Effects of Eggshell Calcium Supplementation on Bone Mass in Postmenopausal Vietnamese Women

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(Received July 13, 2016)

Summary Bone mass decreases along with aging, especially for women after menopause because of lower estrogen secretion together with low calcium intake. This study was conducted to study the effect of eggshell calcium supplementation on bone mass in 54 postmenopausal Vietnamese women living in a farming area about 60 km from Hanoi, Vietnam. Sets of 3 subjects matched by age, bone mass, BMI and calcium intake were divided randomly into 3 groups with 18 subjects in each group. The eggshell calcium group was administered 300 mg/d calcium from eggshell, the calcium carbonate group 300 mg/d calcium from calcium carbonate and the placebo group received no calcium supplementation. Bone mass (Speed of Sound (SOS)) was measured at the beginning (the baseline), the middle (6th month) and the end of the study (12th month) by the single blind method. SOS of the eggshell group increased significantly at 12 mo (p < 0.05) and was significantly higher than that of the placebo and calcium carbonate groups at 12 mo (p < 0.05). The SOS of the calcium carbonate group tended to be higher than that of the placebo group but without a significant difference (p > 0.05). In conclusion, eggshell calcium was more effective in increasing bone mass than calcium carbonate in postmenopausal Vietnamese women.

Key Words eggshell calcium, calcium carbonate, bone mass, postmenopausal women, Vietnamese

The risk of osteoporosis, especially in women, increases as part of the aging process and the measurement of their bone density is important (1). Asian women, who tend to have a smaller body size, have been reported as having low bone density (2). In Vietnam as well as other Asian countries, an increase in osteoporosis is a growing problem because the elderly population has been growing (3). The lack of calcium intake accelerates the problem and higher calcium intake is recommended (4–10). Recommended intake for adults in Vietnam is 1,000 mg/d (11). However, a nutrition survey conducted by both FFQ and 24-h recall methods by Khan et al. showed that the average calcium intake was about 350 mg/d in Vietnamese women (12). The prevalence of osteoporosis in postmenopausal Vietnamese women was about 30% in 2015 and various methods of prevention were examined (13). Hien et al. provided nutrition education to 108 Vietnamese postmenopausal women for 18 mo, advising them to use cheap calcium-rich food sources such as small crabs, shrimp and small fish commonly abundant and not fully utilized (14). The calcium intake increased by about 300 mg and total intake became about 600 mg. Improving the calcium intake could result in bone loss retardation (14). Another advantage of a calcium-rich diet is a decrease in blood pressure. It is suggested that this positive effect is due to a blood calcium suppression by decreased parathormone secretion with a subsequently decreased calcium content in the vascular smooth muscle cells (15).

In attempts to prevent osteoporosis, various calcium sources have been administered (16, 17). Omi and Ezawa compared the effects of eggshell calcium and calcium carbonate supplementation in ovariectomized rats and found that lumbar bone density was higher in the eggshell calcium group than in the calcium carbonate group (18). Shizuka also showed that in rats the calcium absorption from eggshell was higher than that from calcium carbonate with higher femur fracture strength and femur calcium content with eggshell calcium than with calcium carbonate (19). Better absorption with eggshell than with calcium carbonate may be because of eggshell’s easier digestion/absorption due to its porous structure (20–22). As described above, although eggshell calcium has been suggested as a more efficient source of calcium than calcium carbonate, investigation in humans has been limited, especially the effect on recovery from osteoporosis. Therefore, this study was designed to study the effects of eggshell calcium on bone mass in postmenopausal Vietnamese women.

MATERIALS AND METHODS

Calcium supplements were given with hard capsules
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containing 100 mg calcium except for the placebo. The composition of each type of capsule is shown in Table 1. Eggshell calcium capsules contained 100 mg eggshell calcium (Cal-Hope, Kewpie Co., Ltd.) and calcium carbonate capsules contained 100 mg of elemental calcium (CalciF#2000, Sankyo Seifun Co., Ltd.). Capsules were filled with cellulose (CEOLUS FD-101, Asahi Kasei Chemicals Corporation) and the quantity of each capsule is shown in Table 1. In order to provide a benefit to the placebo group, all of the capsules contained the same amounts of thiamine and riboflavin, which do not affect bone metabolism. Each capsule contained the same volume.

