Clinical Study

Vitamin K2 (menaquinone-7) prevents age-related deterioration of trabecular bone microarchitecture at the tibia in postmenopausal women

Sofie Hertz Rønn, Torben Harsløf, Steen Bønløkke Pedersen and Bente Lomholt Langdahl
Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus C, Denmark

Abstract

Objective: Clinical studies suggest that vitamin K2 protects against bone loss and fractures; however, its effect on bone quality has never been investigated. We investigated the effect of vitamin MK-7 on undercarboxylated osteocalcin (ucOC), and bone mass and quality.

Design: We conducted a randomised, placebo-controlled, double-blinded clinical trial.

Methods: We investigated the effect of MK-7 375 µg for 12 months on bone mineral density (BMD) measured by dual X-ray absorptiometry (DXA), bone microarchitecture measured by high-resolution peripheral quantitative computed tomography (HRpQCT) and biochemical bone turnover markers in 148 postmenopausal women with osteopenia. All of them were supplemented with calcium and vitamin D.

Results: ucOC decreased in the MK-7 group (−65.6 (59.1; 71.0) %) (median (CI)) compared with the placebo group (−6.4 (−13.5; 1.2) %) after 3 months (P < 0.01). HRpQCT after 12 months demonstrated that trabecular number in tibia was unchanged in the MK-7 group (−0.1 ± 1.9%) (mean ± s.d.) and decreased in the placebo group (−3.5 ± 2.2%), trabecular spacing was unchanged in the MK-7 group (+1.2 ± 8.0%) and increased in the placebo group (+4.5 ± 9.7%), and trabecular thickness was unchanged in the MK-7 group (+0.2 ± 1.7%) and increased in the placebo group (+4.0 ± 2.2%) (between-group changes for all: P < 0.05). There were no significant differences between the groups in HRpQCT-derived parameters at the radius or in BMD at any site.

Conclusion: The changes in bone microarchitecture in the placebo group are consistent with the age-related deterioration of trabecular structure, with a loss of trabeculae and a greater mean thickness of the remaining trabeculae. This suggests that vitamin MK-7 preserves trabecular bone structure at the tibia.

Introduction

Osteoporosis and the consequent increased risk of fractures is a major health problem in the western world, leading to morbidity, mortality and health costs (1, 2). Postmenopausal women are especially at risk due to the rapid bone loss during and after menopause (3, 4).

Osteocalcin is a matrix protein produced by the osteoblasts in an undercarboxylated form. Vitamin K is a cofactor in the carboxylation of osteocalcin, and the availability of vitamin K controls the carboxylation of osteocalcin. Carboxylated osteocalcin (cOC) is believed to promote mineralisation of bone (5).

Vitamin K exists in several natural forms; vitamin K1 (phylloquinone), synthesised by plants, and vitamin K2 (menaquinones), mainly synthesised by bacteria. The
Menaphthenequinones are divided into more than 10 subtypes based on the isoprenoid side chain, denominated MK-n. In the western diet, vitamin K1 is the major contributor to vitamin K, whereas in Japan, fermented soy beans, Natto, rich in MK-7, is a major contributor to vitamin K. MK-7 has one of the longest half-lives of the menaquinones of approximately 3 days (6).

Epidemiological studies have found associations between serum ucOC and the risk of hip fracture in elderly women (7) and between low intake of vitamin K1 and risk of hip fracture in older men and women (8). A number of clinical studies have investigated the effects of vitamin K on bone phenotypes. Five studies have investigated vitamin K1 in Caucasian populations, and all but one (9) found no effect on BMD (10, 11, 12, 13). Most of the studies investigating the effects of MKs have used MK-4 in Japanese populations. In general they have failed to show an effect on bone mineral density (BMD) but some studies suggest that MK-4 may prevent fractures (14, 15, 16, 17). Only three studies have investigated the effect of MK-7 on bone metabolism in Caucasian postmenopausal women. One study showed no effect on BMD (18), another study found BMD at the femoral neck to be preserved (19), and the third study found a positive effect of MK-7 in combination with calcium and vitamin D on BMD (20). Thus, data on the effect of vitamin K are so far somewhat equivocal; furthermore, the effect of vitamin MK-7 on bone structure has never been investigated.

