ORIGINAL ARTICLE

# Three-year low-dose menaquinone-7 supplementation helps decrease bone loss in healthy postmenopausal women

**M. H. J. Knapen · N. E. Drummen · E. Smit · C. Vermeer · E. Theuwissen**

**Abstract**

*Summary* We have investigated whether low-dose vitamin K2 supplements (menaquinone-7, MK-7) could beneficially affect bone health. Next to an improved vitamin K status, MK-7 supplementation significantly decreased the age-related decline in bone mineral density and bone strength. Low-dose MK-7 supplements may therefore help postmenopausal women prevent bone loss.

*Introduction* Despite contradictory data on vitamin K supplementation and bone health, the European Food Safety Authorities (EFSA) accepted the health claim on vitamin K's role in maintenance of normal bone. In line with EFSA's opinion, we showed that 3-year high-dose vitamin K1 (phylloquinone) and K2 (short-chain menaquinone-4) supplementation improved bone health after menopause. Because of the longer half-life and greater potency of the long-chain MK-7, we have extended these investigations by measuring the effect of low-dose MK-7 supplementation on bone health.

*Methods* Healthy postmenopausal women (*n*=244) received for 3 years placebo or MK-7 (180 μg MK-7/day) capsules. Bone mineral density of lumbar spine, total hip, and femoral neck was measured by DXA; bone strength indices of the femoral neck were calculated. Vertebral fracture assessment was performed by DXA and used as measure for vertebral fractures. Circulating uncarboxylated osteocalcin (ucOC) and carboxylated OC (cOC) were measured; the ucOC/cOC ratio served as marker of vitamin K status. Measurements occurred at baseline and after 1, 2, and 3 years of treatment.

*Results* MK-7 intake significantly improved vitamin K status and decreased the age-related decline in BMC and BMD at the lumbar spine and femoral neck, but not at the total hip. Bone strength was also favorably affected by MK-7. MK-7 significantly decreased the loss in vertebral height of the lower thoracic region at the mid-site of the vertebrae.

*Conclusions* MK-7 supplements may help postmenopausal women to prevent bone loss. Whether these results can be extrapolated to other populations, e.g., children and men, needs further investigation.

**Keywords** Bone mineral density · Bone strength · Osteocalcin · Postmenopausal · Vertebral fracture · Vitamin K

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## Introduction

The prevalence of osteoporosis increases markedly with age, and despite advances in diagnosis and treatment, a minority of patients at high fracture risk is identified for treatment. Long-term vitamin K inadequacy has been indicated as an independent but modifiable risk factor for the development of age-related diseases, including osteoporosis and cardiovascular disease [1]. Vitamin K is required for the posttranslational carboxylation of glutamate into γ-carboxyglutamate (Gla) residues in so-called Gla-proteins [2]. Seventeen Gla-proteins have been identified to date, and the vitamin K-dependent carboxylation is essential for their function. Dietary vitamin K intake is too low, however, to support full carboxylation of at least some of these Gla-proteins, including osteocalcin (OC) and matrix Gla-protein [3]. OC is generally regarded as a local inhibitor of mineralization in bone [4], and the uncarboxylated form (ucOC) is an

accepted marker for poor (bone) vitamin K status. Low vitamin K intake and high circulating ucOC have been associated with low bone mass and increased fracture risk [5–9]. Improvement of vitamin K status as measured by increased carboxylation of OC is readily achievable by dietary supplementation with vitamin K [10–14]. Consistently, the European Food Safety Authorities (EFSA) accepted the health claim on vitamin K's role in maintenance of normal bone [15]. Unfortunately, the molecular mechanism of vitamin K and/or OC on bone health has yet to be elucidated.

Despite the observational link between vitamin K status and bone health, intervention studies have been contradictory regarding supplemental effects on BMD and fracture risk. Most studies have been carried out in Japan with pharmacological doses of synthetic vitamin K2 (short-chain menaquinone-4, MK-4). As summarized in the meta-analysis by Cockayne, these studies showed an overall benefit on reducing fracture risk [16]. A more recent meta-analysis on K1 and K2 supplementation showed modest treatment effects for vitamin K on BMD [17]. Next to the high-dose MK-4 trials, four studies with nutritional amounts of K1 and the long-chain menaquinone-7 (MK-7) were included; these studies showed conflicting results [11, 18–20]. Even with the contradictory findings on BMD, all studies reported a reduction in circulating ucOC in response to vitamin K supplementation. The utility of ucOC as a marker of bone health has however been questioned as improved OC carboxylation did not give concomitant effects on BMD [21]. Still, it could be that improving vitamin K status may have greater effects on bone quality than on bone mass. On the other hand, carboxylation-independent actions have also been reported for vitamin K's action on bone health [22].