**Subjects.** The subjects were 54 women who were mostly farmers and had passed menopause more than 5 y earlier (mean±SD, 14.0±8.7 y) and were living in a farming area, about 60 km from Hanoi, Vietnam. Criteria for subjects were no osteoporosis history, no food allergies, less than 400 mg calcium intake a day, and no supplements to affect bone metabolism. Sets of three subjects matched by age, bone mass, BMI and calcium intake were divided randomly into 3 groups of 18 subjects each. The subject took 3 capsules per day for 12 mo.

**Measurements.** The measurements were conducted by single-blind. Height, weight, blood pressure, and bone mass were measured three times: at the beginning of the study, and after 6 and 12 mo. The nutrition surveys were also implemented three times, at the beginning of the study, and after 6 and 12 mo by semi-quantitative FFQ.

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Table 1. Composition of the capsules containing 100 mg Ca/capsule.

| Composition                  | Eggshell | Calcium carbonate | Placebo |
|------------------------------|----------|-------------------|---------|
| Eggshell calcium (mg)        | 263.3    | —                 | —       |
| Calcium carbonate (mg)      | —        | 257.0             | —       |
| Cellulose (mg)              | 102.5    | 73.8              | 169.8   |
| Thiamine hydrochloride (mg) | 0.1      | 0.1               | 0.1     |
| Riboflavin (mg)             | 0.1      | 0.1               | 0.1     |

Table 2. Baseline characteristics of subjects and changes at 6th and 12th month in 3 groups (mean±SD).

| Characteristics             | Placebo (n=15) | Calcium carbonate (n=14) | Eggshell (n=16) |
|-----------------------------|----------------|--------------------------|-----------------|
| Age (y)                     | 61.7±6.4       | 60.9±6.3                 | 62.6±5.2        |
| No. of children             | 1.9±0.4        | 1.9±0.3                  | 1.8±0.4         |
| Weight (kg)                 |                |                          |                 |
| Baseline                    | 46.7±10.6      | 50.1±5.1                 | 50.9±8.6        |
| 6 mo                        | 47.6±11.3*     | 50.6±5.0                 | 51.9±8.1        |
| 12 mo                       | 47.4±10.6      | 50.4±5.0                 | 51.3±8.6        |
| Height (cm)                 |                |                          |                 |
| Baseline                    | 149.3±6.1      | 151.6±4.5                | 150.5±5.2       |
| 6 mo                        | 148.7±6.3      | 151.5±4.7                | 150.3±5.3       |
| 12 mo                       | 148.8±6.1      | 151.3±4.7                | 150.2±5.0       |
| Systolic blood pressure (mmHg) |              |                          |                 |
| Baseline                    | 137.6±21.2     | 131.4±23.3               | 135.1±20.6      |
| 6 mo                        | 131.8±27.7     | 116.9±13.2*              | 123.6±16.2*     |
| 12 mo                       | 129.5±19.0     | 124.4±13.5               | 123.6±11.1*     |
| Diastolic blood pressure (mmHg) |            |                          |                 |
| Baseline                    | 83.7±11.4      | 83.3±10.0                | 85.2±9.2        |
| 6 mo                        | 75.4±13.2*     | 71.4±6.5*                | 77.9±8.4*       |
| 12 mo                       | 83.6±9.7       | 78.7±7.0                 | 80.0±6.0        |
| Calcium intake from diet (mg/d) |          |                          |                 |
| Baseline                    | 321±94         | 293±61                   | 294±74          |
| 6 mo                        | 463±86*        | 469±63*                  | 445±103*        |
| 12 mo                       | 361±70         | 371±78                   | 343±83*         |
| T-score                     |                |                          |                 |
| Baseline                    | −2.51±1.09     | −2.56±0.89                | −2.44±0.86      |
| 6 mo                        | −2.27±1.13     | −1.83±1.39*               | −1.36±0.55*     |
| 12 mo                       | −2.38±0.84*    | −1.88±0.66*a              | −1.12±0.34*     |

* Significant difference from baseline (p<0.05).
** Different letters indicate significant difference among the groups.
to examine the calcium intake. Bone mass was measured using Achilles Insight, an ultrasonic calcaneus measuring apparatus (GE Healthcare, Barrington, IL). Bone mass was assessed by speed of sound (SOS) (m/s). The T-score was calculated by using the SOS value. A T-score is the difference between the bone mineral density value (in g/cm²) of an individual and the average bone mineral density (expressed in standard deviation units) of a young adult in a reference population. According to The World Health Organization, a T-score of $\geq -1$ indicates normal bone density; a $-2.5 < T$-score $< -1$ indicates low bone mineral density and a $T$-score $\geq -2.5$ indicates osteoporosis (23, 24). We asked subjects to maintain their usual diet and physical activities.

