

Possible site-specific effect of an intervention combining nutrition and lifestyle counselling with consumption of fortified dairy products on bone mass: the Postmenopausal Health Study II

George Moschonis · Spyridon Kanellakis ·
Nikolaos Papaioannou · Anne Schaafsma ·
Yannis Manios

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Abstract The aim of the present study was to examine whether a holistic approach combining nutrition and lifestyle counselling with the consumption of milk and yoghurt enriched with calcium, vitamin D₃ and phylloquinone (vitamin K₁) or menaquinone (vitamin K₂) would have any additional benefit on bone mineral density (BMD) indices measured at various skeletal sites using two different techniques, dual energy X-ray absorptiometry and quantitative ultrasonography (QUS). A sample of 115 postmenopausal women were randomized to three intervention groups, receiving daily via fortified milk and yoghurt and for 12 months, 800 mg calcium and 10 µg vitamin D₃ (CaD group, *n* = 26); 800 mg calcium, 10 µg vitamin D₃ and 100 µg vitamin K₁ (CaDK1 group, *n* = 26); 800 mg calcium, 10 µg vitamin D₃ and 100 µg vitamin K₂ (CaDK2 group, *n* = 24); and a control group (CO group, *n* = 39) following their usual diet. All three intervention groups

attended biweekly nutrition and lifestyle counselling sessions. Total BMD significantly increased in all three intervention groups and these changes were significantly higher compared to the CO (*P* < 0.001). Furthermore, the significant increases observed for L2–L4 BMD in the CaDK1 and CaDK2 groups were found to be significantly higher compared to the decrease observed in the CO (*P* = 0.001). No significant differences were observed for QUS parameters. The combined approach used in the current study led to favourable changes for all three intervention groups in total body BMD, while an additional benefit was observed for L2–L4 BMD in CaDK1 and CaDK2 groups. No significant differences were observed among groups in any of the QUS parameters.

Keywords Vitamin K · Vitamin D · Calcium · Fortified milk · Nutrition counselling

All authors contributed in the study design, writing and revising the manuscript; GM, SK and YM were responsible for data collection, management and statistical analyses.

G. Moschonis · S. Kanellakis · Y. Manios (✉)
Department of Nutrition and Dietetics,
Harokopio University of Athens, 70, El.Venizelou Ave,
Kallithea, 176 71 Athens, Greece
e-mail: manios@hua.gr

G. Moschonis
e-mail: gmoschi@hua.gr

N. Papaioannou
Laboratory for the Research of the Musculoskeletal System,
School of Medicine, University of Athens, Athens, Greece

A. Schaafsma
FrieslandCampina, Innovation International,
P. Stuyvesantweg 1, 8937 AC Leeuwarden, The Netherlands

Introduction

Osteoporosis and the subsequent risk of bone fracture occur most commonly among postmenopausal women. These disorders account for a significant burden of morbidity and mortality worldwide and have become a major public health problem [1]. Adequate intake of a list of nutrients such as calcium, vitamin D, magnesium and phosphorus is essential for bone metabolism. However, decreased calcium bioavailability [2] and cutaneous vitamin D biosynthesis [3] in the elderly, combined with low dietary intake of vitamin D from staple foods, especially in countries without a mandatory fortification policy [4], contribute to lower concentrations of 25(OH)D and consequently to accelerated bone loss and greater risk of bone fracture [5].

In addition to calcium and vitamin D, recent evidence suggests that supplementation with vitamin K also seems to have a favourable role in bone metabolism and bone mass maintenance [6–9]. Although supplementation of all these nutrients essential for bone metabolism is one approach in achieving adequate intakes, consumption of fortified staple foods, such as dairy products, has also been proven to be an effective approach [10–13].

The aim of the present study was to examine whether a holistic approach combining nutrition and lifestyle counselling with consumption of milk and yoghurt enriched with calcium, vitamin D₃ and vitamin K₁ or vitamin K₂ would have any additional benefit on BMD, measured at various skeletal sites using two different techniques, dual energy X-ray absorptiometry (DXA) and quantitative ultrasonography (QUS).

