Journal of Food and Nutrition Research, 2023, Vol. 11, No. 12, 719-725
Available online at http://pubs.sciepub.com/jfnr/11/12/3
Published by Science and Education Publishing
DOI:10.12691/jfnr-11-12-3

Effect of Eggshell and Vitamin D Fortified Meal Kit on Bone Mineral Density in Postmenopausal Women: A Randomized Clinical Trial

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Received November 05, 2023; Revised December 06, 2023; Accepted December 12, 2023

Abstract People in some countries have traditionally low consumption of fresh milk and dairy products in their diets. The purpose of this study was to evaluate the effect of fortified meal with eggshell calcium and vitamin D on bone health between in postmenopausal women with osteopenia and those with normal bone mineral density. Twenty-five postmenopausal women were recruited in a six-month intervention study. Fortified meal kit (800 mg of calcium plus 2,000 IU of vitamin D per week) or conventional meal kit was applied to an osteopenia group (n = 18) and a normal bone density group (n = 7) by random allocation. Participants consumed a delivered meal kit three times a week. Mann-Whitney U test, non-parametric X^2 test, and non-parametric correlation analysis was performed to compare between two groups of bone mineral density and bone markers. Before and after six months, bone mineral density and bone markers were compared between the two groups. Bone mineral density of the femur neck was significantly increased in the osteopenia group (P = 0.043) than in the normal bone mineral density group (P = 0.629). However, there was no significant change in bone mineral density of lumbar vertebrae or total hip in either group (P = 0.829 vs. P = 0.173 or P = 0.229 vs. P = 0.400, respectively). In analysis between the changes of femur neck bone mineral density and bone markers in the osteopenia group, only parathyroid hormone showed a little tendency (P = 0.081). Six months of supplementation with eggshell calcium and vitamin D fortified meal increased bone mineral density of the femur neck in postmenopausal women with osteopenia. Adding dietary calcium and vitamin D to meal may improve the bone mineral density in postmenopausal women with osteopenia

Keywords: *dietary supplements, egg shell, calcium, vitamin D, osteopenia, menopause*

Cite This Article: Seok-Hoon Lee, Kyung-Jin Yeum, and Nam-Seok Joo, "Effect of Eggshell and Vitamin D Fortified Meal Kit on Bone Mineral Density in Postmenopausal Women: A Randomized Clinical Trial." Journal of Food and Nutrition Research, vol. 11, no. 12 (2023): 719-725. doi: 10.12691/jfnr-11-12-3.

1. Introduction

Osteoporosis is caused by a reduction in bone mass. By the time of menopause in women, bone loss accelerates as a result of a cessation of estrogen synthesis. The growth of the elderly population can cause an increase in the prevalence of osteoporosis. In addition, osteoporotic fracture is costly to treat. It can potentially reduce the quality of life to patients and increase mortality. Therefore, it is essential to prevent a substantial decrease in bone mineral density in the aging population.

Calcium and vitamin D are essential nutrients for bone health. Proper dietary intake is one of the important factors that can affect bone mineral density. It has been documented that sufficient intake of calcium and vitamin D is vital to maintain bone strength and to prevent osteoporosis, particularly in postmenopausal women [1]. Moreover, low dietary calcium intake is related to low bone density. Calcium supplementation can reduce age-related bone loss.

Every day, millions of tons of eggshells are produced as biological waste around the world. Thus, processing eggshell waste into food additives is very beneficial [2]. Chicken eggshells are a good natural source of calcium with potential for use in food. Indeed, chicken eggshell powder is a natural source of calcium. It contains about 38-39 % calcium as calcium carbonate [2]. It can be added to food products. Several studies have shown that eggshell powder has positive effects on bone mineral density of the lumbar spine and hip in patients with osteoporosis [3,4]. Schaafsma et al. have shown that eggshell powder supplement is more effective than purified calcium carbonate in improving bone mineral density in the femoral neck of postmenopausal women [4].