This study complied with the provisions of the Declaration of Helsinki and was approved by the Ethical Committee of the National Institute of Nutrition in Hanoi, Vietnam, on September 15th, 2014. Written informed consent was obtained from all the subjects.

**Statistical analyses.** We used SPSS, version 20, software (IBM Corp., Armonk, NY) to perform statistical analyses. For the comparison of the 3 groups, one-way ANOVA was used, followed by Tukey’s multiple comparisons method. For the comparison of baseline, 6th and 12th month data, repeated ANOVA was used.

**RESULTS**

We had some dropouts and the number of subjects at completion was 16 in the eggshell calcium group, 14 in the calcium carbonate group, and 15 in the placebo group. The causes of dropouts were personal reasons (6 subjects), hospitalization (1 subject), and refusing blood drawing (2 subjects). The baseline characteristics of subjects and measurement results are shown in Table 2.

From the interviews and the measurement results adverse effects of calcium supplementations were not observed. Average height of subjects was about 150 cm and body weight was about 50 kg (BMI about 22). Blood pressure in the eggshell calcium group decreased significantly after 6 and 12 mo ($p<0.05$). Differences were observed in the other two groups but were inconsistent.

Calcium intake from diet in each group significantly increased after 6 mo but decreased at 12 mo. T-score at the baseline was nearly the osteoporosis level. After 12 mo, that of the eggshell calcium group and calcium carbonate group significantly increased ($p<0.05$). The score of the eggshell calcium group was close to normal bone mass. T-scores of the eggshell calcium group were significantly higher than those of the calcium carbonate group and placebo group at 12 mo ($p<0.05$). Additionally, the eggshell calcium and calcium carbonate groups took 300 mg from supplementation. The subjects were seen every 6 mo and their compliance with treatment (as assessed by tablet counts) were recorded. The subjects consumed 95% of the tablets we provided.

Figure 1 shows the results of the bone mass (SOS) relative to baseline data. The results of SOS (mean±SD) at baseline, after 6 mo and 12 mo were $1462.9\pm25.8$, $1495.1\pm16.6$ and $1502.4\pm10.3$ in the eggshell calcium group, $1459.3\pm26.7$, $1481.1\pm41.6$ and $1479.6\pm19.7$ in the calcium carbonate group and $1460.6\pm32.7$, $1467.8\pm33.8$ and $1464.6\pm25.3$ in the placebo group, respectively. SOS of the eggshell calcium group was significantly higher than that of the calcium carbonate group and placebo group at 12 mo ($p<0.05$).

**DISCUSSION**

Bone mass was measured three times, at baseline, and after 6 mo and 12 mo. The eggshell calcium group increased in bone mass as compared with the calcium carbonate group and the placebo group ($p<0.05$) after 12 mo. The calcium carbonate group tended to increase in bone mass but did not differ significantly from the placebo group ($p>0.05$).

Our finding is supported by some previous reports. Eggshell consists of 97% calcium carbonate, which must be broken down in the digestive system (25). The eggshell also contributes to developing high calcium-consuming organs of the chicken such as skeleton, muscles, and brain (26). Calcium absorption rates of
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Eggshell powder were 34.8%, which was greater than those of coarse powder and calcium carbonate (27). A study in rats also showed that absorption of eggshell calcium was better than that of calcium carbonate (28). There was also a study which was conducted in older women for 3 mo by giving 2 g calcium from eggshell and calcium carbonate and which showed that those given eggshell calcium significantly suppressed parathyroid hormone secretion compared to those given calcium carbonate (29). The porous eggshell structure might contribute in its high solubility in the gastrointestinal tract (30). These studies suggest that higher bone mass from eggshell calcium was contributed by results from its higher calcium absorption rate and lower bone resorption compared to those for calcium carbonate.

The decrease in blood pressure during the study was considered to be caused by the calcium intake. It suppressed calcium elution into the blood from the bone and lowered the blood calcium concentration (31). Calcium can mediate vascular smooth muscle contraction and its channel blockers lower blood pressure, which suggests that increased dietary calcium intake can decrease high blood pressure (32). Furthermore, there was a positive correlation between blood pressure and parathormone, which regulates calcium influx to the cells and increases the vasoconstrictive effect (15).

