Original

Determining Calcium requirement of Vietnamese women aged 55-65 years old

Vu Thi Thu Hien[1*], Nguyen Anh Tu[1], Nguyen Doan Dung[1], Nguyen Thi Diep Anh[1], Luu Hong Anh[1], Le Hoang Dan[1], Pham Thuy Mai[2], Lai Thi Cam Nhung[2], Le Anh Hoa[2], Ryosuke Matsuoka[2], Yamamoto Shigeru[2]

[1]*Vietnam National Institute of Nutrition (NIN), Hanoi City, Vietnam*
[2]*Jumonji University, Asian Nutrition and Food Culture Research Center, Niiza City, Saitama, Japan*

ABSTRACT *Purpose.* Research was conducted to determine Calcium (Ca) requirement for Vietnamese women aged 55-65 years old, using the Ca balance method. *Methods.* There were 14 women participated in the study. Each subject was provided with a controlled diet in 2 stages: the first 15 days with a Ca intake of about 300 mg/day, the next 15 days with a Ca intake of about 600 mg/day. Ca intake is calculated based on the database of Vietnamese Food Composition Table. Between the two stages is a 15-days of washing out, in which subjects ate their usual diet at home. Dietary intake, feces samples and urine samples were collected during both stages for quantitative Ca analysis using atomic absorption spectrometry (AAS). Ca metabolic balance was calculated for each dietary Ca level. Ca requirements were estimated based on the linear regression correlation between Ca intake and Ca metabolic balance. *Results and conclusion*. Research results show that the Ca requirement of the subjects is 625 mg/day. The Recommended Dietary Allowance (RDA) of Ca for postmenopausal women aged 55-65 years in rural areas is 750 mg/day. More research is needed to determine RDA of Ca for different age groups of Vietnamese.

INTRODUCTION

In recent years, there has been increasing evidence of an association between Ca intake and bone health. Adequate and continuous Ca intake throughout life will contribute to strong bones, increase peak bone mass in adulthood and reduce bone loss in old age, thereby contributing to the prevention of rickets in children, and osteoporosis in the elderly (1). Therefore, research on Ca requirements for each age group and each stage of life is a matter of great concern (2, 3).

Ca requirements for each age group have been studied decades ago. There have been many scientific research projects published and widely applied in many countries around the world (1, 4, 5). However, with current knowledge, Ca requirements depend greatly on physical activity level, nutritional status, age, gender and race. The Ca RDA for people over 50 years old in the US and Canada is 1200 mg/day, in France it is 900 mg/day, in England it is 700 mg/day and in Australia it is 800 mg/day (6-8).

There are many methods to determine Ca requirement such as: determining the requirement by calculating the total amount of Ca excreted in urine and feces (factorial method), the epidemiological investigation method and the balance method. Among them, the balance method is accepted and used the most by scientists because of its high accuracy and feasibility (4). According to this method, the average Ca requirement in adults is determined by the linear regression correlation between Ca intake and Ca metabolic balance. Accordingly, Ca requirement is calculated as the diet at which intake and output are equal (balance=0) (1).

In Vietnam, the rate of osteoporosis in adults is 9%, relatively high compared to other countries in the region (9). Meanwhile, the average Ca intake in adults is still <500 mg/day (10). Ca requirements have been recommended by the National Institute of Nutrition (NIN) based on scientific evidence recommended by the World Health Organization and consultation with other countries in the region (11). Until now, there has been no publication about results of researches on Ca requirements in Vietnamese's adults. Therefore, the authors conducted research to find the Ca requirement in women aged 55-65 years, an age group at high risk of osteoporosis, with the hope that the research results will contribute to enrich the scientific evidence in order to develop Dietary Reference Intake (DRI) for Vietnamese people.

MATERIALS AND METHODS

1. Location: Viet Hung commune, Kim Thanh district, Hai Duong province, Vietnam

2. Time: from November 2007 to February 2008

3. Design: Estimated Ca requirement in subjects based on the linear relationship between Ca intake and Ca metabolic balance, with 2 Ca intake levels of 300 and 600 mg/day corresponding to 2 study stages. Using a linear regression analysis model, Ca requirement was determined as the Ca intake at which balance = 0 (**Fig 1**).

