Original

Effects of Eggshell Supplementation on Blood Pressure in Normotensive and Mildly Hypertensive Females from Vietnam: A Match-Paired Single-Blinded Placebo-Controlled Study

Ryosuke Matsuoka[1], Vu Thi Thu Hien[2], Mai Thuy Pham [2,3], Lai Thi Cam Nhung[2,3], Linh Nhat Nguyen [2], An Thanh Truong [3], Yen Thi Hai Hoang [3], Thu Trang Nguyen[3], Thu Thi Truong [4], Anh Hong Luu[2,3], Le Thi Hang[2], Le Danh Tuyen,[2,3],
and Shigeru Yamamoto[3*]

[1] *R&D Division, Kewpie Corporation, Chofu, Tokyo, Japan*
[2] *National Institute of Nutrition, Hanoi, Vietnam*
[3] *Asian Nutrition and Food Culture Research Center, Jumonji University, Saitama, Japan;*
[4] *Haiduong Medical Technical University, Haiduong, Vietnam*

***Correspondence author**: Shigeru Yamamoto; yamamotoshigeru426@gmail.com

Abstract: For more than half a century, areas with high calcium contents in their water have lower rates of high blood pressure than those with lower calcium levels. To date, calcium-containing foods have not been used for hypertension prevention. Eggshell is a source of calcium, and eggshell-derived calcium has been reported to be easily absorbable. Ninety-four female participants were randomly categorized into two groups, including the control and eggshell groups. Participants received the following test diets. One type was made from eggshell containing 100-mg calcium (eggshell group) and the other type contained 100-mg cellulose (control group). Participants were fed six capsules daily for 6 months. I Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were significantly decreased in the eggshell group ($p < 0.05$). However, these values did not change in the control group ($p > 0.05$). Furthermore, the eggshell group demonstrated a significantly greater SBP and DBP reduction than the control group. These results indicated that eggshell (calcium intake of 600 mg daily) was effective in lowering SBP and DBP in older adult females from Vietnam at a high risk of hypertension.

Keywords: eggshell, blood pressure, prehypertension, Vietnamese women, intervention study

Introduction

In 1952, Jun Kobayashi of Okayama University measured the acidity and alkalinity of river water across Japan and noted that areas with acidic water had a high stroke mortality rate and those with alkaline water had a low stroke mortality rate (1). Alkaline water is hard water containing high calcium and magnesium levels. This report attracted worldwide attention, and a follow-up investigation was conducted. In 1960, Schroeder in the United States investigated the association between drinking water hardness and age-adjusted mortality rates from cardiovascular disease in 49 states(2). The results revealed that states with high drinking water hardness had lower mortality rates from cardiovascular disease, whereas states with lower drinking water hardness had higher mortality rates.

In Vietnam, stroke is the leading cause of death, accounting for 21.5% of deaths in 2019 (3). Among all hypertension-related complications, stroke is the most frequent. In Vietnam, the recommended calcium intake for adults is 1,000 mg/day (4). However, a nutritional survey conducted by Khan et al. using both his FFQ (Food Frequency Questionnaire) and 24-h recall methods reported that the average calcium intake of females from Vietnam was approximately 350 mg/day (5). LE Griffith et al. in 1999 conducted a meta-analysis of intervention studies regarding calcium intake and blood pressure (6). This review article (6) summarized studies using several calcium sources; however, it did not describe studies using eggshell as a calcium source. They identified and analyzed 42 studies, including eight studies with interventions of ≥6 months and the remaining studies with interventions

of 4–14 weeks. Calcium intake was >1,500, 1,000–1,500, and <1,000 mg/day in 17, 19, and 4 studies, respectively. This review article also encompassed studies conducted in developed countries.

Eggshell contains high levels of calcium carbonate with a porous structure (Figure 1)., whereas most other forms of calcium carbonate have a nonporous structure. Eggshell has a large surface area that comes into contact with stomach acid, enabling calcium to dissolve easily. The solubility of calcium in stomach acid is crucial for its absorption in the digestive tract. Previously, we evaluated the effects of ingesting 300 mg of calcium from eggshell per day on bone density for 12 weeks and observed that it increased bone density compared with controls (7). Simultaneously, blood pressure was lowered compared with that before ingestion; therefore, eggshell has the potential to lower blood pressure. In this study, the absence of significant differences observed in the control group may be attributed to the following reasons: some participants had normal blood pressure, the amount of calcium administered was low, and the test system was designed for improving bone density.

Therefore, this study aimed to prove the hypothesis that eggshell supplementation could exert a preventive effect in older adult females from Vietnam with normotension and mild hypertension taking a daily calcium dose of <500 mg.

In Vietnam, stroke is the leading cause of death and in 2019 (21.5%) deaths (3). Stroke is the most frequent of hypertension-related complications. The recommended amount of calcium for adults in Vietnam is 1,000 mg/day (4). However, a nutritional survey conducted by Khan et al. using both his FFQ method and his 24-hour recall method reported that the average calcium intake of Vietnamese women was approximately 350 mg/day(5). A detailed analysis of intervention studies regarding calcium intake and blood pressure can be found in a 1999 Meta-analysis by LE Griffith et al. (6). They selected/identified and analyzed 42 studies. Eight studies had interventions of 6 months or more, and the others had interventions of 4 to 14 weeks. calcium intake was >1500 mg/day in 17 studies, 1000-1500 mg/day in 19 studies, and <1000 mg/day in 4 studies.