At the baseline, all subjects regularly ingested about 300 mg calcium per day from food intake. This reached almost 500 mg after 6 mo and then dropped to about 400 mg at 12 mo. Perhaps this was due to nutrition education at the beginning of the study but the subjects tended to return to their usual dietary habits after 6 mo. This indicates the difficulty of changing dietary habits and therefore, the usefulness of supplementation.

In this study, we believe that the criteria management of our subjects was good and reliable. Along with the study duration, which was adequate, the matched subjects made the data reliable. We believe that the study was well designed because the baseline data of all 3 groups were similar. Subject number in each group was rather insufficient; however, because the study period was 12 mo, the conclusion may be considered reasonable.

The limitation of the study was that the examination of other nutrients related to calcium metabolism such as vitamin D and proteins was not possible because only the semi-quantitative FFQ method was used to examine calcium intake from foods.

In conclusion, the fact that the bone mass at the baseline was nearly at the osteoporosis level but after 12 mo that of the eggshell calcium group increased nearly to the normal level, more than that of the calcium carbonate group, suggests the usefulness of eggshell for the treatment of osteoporosis in elderly women.

Acknowledgments

We deeply thank all the subjects for participating in the study. We are greatly indebted to the staff of Vietnam National Institute of Nutrition and local officials, especially collaborators, for their efforts in implementing the study. The authors would like to thank Andrew R. Durkin, Professor Emeritus of Indiana University, Bloomington, USA, and Ms. Indri Kartico Sari of Jumonji University for their careful editing of the English for this article.

REFERENCES

1) Delmas PD, Fraser M. 1999. Strong bones in later life: luxury or necessity? Bull World Health Organ 77: 416–422.
2) Bhudhikanok GS, Wang MC, Eckert K, Matkin C, Marcus R, Bachrach LK. 1996. Differences in bone mineral in young Asian and Caucasian Americans may reflect differences in bone size. J Bone Miner Res 11: 1545–1556.
3) United Nations. 2013. Selected statistics about Viet Nam. United Nations Development Programme. [Online]. Available: http://www.un.org/vn/en/about-viet-nam/basic-statistics.html (accessed December 14, 2016).
4) Reid IR, Ames RW, Evans MC, Gamble GD, Sharpe SJ. 1993. Effect of calcium supplementation on bone loss in postmenopausal women. N Engl J Med 328: 460–464.
5) Elders PJ, Netelenbos JC, Lips P, van Ginkel FC, Kohe E, Leeuwenkamp OR, Hackeng WH, van der Stelt PF. 1991. Calcium supplementation reduces vertebral bone loss in perimenopausal women: A controlled trial in 248 women between 46 and 55 years of age. J Clin Endocrinol Metab 73: 533–540.
6) Cadogan J, Eastell R, Jones N, Barker ME. 1997. Milk intake and bone mineral acquisition in adolescent girls: randomized, controlled intervention trial. BMJ 315: 1255–1260.
7) Kamel S, Fardellone P, Meddah B, Lorget-Gondelmann F, Sebert JL, Brazier M. 1998. Response of several markers of bone collagen degradation to calcium supplementation in postmenopausal women with low calcium intake. Clin Chem 44: 1437–1442.
8) Guilleman J, Le HT, Accarie C, du Montcel SL, Delabroise AM, Arnaud MJ, Guilleman S. 2000. Mineral water as a source of dietary calcium: acute effects on parathyroid function and bone resorption in young men. Am J Clin Nutr 71: 999–1002.
9) Sadideon H, Swaminathan R. 2004. Effect of acute oral calcium load on serum PTH and bone resorption in young healthy subjects: an overnight study. Eur J Clin Nutr 58: 1661–1665.
10) Meunier PJ, Jenvrin C, Munoz F, de la Gueronnière V, Garnero P, Menz M. 2005. Consumption of a high calcium mineral water lowers biochemical indices of bone remodeling in postmenopausal women with low calcium intake. Osteoporos Int 16: 1203–1209.
11) Ministry of Health Vietnam. 2006. Vietnamese Recommended Dietary Allowances.
12) Khan NC, Mai le B, Hien VT, Lam NT, Hoa VQ, Phuong TM, Nhung BT, Nakamori M, Shimizu Y, Yamamoto S. 2008. Development and validation of food frequency questionnaire to assess calcium intake in postmenopausal Vietnamese women. J Nutr Sci Vitaminol 54: 124–129.
13) Ho-Pham LT, Nguyen UD, Pham HN, Nguyen ND, Nguyen TV. 2011. Reference ranges for bone mineral density and prevalence of osteoporosis in Vietnamese men and women. BMC Musculoskelet Disord 12: 182. doi: 10.1186/1471-2474-12-182
14) Hien VT, Khan NC, Mai le B, Lam NT, Phuong TM, Nhung BT, Nhuie NV, Nakamori M, Yamamoto S. 2009. Effect of community-based nutrition education intervention on calcium intake and bone mass in postmenopausal Vietnamese women. Public Health Nutr 12: 674–679.