*To whom correspondence should be addressed: hienvuthithu.nin@gmail.com

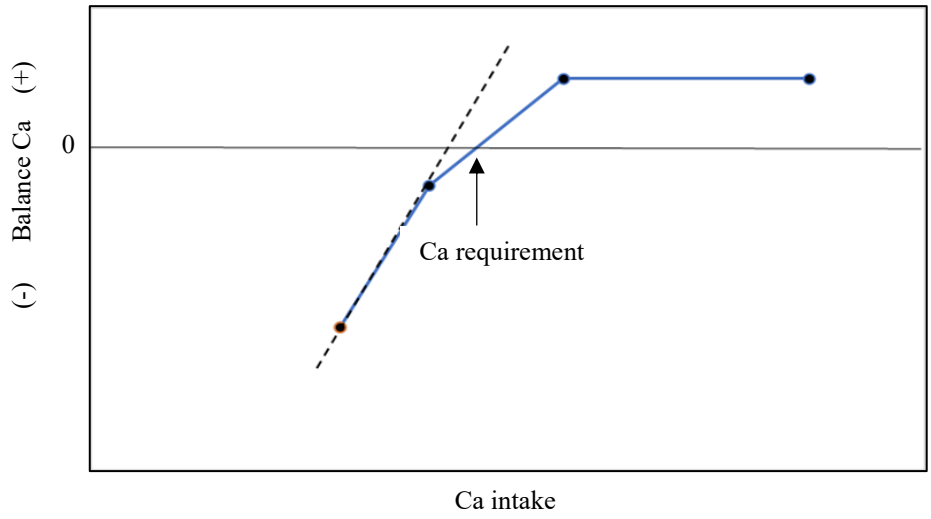

Fig 1. Linear regression analysis model between Ca intake and Ca metabolic balance to estimate Ca requirement

Stage 1: Subject were taken a diet with Ca concentration of about 300 mg/day for 15 days according to a calculated menu, based on the database of Vietnamese Food Composition table (12). The first 10 days (adaptation period) is the time for the subject to adapt to the new menu.

5 days later was the sample collection time: subject still ate the same diet as above. In addition, 24-hour feces and 24-hour urine were collected for analysing Ca concentration.

Stage 2: Subject ate a Ca diet of about 600 mg/day for 15 days, including 10 days of adaptation period and a 5-day of collecting feces and urine samples.

Between the two stages was a 2-weeks of washing out, the subject ate the usual diet at home (4).

4. Subject and sample size

Selection criteria: Women 55 - 65 years old, more than 5 years postmenopausal, voluntarily participating in the study, BMI from 18.5 to 23, has 1-2 children, bone density was normal, did not take drugs that affect Ca metabolism/products containing Ca within 1 month before the study, Ca intake in the past 3 months was 300-400 mg/day.

Exclusion criteria: Have one of these diseases: cirrhosis, duodenal ulcer, high blood pressure, colitis, kidney disease, kidney stones, often constipated.

Sample size: 12 people (4). After adding 15% of the subject who might give up, the estimated study sample size is 14 people.

Selecting subject: Women aged 55-65 years in the same village were invited to participate in screening survey to select 14 eligible subjects for the study.

5. Methods and applied techniques

5.1. Screening and selecting subjects:

Interviewed with general information about age, number of children, menstrual status, medical history... Collected anthropometric measurements: weight, height to calculate BMI. Measured bone density using quantitative ultrasound through the heel bone to identify subjects with osteoporosis (speed of sound index <1503 m/s). Assessed general diet by 24 hours recording method for 3 non-consecutive days, plus asking. Assessed Ca intake over the past 3 months using the semi-quantitative questionnaire (SQFFQ) method.

5.2. Analysis of Ca content in local drinking water: By atomic absorption spectroscopy (AAS) method.

5.3. Preparing menus to achieve 2 levels of Ca intake of approximately 300 mg/day and 600 mg/day, taking into account the amount of Ca from drinking water and water used to prepare dishes. Other nutritional values of the menus (energy, protein, lipid, carbohydrate) were equivalent to usual diets of subjects.