Eggshell calcium is calcium carbonate that has a porous structure, while most other forms of calcium carbonate have a non-porous structure (Figure 1). Eggshell calcium has a large surface area that comes into contact with stomach acid, so it easily dissolves. The key to absorption of calcium from the digestive tract is its solubility in stomach acid. Previously, we evaluated the effect of ingesting 300 mg of eggshell calcium per day on bone density for 12 weeks and found that it increased bone density compared to controls (7) At the same time, blood pressure was also lowered compared to before ingestion, so eggshell calcium can be expected to have a blood pressure lowering effect. The reasons why no significant differences from the control group were observed in this study may be that the subjects included some with normal blood pressure, the amount of calcium administered was low, and the test system was designed to improve bone density.

Based on the above, this study was conducted to prove the hypothesis that eggshell calcium supplementation can be expected to have a preventive effect in elderly Vietnamese women with high risk for hypertension and a daily calcium intake less than 500 mg.

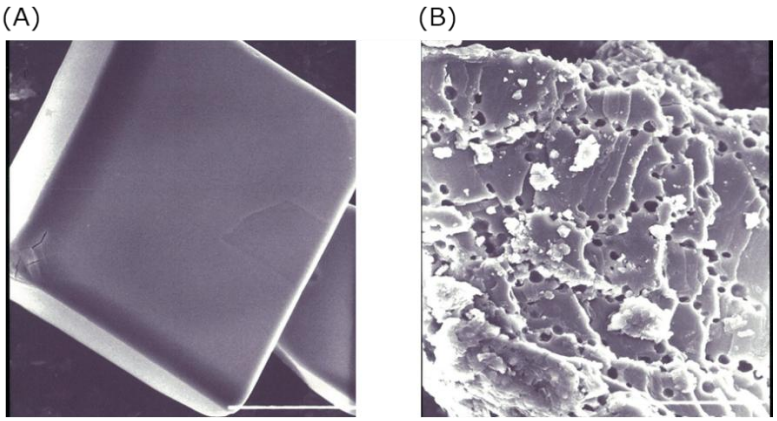

Figure 1. Microscopic photograph of eggshell calcium and calcium carbonate (8000x). A: Calcium bicarbonate B: Eggshell calcium.

Materials and Methods

Materials Calhope from Kewpie Egg Corporation. (Tokyo, Japan) was used as eggshells. Eggshells replaced with crystalline cellulose (CEOLUS FD-101; Asahi Kasei Corporation, Tokyo, Japan) were used as controls. The test food was formulated into capsules, and the composition is shown in Table 1. To support the control group, all capsules were supplemented with thiamin and riboflavin, which are not believed to influence calcium absorption or metabolism. The treatment/dose/intake was designed to provide 600 mg of calcium by consuming six capsules daily. The capsules used in the test were prepared by Aliment Industries Co., Ltd. (Shizuoka, Japan),.

Table 1. Composition of the capsules containing (mg/capsule).

Material	Egg shell Group	Control Group
Egg Shell	263 (100mg Calcium)	0
Cellulose	143	216
Thiamine hydrochloride	0.10	0.10
Riboflavin	0.10	0.10
Sucrose fatty acid ester	8.40	8.40

Subjects and test protocol We recruited 200 females aged ≥60 years. The blood pressure of each participant was measured using a manual manometer, and 120 candidates with a SBP of 120–139 mmHg or a DBP of 80–89 mmHg and were not taking any drugs were selected. Considering the potential use in disease prevention, the participants were with high-normal blood pressure and high-value blood pressure according to the 2019 Hypertension Treatment Guidelines of the Japanese Society of Hypertension (7)

The following were the rationale for selecting females: postmenopausal females are at a higher risk of heart disease, and bone density decreases owing to the effects of estrogen; therefore, the participants of this study were females with the aim that calcium supplementation would improve calcium metabolism overall. We conducted a nutritional survey on these 120 females and selected 94 females whose calcium intake was <500 mg/day)

Participants were instructed to maintain their usual diet, physical activity, sunlight exposure time, and all other activities.

The following were the inclusion criteria: [1] females aged >60 years who were postmenopausal for at least 5 years [2] blood pressure was in the prehypertension range (SBP, 120–139 mmHg; DBP, 80–89 mmHg); [3]) daily calcium intake was <500 mg/day; [4] body mass index (BMI) of 18.5–24.9 kg/m^2; and [5] agreed to participate in this study.

The following were the exclusion criteria: [1] had kidney diseases and (2) were taking other calcium-containing functional foods.

Pairs of participants matched for age, BMI, and calcium intake were randomly categorized into a control group and an eggshell group, each comprising 47 participants. Smokers and drinkers were excluded during screening.