The efficacy of MK-7 to improve bone health has received far less attention than K1 and MK-4. In view of its longer half-life in the circulation and its higher efficacy [23, 24], it is important to study the effects of long-term low-dose MK-7 supplementation on bone health. Small amounts of MK-7 can be found in fermented (curd) cheeses, but large amounts occur in natto, i.e., Japanese fermented soybeans. Lower fracture rates were reported in Japanese regions with high natto intake [25]. Additionally, observational studies suggest that natto may prevent osteoporosis in Japanese women [26]. Only two 1-year intervention studies are known on supplemental MK-7 intake and bone health in healthy elderly, but both lacked a beneficial effect. Similar to other forms of vitamin K, MK-7 supplementation significantly improved OC carboxylation in these studies. The short treatment period may explain the lack of effect of supplemental MK-7 intake on BMD. We hypothesized that long-term supplementation with MK-7 at a nutritionally relevant dosage will beneficially affect bone health.

## Methods

### Study participants

Healthy postmenopausal women aged between 55 and 65 years were recruited from the southern region of the province of Limburg (The Netherlands). Exclusion criteria were <2 years postmenopausal, BMI >30 kg/m$^2$, osteoporotic at baseline (T-score≤−2.5 SD), coagulation disorders, chronic diseases, metabolic bone diseases, gastrointestinal diseases, medication that interferes with vitamin K and/or blood coagulation, use of corticosteroids, bisphosphonates, or hormone replacement therapy, use of supplements containing vitamin K, participation in a clinical study 3 months prior to this study, and soy allergy. Based on these exclusion criteria and a prior health check (interviews and questionnaires), 244 women were included in the study and randomly assigned to either nontreatment ($n=124$) or treatment ($n=120$). Figure 1 represents the flow diagram of the study.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Medical Ethics Committee of the Maastricht University (Maastricht, The Netherlands). Written informed consent was obtained from all subjects before entering the study. Trial registration code: clinicaltrials.gov NCT00642551.

### Study design

The study had a double-blind, randomized, placebo-controlled, parallel design. Participants were randomized into two groups to receive either placebo capsules or capsules containing 180 μg MK-7 (MenaQ7, NattoPharma ASA, Høvik, Norway) per capsule. One capsule was taken daily either with breakfast or dinner during a period of 36 months. Participants came to the research site every year for measurements of body weight and height, blood sampling, and DXA measurements. During the visits to our research site, all noticeable changes in health, dietary pattern, physical activity, and/or medication use were recorded.

