60 min and can therefore be completed over a short time period.¹¹ As calcium load increases absorption time and reduces test sensitivity,¹¹ a small (44 mg) calcium

Table 1. Percentage of ^{40}Ca and ^{45}Ca isotope in the supernatant fraction of calcium-fortified soymilk. Data are given as mean values with their standard deviation

Percent in supernatant	0 h						12 h						24h					
	Ca-40		Ca-45		Difference†		Ca-40		Ca-45		Difference†		Ca-40		Ca-45		Difference†	
Fortificant labelling: before soy milk manufacture	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	21.4	2.4	17.2*	0.5	4.2 ^{*A}	1.8	21.4	2.4	15.9*	3.4	5.5 ^{*A}	3.3	21.4	2.4	15.9*	3.4	5.5 ^{*A}	3.3
After soy milk manufacture – No heat treatment	21.8	3.1	44.0**	3.1	22.1 ^{**B}	5.0	22.1	3.1	45.7**	2.5	23.6 ^{**B}	5.2	21.4	3.0	47.3**	7.2	25.9 ^{**B}	8.2
After soy milk manufacture – Heat treatment 90°C for 30 min	21.3	3.0	28.1***	1.3	6.8 ^{*A}	4.1	22.0	3.1	28.8***	5.2	6.9 ^{*A}	5.1	22.0	3.1	25.0*	3.7	4.0 ^{*A}	1.3

† percent carrier (Ca^{40}) minus percent tracer (Ca^{45}) in the supernatant fraction

‡ mean percentages or mean differences in the same column with *, **, *** are significantly different ($p < 0.05$). Univariate analysis of variance used to compare means and differences between treatments

§. mean percentages or mean differences in percentage in the same row, that have uppercase alphabets are significantly different ($p < 0.05$). Univariate analysis of variance used to compare means over time.

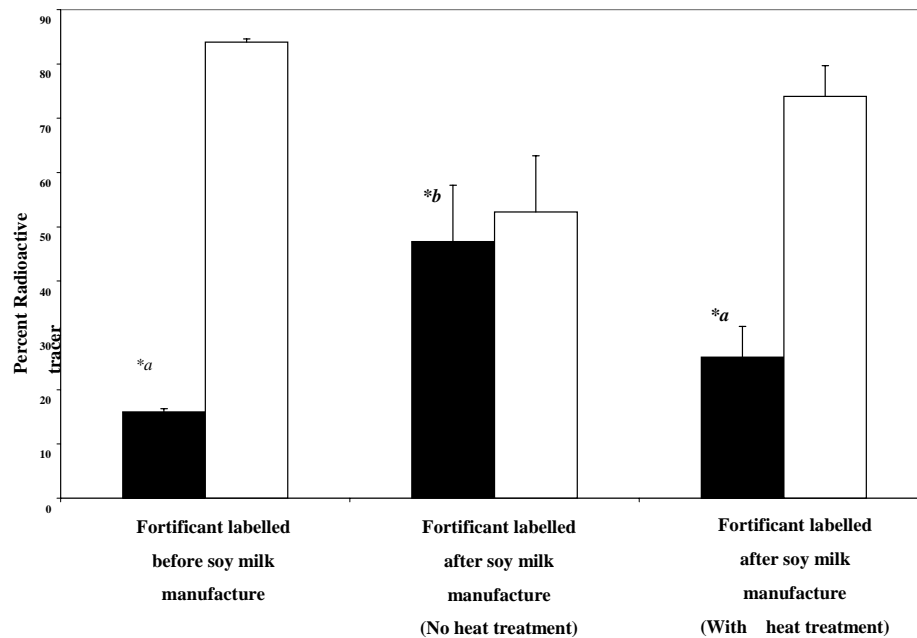


Figure 1. Percentage (percent) of radioactive tracer in supernatant (solid bars) and residue portion (empty bars) of radiolabelled calcium-fortified soymilk (CFSM) samples after 24h equilibrium at 4°C. Heat treatment was 30 min at 90°C. Mean values with different lower letters are significantly different ($p < 0.05$)