For 6 months, participants took six capsules daily, including two capsules each after breakfast, lunch, and dinner. Height, weight, blood pressure, and bone mass were measured at the start of the study and at 6 months. Blood pressure measurements were performed at the local health center after waking up and without having breakfast at 15-min intervals. When a large difference was noted, a third measurement was taken 15 min later, and the average value of the two closest-matching results was used.

This study was conducted between April 2023 and October 2023 at Kim Thanh, Hai Duong, Vietnam. The weather conditions from April to October were almost similar.

This study complied with the guidelines of the Declaration of Helsinki and was approved by the Ethical Committee of the National Institute of Nutrition in Hanoi, Vietnam (784/VDD-QLKH). All participants provided written informed consent. This study was registered at the University Hospital Medical Information Center (UMIN; ID: UMIN000057942).

Dietary analysis This study employed the 24-h dietary recall method. Participants comprehensively recounted what they ate the day before the interview. We used the Institute of Nutrition's Album of popular dishes to help participants recall and accurately answer during the interview. The nutritional value of the diet was calculated on the basis of the Nutritional Composition Table of Vietnamese Foods 2007.

Sample size Based on the results of a previous trial wherein eggshell ingestion led to blood pressure reduction (8), the sample size was calculated using power analysis by the statistical software R ($1 - \beta = 0.80$, $\alpha = 0.05$, effect size = 0.42); 45 or more participants were required per group, and the number of participants in this study satisfied this number.

Statistical analysis Data were presented as means ± standard deviations (SDs). Comparisons of values before and after study initiation within each group were performed using the Wilcoxon signed-rank test. The Mann–Whitney U test was performed to compare both groups. Moreover, a comparison of the changes in blood pressure over a 6-month period between the two groups was performed using the Mann–Whitney U test. In all the abovementioned statistical analyses, $p < 0.05$ was considered statistically significant. All statistical analyses were performed using Statistical Package for the Social Sciences (version 20; IBM Corp., Armonk, NY, USA).

Results

Subject background Of the participants, five withdrew from both the control and eggshell groups owing to personal reasons, leaving 42 participants in the final analysis. The detailed flow diagram of the study is depicted in Figure 2. The background characteristics of the participants in both groups are presented in Table 2. Characteristics encompassed BMI, waist-to-hip ratio, SBP, DBP, and calcium intake. No significant differences were noted between the two groups ($p > 0.05$)

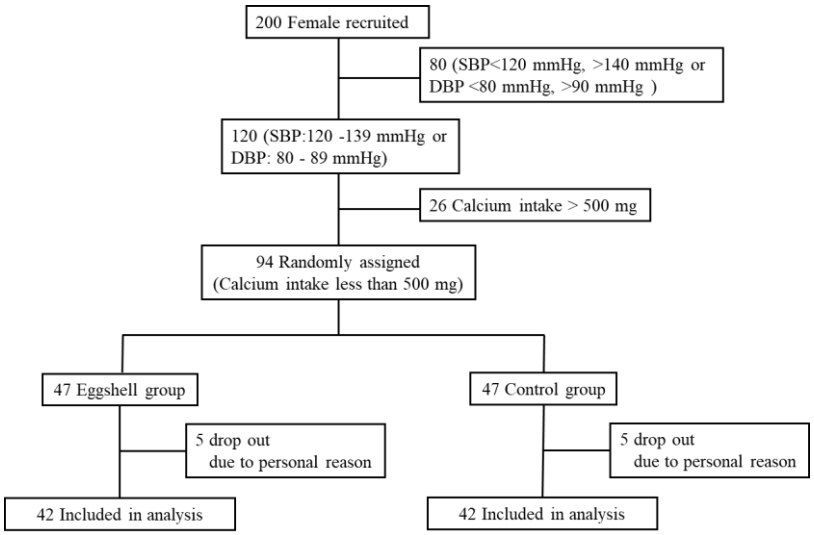

Figure 2. Participant flow through a randomized trial.

Table 2. Background characteristics of subjects between the two groups (mean±SD)

Characteristics	Eggshell Group (n=42)	Control Group (n=42)	*p* value
Age (years)	64.7 ± 3.6	65.1 ± 5.2	0.986
Weight (kg)	52.1 ± 5.7	52.4 ± 6.5	0.785
Height (cm)	152 ± 4	153 ± 5	0.704
BMI (kg/m2)	22.7 ± 2.6	22.5 ± 2.4	0.844
WHR	0.9 ± 0.1	0.9 ± 0.1	0.130
SBP (mmHg)	127 ± 7	126 ± 8	0.447
DBP (mmHg)	79.0 ± 5.9	77.0 ± 6.4	0.188
Education level			
Primary	8	8	
Secondary	29	28	
High school	5	6	
College/University	0	0	
Postgraduate	0	0	
Occupation			
Government staff /Intellectual worker	2	1	
Factory worker	1	2	
Farmer	36	35	
Trader/Business person /Self-employed in trade	1	1	
Housewife	2	3	

Dietary analysis The dietary analysis results are shown in Table 3. No significant differences in intakes of energy, protein, carbohydrates, lipids, calcium, phosphorus, sodium, potassium, and vitamins D and K1 were observed between both groups. After the test period, the bottles containing each supplement were collected from the participants, and the remaining amount was analyzed. The intake rate of the test sample was 100% across all participants.