The demand for meal kit has been increased as changes in lifestyle and increase in single-person families [5].

Eggshell-containing meal kit has been produced and studied for years [6,7]. As calcium is mainly absorbed from the intestine by passive diffusion and vitamin D-dependent active transport [8], vitamin D fortified supplementation should be combined. An animal study has shown that the addition of vitamin D can improve bone mineral density of the lumbar spine and the proximal tibia in rats treated with eggshell [9].

Some randomized controlled trials have shown increases in bone mineral density and a reduction in fracture risk in older patients supplemented with calcium and vitamin D [10,11,12]. However, to the best of our knowledge, studies comparing improvement in bone mineral density in people with normal bone mineral density and those with osteopenia (low bone mass) by calcium and vitamin D supplementation using meal kit for a long-term have not been reported yet. Thus, the purpose of this study was to evaluate effects of meal kit with fortified eggshell and vitamin D on bone mineral density and bone markers in postmenopausal women with osteopenia and those with normal bone mineral density.

2. Material and Methods

This was a secondary analysis study. This double-blind, randomized, placebo-controlled study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the Institutional Review Board of Ajou University School of Medicine (AJOUIRB-FOD-2020-094). Informed consent was obtained from all subjects.

2.1. Study Participants

A total of 33 subjects volunteered for study inclusion. Of them, eight subjects were excluded from the final analysis due to non-compliance such as difficulties in eating convenience food and relocation of residence. Among a total of 25 patients, 7 patients in the normal bone mineral density group and 18 patients in the osteopenia group were analyzed (Figure 1).

2.1.1. Inclusion Criteria

Subjects with age between 50 and 64 years; Those with body mass index between 18.5 and 30.0kg/m2; and postmenopausal women. Menopause was defined as 12 months of spontaneous amenorrhea, if the last menstrual period was less than 6 months to 1 year, and blood follicular stimulating hormone > 40 mIU/mL.

2.1.2. Exclusion Criteria

Patients with osteoporosis (T-score < -2.5), thyroid disease, uncontrolled hypertension (> 160/100 mmHg), diabetes mellitus (fasting blood sugar >180 mg/dL), uncontrolled chronic diseases that could affect metabolism (chronic, liver diseases, alcoholism, primary hyperparathyroidism), malignancy, an esophageal disease that delayed esophageal emptying, uncorrected hypercalcemia, or hypocalcemia; those who took hormone replacement therapy within 3 months prior to participate in the intervention; subjects who had consumed calcium or vitamin D (> 200 IU/day) supplements or medications that might affect bone or calcium metabolism (bisphosphonates, steroids, diuretics, etc.); those who had laboratory test abnormalities in baseline screening; those with aspartate aminotransferase or alanine aminotransferase > 120 U/L, serum creatinine > 2.0mg/dL, thyroid stimulating hormone > 10 μIU/mL or < 0.15 μIU/mL; and those who had egg allergy.

2.2. Intervention

If subjects consented to registration, they were randomly assigned using a random number table (stratified by age and body mass index) in the regional clinical trial center of Ajou University Hospital. and provided the subject's information to Konkuk University. Eggshell-containing meal kit contained additional eggshells (1.0-1.7%) (800 mg of calcium) and 2,000 IU of vitamin D per week compared to the conventional meal kit. One of two kinds of meal was delivered to subjects according to the allocation information. Double-blind could be maintained because it was difficult to distinguish between eggshell-containing meals and regular meal kit as they were packaged identically with similar taste. Since the intervention period was relatively long (six months), the frequency of taking meal kit was three meals a week and three different types of meal kit (curry, black bean source, pumpkin porridge; we educated them to eat curry and black bean sauce mix with rice) were served in rotation [for example; Monday lunch (curry with rice), Wednesday lunch (rice with black bean source), Friday lunch (pumpkin porridge)]. The rest of the meals were eaten as usual and analyzed through meal diaries and food frequency surveys.