5.4. Deployment:

5.4.1. Providing subjects a controlled diet:

In each stage of study, subjects were provided with free meals for 15 days (3 meals/day), at a centralized dining location, under the supervision of NIN's staff. Subjects were asked not to eat any food or drink other than provided meals.

5.4.2. Collecting food samples

For each meal, the researchers prepared 15 meals (14 meals for subjects, 1 meal for analysing Ca concentration in the meal). The 15th portion of 3 meals a day were collected, mixed well and ground with a food processor. After grinding, 50g of food was put into a jar with a tight lid, stored in the freezer and weekly transferred to the NIN for storage at -20°C until analysing Ca concentration.

5.4.3. Collecting feces samples

Before the first meal of day 11, each subject ingested 1 g of carmine marker (a red substance that is not involved in metabolic processes in the body). When the red color of Carmine appears in the subject's feces, sampling begins and the red-colored portion of the stool is removed. The subject's feces are also collected in the following days. On day 15, after the last meal, 1 g of carmine was administered to each subject. Sampling ended when the feces turned the red color of carmine. The red feces were later removed. The fecal samples from all subjects were stored for 5 days at room temperature in sealed boxes labelled with the code of each subject (each subject had 5 boxes for 5 days). After the fecal samples were collected for all 5 days, all samples in each subject's 5 boxes were collected, shredded and mixed well. Approximately 50 g of feces were placed in a jar with a tight-fitting lid and taken to the NIN, where they were stored at -20 °C until analysis.

5.4.4. Collecting urine samples

24-hour urine samples were collected immediately after the first intake of carmine over a period of about 2 hours (subjects were asked to urinate completely before taking carmine) until the end of the night on the 15th day. Using a special sampling device (product made in Japan), 1/50th of the urine volume was stored after each urination. The subjects could carry the device with them day and night. After each day, this device was returned to the researcher, who collected, stored and analysed the urine samples. Each subject's urine was stored in a separate bottle (labelled with date and code). The urine was preserved with a 6N-HCL acid solution. (20 - 30 ml for a 24-hour urine sample) at a temperature of 5-8°C. The 5-day urine of each subject was collected, mixed well, 50 ml was poured into a bottle with a large lid and taken to the NIN where it was stored at -20°C until analysis.

5.4.5 Monitoring and evaluation.

Daily monitoring of water intake, body weight, feces and urine, and unusual health manifestations of the subjects during the research process. Body weight was measured in the morning on an empty stomach. The subjects' diet was monitored throughout the study. The subjects' physical activity was maintained as usual throughout the study period.

5.4.6 Analysing Ca concentration in food, feces and urine.

Food, feces, and urine samples were spiked with HNO3 acid prior to analysis. After inorganic combustion, the Ca concentration of the sample was analysed using the AAS method.

5.4.7. Method for estimating Ca requirement:

Balance of Ca metabolism (Ca balance) was estimated based on the equation:

Balance Ca = Ca intake – Ca output

Ca output = Ca in feces + Ca in urine

$$\text{Ca absorption rate (\%)} = \frac{\text{Ca intake} - \text{Ca in feces}}{\text{Ca intake}} \times 100$$

Use a linear regression model to estimate Ca requirement based on two dietary levels of 300 and 600 mg/day, refer to the method of Jackman and CS (4, 13)

Recommended Ca requirement (RDA) is estimated according to the following equation (4, 14): RDA = Ca requirement x 1.2

6. Data analysis

Dietary data and menus are calculated using ACCESS software, with the database of Vietnamese Food Composition Table.

Data was analysed by using SPSS software. Linear regression analysis was used to estimate Ca requirements.

RESULTS

After screening, 14 eligible subjects were invited to participate in the study. Throughout the study, all subjects consumed no food or drink other than the meals offered, physical activity remained as usual, and all followed the instructions for collection of feces and urine samples. All subjects consumed the offered meals every day and had no health problems.

