VITAMIN K2
VITAL FOR HEALTH AND WELLBEING

CHAPTER 6 Vitamin K2 and Bone Health
by Niels Erik Frandsen and Jan Oxholm Gordeladze

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Abstract

During the last 20 years, the main clinical effects of vitamin K2 on bone homeostasis have been investigated in both indirect and direct vitamin K treatment regimens. This chapter is mainly based on randomized clinical trials (RCT) lasting for more than 1 year. As for vitamin K1 (phylloquinone, indirect treatment) and vitamin K2 (menaquinone MK-4 and MK-7 direct treatment), respectively, the clinical trials have consistently shown decreased fracture rate incidents, however, mainly in Asian populations. In 2013, a major breakthrough was observed by Knapen et al. in the Netherlands, where menaquinone MK-7 supplementation of 180 μg/day for 3 years to healthy postmeno-pausal women significantly decreased the age-related decline in BMC (bone mineral contents) and BMD (bone mineral density) at the lumbar spine and femoral neck, but not at the total hip, as compared to placebo. Thus, MK-7 supplementation has shown a significant "double"-positive action through (1) increased bone building and (2) decreased bone resorption. We look forward to seeing the clinical effects on low bone mass and osteoporosis as well as other bone diseases.

Keywords: bone health, RCT trials, vitamin K1, vitamin K2 (MK-4, menatetrenone), menaquinone-7 (MK-7)

1. Introduction

Vitamin K2 (menaquinone-4, MK-4, or menatetrenone) is a very important vitamin K species serving special functions in several extrahepatic organs, like bone tissue, heart, blood vessels, kidneys, brain, and cartilage. MK-4 is a member of a sub-family with eliciting the same cellular reactions, but with different effects. MK-4 is deemed necessary for γ-carboxylation of proteins, and activation of the vitamin K-dependent proteins, i.e., Osteocalcin (bone-Gla-protein), matrix-Gla proteins (MGPs), Periostin, as well as protein S. Without these activated proteins, the body

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is not able to regulate the process of calcium uptake and bone mineralization. To date, we know of 19 different vitamin K-dependent proteins. The process also ensures the access of energy for a plethora of cellular reactions. The most common of the K vitamins is phylloquinine-1 (K1). K1 is found in leafy green vegetables, olive oil, and soybean oil, while MK-4 is found in small amounts in egg yolks, butter, and hard cheese. Fermented soybeans, known as natto, a special Japanese food, contain another subfamily member menaquinone-7 (MK-7). It is the richest dietary source of menaquinones (yielding about 1100 µg/100 g). In Western diets, MK-7 in fermented cheeses amounts to only 62 µg/100 g, approximately. Very small amounts of MK-4 are produced by intestinal bacteria. The adequate intake (AI) for vitamin K1 from 2001 in the United States and Canada was recommended to be 90 µg/day for women, 120 µg/day for men. These values are based on the need for hepatic vitamin K1 to ensure sufficient clotting factor synthesis. To date, we know that the early AI is not enough to meet the request for a complete carboxylation of all the extrahepatic vitamin K-dependent proteins. When MK-4 ingestion is insufficient, the serum concentration of uncarboxylated MGP (ucMGP) or uncarboxylated Osteocalcin (ucOC) is increased. This serves as the major sign of a deficient intake of K1 or a deficient hepatic transformation of vitamin K1 to MK-4. High serum concentrations of MK-1 and MK-4 reflect only the recent intake of vitamin K, not the accumulation or steady state levels of a "chronic" intake. The bioavailability of K1 and MK-4 is very short, i.e., about 1.5–3 h in contrast to 72 h for MK-7. To date, K1, MK-4, and MK-7 are available as dietary supplements [1, 2].