### Study products

The capsules were manufactured by EuroPharma Alliance (Wroclaw, Poland) for Nattopharma (Høvik, Norway). The study products, containing 180 μg MK-7 in the form of MenaQ7 (Nattopharma, Høvik, Norway) and matching placebo capsules, were delivered to VitaK (Maastricht, The Netherlands) as complete ready-to-use end products (closed bottles). The proprietary product MenaQ7 utilized in the study was provided by NattoPharma. To verify the stability of MK-7, three capsules of each batch were analyzed at the
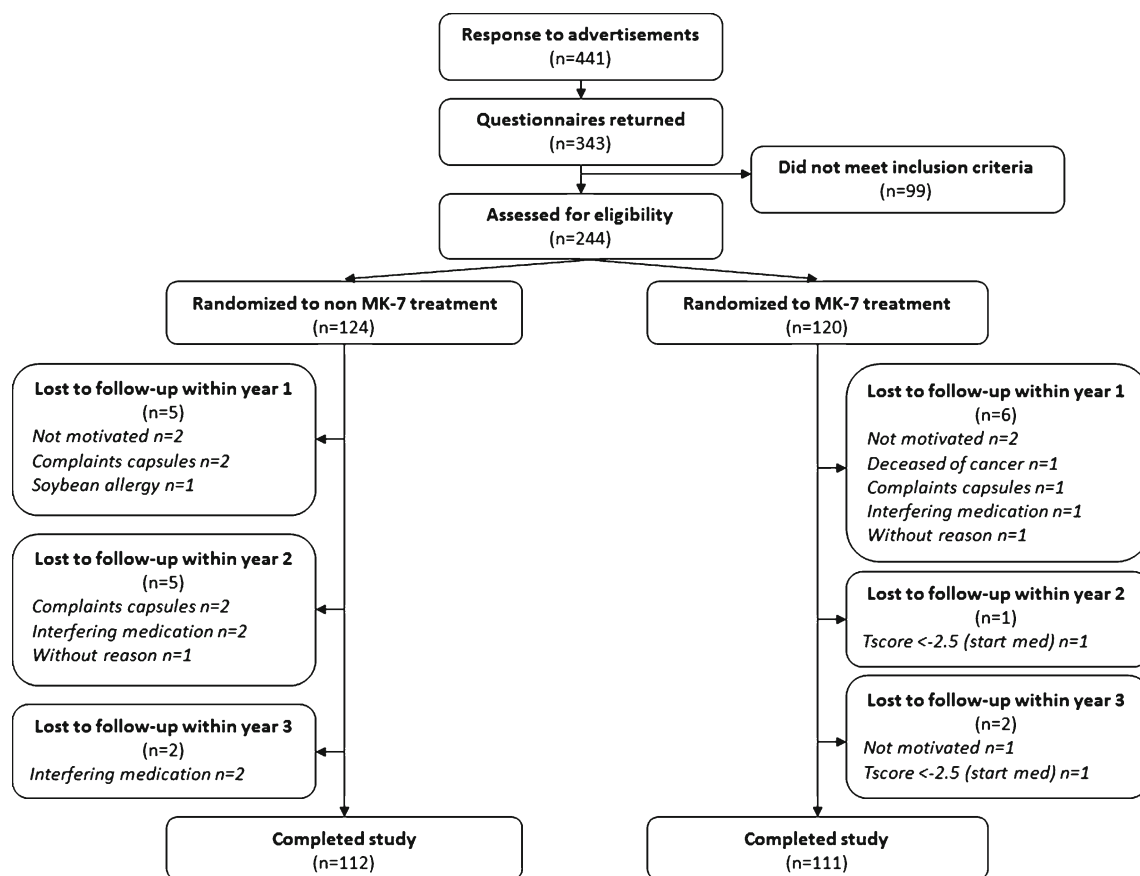
**Fig. 1** Flow diagram

start, half-way, and at the end of the intervention period. Before, during, and after the study, the MK-7 content of the capsules was determined to be stable, and the mean content was $180 \pm 3$ μg/capsule.

Blood sampling

Fasting venous blood was collected once a year for the preparation of serum and plasma (Vacutainers, Greiner Bio-One BV, Alphen a/d Rijn, The Netherlands). All blood samples were drawn between 8:00 a.m. and 11:00 a.m. by experienced research nurses. For plasma preparation, blood (10 ml) was collected in citrate tubes, centrifuged for 15 min at $3,000 \times g$, aliquoted, and stored at $-80$ °C until analysis. For serum preparation, blood (10 ml) was allowed to clot for 30 min at room temperature, centrifuged, and stored as described above.

Circulating markers

Serum uncarboxylated and carboxylated OC (ucOC and cOC) concentrations were determined by separate commercial dual-antibody ELISA tests (Takara Shuzo Co.

Ltd., Otsu, Shiga, Japan). An in-house control serum pool was run on all ELISA plates. The ucOC/cOC ratio was calculated from circulating ucOC and cOC values, and used as marker for (bone) vitamin K status. To minimize scattering, samples from four different time points of each subject were analyzed on the same ELISA plate. Baseline plasma 25(OH)D and serum *i*PTH were determined by using the automated chemiluminescent immunoassays on an iSYS system (IDS Ltd., Boldon, UK).

Bone measurements

BMC and BMD of the femoral neck, total hip, and lumbar spine (L1–L4) were determined at baseline and at years 1, 2, and 3 by DXA (Hologic Discovery A, Waltham, MA, USA). APEX software version 12.7 was used for acquisition and analysis. The geometry of the hip bone comprising femoral neck width (FNW) and hip-axis length (HAL) were assessed by the scanner software. Indices of femoral neck bone strength (compression strength, CSI; bending strength, BSI; and impact strength, ISI) were calculated from body weight and height, BMD, FNW, and HAL data [12, 27].

Vertebral fracture (VF) assessment (VFA) was performed immediately after BMC and BMD measurements. Making use of Hologic's software, six markers were placed on the cranial and caudal sides of the vertebral bodies in anterior, posterior, and middle positions. Subsequently, the anterior, posterior, and middle vertebral heights were determined from the marker points. The Genant's semiquantitative method was used to define VF as mild (height reduction 20–25 %), moderate (height reduction 25–40 %), or severe (height reduction >40 %) [28]. Furthermore, a distinction was made in fracture site (mid thoracic, T7–T9; low thoracic, T10–T12; lumbar, L1–L3) and type of VF (wedge, biconcave, crush). L4 was excluded from data analyses, because the accurate positioning of the markers on the sides of L4 was difficult in 32 women due to the disappearance of the disk space between L4 and L5.