Materials and methods

The recruitment of the study participants followed two screenings, after the approval of the Ethical Committee of Harokopio University of Athens. These two screenings yielded 173 apparently healthy self-dependent postmenopausal women (55–65 years old) who were randomly assigned into three intervention and one control (CO) group. More specifically, the three intervention groups comprised a group receiving 800 mg of calcium and 10 µg vitamin D₃ (CaD group, *n* = 38), a group receiving 800 mg calcium, 10 µg vitamin D₃ and 100 µg vitamin K₁ (CaDK1 group, *n* = 38); and a group receiving 800 mg calcium, 10 µg vitamin D₃ and 100 µg vitamin K₂ (MK-7) (CaDK2 group, *n* = 39) via fortified milk and yoghurt. The major reasons for choosing MK-7 instead of another active menaquinone, MK-4, for the fortification of milk are: (1) MK-7 has an extended half-life time compared to MK-4 (approximately 3 days vs. 1 h) and because of that vitamin K status improves during the period of consumption and shows less daily variation [14]; and (2) MK-7 seems to be effective in much lower daily amounts (100 µg) than MK-4 (45 mg). From a safety point of view and mainly considering the effect of high doses of vitamin K on hemostasis, the dose–effect difference between MK-7 and MK-4 is of great importance when vitamin K is used to fortify staple food products [14].

Subjects in the three intervention groups were advised to consume two portions of fortified low fat milk and yoghurt on a daily basis and attended biweekly nutrition and lifestyle counselling sessions. The intervention scheme was based on a combined application of the Health Belief Model [15] and the Social Cognitive Theory [16] aiming to increase subjects' awareness of health issues, primarily related to osteoporosis, but also to improve their self-

efficacy in adopting healthier lifestyle and dietary patterns. On the other hand, no intervention was delivered to the CO group. More information on the sampling procedures and inclusion criteria can be found elsewhere [10].

At baseline and follow-up examinations, the following measurements were obtained. Dietary intake data were collected at baseline and follow-up with the use of three 24-h recalls [10]. Furthermore, physical activity levels were assessed by recording the number of steps for 1 week with the use of waist-mounted pedometers (Yamax SW-200 Digiwalker). Body weight and standing height were measured in light clothing and with no shoes using a digital scale (Seca Alpha, model 770; Seca, Hamburg, Germany) with an accuracy of ±100 g and a commercial stadiometer (Leicester Height Measure; Invicta Plastics Ltd, Oadby, Leics, UK) to the nearest 0.5 cm, respectively. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m²). Quantitative data on bone mineral density (BMD, g/cm²) in the lumbar spine (L2–L4), hip (i.e. total proximal femur, femoral neck, trochanter, intertrochanter, Ward's triangle) and total body (i.e. total and segmental BMD, lean and fat mass) were collected using DXA (Lunar DPX-MD, Madison, USA) with the analysis software version 4.6. Furthermore, calcaneal QUS measurements were conducted for both the left and right heel with the SAHARA Clinical Bone Sonometer (Hologic, Inc., Waltham, MA, USA). Broadband ultrasound attenuation and speed of sound were measured at a fixed region in the mid-calcaneus. An estimate of heel BMD (eBMD, g/cm²) was derived from these measurements. More details on QUS measurements, calculations, and standardization procedures are presented elsewhere [17].

Subjects with less than 75% compliance for any of the supplemented key nutrients were excluded, resulting to a total of 115 subjects. Repeated measures analysis of variance (ANOVA) was used to compare the differences with respect to the QUS and BMD changes among the four study groups. All statistical analysis was conducted with SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). The level of statistical significance was set at *P* < 0.05.