Table 1. General characteristics of the subjects at the beginning of the study

Variables	Mean ± SD
Age (years)	58 ± 3,8
Height (cm)	154,5 ± 1,6
Weight (kg)	47,1 ± 3,4
BMI	19,8 ± 1,3
Ca intake in the past 3 months (mg/day)	346,8 ± 40,5
Bone density (SOS, m/s)	1521,9 ± 18,1

Table 1 shows the general characteristics of the subjects at the beginning of the study. The subjects were on average 58 years old and had a normal BMI of 19.8. The average Ca intake of the subjects in the 3 months prior to the study was 346.8 ± 40.5 (mg/day).

Table 2. Dietary characteristics of subjects *(24-hour recording method in 3 days)*

Nutritional value	Mean ± SD
Energy (kcal)	1813 ± 232.1
Protein	
Total (g)	56.0 ± 21.6
Animal protein (g)	29.0 ± 18.5
Lipid	
Total lipid (g)	25.3 ± 11.3
Animal lipid/ Total lipid (%)	52.8 ± 17.8
Mineral	
Ca (mg)	377.5 ± 134
Fe (mg)	12.9 ± 7.8
Vitamin	
Vitamin A (mcg)	0.19 ± 0.94
Carotene (mg)	4.7 ± 3.9
Vitamin D (mcg)	0.28 ± 0.55
% Energy	
Protein	12.3
Lipid	12.9
Carbohydrate	74.8

Table 2 shows the general diet of the subjects, with average energy intake is 1813 ± 232.1 (kcal/day), protein intake and lipid intake are 56.0 ± 21.6 g/day and 25.3 ± 11.3 g/day, respectively. The subjects' dietary energy mainly came from carbohydrates, with a P: L: C energy ratio of 12.3%:12.9%:74.8%.

3 menus for Ca levels of approximately 300 mg/day and 3 menus for Ca levels of approximately 600 mg/day had been designed with energy, carbohydrate, protein, and lipid values approximately equivalent to those shown in Table 2 to ensure the experimental diet is diverse and similar to the subject's usual diet.

Table 3. Results of analysing Ca concentration in the diet

Menu	Ca concentration in the diet (mg/day)	
	Stage 1	Stage 2
Menu A (n=5)	327.6 ± 15.7	605.6 ± 7.7
Menu B (n=5)	306.1 ± 21.2	595.8 ± 6.7
Menu C (n=5)	317.6 ± 8.8	605.8 ± 13.4
Average (n=15)	317.1 ± 18.1	602.4 ± 10.1

Data are presented as mean ± SD

Table 3 shows the results of analysing Ca concentration in the diet provided to the subjects. Results shown that Ca concentration in the diet assessed directly by food sample analysis has a value nearly equivalent to the Ca diet estimated based on the Vietnamese Food Composition Table (12).

Table 4. Ca balance of subjects

	Stage 1	Stage 2
Ca intake (mg/day)	317.1 ± 18.1	602.4 ± 10.1
Amount of Ca in feces (mg/day)	309.7 ± 95.2	498.2 ± 193.6
Amount of Ca in urine (mg/day)	150.3 ± 36.4	146.0 ± 53.7
Metabolism balance= Intake-Output (mg/day)	-130.0 ± 101.6	-10.1 ± 203.6
Amount of Ca absorbed (mg/day)	20.2 ± 95.2	136.1 ± 193.1
Ca absorption rate (%)	6.1 ± 28.8	21.4 ± 30.5

Data are presented as mean ± SD or %

Table 4 shows the average amount of Ca excreted in feces of subjects in stage 1 is 309.7 ± 95.2 mg/day and stage 2 and 498.2 ± 193.6 mg/day. Meanwhile, the amount excreted in urine is less, in stage 1 is 150.3 ± 36.4 mg/day and stage 2 and 146,0 ± 53.7 mg/day. Ca absorption rate in stage 1 (6.1%) is lower than stage 2 (21.4%). There is a remarkable point that, Ca metabolic balance in both stages has negative values (-130.0 ± 101.6 mg/day in stage 1 and -10.1 ± 203.6 in stage 2).

The results of linear regression analysis are illustrated in Fig 2, according to which the Ca metabolic balance is 0 when the Ca diet reaches 625mg/day. Therefore, the subject's Ca requirement is estimated to be 625mg/day. From that result, the RDA of Ca for postmenopausal women aged 55-65 years in rural areas was estimated according to the method of K. Uenishi et al (4, 14) are as follow: 625 x 1.2 = 750 mg/ day.