Table 2. Hourly fractional calcium absorption rate (α) of cows' milk and fortified soymilk in 12 osteopenic post-menopausal women. Data given are mean values with their standard deviation

Participant code	Hourly fractional absorption rate (α)	
	Cows' Milk	Soymilk (CFSM)
101	0.77	0.72
102	0.62	0.90
103	0.72	1.00
104	0.76	0.94
105	0.51	0.64
106	0.68	0.67
107	0.90	0.53
108	0.68	0.74
109	0.55	0.67
110	0.38	0.37
111	0.93	0.43
112	0.32	0.31
Mean±SD	0.66±0.22*	0.65±0.19*

* no significant difference ($p < 0.05$) between means

load was chosen for the present study. If the calcium load had been greater than 50 mg, the double isotope method would have had to be used. The larger carrier dose, the more interference during the calcium absorption diffusion process and the less valid the single isotope procedure.¹¹ A low dose has the advantage in that it appears to maximise differences between low calcium absorbers and high calcium absorbers.²⁸

Calcium bioavailability is highly determined by the type of fortificant used. A recent study found that calcium fortificants added to beverages sold in the US had very different settling properties. While some formed stable suspensions, others settled quickly on the bottom of the container.⁷ A new calcium-enriched orange beverage had similar calcium absorption to that of cows' milk.²⁹ In soymilks, fortification with a type of calcium carbonate fortificant appears to yield similar calcium absorption to that of cows' milk, whereas CFSM fortified with tricalcium phosphate often has lower calcium absorption.^{6,9} In the present study, the calcium fortificant in the CFSM used was a proprietary form of phosphate of calcium which circumvents this problem. The fractional calcium absorption from CFSM with this particular fortificant was equivalent to the fractional calcium absorption from normal cows' milk.

Apart from type of fortificant, calcium absorption may also be affected by the calcium/phosphorus ratio in foods, a determinant factor for mineral absorption and deposition into bone.³⁰ High levels of phosphate may precipitate calcium in the intestine rendering it unavailable.²⁵ Differences in the calcium/phosphate ratio may thus potentially cause differences in calcium bioavailability between soy drinks apparently fortified with similar amounts of calcium. In the present study, this factor would appear to be minimised since the CFSM used in this study appears to have a calcium/phosphorus ratio similar to that of cows' milk (0.8:1).

Phytic acid, which is present in many legumes including soybeans, is known to contribute to the poor bioavailability of many minerals including calcium.^{31,32} Soymilk has minimal amounts of phytic acid.⁵ This is particularly true for the soymilk used in this study, which is manufactured from isolated soy protein rather than from whole soybeans. This CFSM has a phytic acid content of only <0.1 percent, which is unlikely to impact on calcium absorption (Ashton, JF, 2009, unpublished results).

Soy is the major source of dietary phytoestrogen in the form of isoflavones which may potentially relieve some post-menopausal symptoms and may also reduce urinary calcium excretion thereby potentially minimising bone loss.³³⁻³⁵ Many epidemiological and some clinical studies have suggested that dietary phytoestrogens may also be helpful with respect to menopausal symptoms such as hot flashes.³⁶ Isoflavone content however, appears to have little effect on calcium bioavailability.^{37,38}

While these findings indicate that calcium absorption was similar in this Australian CFSM and cows' milk, investigations are needed over the longer term to show whether similar benefit accrues in bone building and maintenance. Similarly, further studies are needed to ascertain whether these benefits extend to other brands of soymilk supplemented with calcium.