Results

No significant differences were observed between study groups with respect to baseline characteristics, such as age, elapsed time since menopause, weight, height, BMI, fat and lean body mass (Table 1). However, drop-out of subjects from baseline to follow-up inevitably led to significant baseline differences in L2–L4 BMD among the four study groups (*P* = 0.028) (Table 2). Furthermore, no significant differences were observed in the changes of BMI and percent of fat mass levels among the four study groups

from baseline to follow-up (data not shown in Tables). On the contrary, the increases in the average daily number of steps recorded by subjects in the three dietary intervention groups were found to differ significantly compared to the change observed in the CO group ($P = 0.003$) (Table 2). As far as dietary indices were concerned, no significant differences were observed between groups with regard to the changes in energy and macronutrient (i.e. total fat, carbohydrates and protein) intake (data not shown in Tables), while all three dietary intervention groups significantly increased their intakes of calcium and vitamin D to higher levels compared to the CO group ($P = 0.001$). Similarly, the increases observed for vitamin K intake were significantly higher in the CaDK1 and CaDK2 than the changes in the CaD and CO groups ($P = 0.001$) (Table 2). Regarding BMD changes assessed by DXA (Table 2), all three dietary intervention groups were found to have significantly higher increases in total body BMD compared to the decrease observed in the CO group ($P = 0.001$). Furthermore, the significant decrease observed for L2–L4 BMD in the CO group was found to differ significantly from the changes observed in the CaDK1 and CaDK2 groups ($P = 0.001$). Finally, no significant differences were observed between groups with respect to changes in QUS parameters, such as eBMD (Table 2).

Discussion

The present study showed that a 12-month intervention program, which combined nutrition and lifestyle counselling with consumption of fortified milk and yoghurt, managed to induce certain favourable changes in behavioral (i.e. dietary and physical activity) and bone mass indices of postmenopausal women. Specifically, the intakes of calcium and vitamin D significantly increased in all three dietary intervention groups, reaching the recommended Adequate Intake (AI) levels (i.e. 1200 mg and 10 μg per day, respectively) [18] throughout the

intervention period. Additionally, vitamin K intake significantly increased in the CaDK1 and CaDK2 groups, leading to doses that were twice as high as the recommended AI level of 90 μg per day [18]. As far as physical activity levels were concerned, significant increases were observed in all three intervention groups by more than 2000 steps per day, reaching an average of 8000 to 9000 steps per day. However, other similar studies have confronted difficulties in motivating middle-aged women already having a sedentary lifestyle to become more active [10, 12], possibly due to the use of subjective methods (i.e. questionnaires) to assess changes in physical activity levels.

The effect of the intervention on BMD should be attributed to the changes induced both in diet and in physical activity levels of the intervention subjects. According to the results derived from the DXA measurements, the findings of the present study revealed more favourable changes over the intervention period in total body BMD in the three intervention groups compared to the CO group (Table 2). Similarly, other intervention studies showed that total dietary calcium intake of 1200 mg per day or higher was adequate to prevent bone loss from the total body, lumbar spine, femoral neck and greater trochanter in postmenopausal women [13, 19], especially when combined with increased vitamin D intake [10, 20]. However, there is limited scientific evidence available regarding the exact effect of vitamin K supplementation in low concentrations on bone mass in humans [6–9, 21–24]. The present study showed more favourable changes in lumbar spine (L2–L4) BMD for the CaDK1 and CaDK2 groups compared to the CaD and CO groups. These changes could probably point to additional site-specific skeletal benefits, derived from increased vitamin K intakes, which mainly apply to the highly metabolically active trabecular (cancellous) but not to the cortical (compact) bone tissue [21]. Similar changes have been reported by another 2-year randomized controlled intervention study that combined vitamin K₁ and vitamin D supplementation [21]. Stimulation of γ -carboxylation of

Table 1 Baseline descriptive characteristics of the study participants presented by study group