Table 3. Energy and nutrient intakes (mean±SD)

Characteristics	Eggshell Group (n=42)	Control Group (n=42)	*p* value
Energy (Kcal)	1292 ± 433	1232 ± 447	0.499
Protein (g)	49.0 ± 22.4	47.1 ± 18.7	0.799
Carbohydrate(g)	228 ± 80	220 ± 100	0.778
Lipids (g)	20.4 ± 11.7	18.2 ± 11.6	0.258
Calcium (mg)	377 ± 254	396 ± 224	0.474
Phosphorus (mg)	642 ± 351	606 ± 253	0.932
Sodium (mg)	1369 ± 894	1217 ± 787	0.471
Potassium (mg)	1538 ± 708	1450 ± 745	0.483
Vitamin D (mg)	1.77 ± 4.08	3.47 ± 9.45	0.238
Vitamin K1 (mg)	192 ± 355	169 ± 326	0.669

Blood pressure The SBP (left chart) and DBP (right chart) at baseline and at 6 months for the eggshell and control groups are illustrated in Figure 3. SBP and DBP values significantly decreased in the eggshell group but remained unchanged in the control group.

The changes (mmHg) in SBP (left) and DBP (right) values over 6 months in the eggshell and control groups are shown in Figure 4. The eggshell group demonstrated significantly decreased SBP values compared with the control group ($p < 0.05$). Furthermore, the eggshell group exhibited significantly decreased DBP values compared with the control group ($p < 0.05$).

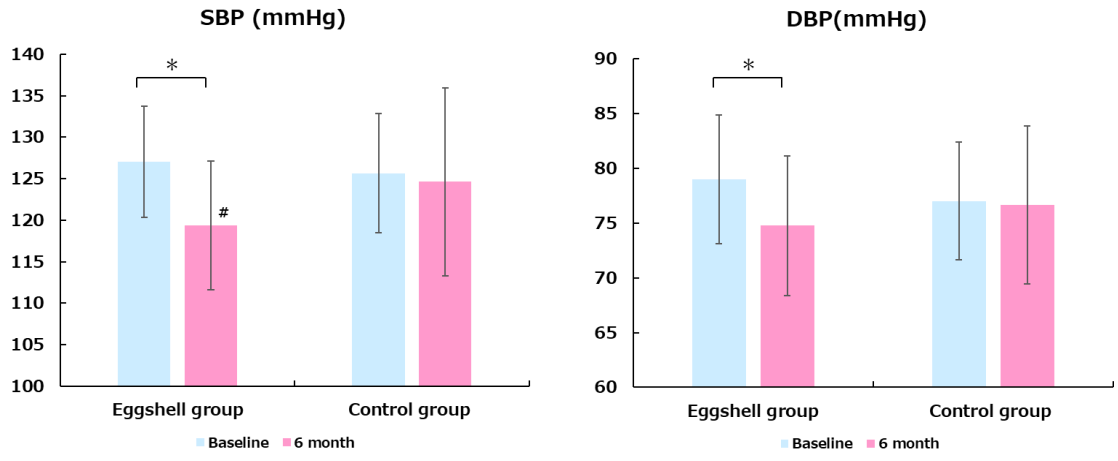

Figure 3. Changes in blood pressure after 6 months in the eggshell group and control groups (n = 42 in each group; mean ± standard deviation)

*: Significant difference compared with baseline by Wilcoxon signed-rank test ($p < 0.05$), #: Significant difference compared with the control group by the Mann–Whitney U test ($p < 0.05$)

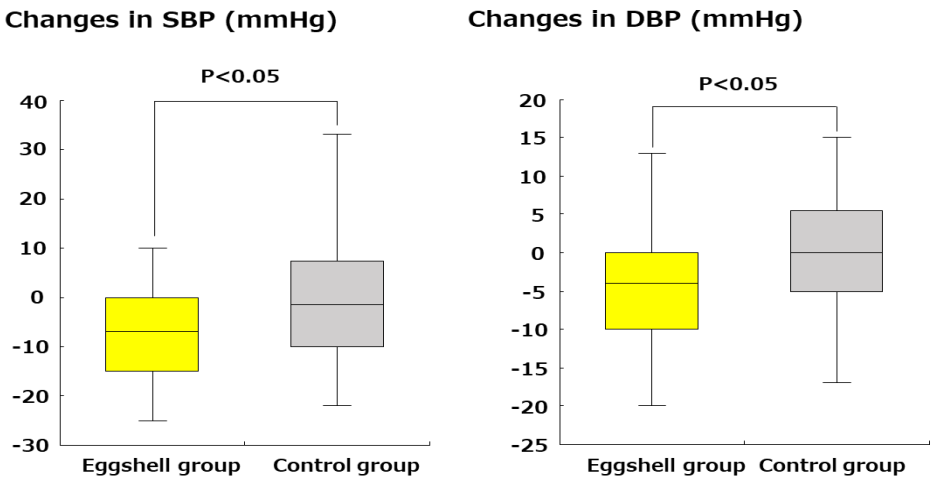

Figure 4. Boxplots comparing changes in blood pressure over 6 months. *P* <0.05 indicates a significant difference by the Mann–Whitney U test.