## Statistics

Based on preliminary estimates of SD in bone strength in postmenopausal women, we determined that 120 participants were required in each group for the study to have a statistical power of 90 % to detect clinically meaningful differences of 20 % between treatment groups while allowing for a projected withdrawal of 10 % per year. Normality was tested by the Kolmogorov–Smirnov test. Data that were not normally distributed were log-transformed before statistical analyses (ucOC/cOC). Between-group differences were tested by the independent samples $t$ test. The paired samples $t$ test was used to study within-group effects. Linear regression analysis was performed to study associations between supplemental MK-7 intake and the measures of interest (ucOC, cOC, ucOC/cOC, BMD, BMC, and bone strength indices). The measure of interest was used as the dependent variable. The concomitant baseline value and the treatment code were included as independent variables. Age and BMI (if applicable) were included as covariates. Years since menopause, smoking, 25(OH)D, and $i$PTH were non-significant contributors to the statistical model and were therefore not included in the analyses. Data are presented as means with SD (SE in figures). A $p < 0.05$ was considered statistically significant. Statistics were performed using SPSS for Windows, version 19 (SPSS Inc., Chicago, Illinois, USA).

## Results

### Baseline characteristics

Baseline characteristics of the total group as well as the placebo and MK-7 groups are presented in Table 1. Mean

values of the variables at baseline were similar ($p > 0.05$) in both treatment groups.

At the end of the study, twelve women in the placebo group and nine women in the MK-7 group had withdrawn from the study. The overall drop-out rate was 8.6 %. Only a few women reported complaints during the study. The complaints in the placebo group were: hair loss and/or brittle nails ($n=2$), hot flashes ($n=1$), knee pain ($n=1$), numb sensation in arms and legs, washed-out ($n=1$), and weight gain ($n=2$) and in the MK-7 group: bone pain ($n=1$), hot flashes ($n=1$), rash around eyes and ears ($n=1$), smelly capsules ($n=1$), and weight gain ($n=1$). Five women withdrew due to these complaints; four women in the placebo group and one in the MK-7 group. Compliance was measured by capsule counts at the end of every half-year period; the mean compliance for both treatment groups was 97 %.

There were no crush deformations at baseline in this cohort. Within the total group, the prevalence of biconcave deformations was the highest in the vertebrae T11 and T12, whereas wedge deformations occurred mainly in T7 and T8.

### Effects of treatment on circulating osteocalcin

MK-7 supplementation significantly decreased circulating ucOC levels by $51\pm21$ % as compared to placebo ($+ 4\pm 49$ %) ($p < 0.001$; Fig. 2a). Circulating cOC was increased by $21\pm19$ % as compared to placebo ($+ 3\pm16$ %) ($p < 0.001$; Fig. 2b). Vitamin K status of bone, determined as the ucOC/cOC ratio, was significantly improved by $58\pm18$ % after MK-7 supplementation, whereas placebo supplements did not alter the ratio ($+ 2\pm47$ %) ($p < 0.001$; data not shown). The maximal effect on osteocalcin carboxylation was already reached during the first year and was maintained throughout the next 2 years of supplementation.

### Effects of treatment on BMC and BMD

Both the placebo group and the MK-7 group experienced bone loss (decrease in BMC and BMD) at the site of the femoral neck (Fig. 2c, d). During the first year, the rate of bone loss was similar in both groups. After the first year, the lines started to diverge with slower bone loss in the MK-7 group. Only after 3 years, MK-7 intake beneficially affected bone health as compared to the placebo group ($p=0.011$ for BMC, $p=0.012$ for BMD). After adjusting for age and BMI, the effect of MK-7 was still significant ($p=0.023$ for BMC and $p=0.014$ for BMD). BMC and BMD of the total hip steadily decreased during the 3-year study period in both treatment groups with no significant differences between the groups (data not shown). In the MK-7 group, the decrease in BMC and BMD at the lumbar spine was less than 1 % after the 3-year supplementation period compared to baseline ($p=0.001$ and $p=0.111$, respectively; paired samples $t$ test). The