We therefore conducted a randomised placebo-controlled trial in which postmenopausal women with osteopenia were randomised to MK-7 (375 µg/day) or placebo as an add-on to calcium and vitamin D and evaluated the effect on bone turnover measured by biochemical markers, areal BMD measured by DXA and bone structure evaluated by high-resolution peripheral quantitative computed tomography (HRpQCT).

Methods

Participants and recruitment

We included 148 healthy postmenopausal women. Inclusion criteria were postmenopausal status defined as at least 2 years past the last menstrual period, age between 60 and 80 years, and osteopenia (T-score of hip or lumbar spine ≤−1, but >−2.5). Exclusion criteria were smoking, diseases, or use of medication which affect bone metabolism, intake of vitamin K supplements or vitamin K antagonists, or vitamin D insufficiency (s-25-OH vitamin D <50 nmol/L). Women with vitamin D insufficiency had the option of receiving vitamin D, 38 µg daily, for 3 months and go through re-screening. This applied to one woman.

We recruited study participants among women previously examined for osteoporosis at the Osteoporosis Clinic from August 2013 to May 2014. We invited 455 women by mail and of these 242 replied and 148 were eligible and provided written informed consent. One hundred and forty-two women completed the first year of the study (71 in the MK-7 group and 71 in the placebo group). Six women either dropped out (n=4), one due to illness and three due to lack of time to participate, or were excluded (n=2), both due to illness.

Design

The study was a randomised, placebo-controlled, double-blinded, clinical trial investigating the effect of MK-7 on bone mass, structure and turnover. The primary endpoint was change in s-undercarboxylated osteocalcin after 3 months; secondary endpoints were changes in areal bone mineral density (aBMD), bone microarchitecture, bone strength and bone turnover markers at 1 year. The study was a single-centre study which took place at the Osteoporosis Clinic, Department of Endocrinology and Internal Medicine, Aarhus University Hospital. The study was approved by the Regional Ethics Committee and the Danish Data Protection Agency, and registered at Clinicaltrials.gov (NCT01922804) before recruitment was initialised. We conducted the study in accordance with the Declaration of Helsinki II and the guidelines on Good Clinical Practice (GCP).

Study medication

The study medication was one tablet containing 375 µg menaquinone-7 (MK-7) or similar placebo, taken once daily. Also, all the participants received supplementation of calcium (800 mg) and vitamin D (38 µg), contained in two daily tablets. The study medication was produced and supplied by Orkla Health (Ishøj, Denmark). The randomisation was done by the pharmacy at Aarhus University Hospital, in blocks of 10 participants (five in each group). The study investigators and participants were blinded to study drug allocation. After randomisation the participants were seen for study visits after 1, 3, 6, 9 and 12 months. Study medication was provided for 3 months at a time (100 tablets of MK-7/placebo, and 180 tablets of calcium and vitamin D), and compliance was estimated based on tablet counts at each visit.
General measurements

We measured standing height and weight on a wall-mounted stadiometer with the participants lightly clothed at baseline, and at months 3, 6 and 12. Furthermore, we performed physical examination at baseline and after 12 months.

Dual X-ray absorptiometry (DXA)

We evaluated aBMD by DXA of the lumbar spine (L1–L4) and left hip (Hologic Discovery, Hologic Inc, Waltham, MA, USA). The coefficient of variation (CV) of repositioning is 1% at the lumbar spine and 2% at the total hip. If the left hip was not eligible for evaluation, we evaluated the right hip. We performed DXA at screening and after 3, 6 and 12 months. We used the same scanner for each individual throughout the study. If a participant had been examined by DXA within 2 months of randomisation, this was used as the baseline scan. Due to metal implants in the lumbar spine, it was not possible to perform DXA of the spine in one patient.