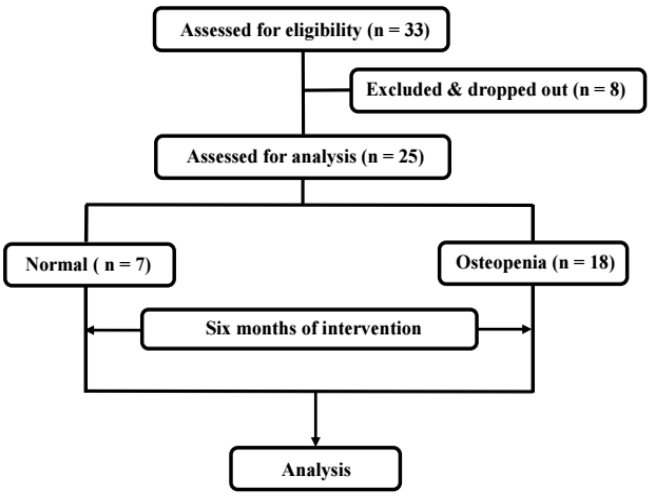

Figure 1. Enrollment of study subjects

2.3. Measurements

2.3.1. Anthropometric, Biochemical, and Bone Mineral Density Measurements

Height, weight, and abdominal circumference were measured by a trained research coordinator. Body mass index was calculated as weight (kg) divided by height squared (m^2). Blood pressure was measured using a standard automated sphygmomanometer after a resting period of 5 minutes. Blood samples were collected after subjects had fasted overnight. Venous blood samples were

collected for measuring fasting blood glucose, aminotransferases, total cholesterol, high-density lipoprotein-cholesterol, triglycerides, low-density lipoprotein-cholesterol, hemoglobin A1c, insulin, serum calcium, thyroid stimulating hormone, thyroxine, parathyroid hormone, and serum osteocalcin. Urine samples were also collected for measuring urine n-telopeptide, urine calcium, and urine creatinine. Serum 25(OH)D was assessed with a radioimmunoassay kit (DiaSorin Inc., Stillwater, MN, USA) using a γ-counter (1470 Wizard; PerkinElmer, Turku, Finland). Serum intact parathyroid hormone was gauged using a chemiluminescence assay (DiaSorin, USA). Bone mineral density of the lumbar spine (L1-4) and total hip, femoral neck was measured using dual-energy X-ray absorptiometry (DXA, DISCOVERY-W fan-beam densitometer, Hologic Inc., USA) with coefficient of variations of 1.9% and 2.5%, respectively.

2.3.2. Nutritional Assessments

Nutrient intakes were assessed with three days of dietary recall questionnaires conducted by a trained dietician in Konkuk University. Results were calculated using the Food Composition Table developed by the National Rural Resources Development Institute (7th revision) [13].

2.3.3. Behavioral Assessments

Current smoker was defined as someone who had smoked more than a hundred cigarettes during their whole life. All others were regarded as ex-smokers or non-smokers. Regular alcohol drinkers were defined as those who drank alcohol more than once a month. All others were regarded as non-drinkers. Aerobic exercises were defined as the practice of more than 150 minutes of moderate-intensity physical activity or 75 minutes of high-intensity physical activity, or a mixture of them.

Table 1. Baseline Characteristics of the Study Subjects (n = 25)