In summary, this study shows that the calcium bioavailability of marketed fortified soymilk could be assessed using the tracer method by labelling the fortificant after soy milk manufacture provided that a heat treatment was applied. Calcium absorption from a popular Australian brand of CFSM fortified with a proprietary phosphate of calcium was found to be similar to that of cows' milk in osteopenic post-menopausal women. Soymilk can therefore be successfully fortified to deliver the same levels of calcium as cows' milk and can thus be confidently used as a substitute for cows' milk in the diets of post-menopausal women, vegetarians and of individuals with lactose or other forms of dairy food intolerance.

ACKNOWLEDGMENTS

We thank members of the Department of Nuclear Medicine, the Body Composition Laboratory from the Monash Medical Centre, Victoria, Australia and the Department of Medicine, Monash University, Victoria, Australia for their kind support during the study. This study was supported by the Sanitarium Health Food Company for the Australian Research Council Sanitarium linkage grant funding. ALT contributed to the overall study from experimental design, collection and analysing of data to the writing of the manuscript; KZW, GW, JFA and LS conceived the idea, designed the experiment, contributed in the implementation of the study, provided help with data interpretation and editing of manuscript, BJS contributed with significant advice and consultation; JFA is currently employed by Sanitarium Health Food Company, Australia. All authors read and contributed to the finalisation of the manuscript.

AUTHOR DISCLOSURES

Anne Lise Tang, Karen Z Walker, Gisela Wilcox, Boyd J Strauss, Lily Stojanovska, no conflict of interest. John F Ashton is currently employed at Sanitarium Development and Innovation as the Strategic Research Manager and is also Adjunct Associate Professor at Victoria University.

REFERENCES

1. Nordin BEC. Calcium in health and disease. In: Food, Nutrition and Agriculture; 1997. pp. 13-26.
2. Nordin BEC. Redefining osteoporosis. *Calcif Tissue Int.* 2008;83:365-7.
3. The North American Menopause Society. Management of osteoporosis in postmenopausal women: 2010 position statement of The North American Menopause Society. *Menopause.* 2010;17:25-54.
4. Osteoporosis Australia. Calcium, Vitamin D and Osteoporosis - A guide for consumers. In: *Osteoporosis Australia*; 2008:1-10.
5. Setchell KDR, Cassidy A. Dietary isoflavones: biological effects and relevance to human health. *J Nutr.* 1999;129:758S-767S.
6. Heaney RP, Dowell MS, Rafferty K, Bierman J. Bioavailability of the calcium in fortified milk, with some observations on method. *Am J Clin Nutr.* 2000;71:1166-9.
7. Heaney RP, Rafferty K, Bierman J. Not all calcium-fortified beverages are equal. *Nutrition Today.* 2005;40:39-44.
8. Lopez-Huertas E, Teucher B, Boza JJ, Martinez-Ferez A, Majsak-Newman G, Baro L, Carrero JJ, Gonzalez-Santiago M, Fonolla J, Fairweather-Tait S. Absorption of calcium from milks enriched with fructo-oligosaccharides, tricalcium phosphate, and milk solids. *Am J Clin Nutr.* 2006;83:310-6.
9. Zhao Y, Martin BR, Weaver CM. Calcium bioavailability of calcium carbonate fortified soymilk is equivalent to cow's milk in young women. *J Nutr.* 2005;135:2379-82.