**Table 1** Baseline characteristics of the subjects

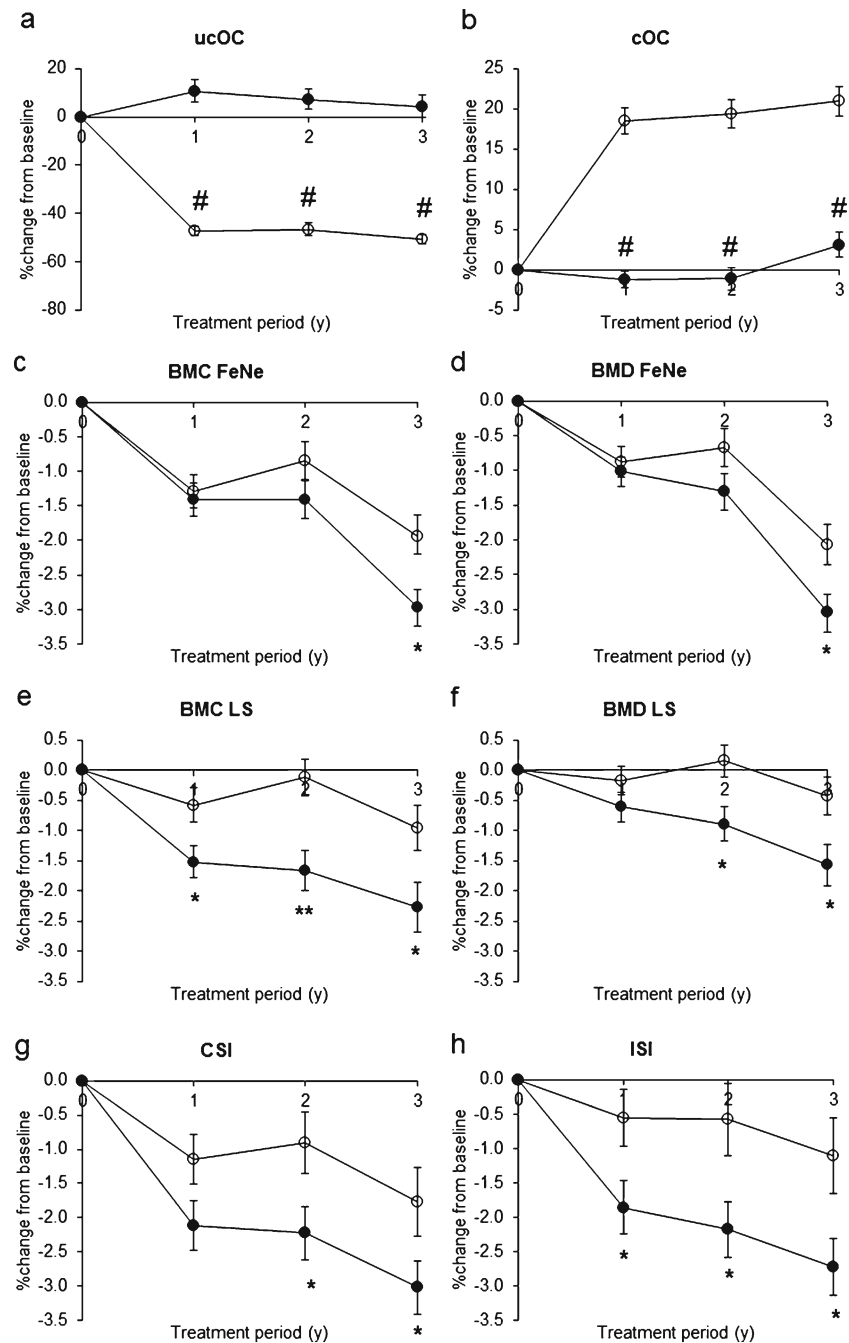|  | Total (n=244) | Placebo (n=124) | MK-7 (n=120) |
|---|---|---|---|
| Clinical parameters |  |  |  |
| Age (years) | 60±3 | 59±3 | 60±4 |
| Years since menopause | 9±6 | 8±5 | 9±6 |
| Weight (kg) | 69±10 | 68±9 | 69±10 |
| Height (cm) | 165±6 | 165±6 | 166±6 |
| BMI (kg/m$^2$) | 25±3 | 25±3 | 25±3 |
| Current smoker (%) | 13 (n=32) | 15 (n=19) | 11 (n=13) |
| Cigarettes per day | 9±7 | 11±7 | 7±8 |
| Former smoker (%) | 55 (n=135) | 58 (n=72) | 53 (n=63) |
| Alcohol consumption (%) | 76 (n=185) | 78 (n=97) | 73 (n=88) |
| Units of alcohol per week | 5.6±5.5 | 5.6±5.0 | 5.5±6.0 |
| Circulating markers |  |  |  |
| cOC (ng/ml) | 5.5±1.3 | 5.6±1.3 | 5.4±1.2 |
| ucOC (ng/ml) | 3.6±1.9 | 3.6±1.9 | 3.6±1.9 |
| ucOC/cOC ratio | 0.69±0.41 | 0.69±0.41 | 0.69±0.41 |
| 25(OH)D (ng/ml) | 30.3±11.0 | 30.8±11.2 | 29.8±10.9 |
| iPTH (pg/ml) | 43.0±15.6 | 42.5±15.1 | 43.4±16.2 |
| Bone density parameters |  |  |  |
| BMC femoral neck (g) | 3.77±0.60 | 3.74±0.53 | 3.79±0.67 |
| BMC total hip (g) | 32.1±4.9 | 31.9±4.3 | 32.4±5.4 |
| BMC lumbar spine (g) | 56.0±8.7 | 55.5±7.8 | 56.5±9.6 |
| BMD femoral neck (g/cm$^2$) | 0.732±0.101 | 0.728±0.085 | 0.737±0.115 |
| BMD total hip (g/cm$^2$) | 0.872±0.102 | 0.867±0.088 | 0.877±0.115 |
| BMD lumbar spine (g/cm$^2$) | 0.933±0.119 | 0.926±0.105 | 0.939±0.132 |
| Hip geometry |  |  |  |
| FNW (cm) | 3.43±0.27 | 3.43±0.28 | 3.43±0.26 |
| HAL (cm) | 11.2±0.7 | 11.2±0.7 | 11.3±0.7 |
| BSI (g/kg.M) | 1.13±0.21 | 1.13±0.21 | 1.13±0.21 |
| CSI (g/kg.m) | 3.70±0.58 | 3.69±0.54 | 3.70±0.63 |
| ISI (g/kg.M) | 0.25±0.04 | 0.25±0.04 | 0.25±0.05 |
| Vertebral Fracture Assessment |  |  |  |
| Height (mm) at the posterior site of |  |  |  |
| T7–T9 | 61.5±3.8 | 61.3±3.9 | 61.7±3.7 |
| T10–T12 | 71.9±4.0 | 71.6±3.8 | 72.1±4.2 |
| L1–L3 | 83.1±4.6 | 82.7±4.6 | 83.5±4.5 |
| Height (mm) at the midst site of |  |  |  |
| T7–T9 | 55.5±2.9 | 55.3±2.6 | 55.6±3.2 |
| T10–T12 | 64.4±3.7 | 64.3±3.3 | 64.4±4.1 |
| L1–L3 | 75.3±4.5 | 75.1±4.2 | 75.6±4.7 |
| Height (mm) at the anterior site of |  |  |  |
| T7–T9 | 55.4±3.5 | 55.5±3.4 | 55.3±3.6 |
| T10–T12 | 66.0±4.6 | 65.9±4.3 | 66.1±4.6 |
| L1–L3 | 80.2±4.5 | 80.0±4.7 | 80.5±4.2 |