Variables	Total (n = 25)	Normal (n = 7)	Osteopenia (n = 18)	*P*-value
Age (years)	55.7 (0.7)	53.4 (0.9)	56.6 (0.8)	0.035
Body weight (kg)	60.1 (1.6)	60.7 (2.9)	60.1 (2.0)	0.904
Height (cm)	158.0 (1.0)	158.9 (2.7)	157.6 (1.5)	0.809
BMI (kg/m^2)	24.1 (0.6)	24.1 (1.1)	24.5(0.9)	0.809
Waist (cm)	82.7 (1.5)	81.5 (2.4)	83.1 (2.0)	0.628
SBP (mmHg)	120.6 (2.0)	117.6 (4.8)	121.8 (2.2)	0.525
DBP (mmHg)	76.1 (1.9)	72.9 (4.3)	77.3 (2.1)	0.380
FBS (mg/dl)	96.0 (2.3)	98.1 (5.4)	95.2 (2.6)	0.762
Insulin (uIU/ml)	6.1 (0.7)	7.8 (2.0)	5.5 (0.7)	0.430
Serum creatinine (mg/dl)	0.69 (0.18)	0.69 (0.03)	0.68 (0.02)	0.879
Total cholesterol (mg/dl)	214.2 (7.6)	206.4 (10.3)	217.2 (9.9)	0.449
Triglyceride (mg/dl)	113.8 (10.3)	106.1 (16.1)	116.8 (13.0)	0.565
HDL (mg/dl)	63.9 (3.4)	65.6 (5.7)	63.2 (4.2)	0.585
LDL (mg/dl)	127.5 (7.2)	119.6 (8.2)	130.6 (9.5)	0.430
Serum calcium (mg/dl)	9.4 (0.1)	9.7 (0.2)	9.4 (0.1)	0.084
TSH (uIU/m)	2.61 (0.25)	2.52 (0.53)	2.65 (0.29)	0.739
FT4 (ng/dl)	1.25 (0.03)	1.25 (0.06)	1.24 (0.03)	0.832
PTH (pg/ml)	36.0 (4.2)	24.7 (4.4)	40.4 (5.3)	0.084
25(OH)D (ng/ml)	25.1 (2.1)	28.6 (6.6)	23.7 (1.5)	0.785
Osteocalcin (ng/ml)	24.2 (1.6)	25.1 (1.8)	23.9 (2.2)	0.716
NTx (mMBCE/mM Cr)	55.1 (4.8)	58.4 (11.3)	53.8 (5.3)	0.785
Urine calcium (mg/dl)	14.5 (1.7)	15.4 (3.1)	14.1 (2.0)	0.506
Urine creatinine (mg/dl)	103.3 (9.5)	79.0 (9.8)	112.8 (12.1)	0.146
Urine Calcium/Creatinine	0.14 (0.01)	0.19 (0.03)	0.12 (0.01)	0.014
Lumbar BMD (g/cm^3)	1.086 (0.031)	1.219 (0.054)	1.035 (0.030)	<0.001
Femur Neck BMD (g/cm^3)	0.859 (0.028)	1.041 (0.044)	0.788 (0.016)	<0.001
Total Hip BMD (g/cm^3)	0.930 (0.027)	1.090 (0.046)	0.868 (0.018)	<0.001
Exercise/day (min)	60.9 (6.8)	74.0 (26.8)	57.1 (4.6)	0.871
Calorie intake (kcal/day), 0m	2216.7 (770.1)	2439.5 (662.4)	2075.1 (905.2)	0.345
Calorie intake (kcal/day), 6m	2227.8 (754.4)	2395.7 (812.6)	2087.4 (732.9)	0.369
Smoking, Yes (n)	0	0	0	
Alcohol drinking, Yes (n)	8	2	6	0.072*
Regular exercise, Yes (n)	22	5	17	<0.001*

Normal, normal bond density group; Osteopenia, osteopenia group; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBS, fasting blood sugar; HDL, high density lipoprotein; LDL, low density lipoprotein; TSH, thyroid stimulating hormone; FT4, free T4; PTH, parathyroid hormone; NTx, n-telopeptide; BMD, bone mineral density; Exercise, aerobic exercise; 0m, before intervention, 6m, 6 months after initiating intervention. Data represents mean (standard error) and P-values were calculated from non-parametric comparison by Mann-Whitney U test. Data represents numbers P-values* were calculated from non-parametric X^2 test.