High-Resolution peripheral Quantitative Computed Tomography (HRpQCT)

We investigated bone microarchitecture and performed finite element analysis by HRpQCT (XtremeCT, Scanco Medical, Switzerland). We examined the distal right radius and the distal right tibia at baseline and after 12 months. If there had been a previous fracture at the right side, the left was preferred (n = 10). We immobilised the limb in a carbon cast, and used a scout view to define the region to scan, which was 9.5 and 22.5 mm from the endplate of radius and tibia respectively. At both sites we obtained 110 cross-sectional slices, giving a 9 mm long 3D representation of the bone. We checked image quality after each scan, and conducted a rescans if necessary. Analyses were performed solely by SH Rønn. We calculated the coefficient of variation by repeated scans after repositioning and evaluation for 10 participants at tibia and 8 participants at radius. Coefficient of variation of repositioning was for vBMD 0.2% in tibia and 0.8% in radius. This is in agreement with the reproducibility reported by others (21, 22).

Safety

Information about adverse events was obtained at each visit.

Statistical analysis

We conducted the statistical analyses as per the protocol and five participants with early termination were not included in the statistical analyses.

The primary endpoint was change in undercarboxylated osteocalcin after 3 months. Previous short-term studies have demonstrated decrease of 29% in serum levels of ucOC after treatment with 180 µg MK-7 daily (26). The minimum number of participants was determined by power calculations to be 51 participants in each group, assuming a change in ucOC of 29%, a mean

Due to technical difficulties, tibia could not be analysed in two participants (both allocated to placebo), radius could not be analysed in 6 participants (3 in each group), and finite element analysis of the radius could not be performed in one participant in the placebo group.

Blood sampling and biochemistry

All blood samples were collected between 0730 and 0000 h after an overnight fast. After centrifugation, we stored serum and plasma at −80°C until analysis. Blood samples for routine safety biochemistry were collected at the screening visit, and every 6 months, and analysed the same day at the hospital clinical laboratory.

As bone formation markers we measured N-terminal midfragment osteocalcin (OC), undercarboxylated osteocalcin (ucOC), procollagen 1 N-terminal propeptide (P1NP) and bone-specific alkaline phosphatase (BAP) and as bone resorption marker we measured C-terminal telopeptide (CTX). We analysed OC, P1NP and CTX using a chemiluminometric method on a Cobas 6000 E reader (Roche Diagnostics, Lewes, UK) with interassay CVs of 2.9–3.0%, 3.7% and 5.1–5.8% in the ranges of 19–92 µg/L, 30–205 µg/L and 0.26–0.59 µg/L respectively. We analysed ucOC using an ELISA kit (Takara Bio) with an interassay CV < 6.7% and cross-reactivity with carboxylated osteocalcin was 5%. We analysed BAP using a chemiluminometric method on iSYS (Immunodiagnostic Systems, Tyne and Wear, UK), with an interassay CV of 10% in the range of 4.5–45.9 µg/L.
level of ucOC of 2.25 ± 1.16 ng/mL, a power of 80%, and a level of significance of 5%, and 54 participants in each group with a mean level of ucOC of 1.71 ± 0.92 ng/mL (27). To compensate for drop-outs we aimed at including 75 participants in each group.

We used STATA for statistical analysis. We checked normality using QQ plots for the two groups separately. Data were normally distributed except for ucOC, which was logarithmic transformed to achieve normal distribution. We compared baseline characteristics and changes from baseline to 12 months between the two treatment groups using unpaired t-test. Binomial data were analysed using Chi-square tests. We investigated serial changes using mixed models. These analyses comprised data from 0, 3, 6 and 12 months for BMD and from 0, 6 and 12 months for bone turnover markers. In case of a significant between-group difference by mixed models, we analysed differences between groups at each time point of measurements by a posteriori analysis using Bonferroni correction. Pearson’s correlation was performed to investigate the correlations between changes in s-ucOC and changes in trabecular microstructure. We investigated the potential effect of baseline bone turnover on treatment outcome by ANCOVA. Level of significance was 0.05. Results are reported as mean ± s.d. or median and confidence interval.