Data are means±SD or percentage of total. No significant differences were found between the treatment groups

*BSI* bending strength index, *CSI* compression strength index, *cOC* carboxylated osteocalcin, *FNW* femoral neck width, *HAL* hip axes length, *ISI* impact strength index, *ucOC* uncarboxylated osteocalcin

placebo group confirmed the expected age-related decline in BMC and BMD (Fig. 2e, f). Between-group analysis showed significant differences at the site of the lumbar spine after the first year of supplementation for BMC ($p$=0.019) and after the second year for BMD ($p$=0.008). After adjusting for age and BMI, the beneficial effect of MK-7 on BMC remained significant ($p$=0.042), whereas the effect on BMD became borderline significant ($p$=0.070).

**Fig. 2** Effect of MK-7 supplementation on osteocalcin, BMC and BMD of the femoral neck and lumbar spine, and bone strength indices. *CSI* compression strength index, *ISI* impact strength index, *cOC* carboxylated osteocalcin, *ucOC* uncarboxylated osteocalcin, *FeNe* femoral neck, *LS* lumbar spine. Mean (SE) % change in osteocalcin, BMC and BMD of femoral neck and lumbar spine and bone strength indices relative to baseline during 3 years of supplementation with MK-7 (*white circles*) and placebo (*black circles*). **a** and **b** circulating ucOC and cOC; **c** and **d** BMC and BMD of the femoral neck; **e** and **f** BMC and BMD of the lumbar spine; **g** and **h** CSI and ISI of the femoral neck. *$p<0.05$, **$p<0.005$, #$p<0.0001$ as compared to placebo