10. Heaney RP. Factors Influencing the measurement of bioavailability, taking calcium as a model. *J Nutr.* 2001; 131(4):1344S-8S.
11. Nordin BEC, Morris HA, Wishart JM, Scopacasa F, Horowitz M, Need AG, Clifton PM. Modification and validation of a single-isotope radiocalcium absorption test. *J Nucl Med.* 1998;39:108-13.
12. Nordin BEC, Morris HA, Horowitz M, Coates PS, O'Loughlin PD, Need AG. Calcium malabsorption does not cause secondary hyperparathyroidism. *Calcif Tissue Int.* 2009;85: 31-6.
13. Marshall DH, Nordin BEC. A comparison of radioactive calcium absorption tests with net calcium absorption. *Clin Sci.* 1981;61:477-81.
14. Heaney RP. Quantifying human calcium absorption using pharmacokinetic methods. *J Nutr.* 2003;133:1224-6.
15. Nickel KP, Berdine MR, Smith DL, Smith JB, Miller GD, Weaver CM. Calcium bioavailability from bovine milk and dairy products in premenopausal women using intrinsic and extrinsic labeling techniques. *J Nutr.* 1996;126:1406-11.
16. Weaver CM, Heaney RP, Martin BR, Fitzsimmons ML. Extrinsic vs intrinsic labeling of the calcium in whole-wheat flour. *Am J Clin Nutr.* 1992;55:452-4.
17. Heaney RP, Weaver CM, Recker RR. Calcium absorbability from spinach. *Am J Clin Nutr.* 1988;47:707-9.
18. Kruger MC HD. Calcium metabolism, osteoporosis and essential fatty acids: A review. *Prog Lipid Res.* 1997;36: 131-51.
19. Bronner F. Calcium absorption - a paradigm for mineral absorption. *J Nutr.* 1998;128:917-20.
20. Abrams SA. Using stable isotopes to assess mineral absorption and utilization by children. *Am J Clin Nutr.* 1999;70: 955-64.
21. Abrams SA, Yergey AL, Heaney RP. Relationship between balance and dual tracer isotopic measurements of calcium absorption and excretion. *J Clin Endocrinol Metab.* 1994;79: 965-9.
22. Heaney RP, Dowell MS, Wolf RL. Estimation of true calcium absorption in men. *Clin Chem.* 2002;48:786-8.
23. Moser-Veillon PB, Mangels AR, Vieira NE, Yergey AL, Patterson KY, Hill AD, Veillon C. Calcium fractional absorption and metabolism assessed using stable isotopes differ between postpartum and never pregnant women. *J Nutr.* 2001;131:2295-9.
24. Hambridge KM, Krebs NF, Westcott JL, Sian L, Miller LV, Peterson KL, Raboy V. Absorption of calcium from tortilla meals prepared from low-phytate maize. *Am J Clin Nutr.* 2005;82:84-7.
25. Schroder BG, Griffin IJ, Specker BL, Adrams SA. Absorption of calcium from the carbonated dairy soft drink is greater than that from fat-free milk and calcium-fortified orange juice in women. *Nutr Res.* 2005;25:737-42.
26. Marshall DH. Calcium and phosphate kinetics. In: Nordin BEC, editor. *Calcium, phosphate and magnesium metabolism.* Edinburgh: Churchill Livingstone; 1976. pp. 257-97.
27. Nordin BEC. *Calcium, phosphate and magnesium metabolism: Clinical physiology and diagnostic procedures.* 3rd ed. London: Churchill Livingstone; 1976.
28. Wilkinson R. Absorption of calcium, phosphorus and magnesium. In: Nordin BEC, editor. *Calcium, phosphate and magnesium metabolism.* Edinburgh: Churchill Livingstone; 1976. pp. 36-112.
29. Gonnelli S, Campagna MS, Montagnani A, Caffarelli A, Cadirni A, Giorgi G, Nuti R. Calcium bioavailability from a new calcium-fortified orange beverage, compared with milk, in health volunteers. *Int J Vitam Nutr Res.* 2007;77:249-54.
30. Shapiro R, Heaney RP. Co-dependence of calcium and phosphorus for growth and bone development under varying deficiency. *Bone.* 2003;32:532-40.
31. Messina MJ. Legumes and soybeans: overview of their nutritional profiles and health effects. *Am J Clin Nutr.* 1999; 70:439-50.
32. Charoenkiatkul S, Kriengsinyos W, Tuntipopipat S, Suthutvoravut U, Weaver CM. Calcium absorption from commonly consumed vegetables in healthy Thai women. *J Food Sci.* 2008;73:218-21.
33. Adlercreutz CH, Goldin B, Gorbach S, Hockerstedt K, Watanabe S, Hamalainen E, Markkanen M, Makela T, Wahala K, Aldercreutz T. Soybean phytoestrogen intake and cancer risk. *J Nutr.* 1995;125(suppl3):757S-70S.
34. Jefferson A. Phytoestrogens and the menopause - do they really help? *Nutr Bull.* 2005;30:370-3.
35. Setchell KDR, Lydeking-Olsen E. Dietary phytoestrogen and their effect on bone: evidence from in vitro and in vivo observational and dietary intervention studies. *Am J Clin Nutr.* 2003;78:593-609.
36. Murkies AL, Wilcox G, Davis SR. Phytoestrogens. *J Clin Endocrinol Metab.* 1998;83:297-303.
37. Cai DJ, Zhao YD, Glasier J, Cullen D, Barnes S, Turner CH, Wastney M, Weaver CM. Comparative effect of soy protein, soy isoflavones, and 17 beta-estradiol on bone metabolism in adult ovariectomized rats. *J Bone Miner Res.* 2005;20: 828-39.
38. Spence LA, Lipscomb ER, Cadogan J, Martin B, Wastney ME, Peacock M, Weaver CM. The effect of soy protein and soy isoflavones on calcium metabolism in postmenopausal women: a randomized crossover study. *Am J Clin Nutr.* 2005;81:916-22.