Results

Baseline characteristics, compliance and tolerability

At baseline the two groups were well matched with respect to age, years since menopause, measures of body composition, previous fractures, smoking status, vitamin D status, BMD and bone structure (Table 1). Regarding bone turnover markers, however, there was a slight imbalance as s-P1NP was 55.5 ± 17.1 µg/L (mean ± s.d.) in the MK-7 group and 63.0 ± 18.9 µg/L in the placebo group (P=0.02) and similarly s-CTX was 0.40 ± 0.14 ng/mL in the MK-7 group and 0.46 ± 0.15 ng/mL in the placebo group (P=0.03). There were no differences between the groups for BAP, OC or ucOC.

Compliance was 97.2% and 97.8% in the MK-7 and placebo groups respectively (P=0.37) and all but one participant were more than 80% compliant. There were no differences between the groups regarding adverse events or serious adverse events (P>0.05 for both, data not shown).

Effect of MK-7 on bone microarchitecture

Changes in bone microarchitecture and geometry from baseline to 12 months are presented in Fig. 1, Table 1  Baseline characteristics for the MK-7 and placebo groups.*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>MK-7 (n=71)</th>
<th>Placebo (n=71)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67.9 ± 4.6</td>
<td>67.0 ± 4.5</td>
<td>0.26</td>
</tr>
<tr>
<td>Years since menopause (years)</td>
<td>17.5 ± 6.6</td>
<td>16.9 ± 6.5</td>
<td>0.58</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.7 ± 3.5</td>
<td>24.2 ± 3.5</td>
<td>0.40</td>
</tr>
<tr>
<td>Family history of osteoporosis (yes/no)</td>
<td>37/34</td>
<td>35/36</td>
<td>0.74</td>
</tr>
<tr>
<td>Low-energy fracture (yes/no)</td>
<td>11/60</td>
<td>10/61</td>
<td>0.30</td>
</tr>
<tr>
<td>Smoking (previous/never)</td>
<td>23/48</td>
<td>29/42</td>
<td>0.32</td>
</tr>
<tr>
<td>S-25-OH-vitamin D (nmol/L)</td>
<td>88 ± 19</td>
<td>86 ± 23</td>
<td>0.59</td>
</tr>
<tr>
<td>S-P1NP (µg/L)</td>
<td>55.5 ± 17.1</td>
<td>63.0 ± 18.9</td>
<td>0.02</td>
</tr>
<tr>
<td>S-CTX (ng/mL)</td>
<td>0.40 ± 0.14</td>
<td>0.46 ± 0.15</td>
<td>0.03</td>
</tr>
<tr>
<td>S-BAP (µg/L)</td>
<td>15.6 ± 4.0</td>
<td>17.0 ± 4.7</td>
<td>0.06</td>
</tr>
<tr>
<td>S-OC (µg/L)</td>
<td>26.4 ± 8.6</td>
<td>28.1 ± 8.0</td>
<td>0.24</td>
</tr>
<tr>
<td>S-ucOC (ng/mL)*</td>
<td>6.6 (5.2; 8.5)</td>
<td>6.0 (4.8; 7.5)</td>
<td>0.56</td>
</tr>
<tr>
<td>aBMD hip total (g/cm²)</td>
<td>0.782 ± 0.070</td>
<td>0.779 ± 0.066</td>
<td>0.79</td>
</tr>
<tr>
<td>aBMD total spine (g/cm²)</td>
<td>0.873 ± 0.060</td>
<td>0.871 ± 0.062</td>
<td>0.80</td>
</tr>
<tr>
<td>vBMD tibia (g/cm²)</td>
<td>0.239 ± 0.046</td>
<td>0.238 ± 0.036</td>
<td>0.87</td>
</tr>
<tr>
<td>Trabecular numbers tibia (per mm)</td>
<td>1.7 ± 0.3</td>
<td>1.7 ± 0.3</td>
<td>0.47</td>
</tr>
<tr>
<td>Trabecular thickness tibia (µm)</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>0.95</td>
</tr>
<tr>
<td>Trabecular spacing tibia (µm)</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.79</td>
</tr>
<tr>
<td>Trabecular numbers radius (per mm)</td>
<td>1.8 ± 0.4</td>
<td>1.7 ± 0.4</td>
<td>0.57</td>
</tr>
<tr>
<td>Trabecular thickness radius (µm)</td>
<td>0.06 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.25</td>
</tr>
<tr>
<td>Trabecular spacing radius (µm)</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>0.30</td>
</tr>
<tr>
<td>Estimated ultimate failure load tibia (N)</td>
<td>8219 ± 946</td>
<td>8384 ± 1056</td>
<td>0.33</td>
</tr>
<tr>
<td>Estimated ultimate failure load radius (N)</td>
<td>2810 ± 474</td>
<td>2849 ± 463</td>
<td>0.31</td>
</tr>
</tbody>
</table>