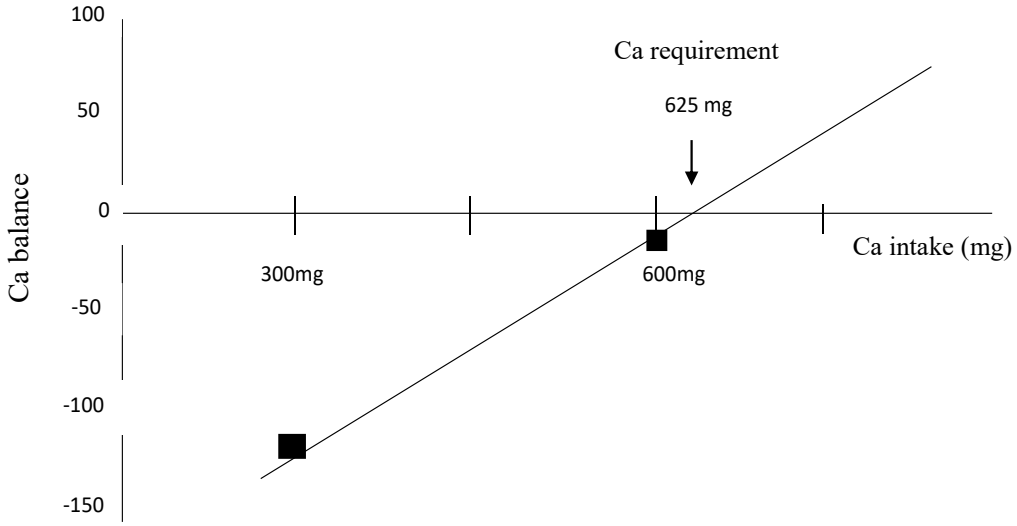

Fig 2. Linear relationship between Ca intake and Ca metabolic balance to estimate the subject's Ca requirement

Body weight changes:

The average body weight of the subject increased from 47.1 kg (before intervention) to 47.5 kg (after stage 1) and 47.8 kg (after stage 2). The change in body weight of subjects from before the study to the end of the study was not statistically significant.

DISCUSSION

The balance method (determination of requirements via the metabolic balance in the body) has proven to be very accurate and practicable in studies on nutrient requirements (4). Ideally, 3 points are used to determine nutrient requirements, which correspond to 3 nutritional levels (Figure 1). In Ca requirement studies, the method of using 2 points corresponding to 2 intakes is still scientific if the point corresponding to a high intake is close to the estimated intake, as Ca balance is linearly related to Ca intake (13). The 2-point method has also been used in many previous studies and has been shown to be reliable (4,13). In this study, we used two Ca intakes: 300 mg/day and 600 mg/day. The results show that the estimated Ca requirement is 625 mg/day, which corresponds to a close point of 600 mg/day (Figure 1). Therefore, we believe that the estimated results of the study are relatively close to reality.

Compared to women of the same age in other countries, the Ca requirement of the subjects in this study was lower. Using the same method of utilisation, the Ca requirement of women in some white communities is 900 mg/day, while that of Japanese women is 788 mg/day, while that of Vietnamese women is 625 mg/day (4, 15). This finding is consistent with the conclusion of previous studies that Ca requirements vary by race (8).

The results regarding the amount of Ca absorbed by the body are almost identical to the study by K. Uenishi et al. on Japanese women of the same age (4). K. Uenishi used 2 levels of Ca intake (250 mg/day and 850 mg/day) to determine the Ca requirement. The results show that the amount of Ca ingested at low Ca intake of Vietnamese people (20.2 mg/day) is similar to that of Japanese women (21 mg/day), but at high Ca intake, the Ca requirement of Vietnamese women (136.1 mg/day) is much higher than that of Japanese women (113 mg/day). This indicates that the Ca requirement of Vietnamese is lower than that of Japanese because the Ca saturation point is lower.