2.4. Statistical Analysis

All continuous variables are presented as mean ± error by non-parametric comparison with Mann-Whitney U test in both two groups due to a small sample size. In the case of categorical variables, a non-parametric X^2 test was used. Non-parametric correlation analysis was performed to investigate the relationship of bone mineral density with bone markers. All statistical analyses were performed with SPSS version 25.0 (SPSS Inc., Armonk, NY, USA). *P*-value < 0.05 was considered statistically significant.

3. Results

Baseline characteristics of subjects are shown in Table 1. Subjects were divided into two groups: a normal bone mineral density group and an osteopenia (low bone mass) group. Mann-Whitney U test and non-parametric X^2 test were performed to identify significantly different parameters between the two groups. We found that parameters including age, body mass index, parathyroid hormones, 25(OH)D, osteocalcin, and n-telopeptide were not significantly different between the two groups (P = 0.035, P = 0.809, P = 0.084, P = 0.785, P = 0.716, and P = 0.785, respectively). Bone mineral density of lumbar vertebrae and femur were significantly different between the two groups (P < 0.001). Urine calcium/creatinine ratio and regular exercise were also significantly different between the two groups (P = 0.014 and P < 0.001, respectively).

Table 2 shows changes of bone mineral density after intervention in the normal bone mineral density group and osteopenia group according to the kind of MEAL KIT. In the osteopenia group, we found a statistically significant change in bone mineral density of the femur neck (P = 0.043) in the osteopenia group compared to the normal

group. However, we did not observe any significant difference in bone mineral density change of lumbar vertebrae or total hip in the osteopenia group (P = 0.829 and P = 0.173, respectively). Moreover, there were no statistically significant changes of bone mineral density in the normal bone mineral density group (P = 0.229, P = 0.629, and P = 0.400, respectively).

Spearman's correlation coefficients were calculated to evaluate relationships between changes of femur neck bone mineral density and bone markers. Results are presented in Table 3. The correlation between changes of parathyroid hormone and femoral neck bone mineral density showed a little tendency (P = 0.081) (Figure 2) without reaching statistical significance. Contrary to expectation, changes of femur neck bone mineral density were not statistically correlated with changes of serum 25(OH)D, osteocalcin, n-telopeptides, or urine calcium/creatinine ratio (P = 0.740, P = 0.189, P = 0.223, or P = 0.291, respectively).