*Results are shown as mean ± s.d. except for *that is shown as median and CI. Significant P-values are shown in boldface.
Tables 2 and 3. At the tibia trabecular number was unchanged in the MK-7 group – $-0.6 \pm 8.2\%$ ($P=0.52$), whereas it decreased significantly by $-3.5 \pm 8.6\%$ ($P<0.001$) in the placebo group. These changes were significantly different between the groups ($P=0.04$). In addition, trabecular spacing was unchanged in the MK-7 group – $+1.2 \pm 8.0\%$ ($P=0.21$), but increased significantly in the placebo group by $4.5 \pm 9.7\%$ ($P=0.001$) which was significantly different between groups ($P=0.03$). Trabecular thickness was unchanged in the MK-7 group – $+0.7 \pm 7.7\%$ ($P=0.58$), but increased in the placebo group by $4.0 \pm 8.7\%$ ($P<0.001$), which was significantly different between groups ($P=0.02$).

At the radius, there were no differences between the two groups in changes in the structural parameters ($P>0.05$ for all). There were no differences between the two groups in changes in any measure of BMD or failure load at the tibia or at the radius ($P>0.05$ for all).

### Effect of MK-7 on biochemical markers of bone turnover

Changes in s-ucOC over time differed significantly between the MK-7 group and the placebo group ($P<0.01$) (Fig. 2). After 3 months s-ucOC was reduced by $65.6$ (59.1; 71.0) % (median (95% CI)) in the MK-7 group compared with no changes in the placebo group ($-6.4$ ($-13.5$; 1.2) %) ($P<0.01$). After 6 months the changes were $-72.3$ ($-77.0$; $-66.6$) % in the MK-7 group and $-8.0$ ($-15.3$; $-0.1$) % in the placebo group ($P<0.01$) and after 12 months the changes were $-70.3$ ($-63.8$; $-75.6$) % in the MK-7 group and $-7.2$ ($-15.9$; 2.0) % in the placebo group ($P<0.01$).

Likewise, changes in total osteocalcin over time differed significantly between the groups ($P<0.01$). After 6 months, s-OC had decreased by $23 \pm 14.8\%$ in the MK-7 group compared with the placebo group – $6.7 \pm 14.8\%$ ($P=0.02$).

### Table 2: Percent changes in vBMD, microarchitecture and bone strength at the distal tibia and after 12 months evaluated by HRpQCT.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MK-7 (n=71)</th>
<th>Placebo (n=69)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>vBMD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMD</td>
<td>$-0.5 \pm 1.9$</td>
<td>$-0.3 \pm 1.8$</td>
<td>0.57</td>
</tr>
<tr>
<td>Cort. BMD</td>
<td>$0.1 \pm 0.9$</td>
<td>$0.2 \pm 1.1$</td>
<td>0.74</td>
</tr>
<tr>
<td>Trab. BMD</td>
<td>$-0.4 \pm 2.5$</td>
<td>$-0.3 \pm 2.1$</td>
<td>0.66</td>
</tr>
<tr>
<td>Microarchitecture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trab. numbers</td>
<td>$-0.6 \pm 8.2$</td>
<td>$-3.5 \pm 8.6$</td>
<td>0.04</td>
</tr>
<tr>
<td>Trab. Thickness</td>
<td>$0.7 \pm 7.7$</td>
<td>$4.0 \pm 8.7$</td>
<td>0.02</td>
</tr>
<tr>
<td>Trab. spacing</td>
<td>$1.2 \pm 8.0$</td>
<td>$4.5 \pm 9.7$</td>
<td>0.03</td>
</tr>
<tr>
<td>Finite element</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stiffness</td>
<td>$-0.4 \pm 3.8$</td>
<td>$0.7 \pm 4.4$</td>
<td>0.11</td>
</tr>
<tr>
<td>Failure load</td>
<td>$-0.4 \pm 3.0$</td>
<td>$0.4 \pm 3.6$</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Changes are shown in percent $\pm$ s.d. P-values refer to unpaired t-test between MK-7 and placebo groups. Significant P-values are shown in boldface.