	CO ($n = 39$)	CaD ($n = 26$)	CaDK1 ($n = 26$)	CaDK2 ($n = 24$)	P value*
Age (years)	62.4 (5.3)	62.5 (6.5)	60.9 (5.3)	62.0 (6.4)	0.728
Time since menopause (years)	12.8 (6.4)	11.4 (6.6)	10.4 (6.0)	12.9 (8.8)	0.424
Weight (kg)	72.2 (10.5)	69.7 (11.8)	75.2 (13.0)	71.5 (9.9)	0.372
Height (cm)	155.3 (5.6)	154.4 (5.2)	158.1 (4.4)	156.3 (5.3)	0.056
BMI (kg/m^2)	29.9 (4.2)	29.4 (4.4)	30.1 (5.2)	29.4 (3.8)	0.921
Lean body mass (% of body weight)	51.4 (7.2)	52.6 (5.7)	53.0 (4.9)	52.3 (4.7)	0.727
Fat body mass (% of body weight)	42.4 (5.9)	42.7 (6.7)	42.7 (4.8)	42.9 (5.4)	0.991

All data are presented as mean (SD)

* P values were evaluated by using one-way ANOVA

Table 2 Changes in dietary, physical activity and bone mineral density in the four study groups based on dual energy X-ray absorptiometry and quantitative ultrasound measurements

	Baseline [mean (SD)]	Follow-up [mean (SD)]	12-Month change [mean change (95% CI)]	<i>P</i> value (treatment × time)
Dietary changes				
Calcium (mg/day)				0.015
CO (<i>n</i> = 39)	789.6 (213.5)	781.3 (475.5)	−8.3 (−151.5 to 134.9)	
CaD (<i>n</i> = 26)	860.8 (230.7)	1188.5 [‡] (363.4)	327.7 (152.4 to 503.1)	
CaDK1 (<i>n</i> = 26)	831.7 (362.6)	1077.2 [‡] (356.5)	245.4 (70.1 to 420.8)	
CaDK2 (<i>n</i> = 24)	849.7 (250.9)	1068.5 [‡] (313.5)	218.8 (36.3 to 401.3)	
<i>P</i> value (treatment effect)	0.711	0.001		
Vitamin D (μg/day)				0.001
CO (<i>n</i> = 39)	0.890 (0.660)	0.954 (1.243)	0.064 (−1.199 to 1.328)	
CaD (<i>n</i> = 26)	1.021 (0.815)	9.773 [‡] (4.722)	8.752 (7.204 to 10.29)	
CaDK1 (<i>n</i> = 26)	1.015 (1.139)	9.414 [‡] (4.886)	8.399 (6.851 to 9.947)	
CaDK2 (<i>n</i> = 24)	1.027 (0.748)	10.179 [‡] (4.390)	9.152 (7.541 to 10.76)	
<i>P</i> value (treatment effect)	0.889	0.001		
Vitamin K (μg/day)				0.001
CO (<i>n</i> = 39)	80.2 (43.0)	145.3 (184.8)	65.2 (−1.1 to 129.3)	
CaD (<i>n</i> = 26)	112.3 (68.1)	140.3 (159.0)	28.0 (−50.5 to 106.6)	
CaDK1 (<i>n</i> = 26)	119.1 (119.8)	255.7 ^{‡,*} (252.8)	136.7 (58.1 to 215.2)	
CaDK2 (<i>n</i> = 24)	121.2 (175.8)	209.1 ^{‡,*} (111.0)	87.9 (6.2 to 169.7)	
<i>P</i> value (treatment effect)	0.358	0.001		
Physical activity change				
Steps (no/day)				0.003
CO (<i>n</i> = 39)	6356 (2655)	6832 (2364)	476.2 (−463.4 to 1415.8)	
CaD (<i>n</i> = 26)	7004 (4325)	9967 [‡] (3953)	2962.8 (1812.1 to 4113.6)	
CaDK1 (<i>n</i> = 26)	6977 (3242)	9393 [‡] (2688)	2415.7 (1264.9 to 3566.4)	
CaDK2 (<i>n</i> = 24)	5796 (2769)	8534 [‡] (1979)	2738.4 (1540.7 to 3936.2)	
<i>P</i> value (treatment effect)	0.500	0.001		
BMD changes derived from DXA				
Lumbar spine L2–L4 BMD (g/cm ²)				0.001
CO (<i>n</i> = 39)	1.134 (0.176)	1.101 (0.167)	−0.032 (−0.046 to −0.011)	
CaD (<i>n</i> = 26)	1.121 (0.158)	1.113 (0.160)	−0.008 (−0.011 to 0.030)	
CaDK1 (<i>n</i> = 26)	1.256 (0.192)	1.273 [‡] (0.195)	0.016 [‡] (−0.005 to 0.036)	
CaDK2 (<i>n</i> = 24)	1.165 (0.169)	1.171 [‡] (0.158)	0.006 [‡] (−0.018 to 0.026)	
<i>P</i> value (treatment effect)	0.028	0.001		
Total body BMD (g/cm ²)				0.001
CO (<i>n</i> = 39)	1.095 (0.079)	1.094 (0.079)	−0.001 (−0.008 to 0.005)	
CaD (<i>n</i> = 26)	1.112 (0.077)	1.135 [‡] (0.083)	0.024 [‡] (0.016 to 0.032)	
CaDK1 (<i>n</i> = 26)	1.117 (0.101)	1.129 [‡] (0.099)	0.013 [‡] (0.006 to 0.021)	
CaDK2 (<i>n</i> = 24)	1.105 (0.078)	1.117 [‡] (0.077)	0.013 [‡] (0.005 to 0.021)	
<i>P</i> value (treatment effect)	0.892	0.001		
BMD change derived from QUS				
eBMD (g/cm ²)				0.762
CO (<i>n</i> = 39)	0.472 (0.083)	0.461 (0.083)	−0.011 (−0.026 to 0.004)	
CaD (<i>n</i> = 26)	0.476 (0.091)	0.459 (0.081)	−0.016 (−0.035 to 0.002)	
CaDK1 (<i>n</i> = 26)	0.477 (0.116)	0.468 (0.110)	−0.008 (−0.026 to 0.010)	
CaDK2 (<i>n</i> = 24)	0.468 (0.115)	0.465 (0.097)	−0.003 (−0.021 to 0.016)	
<i>P</i> value (treatment effect)	0.990	0.988		