Discussion

We here investigated the effects of a 6-month eggshell intake on blood pressure in females from Vietnam with prehypertension (SBP of 120–139 mmHg and/or DBP of 80–89 mmHg). Two types of capsules were prepared. One contained 263 mg of eggshell (100-mg calcium) per capsule (eggshell group), and the other contained 263 mg of cellulose (control group). In the eggshell group, SBP (mmHg) significantly decreased from 127 to 119 mmHg, and DBP (mmHg) significantly decreased from 79 to 75 mmHg ($p < 0.05$). The control group did not exhibit changes in blood pressure ($p > 0.05$). In Vietnam, the recommended calcium intake for adults is 1,000 mg/day. The average daily calcium intake of the study participants was 350 mg; therefore, providing those 600 mg daily brought their intake near the recommended amount.

Here, the degrees of eggshell intake-induced blood pressure reduction were 8 and 4 mmHg for SBP and DBP, respectively. The degree of blood pressure reduction caused by materials other than calcium was as follows: sardine peptides in 13 weeks (SBP, −6.2 mmHg; DBP, −3.1 mmHg) (9) flaxseed oil in 12 weeks (SBP, −7.6 mmHg; DBP, −4.5 mmHg) (10), and milk protein tripeptides in 4 weeks (SBP, −8.0 mmHg; DBP, −3.8 mmHg) (11). Therefore, the blood pressure-lowering effect of eggshell was deemed effective, given its significant difference from the control group and the degree of reduction.

Epidemiological studies have demonstrated that a 10-mmHg reduction in SBP decreases the risk of major cardiovascular disease, coronary heart disease, stroke, and heart failure by approximately 20%, 17%, 27%, and 28%, respectively (12). In this study, an 8-mmHg reduction in SBP may reduce the risk of these diseases.

In particular, in Vietnam, stroke is the leading cause of death, accounting for 21.5% of mortalities (3); considering that calcium intake is only approximately one-third (350 mg/day) of the recommended amount (1,000 mg/day), the blood pressure-lowering effect of 600-mg/day eggshell calcium supplementation is of great public health significance. Interventions using safe and readily available food-derived ingredients, including eggshell calcium, before initiating drug therapy, may be valuable from the perspectives of reducing medical costs and avoiding drug side effects. Furthermore, as the study participants were older adult females at a high risk of osteoporosis, eggshell calcium intake may exert the dual health benefits of reducing blood pressure and improving bone density. These outcomes could contribute to extending healthy lifespan and reducing medical and nursing care-related burden in an aging society.

The following mechanism is believed to be caused by calcium intake on hypertension prevention and management. Abnormal calcium accumulation may develop in the arteries of older adults with arteriosclerosis, which initially appears to be caused by excessive calcium intake. However, this accumulation was actually due to inadequate calcium intake, known as the calcium paradox (13).

The mechanism for this is believed to be that parathyroid hormone secretion increases secondary to deficient calcium intake, releasing calcium from bones and promoting its uptake into cells (13. In other words, when calcium is insufficient, it abnormally enters the cells, keeping them constantly switched on. Muscle contraction occurs when calcium abnormally enters muscle cells due to calcium deficiency. Smooth muscles surround arteries; therefore, excessive calcium concentration causes the muscles to contract, narrowing the arteries and increasing

blood pressure. A similar process affecting the heart causes angina pectoris. The effectiveness of calcium blockers, which inhibit calcium uptake into cells, for hypertension and angina pectoris represents a good example of the strong association between calcium and cardiovascular disease. Moreover, high blood pressure can damage blood vessel walls and cause arteriosclerosis and ischemic heart disease.

To date, most studies that have investigated the effects of calcium interventions on blood pressure have been conducted in Western populations. The meta-analysis by LE Griffith et al. (6) in 1999 analyzed 42 studies. Eight studies had interventions of ≥6 months, and the remaining studies had interventions of 4–14 weeks. Calcium intake was >1,500, 1,000–1,500, and <1,000 mg/day in 17, 19, and 4 studies, respectively. Several studies have reported favorable outcomes. Moreover, a previous study in Japan demonstrated similar effects. In 2013, an epidemiological study by Umezawa et al. indicated that dietary calcium intake reduces the incidence of stroke in middle-aged individuals from Japan (14,15).