### Table 3: Percent changes in vBMD, microarchitecture and bone strength at the radius after 12 months evaluated by HRpQCT.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MK-7 (n=68)</th>
<th>Placebo (n=68)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>vBMD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMD</td>
<td>$-1.2 \pm 4.0$</td>
<td>$-1.0 \pm 3.1$</td>
<td>0.67</td>
</tr>
<tr>
<td>Cort. BMD</td>
<td>$-0.3 \pm 2.1$</td>
<td>$-0.2 \pm 2.3$</td>
<td>0.86</td>
</tr>
<tr>
<td>Trab. BMD</td>
<td>$0.1 \pm 5.5$</td>
<td>$0.4 \pm 3.1$</td>
<td>0.69</td>
</tr>
<tr>
<td>Microarchitecture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trab. numbers</td>
<td>$-0.2 \pm 9.2$</td>
<td>$-0.2 \pm 9.2$</td>
<td>0.99</td>
</tr>
<tr>
<td>Trab. thickness</td>
<td>$1.0 \pm 8.6$</td>
<td>$1.4 \pm 9.1$</td>
<td>0.79</td>
</tr>
<tr>
<td>Trab. spacing</td>
<td>$1.1 \pm 9.9$</td>
<td>$1.1 \pm 9.6$</td>
<td>0.96</td>
</tr>
<tr>
<td>Finite element</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stiffness</td>
<td>$0.9 \pm 7.0$</td>
<td>$1.9 \pm 6.7$</td>
<td>0.39</td>
</tr>
<tr>
<td>Failure load</td>
<td>$0.7 \pm 6.0$</td>
<td>$1.6 \pm 5.8$</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Changes are shown in percent $\pm$ s.d. P-values refer to unpaired t-test between MK-7 and placebo groups.

**Figure 1**

Changes in microarchitecture by HRpQCT from baseline to 12 months. (A) Tibia. (B) Radius. Mean (%) change $\pm$ SE in trabecular numbers, trabecular spacing and trabecular thickness. *$P<0.05$ compared with placebo.
group compared with no change (−4.3 ± 11.1%) in the placebo group (P < 0.01). After 12 months the changes were −18.8 ± 13.9% in the MK-7 group and −2.5 ± 12.8% in the placebo group (P < 0.01).

In addition, the changes in the ratio between s-ucOC and s-OC differed significantly between the groups (P < 0.01). After 6 months the ratio had decreased by −63.3% (−69.5; −55.9) % in the MK-7 group compared with −3.2% (−10.0; 4.0) in the placebo group (P < 0.01), and after 12 months by −62.9% (−69.3; −55.1) in the MK-7 group compared with −4.2% (−12.4; 4.8) in the placebo group (P < 0.01).

Correlations between change in s-ucOC and changes in trabecular microarchitecture

Percentage change in s-ucOC after 12 months was negatively correlated to percentage change in trabecular numbers (r = −0.19) (P = 0.02), but positively correlated to percentage change in trabecular thickness (r = 0.21) (P < 0.01) and trabecular spacing (r = 0.19) (P = 0.02). Finally, S-BAP increased in the MK-7 group (4.1 ± 13.7%).