All *P* values are corrected for changes in physical activity levels. In the case of lumbar spine, BMD adjustment was also made for baseline values

[‡] Statistically significant difference from CO group

* Statistically significant difference from CaD group

osteocalcin by vitamin K, a process that is necessary for proper bone formation and protection [25], could provide an explanation for the possible site-specific skeletal effect of vitamin K.

The significant BMD changes observed for subjects in the three intervention groups were not confirmed by changes in QUS parameters. Likewise, another similar dietary intervention study conducted with Greek postmenopausal women showed no significant changes in QUS parameters after 12 months of nutrition counselling combined with consumption of dairy products enriched with calcium and vitamin D [26], probably because QUS is less sensitive than DXA and more time is required for a significant response in QUS parameters [27]. Some researchers indicate that although QUS has a potential role in long-term monitoring of skeletal status changes, the period of time required to follow individual subjects remains 1.5–3 times that for conventional DXA measurements [28].

In conclusion, our findings showed that the holistic approach followed in the current study, combining nutrition and lifestyle counselling with the consumption of fortified milk and yoghurt, managed to induce favourable changes in dietary intake indices (i.e. calcium, vitamin D and vitamin K) and physical activity levels, as well as in total body BMD assessed by DXA, but not in QUS parameters. Furthermore, an additional benefit was observed for lumbar spine BMD for the groups supplemented with vitamin K₁ and K₂, probably indicating a site-specific skeletal effect of this nutrient. Further research is necessary to clarify the exact role of each one of these two vitamin K isomers on bone metabolism and BMD.

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Conflict of interest YM also works as a part-time science and nutrition consultant for FrieslandCampina Hellas. AS also works as a cluster leader Nutrition for FrieslandCampina BV. The study sponsor had no role in the collection and analysis of the data, the writing of the manuscript, and the submission of the paper. None of the other authors had any potential conflict of interest.

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