Original Article

Calcium absorption in Australian osteopenic postmenopausal women: an acute comparative study of fortified soymilk to cows' milk

Anne Lise Tang BSc(Hons)¹, Karen Z Walker PhD², Gisela Wilcox MD³, Boyd J Strauss MD³, John F Ashton PhD⁴, Lily Stojanovska PhD¹

¹From the School of Biomedical and Health Sciences, Victoria University, St Albans Campus, Melbourne, Australia

²Nutrition and Dietetics Unit, the Department of Medicine, Monash University, Clayton, Australia

³Monash University Department of Medicine and Clinical Nutrition and Metabolism Unit, Monash Medical Centre, Clayton, Australia

⁴Sanitarium Development and Innovation, Cooranbong, Australia

澳洲骨質減少的停經婦女之鈣吸收：比較強化豆漿與牛奶的極短期試驗

停經後的鈣流失，增加老年女性罹患骨鬆的危險。雖然比起牛奶含有較少的鈣，食用豆漿通常被用來減緩更年期症狀。此外，當在豆漿內添加鈣，也許不會被有效率的吸收。本篇研究比較豆漿中添加的鈣(以專利磷酸鈣形式)與牛奶中鈣的吸收率。初步的研究先比較鈣的標記方法(加入豆漿前與加入豆漿後)。初步確定，磷酸鈣在加入豆漿前就先標記鈣的方法，與磷酸鈣加入豆漿之後才標記鈣，再經 90°C 加熱 30 分鐘的方法，有非常相似的追蹤劑分布型態。因此後一方法就被用於之後的生物利用率研究中。強化豆漿與牛奶的鈣吸收比較，是利用一個隨機、單盲的極短期交叉實驗設計，對象為 12 位骨質減少的停經後婦女，平均年齡為 56.7 ± 5.3 歲，身體質量指數平均為 $26.5 \pm 5.6 \text{ kg/m}^2$ 。參與者攝取 20 mL 標記過的牛奶或強化鈣豆漿(44 mg 的鈣載體內含有 185 kBq 的 ^{45}Ca)，使用單一同位素放射性鈣以測定每小時的鈣吸收分率(α)。鈣強化豆漿其平均每小時的鈣吸收分率與牛奶的相似：分別為 $\alpha = 0.65 \pm 0.19$ 與 $\alpha = 0.66 \pm 0.22$ ， $p > 0.05$ 。

關鍵字：鈣、豆漿、骨質減少、標記、生物利用率