15) Simonetti G, Mohaupt M. 2007. Calcium and blood pressure. Ther Umsch 64: 249–252.

16) Dawson-Hughes B, Dallal GE, Krall EA, Sadowski L, Sahyoun N, Tannenbaum S. 1990. A controlled trial of the effect of calcium supplementation on bone density in postmenopausal women. N Engl J Med 323: 878–883.

17) Gui JC, Brasich JR, Liu XD, Gong GY, Zhang GM, Liu CJ, Gao GQ. 2012. Bone mineral density in postmenopausal Chinese women treated with calcium fortification in soymilk and cow’s milk. Osteoporos Int 23: 1563–1570.

18) Omi N, Ezawa I. 1998. Effect of egg-shell Ca on preventing of bone loss after ovariectomy. J Home Econ Jpn 49: 277–282.

19) Shizuka F. 2011. Bioavailability of some organic calcium products in the rat. J Nagano Pref Coll 66: 1–7 (in Japanese).

20) Igarashi C, Ezawa I, Ogata E. 1990. Effects of whey calcium on bone metabolism in ovariectomized osteoporosis model rats. Nippon Eigo Shokurog Gakkaishi (J Jpn Soc Nutr Food Sci) 43: 437–443 (in Japanese).

21) Omi N, Morikawa N, Ezawa I. 1992. The effect of spiny lobster shell powder on bone metabolism in ovariectomized osteoporotic model rats. J Nutr Sci Vitaminol 38: 555–563.

22) Kikuchi T, Fujiy Y, Fukunaga M. 1994. Effect of eggshell calcium on bone mineral maintenance in lactating rats. Nippon Eigo Shokurog Gakkaishi (J Jpn Soc Nutr Food Sci) 47: 11–14 (in Japanese).

23) World Health Organization. 2004. WHO Scientific Group on the Assessment of Osteoporosis at Primary Health Care Level. Summary Meeting Report. WHO Press, World Health Organization.

24) Kanis JA. 2002. Diagnosis of osteoporosis and assessment of fracture risk. Lancet 359: 1929–1936.

25) Hunt A. 2005. Research on eggshell structure and quality: an historical overview. Brazilian J Poultry Sci (Rev Bras Cienc Avic) 7: 67–74.

26) Karlsson O, Lilja C. 2007. Eggshell structure, mode of development and growth rate in birds. Zoology 111: 494–502.

27) Goto S, Suzuki K, Kanke Y, Kokubu T, Kurakawa T. 1981. The utilization of eggshell as calcium source. Abstracts 35th Annual Mtg Japanese Society of Food and Nutrition, Tokushima, p 124.

28) Niiyama Y, Sakamoto S, Uenishi K. 1985. Effect of soy protein isolate, casein and egg white on calcium utilization in growing rats. Nutr Sci Soy Protein 6: 45–50 (in Japanese).

29) Masaki H, Nakatsu K, Miki T, Takamoto S, Onishi T, Katsuro N, Kunou M, Kawamura M, Nishizawa Y, Morii H. 2000. The effect of eggshell calcium on suppressing bone resorption associated with parathyroid function in the elderly—comparison with the calcium carbonate—. Osteoporos Jpn 8: 245–247 (in Japanese).

30) Kusumi N, Nakamura T, Tando Y, Suda T, Kudo K. 1999. Egg-shell calcium solubility in the stomach. Eijyou-Hyouka to Chiryou 16: 291–296.

31) Griffith LE, Guyatt GH, Cook RJ, Bucher HC, Cook DJ. 1999. The influence of dietary and nondietary calcium supplementation on blood pressure: an updated meta-analysis of randomized controlled trials. Am J Hypertens 12: 84–92.

32) McCarron DA, Morris CD, Bukoski R. 1987. The calcium paradox of essential hypertension. Am J Med 82: 27–33.