**Figure 2**
Changes in bone turnover markers. (A) S-undercarboxylated osteocalcin (ucOC). (B) S-total osteocalcin (OC). (C) Ratio s-undercarboxylated osteocalcin to s-total osteocalcin (ucOC/OC-ratio). (D) S-bone-specific alkaline phosphatase (BSAP). (E) S-Procollagen 1, N-terminal propeptide (P1NP). (F) S-C-terminal telopeptide (CTX). Mean (%) change ± se from baseline after 6 and 12 months. *P < 0.017 compared with placebo.

**Figure 3**
Changes in aBMD by DXA. (A) BMD of total hip. (B) BMD of femoral neck. (C) BMD of lumbar spine. Mean (%) change ± se from baseline after 3, 6 and 12 months.
compared with the placebo group (−1.3±12.7%) after 12 months (P=0.02). S-25-OH-vitamin D increased in the MK-7 group (17.8±20.6%) (P<0.01) and in the placebo group (21.0±16.7%) (P<0.01) after 12 months, with no differences between the two groups (P=0.31).

Mixed models analysis showed no difference in s-P1NP or s-CTX over time between the groups (P=0.86 for s-P1NP and P=0.54 for s-CTX). Furthermore, baseline s-P1NP and s-CTX did not affect the effect of treatment allocation on trabecular bone microarchitecture (data not shown).

**Effect of MK-7 on bone mass and density**

Changes in aBMD after 12 months were small and did not differ between groups (Fig. 3). Mixed models analysis showed no differences in BMD changes, when BMD at all the time points were included in the analysis (P=0.58 for total hip, P=0.76 for femoral neck and P=0.53 for total spine).

At 12 months changes in total hip BMD were −0.3±2.1% and −0.5±1.8% in the MK-7 and placebo group respectively (P=0.42), changes in femoral neck BMD were −0.1±3.2% and −0.1±3.3% in the MK-7 and placebo group respectively (P=0.94), and changes in lumbar spine BMD were −0.8±3.7% and −0.1±3.3% in the MK-7 and placebo group respectively (P=0.23).

**Discussion**

In this randomised, placebo-controlled, double-blinded clinical trial, we found that treatment with vitamin MK-7 375 µg daily, in addition to calcium and vitamin D, for 12 months reduced serum levels of undercarboxylated osteocalcin by more than 70%, reduced the ratio ucOC/OC by more than 60%, increased BAP by 5%, and maintained microarchitecture in trabecular bone in tibia, despite no significant effects on BMD. The reduction in trabecular numbers, increase in trabecular spacing and increase in trabecular thickness observed in the placebo group compared with the MK-7-treated women are in agreement with the age-related changes previously reported in postmenopausal women. Kawalilak et al. found comparable changes also using HRpQCT after 12 months in 31 postmenopausal women (28). The findings represent a deterioration of trabecular bone structure, with a loss of trabeculae, which would explain the decrease in trabecular numbers and increased trabecular separation. In addition, loss of the thinnest trabeculae would lead to an increase in the mean trabecular thickness of the remaining trabeculae. Our data suggest that treatment with vitamin MK-7 counteracts this process, and preserves trabecular structure. We found no significant effects of the treatment with MK-7 at the radius. The tibia is a weightbearing bone, whereas radius is not. Furthermore, because it is technically easier to position a leg than an arm in the HRpQCT scanner the variation of the measurements performed at the tibia is smaller than the variation of measurements of bone structure in the radius which makes it easier to detect differences at the tibia. This difference between the two sites has also been demonstrated by Cheung et al. (29).

Our study like many other studies (18, 19, 20) shows a decrease in s-ucOC in response to MK-7 treatment indicating an increased carboxylation of osteocalcin. S-OC also decreased although to a lesser extent. This has been described before in response to MK-7 treatment (18). One could speculate that the decrease in s-ucOC is merely a consequence of a decreased s-OC; however, cell studies show that carboxylated osteocalcin has greater affinity for calcium and hydroxyapatite, and consequently accumulates in bone matrix (30). Thus, the decrease in s-OC is more likely to be explained by increased carboxylation and thereby a change in distribution from serum to bone matrix. Moreover, the fact that s-ucOC/s-OC also decreases significantly shows that the change in s-ucOC is at least in part independent of the change in s-OC. We speculate that this decrease in s-ucOC promotes mineralisation which may explain the preservation of trabecular structure in response to MK-7. In addition, changes in mineralisation does not affect bone turnover; accordingly we found no changes in s-CTX or s-P1NP with treatment.