Regarding the influence of calcium on blood pressure in Asians, it is interesting to observe the association between diseases and life expectancy in Okinawa, one of Japan's 47 prefectures. Okinawa was the location of Japan's most intense ground battle during World War II. Despite its defeat in the war, Okinawans had the longest global life expectancy(16). At that time, stroke due to high blood pressure was the leading cause of mortality among individuals from Japan; however, the mortality rate for stroke among individuals from Okinawa was low. Yamamoto et al. reported that Okinawa is an island made of raised coral reefs, and the water is hard with a high calcium concentration (16). Sweet potatoes, the people's major energy source back then, and locally grown vegetables had high calcium levels. During that period, the average daily calcium intake for a Japanese adult was <500 mg, whereas for an adult in Okinawa, it was >2,000 mg (17,18) Westerners are already taking the recommended dose in Vietnam in their daily lives and are also administering 500–1,000 mg/day to achieve the desired effect. Conversely, the daily calcium intake of Vietnamese is <500 mg, which is approximately 50% that of Westerners (19). This finding suggests that even with similar additional dose is the same, 500mg, the effect of improving blood pressure can be expected to be higher in Vietnamese than in Westerners. Various calcium sources have been investigated in an attempt to prevent osteoporosis (20,21)

The eggshell is composed of 97% calcium carbonate (22,23) and has a spongy tissue structure with many small holes ((micro-pores: Figure 1). The porous eggshell structure might potentially contribute to its high solubility in the gastrointestinal tract, supporting the rapid development of organs that consume high calcium levels, including the skeleton, muscles, and brain of the chick before hatching. The calcium absorption rate of eggshell powder was 34.8%, which was higher than that of nonmicropore calcium carbonate (24). A study involving rats revealed the superiority of eggshell calcium absorption over other carbonate calcium absorption (25-27) (Figure 5). Additionally, a 3-month study involving older females receiving 2 g of calcium from eggshell and calcium carbonate reported that those who received eggshell calcium exhibited significantly suppressed parathyroid hormone secretion compared with those provided calcium carbonate (13). Furthermore, Shizuka reported that in rats, the absorption of eggshell calcium was higher than that from other carbonate calcium, and eggshell calcium consumption resulted in greater fracture strength of the femur and higher femoral calcium content than the consumption of other carbonate calcium (28). Moreover, Kikuchi et al. cited the low P content as a reason for the excellent eggshell calcium absorption (29). The ratio of calcium to P is 0.3 to 100 calcium in eggshells, and 73.3 in commercially available calcium preparations. Human health research on eggshell calcium is scarce. Hien et al. supplemented two dietary types to females from Vietnam (average age, approximately 60 years) with low bone density, which included 300 mg of eggshell calcium or 300 mg of calcium carbonate, to their daily diet (approximately 400-mg calcium/day) (8). Omi and Ezawa compared the effects of eggshell calcium supplementation with other nonmicropore carbonate calcium supplements in ovariectomized rats and observed that the eggshell calcium group had higher hip bone density than the other carbonate calcium group (30).

Although no significant difference was noted in vitamin D intake, the control group demonstrated a higher value of vitamin D intake. As an essential nutrient, vitamin D promotes calcium absorption. As the eggshell group exhibited a blood pressure-lowering effect despite having a low vitamin D intake, calcium, the main component of eggshells, may have significantly contributed to this effect.

A community health center worker with extensive experience in blood pressure taking performed all measurements using a manometer. Measurements were taken twice at 15-min intervals before breakfast; when a significant difference was noted, a third measurement was taken 15 min later, and the average value of the two similar measurements was used. Through these efforts, we believe that the data obtained were reliable. The required number of participants was approximately 60 but was increased to 94 to account for dropouts. In this study, dropouts were rare that 84 participants (42 in each group) completed the 6-month study. To reduce dropouts, researchers and community health center workers visited the participants once a week to ask about their progress and establish good relationships. The SDs of DBP and SBP changes were small (Figure 1). These findings suggest the universality of the effect of eggshell calcium on improving hypertension.

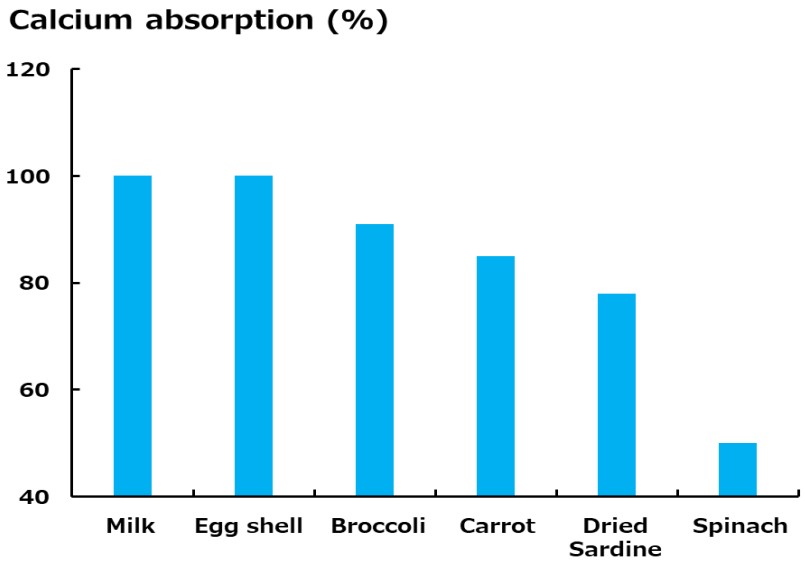

Figure 5. Calcium absorption rate of various foods when the calcium absorption rate of milk is 100% (The figure was made by extracted from the table in Reference (27).