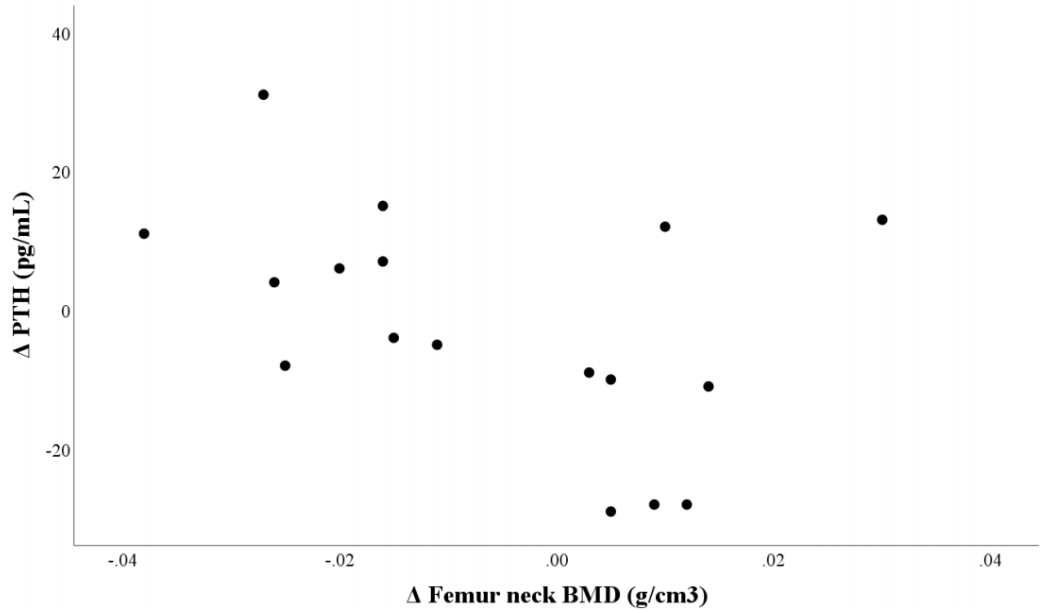

Figure 2. Correlation between changes of parathyroid hormone and femur neck bone mineral density after 6 months of intervention in the osteopenia group

Table 2. Comparison of Bone Mineral Density after 6 months of Intervention in Normal or Osteopenia Group

Variables	Normal group (n = 7)			Osteopenia group (n = 18)		
	Treatment (n = 3)	Control (n = 4)	*P*-value	Treatment (n = 10)	Control (n = 8)	*P*-value
ΔLumbar BMD (g/cm^3)	0.002 (0.012)	-0.010 (0.006)	0.229	-0.007 (0.004)	0.005 (0.005)	0.829
ΔFemur Neck BMD (g/cm^3)	-0.020 (0.015)	-0.036 (0.009)	0.629	0.001 (0.004)	-0.017 (0.007)	0.043
ΔTotal Hip BMD (g/cm^3)	0.002 (0.012)	-0.010 (0.006)	0.400	-0.007 (0.004)	0.005 (0.005)	0.173

BMD, bone mineral density. Data represents mean (standard error) and P-values were calculated from non-parametric comparison by Mann-Whitney U test.

Table 3. Correlation Between Changes of Bone Markers and Femur Neck Bone Mineral Density after 6 Months of Intervention in Osteopenia Group (n = 18)

Variables	Δ Femur neck BMD (g/cm^3)	*P*-value
ΔPTH (pg/ml)	-0.435	0.081
Δ25(OH)D (ng/ml)	-0.084	0.740
ΔOsteocalcin (ng/ml)	-0.324	0.189
ΔNTx (mMBCE/mM Cr)	-0.302	0.223
ΔUrine Calcium/Creatinine	0.263	0.291

PTH, parathyroid hormone; NTx, n-telopeptide; BMD, bone mineral density. Values represent non-parametric partial correlation coefficients.

4. Discussion

The benefit of calcium supplement for bone health in postmenopausal women is still controversial. Some studies have illustrated that calcium supplements could improve bone mineral density. A Chinese study showed that habitual dietary calcium intake had a protective effect on bone loss [14]. A randomized controlled trial on 1,471 postmenopausal women reported that daily intake of calcium citrate resulted in sustained reduction in bone loss and turnover [15]. In addition, several meta-analyses have suggested that calcium intake in combination with vitamin D could prevent osteoporotic fractures and bone loss [16,17,18]. Furthermore, a number of positive effects of eggshell powder have been reported in experimental and clinical studies. An animal study suggested that eggshell calcium positively affected bone metabolism in ovariectomized rats [9]. Eggshell was also reported to have positive effects on bone mineral density values of the lumbar spine and the hip in postmenopausal women with osteoporosis [3,19]. On the other hand, some other studies have found no association between calcium intake and change in bone mineral density [20]. These inconsistent results might be partially due to individual differences of calcium and vitamin intake requirements [10,15]. Different duration of osteoporosis and difference in intake amount of calcium and vitamin D could also lead to different results.