We found no effect of MK-7 on the other bone formation and resorption markers, apart from a small but significant increase in BAP in the MK-7-treated women. In accordance with that, Emans et al. found a borderline significant increase in BAP in the MK-7-treated group compared with placebo (18).

Three other studies have investigated the effect of MK-7 on bone metabolism in postmenopausal Caucasian women. Emans et al. investigated the effect of 360 µg MK-7 or placebo in 334 women without supplementation of vitamin D and calcium and found no effect on BMD after 1 year (18).

Knapen et al. randomised 244 women to 180 µg MK-7 or placebo for 3 years. Vitamin D and calcium were not provided (19). After 1 year, no effect was found on BMD but BMC at the lumbar spine decreased less in the MK-7-treated women compared with the placebo-treated women. After 3 years, the MK-7-treated women had a
smaller decline in BMD at the femoral neck compared with the placebo group. Finally, Kanellakis et al. investigated the effects of vitamin K1 and MK-7. Twenty-four women were treated with 100 µg MK-7 and a second group of 26 women received 100 µg vitamin K1, both these groups received calcium 800 mg, vitamin D 10 µg, and lifestyle counselling. A control group of 39 women did not receive any intervention (20). BMD at the lumbar spine was improved in the MK-7 and K1 groups in a combined analysis after 1 year; however, the isolated effect of MK-7 in that study is not clear.

Disregarding the study by Kanellakis et al. due to its inability to investigate the isolated effect of MK-7, changes in BMD after 1 year were small and there was no difference between groups in all MK-7 studies including this study. These findings would suggest that 1 year is too short to observe a difference in BMD, even with high doses of 360–375 µg MK-7; our results suggest that supplementation with vitamin D and calcium does not alter this.

In a recent meta-analysis by Huang et al. (16) including MK-4 and MK-7 studies only it was shown that in patients with osteoporosis, the treatments prevent vertebral BMD decline. The majority of the osteoporosis patients, however, were of Japanese ethnicity (9 of 10 studies), while the majority of the non-osteoporosis individuals, for whom no effect was shown, were Caucasian (9 of 11 studies). This makes it possible that the different effects in the two groups may also be explained by differences in ethnicity including pharmacogenomics and lifestyle.

This study has strengths and limitations. The strengths include the study design and a high compliance. Furthermore, s-ucOC decreased, confirming intake, absorption and the biological effect of vitamin MK-7. Moreover, calcium and vitamin D were provided to all participants, which is mainstay in the prevention of bone loss and fractures in patients with osteopenia (31, 32). Lastly, this is the first study of vitamin MK-7 to investigate the microarchitectural changes in bone using HRpQCT. Limitations of the study include the short follow-up time of 1 year. The study was powered to show a decrease in ucOC after 3 months, and not a change in BMD after 12 months. Despite randomisation, two out of four bone turnover markers at baseline were higher in the placebo group. This potentially could have affected the different outcome between the two groups; however, the effect of MK-7 does not seem to be mediated through an effect on bone turnover. Furthermore, treatment response was not affected by baseline serum levels of P1NP or CTX. We do not have bone biopsies to further investigate the mechanisms by which MK-7 affects bone microarchitecture. Moreover, our participants had osteopenia, and the meta-analysis by Huong et al. suggests that vitamin K2 has an effect on osteoporosis patients only. Finally, our results may not be representative for younger women, men, children or non-Caucasians.

In conclusion, we investigated the effect of MK-7 on bone mass, bone microarchitecture and bone turnover markers. We found a decrease in s-ucOC, indicating an increased carboxylation of osteocalcin and an increase in s-BAP. HRpQCT analyses demonstrated that MK-7 preserved trabecular bone structure. Longer-term studies are needed to investigate the implications of this, and to investigate the long-term effect of MK-7 in combination with calcium and vitamin D on bone.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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