"Recommended dietary allowance (RDA)" is a different term than "requirements". While the requirements of a nutrient for the body is estimated based on the individual, the RDA is used to make recommendations for the whole community. Therefore, the RDA is usually estimated at 1.2 times the requirement (14). The recommended Ca requirement for some white communities are 1000-1500 mg/day for elderly women. The requirement for Japanese people of the same age is 650 mg/day (15),

while Vietnamese people are 750 mg/day. If computed the requirements by weight, with the average weight of the subjects in this study being 47.1 kg, the Ca requirements is 15.9 mg/kg body weight. This weight-based requirement also remains lower than that of white women (18.5 mg/kg) and Japanese women (18.1 mg/kg) (4, 16, 17, 18). Compared to the recommended dietary allowance in the book "Recommended nutritional dietary allowance for Vietnamese people" published in 2007, our estimated results are lower. Therefore, required studies to confirm the results of this study. Our research results show that, subjects gained weight after 45 days of joining in the study, although this weight gain was not statistically significant. This may be because the study was conducted at a time when farmers were leisured, and the diet provided, although having total energy, carbohydrates, proteins and lipids, was nearly equivalent to the regular diet, the completeness was related to Dietary restrictions that have led to weight gain. However, the menu computed in this study closely follows the recommended dietary allowance for energy-generating substances, so the estimated Ca requirement is applicable.

CONCLUSION

The Ca requirement of women aged 55 to 65 years was estimated at 625 mg/day in this study.

The Ca requirements dietary allowance of women aged 55 to 65 years was estimated at 750 mg/day in this study.

RECOMMENDATION

There is a need to research Ca requirements for different age groups to enrich scientific knowledge and develop a Dietary Reference Intake (DRI) for the Vietnamese population.

REFERENCES

1. FAO/WHO. Calcium. In: Human vitamin and mineral requirement. Report of a Joint Expert Consultation. FAO Rome: 151-171. 2002.
2. Heaney RP. Calcium, dairy products and osteoporosis. J Am Coll Nutr, 2000.
3. Heaney RP. The important of calcium intake for lifelong skeletal Health. Calcif Tissue Int; 70: 70-73. 2002.
4. K. Uenishi, H. Ishida, A. Kamei, et al. Calcium requirement estimated by balance study in elderly Japanese people. Osteoporos Int 12: 858-868. 2001.
5. Commission of the European Communities. Nutrient and energy intakes for the European community. Reports of the Scientific Committee for Food (31st Series). Office for Official Publications of the European Communities, Luxembourg. 1993.
6. Connie M Weaver. Calcium requirements of physically active people. Am J Clin Nutr 72(suppl): 579S-84S. 2000.
7. WHO. Prevention and management of osteoporosis, Technical Report Series, No. 921. 2003.
8. A. Prentice. What are the dietary requirements for calcium and vitamin D? Calcif Tissue Int; 70: 83-88. 2002.
9. Hien VTT; Khan NC, Lam NT, et al. Determining the prevalence of osteoporosis and related factors using quantitative ultrasound in Vietnamese adult women. Am J Epidemol 161(9): 824-830. 2005.
10. National Institute of Nutrition, Ministry of Health. General nutrition survey 2000. Hanoi Medical Publishing House. 2003.
11. National Institute of Nutrition, Ministry of Health (1997). Dietary reference intake of Vietnamese people. Hanoi Medical Publishing House. 1997.
12. National Institute of Nutrition, Ministry of Health. Food composition table. Hanoi Medical Publishing House. 2000.
13. Jackman LA, Millane SS, Martin BR, et al. Calcium retention in relation to calcium intake and postmenarcheal age in adolescent females. Am J Clin Nutr 66:327-33. 1997.
14. Health Promotion and Nutrition Division, Health Service Bureau, Ministry Health and Welfare. Recommended dietary allowances for Japanese. 5th revision. Tokyo: Dai ichi Shuppan. 1996.
15. Ministry of Health, Labour and Welfare. Dietary Reference Intakes for Japanese. 2020.
16. NIH consensus development panel on optimal calcium intake. Optimal calcium intake. JAMA 272: 1942-8. 1994.
17. Heaney RP. Recommended calcium intakes revisited: round table. Nutritional aspect of osteoporosis. Springer 317-25. 1998.
18. Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. National Academy Press 111-7. 1997.