There are several benefits of using eggshell calcium compared with purified calcium supplements. First, calcium in eggshell has a considerably higher level of efficiency in increasing bone mineral density of the femur neck in postmenopausal women than purified calcium carbonate [4]. In addition, eggshell contains additional substances that are helpful for bone health. Matrix proteins present in eggshell are claimed to be effective in enhancing calcium transport across caco-2 cells in the human intestine. Eggshell contains 1% magnesium carbonate, which prevents calcium leakage from bone by regulating the pH of extracellular fluid. Furthermore, it is necessary for the synthesis of calcitriol [21]. Two studies on post-menopausal women have suggested an effect of magnesium on bone mineral density of the hip [22]. Eggshell powder also contains a small amount of strontium that is supposed to have an anabolic effect on bone [19,23]. Moreover, calcium supplements with eggshell might help improve poor compliance, which is a major obstacle to obtaining the full benefit. In clinical practice, some people prefer to consume calcium and vitamin D naturally through food (for example, milk) rather than supplements in tablet form. Indeed, poor long-term compliance of calcium tablets reached 43% during 5 years of the study period [24].

Koreans have a traditionally low consumption of fresh milk and dairy products in diets. This is closely associated with an inadequate intake of dietary calcium and vitamin D among Koreans [25,26]. According to the Korea National Health and Nutrition Examination Survey 2018 [27], the ratio of actual intake to the recommended intake of calcium for adults was 67.8%. Especially, women in their 60s had less calcium intake than other age groups. In addition, the Korean Health Insurance Review & Assessment Service reported that 73.7% of Koreans had vitamin D deficiency [28]. Moreover, Asian populations including Korea present higher prevalence of lactose intolerance than other populations, which results from a high prevalence of lactase deficiency [29]. For these reasons, simply encouraging dairy intake is limited. Therefore, adding dietary calcium and vitamin D to meal kit could help increase consumption.

We found increased density of the femur neck in the osteopenia group, whereas we failed to detect changes of lumbar vertebrae density. The probable explanation for this different response was that material properties of cortical and trabecular bone were different. The spine consists predominantly of trabecular bone (66%), whereas the neck of the femur consists predominantly of cortical bone (75%) [30]. The turnover rate of the trabecular bone is higher than that of the cortical bone. In addition, the trabecular bone has lower calcium content but higher water content than the cortical bone [29]. In addition to differences in compositions, trabecular and cortical bones can be differentially affected by hormones and medications. For example, in patients with chronic kidney disease, higher parathyroid hormone levels are associated with rapid cortical bone loss, whereas trabecular density is preserved [31]. These differences might have caused different results in our study.

Negative correlations between changes of femur neck bone mineral density and bone markers might indicate a higher responsiveness of subjects with low baseline bone mineral density. Although it was statistically insignificant, some tendency was shown. A German study has reported that two months of supplementation with vitamin D and calcium can reduce parathyroid hormone levels in women aged 70 years or more [32]. A randomized controlled trial has shown that after two years of calcium supplementation, plasma parathyroid hormone is decreased in the intervention group but increased in the placebo group [33]. The current study had only 6 months of research period, which might have led to different results from other studies.

Our study has several limitations. First, as the intervention

period of six months is relatively short for evaluating bone mineral density (even though the actual research period was not short for each study subject), cumulative bone mineral density and bone marker changes were relatively small. Second, the number of study subjects was small. In addition, subjects participating in our study were not representative of the general population. Third, since premenopausal women were excluded from this study, the effect of meal kit on these women was unknown. Nonetheless, to the best of our knowledge, clinical trials addressing different effects of fortified meal kit with eggshell calcium and vitamin D on bone mineral density and bone markers in postmenopausal women with normal bone mineral density and those with osteopenia have not been reported yet.

In conclusion, bone mineral density of the femur neck was increased in postmenopausal women with osteopenia after intake of eggshell calcium and vitamin D fortified meal kit for six months. Further large-scale studies are warranted to validate our findings.

ACKNOWLEDGMENTS

This research was supported by Ottogi Ham Taiho Foundation.

Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

Seok-Hoon Lee: formal analysis, writing - original draft, writing - review & editing; Kyung-Jin Yeum: conceptualization, data curation, funding acquisition; Nam-Seok Joo: conceptualization, formal analysis, data curation, funding acquisition, supervision, writing - review & editing. All authors have read and agreed to the published version of the manuscript.

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