This study had some limitations. First, it was conducted on females from Vietnam. Although the effects on males and other races were not explored, it was believed that the abovementioned mechanism would also be effective in these populations. Second, this study was conducted on untreated individuals with high blood pressure, and the effects on patients with hypertension remained unclear; so, its use in treatment is a topic for future investigation.

The large disparity between healthy life expectancy and average life expectancy has become a worldwide social problem. This gap is likely attributed to cardiovascular diseases and fractures. In this study, eggshell has been demonstrated to increase bone density but also reduces blood pressure as shown in the results of this study, so contribute to extending healthy life expectancy.

Conclusions

In conclusion, administration of Eggshell (600mg calcium/day) was effective to decrease SBP and DBP in elderly Vietnamese women with pre-hypertension.

Acknowledgments:

We appreciate all of the subjects and staff of the Community Health Center and Prof. Emeritus of Indiana University, Andrew Durkin for editing the manuscript's English.

References

1. Kobayashi, J. On geographical relationship between the chemical nature of river water and death-rate from apoplexy Preliminary reports). *Berichte des Ohara Instituts für landwirtschaftliche Biologie, Okayama Universität*, **1957**, 11, 12-21. http://ousar.lib.okayama-u.ac.jp/49903 (Accessed on May 1st, 2025)
2. Schroeder, H.A. Relation between mortality from cardiovascular disease and treated water supplies: variations in states and 163 largest municipalities of the United States. *J. Am. Med. Assoc*. **1960**, 172, 1902-1908.
3. Aminde, L.N.; Phung, H.P.; Phung, D.; Linda, J.; Cobiac, J.; Veerman, L. Dietary Salt Reduction, Prevalence of Hypertension and Avoidable Burden of Stroke in Vietnam: Modelling the Health and Economic Impacts, 2021, *Public Health*, 9, 682975.
4. Khan, N.C.; Hoan, P.V. Vietnum recommended dietary allowances 2007. *Asia Pac J Clin Nutr,* **2008**, 17, 409-415
5. Khan, N.C.; Mai, L.B.; Hien, V.T.T.; Lam, N.T.; Hoa, V.Q.; Phuong, T.M.; Nhung, B.T.; 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*, **2008**, 54, 124-129.