### Effects of treatment on bone strength indices

The FNW as well as the HAL did not change significantly during the treatment with either placebo or MK-7 (data not shown). For the CSI (Fig. 2g), significant differences were seen after the second year of MK-7 supplementation ($p=0.026$) and remained significant ($p=0.045$). For the ISI (Fig. 2h), between-group differences were significant after 1, 2, and 3 years of treatment ($p=0.021$, $p=0.016$, $p=0.019$, respectively). After adjusting for age, the difference in ISI between placebo

and MK-7 remained significant ($p<0.05$), whereas the effects on CSI became less pronounced ($p=0.022$ and $p=0.075$ for years 2 and 3, respectively). No significant effects were found on the BSI (data not shown).

### Effects of treatment on vertebral fractures

In six participants of the placebo group, moderate wedge/biconcave VF occurred in T11 ($n=4$), in T12 ($n=1$), and in T7+T10 ($n=1$) during the trial; these vertebrae had normal heights at baseline. Only in one participant from the

MK-7 group, T10 showed a moderate wedge VF after 3 years of treatment. The numbers are however too small to perform statistics.

To investigate the effects of MK-7 supplementation on the vertebral heights, we divided the spine in three parts: mid thoracic (T7–T9), lower thoracic (T10–T12), and lumbar (L1–L3) spine. The losses of height (mean loss in T7–T9, T10–T12, and L1–L3) at the posterior and anterior sites of the spine were comparable for both treatment groups ($p>0.05$; data not shown). The height loss of the middle site of the vertebrae at T10–T12 was significantly lower in the MK-7 group than in the placebo group after 2 (MK-7, $-2.5\pm 1.8$ %; placebo, $-3.1\pm 2.2$ %; $p=0.044$) and 3 years (MK-7, $-3.3\pm 2.0$ %; placebo, $-4.1\pm 2.2$ %; $p=0.003$).

## Discussion

In this paper, we demonstrate that 3-year supplementation of low-dose vitamin K2 as MK-7 significantly decreased the age-related loss in bone mass. Participants taking the MK-7 supplements showed a 3-year preservation of lumbar spine-BMD, while the placebo group confirmed the expected age-related decline in BMD. Though both intervention groups experienced bone loss at the site of the femoral neck, MK-7 intake beneficially affected the rate of bone loss—but the difference became only significant after 3 years of supplementation. Also, bone strength of the femoral neck was positively affected by MK-7 supplementation. These results confirm the hypothesis that long-term supplementation with MK-7 beneficially affects bone health. Our findings support EFSA's acceptance of the health claim that "a cause and effect relationship has been established between vitamin K and maintenance of normal bone" [15]. EFSA's opinion was mainly based on observational data showing a link between vitamin K (status) and bone health, as supplementation studies showed contradictory results. In view of the increasing retail availability of MK-7 supplements intended for bone health, it is also important to substantiate their effectiveness. The only comparable MK-7 trials published thus far are the Postmenopausal Health Study II (PHSII) [29] and a Norwegian study in healthy postmenopausal women [19], which were both 1-year trials showing conflicting results. The PHSII showed that 1-year consumption of milk and yogurt enriched with CaD and MK-7 (100 μg/day) together with nutrition and lifestyle counseling significantly improved lumbar spine-BMD as compared to the control group (no vitamin fortification and no counseling). It should be noted that similar results were seen with products enriched with CaD and products fortified with CaD and K1 (100 μg/day). In the Norwegian study, 1-year supplementation with MK-7 (360 μg/day) capsules did not influence BMD of total hip, femoral neck, lumbar spine, and total

body. The short follow-up period of 1 year was mentioned as a major limitation. Indeed, we did show that effects of MK-7 supplementation on bone mass only became significant after at least 2 years of intervention.

Subgroup analysis in the most recent meta-analysis on vitamin K and bone health revealed that K2, but not K1 supplementation, had favorable effects on lumbar spine-BMD [17]. It is important to note that only two placebo-controlled trials with MK-7 supplementation were included [19, 20], and outcomes were mainly based on MK-4 interventions. Most of the MK-4 trials were performed in Japanese osteoporotic women and/or used a pharmacological dose of MK-4 making direct comparison to trials with nutritional dosages and/or healthy participants difficult. Additionally, two supplementation trials with K1 at nutritional doses were included in the meta-analysis. In healthy older Scottish women, 2-year supplementation of CaD and K1 (200 μg/day) significantly increased distal radius-BMD, but this change did not differ from that in the control group [11]. In line, no beneficial effect was seen on BMD of lumbar spine and femoral neck after 3-year supplementation with CaD and K1 (500 μg/day) in healthy American elderly [13]. K1 and MK-7 differ with respect to their chemical structure and pharmacokinetics [23, 24], which may account for the differences in effects on bone mass when administrated in nutritional dosages. Actually, we previously showed greater bioactivity of MK-7 as compared to K1 when administrating the same molar dose to healthy adults: higher serum levels were reached with MK-7, and MK-7 had a higher efficacy in both hepatic and extra-hepatic protein carboxylation [23]. Recent in vitro data also suggested superiority of MK-7 as compared to MK-4 and K1 in inhibiting NF-κB activation related to osteoclast development [22].

Despite the heterogeneity in bone mass outcomes, all studies that measured ucOC (expressed as total circulating ucOC or percentage ucOC of total circulating osteocalcin) reported a significant reduction of 20–85 % in this bone marker in response to vitamin K supplementation [10–13, 19, 29–32]. In agreement, we found that 3-year MK-7 supplementation at a daily dose of 180 μg significantly lowered circulating ucOC by ~50 %. The maximal effect on osteocalcin carboxylation was already reached during the first year and was maintained over the next 2 years of supplementation. Recently, we described a similar reduction in serum ucOC levels after 3-month supplementation with the same dose of MK-7 [33]. Although significant positive effects were seen on both osteocalcin carboxylation and bone mass, no association was found between the vitamin K-induced changes in serum ucOC levels and bone parameters. Such correlation analyses are however lacking in other published intervention trials studying vitamin K effects on bone mass. On the other hand, cross-sectional analyses have shown an inverse relationship between circulating ucOC

and bone health, measured as BMD or hip fracture [5]. However, the physiological implications for (maximally) improved carboxylation of circulating ucOC have remained unknown. Next to the suggested primary mechanism through OC carboxylation, vitamin K was described to have carboxylation-independent effects on bone health as well. Next to superiority of MK-7, the in vitro data showed that MK-7's action on osteoblast and osteoclast formation and activity was accomplished by downregulating NF-κB activation in a carboxylation-independent manner [22].

VF are the most common osteoporotic fractures; they are mostly asymptomatic, but strongly predict risks for subsequent fractures independent of BMD [34]. VFA by DXA can be used to classify vertebral deformity consistent with fractures, but has not yet become standard practice. Nevertheless, this method was considered a new patient-friendly diagnostic tool as it can detect unknown VF in one out of each six patients [35]. In our study population, the thoracic vertebrae showed more VF as compared to the lumbar spine. Regarding the thoracic spine, biconcave deformations were mostly seen in the lower region (T10–T12) and wedge deformations in the mid region (T7–T9). Low dietary intake of MK-7 significantly reduced the loss in vertebral height of the lower thoracic region (T10–T12) at the mid-site of the vertebrae. This could however be a chance finding. It should be noted that the number of participants was not calculated to detect changes in VF (or fracture risk). More studies are warranted to assess the relation between K2 intake and VF (or fracture risk).

There are certain limitations to consider. Although several bone parameters were investigated in this study, we did not perform measurements of bone quality, including fracture risk or markers of bone formation/resorption. As mentioned before, improving vitamin K status may have more pronounced effects on bone quality than on bone mass. Further, we targeted participants in a group generally known to be at increased risk for accelerated bone loss: healthy early postmenopausal women. Whether these data can be extrapolated to other study populations, including children, men, osteoporotic subjects, or other patient groups needs further investigation. Nevertheless, the targeted study population may explain why we found positive results [12, 30] in contrast to other long-term studies (2–3 years of treatment) in late postmenopausal women [11, 18], and osteopenic/osteoporotic women [10]. Finally, our study products were not enriched with CaD, while most studied K1 supplements did contain CaD. A subject's CaD environment may influence vitamin K's effect on bone health.

## Conclusions

Long-term use of MK-7 supplements significantly decreased the age-related decline in bone mass and strength.

Additionally, the loss in vertebral height of the lower thoracic region was significantly reduced at the mid-site of the vertebrae after intake of the MK-7 capsules. We conclude therefore that postmenopausal women may benefit from taking MK-7 supplements to prevent the age-related bone loss. Since the efficacy of MK-7 supplements is poorly documented, our results significantly contribute to substantiating the marketed anti-osteoporotic properties of vitamin K2.

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