6. Griffith, L.E.; Guyatt, G.H.; Cook, R.J.; Bucher, H.C.; Cook, D.J. The influence of dietary and nondietary Ca supplementation on blood pressure: an updated meta-analysis of randomized controlled trials. *Am J Hypertens*, **1999** 12: 84–92.
7. Umemura, S.; Arima, H.; Arima, S.; Asayama, K.; Dohi, Y.; Hirooka, Y.; Horio, T.; Hoshide, S.; Ikeda, S.; Ishimitsu, T.; Ito, M.; Ito, S.; Iwashima, Y.; Kai, H.; Kamide, K.; Kanno, Y.; Kashihara, N.; Kawano, Y.; Kikuchi, T.; Kitamura, K.; Kitazono. T.; Kohara, K, Kudo M, Kumagai H, Matsumura K, Matsuura H, Miura K, Mukoyama M, Nakamura S, Ohkubo T, Ohya Y, Okura T, Rakugi H, Saitoh S, Shibata H, Shimosawa T, Suzuki H, Takahashi S, Tamura K, Tomiyama H, Tsuchihashi T, Ueda S, Uehara Y.; Urata, H.; Hirawa, N. The Japanese society of hypertension guidelines for the management of hypertension (JSH 2019), *Hypertens Res*, **2019**, 42*,* 235-1481.
8. Sakai, S.; Hien, V.T.T.; Tuyen, L.D.; Duc, H.A.; Masuda, Y.; Yamamoto, S. Effects of Eggshell Calcium Supplementation on bone mass in postmenopausal Vietnamese women, *J Nutr Sci Vitaminol*, **2017**, 63, 120-124.
9. Kawasaki, T.; Jun, C.J.; Fukushima, Y.; Kagei, K.; Osajima, K.; Itoh, K.; Matsui, T.; Matsumoto, K. Antihypertensive effect and safety evaluation of vegetable drink with peptides from sardine protein hydrolysate on mild hypertensive, high-normal and normal blood pressure subjects. *Fukuoka Igaku Zasshi*. **2002**, 93. 208-218.
10. Takeuchi, H.; Sakurai, C.; Noda, R.; Sekine, S.: Murano, Y.; Wanaka. K.; Watanabe, S.; Aoyama, T.; Kondo K. Antihypertensive effect and safety of dietary α-linolenic acid in subjects with high-normal blood pressure and mild hypertension. *J Oleo Sci*, **2007**, 56, 347-60.
11. Ishida. Y.; Shibata, Y.; Fukuhara, I.; Yano. Y.; Takehara. I.; Kaneko. K. Effect of an excess intake of casein hydrolysate Val-Pro-Pro in subjects with normal blood pressure, high-normal blood pressure, or mild hypertension. *Biosci Biotechnol Biochem*. **2011**. 75. 427-433.
12. Ettehad, D.; Emdin, C.A.; Kiran, A.; Anderson, S.G.; Callender, T.; Emberson, J.; Chalmers, J.; Rodgersm, A.; Rahimi, K. Blood pressure lowering for prevention of cardiovascular disease and death: a systematic review and meta-analysis. *Lancet*, **2016**, 387, 957-967
13. Mccarron, D.A.; Morris, C.D.; Bukoski, R. The calcium paradox of essential hypertension. *Am J Med*. **1987**, 82, 27-33.
14. Hien, V.T.T.; Khan, N.C.; Mai, L.B.; Lam, N.T.; Phuong, T.M.; Nhung, B.T.; Nhien, N.V.; Nakamori, M.; Yamamoto. S. Effect of community-based nutrition education intervention on calcium intake and bone mass in postmenopausal Vietnamese women. *Public Health Nutr*, **2009**, 12, 674-679.
15. Umesawa, M.; Iso. H.; Date, C.; Yamamoto, A.; Toyoshima, H.; Watanabe, Y.; Kikuchi, S.; Koizumi, A.; Kondo, T.; Inaba, Y.; Tanabe, N.; Tamakoshi, A. Dietary intake of calcium in relation to mortality from cardiovascular disease: The JPHC study cohort I. *Stroke*, **2006**, 37, 20-26
16. Umesawa , M.; Iso, H.; Ishihara, J.; Saito, I.; Kokubo, Y.; Inoue, M.; Tsugane S.; JPNC Study Group. Dietary calcium intake and risks of stroke, its subtypes, and coronary heart disease in Japanese: The JPHC study cohort I. *Stroke*; **2008**, 39: 2449-2456.
17. Yamamoto, S.; Sinjyo, S.; So, S.; Takahashi, M. Okinawa paradox of nutrition and health. *Long lives of Okinawns* 2nd ed.; Sho, H.; Yamamoto. S. **1999**, pp. 141-163. in Japanese.
18. Takahashi, M. Mineral intake of Okinawan people, *Notre Dame Seishin University Kiyo*, **1998**, 22, 79-90. in Japanese.
19. Yamamoto, S. Mineral concentration of Okinawa foods and its effect on healthy, *Journal of the society tropical resources technologists*, 1996, 12, 9-16. in Japanese.
20. Khan, N.C.; Mai, L.B.; Hien, V.T.T.; Lam, N.T.; Hoa, V.Q.; Phuong, T.M.; Nhung, B.T.; Nakamori, M.; Shimizu, Y.; Yamamoto, S. Development and validation of food frequency questionnaire to assess Ca intake in postmenopausal Vietnamese women. *J Nutr Sci Vitaminol*, **2008**, 54, 124-129.
21. Ho-Pham, L.T.; Nguyen, U.D., Pham, H.N.; Nguyen, N.D.; Nguyen, T.V. Reference ranges for bone mineral density and prevalence of osteoporosis in Vietnamese men and women. *BMC Musculoskelet Disord*, **2011**, 12, 182.
22. Dawson-Hughes, B.; Dallal, G.E.; Krall, E.A.; Sadowski, L.; Sahyoun, N.; Tannenbaum, S. A controlled trial of the effect of calcium supplementation on bone density in postmenopausal women. *N Engl J Med,* **1990**, 323, 878–883.
23. Hunton, P. Research on eggshell structure and quality: an historical overview. *Brazilian J Poultry Sci*, **2005**, 7, 67-74.
24. Karlsson, O.; Lilja, C. Eggshell structure, mode of development and growth rate in birds. *Zoology*, **2007**, 111: 494–502.
25. Niiyama, Y.; Sakamoto, S.; Uenishi, K. Effect of soy protein isolate, casein and egg white on calcium utilization in growing rats. *Nutr Sci Soy Protein*, **1985**, 6, 45-50. in Japanese.

26. Masaki, H.; Nakatsuka, K.: Miki, T.; Takamoto, S.; Onishi, T.; Katsuro, N.; Kunou, M.; Kawamura, M.; Nishizawa, Y.; Morii, H. The effect of eggshell Ca on suppressing bone resorption associated with parathyroid function in the elderly - comparison with the calcium carbonate. *Osteoporos Japan*, **2000**, 8, 245–247. in Japanese.
27. Nakashima, Y.; Esashi, T. Comparative study of calcium absorption rates due to differences in calcium sources 2 - Comparison of calcium in each food with the calcium utilization rate in milk (calcium utilization rate in milk is set at 100) -. Rinsho eiyo, 1994, 85, 81-85. in Japanese.
28. Shizuka, F. Bioavailability of some organic calcium products in the rat. *J Nagano Pref Coll*, **2011**, 66, 1-7. in Japanese.
29. Kikuchi, T.; Fujii, Y.; Fukunaga, M. Effect of eggshell calcium on bone mineral maintenance in lactating rats. *Nippon Eiyo Shokuryo Gakkaishi,* **1994**, 47, 11–14, in Japanese.
30. Omi, N.; Morikawa, N.; Ezawa, I. The effect of spiny lobster shell powder on bone metabolism in ovariecto mized osteoporotic model rats. *J Nutr Sci Vitaminol*, **1992**, 38, 555-563.