Osteoporosis is, on a worldwide basis, the reason for many emerging, spontaneous fractures. On a global basis, osteoporosis causes more than 88.9 million fractures annually. The condition is estimated to affect 200 million women worldwide, i.e., approximately one-tenth of women aged 60, one-fifth of women aged 70, two-fifths of women aged 80, and two-thirds of women aged 90 years. Worldwide, one in three women over the age of 50, will experience osteoporotic fractures, as will one in five of men aged over 50 [3]. From Europe, the number of new fractures in 2010 was estimated at 3.5 million, comprising approximately 620,000 hip fractures, 520,000 vertebral fractures, 560,000 forearm fractures, and 1,800,000 of other fractures (types or sites). For women in the EU: approximately 50% of fractures related deaths, were due to hip fractures, 28% to clinical vertebral fractures, and 22% to other types of fractures. In men, the corresponding figures were 47, 39, and 14%, respectively [3]. From the USA Preventive Services Task Force (USPSTF) for 2013, it is therefore concluded that the current evidence is insufficient to assess the balance of the benefits and harm of combined vitamin D and calcium supplementation for the primary prevention of fractures In the treatment of pre- and postmenopausal women or in men, these indicate that new treatments of osteoporosis are wanted [3, 4].

In 1984, Hart et al. [5] revealed that the serum concentrations of vitamin K1 were very low among patients with hip fractures, and hypothesized that poor vitamin K status is associated with increased rates of osteoporotic fractures. The period of 1991–1993 was regarded as the beginning of the era of vitamin K examinations, since patients-based studies by Hodges et al. confirmed that low serum concentrations of vitamin K1 and K2 were associated with increased risk of spine and hip fracture. The same phenomena were also shown by Szulc et al. in a French study, yielding a positive association between ucOC and fracture risk [6–8]. Other countries,

like the Netherlands, as shown by the group of Knaben et al. [9] and Finland, as shown by Luukinen et al. [10], clearly demonstrated that this association (ucOC versus fracture risk) point to the fact that vitamin K status is a predictor of bone health (fracture-free percentage of a population group). The correlation between low intake of vitamin K and increased fracture rate was also revealed by prospective analysis within the Nurses' Health Study cohort 1984. Here, the diet was assessed in 72,327 women, aged 38–63 years, with a food-frequency baseline-questionnaire. During the subsequent 10 years of follow-up, 270 hip fractures resulting from low or moderate trauma were reported. Results: women in quintiles 2–5 of vitamin K intake had a significantly lower age-adjusted relative risk (RR: 0.70; 95% CI: 0.53, 0.93) of hip fracture than women in the lowest quintile [11].

In Japan, a high dose of menaquinone-4 (MK-4) of 45 mg/day (15 mg × 3/day) was used as therapeutic treatment for osteoporosis. The principle cause-effect of vitamin K2 on osteoporosis is mainly the prevention of bone fractures due to its improvement of bone quality, and not the ensuing increasing bone mineral density. Due to variable contents of vitamin K in the diet, the Japanese Society of Osteoporosis included in 1995 vitamin K2 as Menatetrenone (MK-4), together with vitamin D, in the first line treatment of osteoporosis [12].

Osteoporosis, in its primary form, is characterized by bone loss, and the age of females upon onset or development of menopause, however, the disease also affects a smaller cohort of men. Genetics is the single most important cause for both sexes, however, changes of lifestyle, exercise levels, smoking habits, and low body weight, are important contributors, which may trigger the onset of the disease. Secondary forms of osteoporosis-related chronic diseases are rheumatoid arthritis, chronic lung disease, and anorexia. In addition, it is well known that the use of cortisone or prednisolone can reduce bone mineral density (BMD). Standard examination of bone mineral density (BMD) is the dual energy X-ray absorptiometry (DEXA). BMD values is not the same for all women and men but different due to race. In the last decade, research have combined DEXA scan with more specific geometric hip and vertebra measurements as femoral neck width (FNW) and hip-axis length (HAL) and vertebral fracture (VF) assessment (VFA).

However, it may be asserted to a certain extent, that proper bone 3D-architecture may "make up" for a marginal reduction in BMD-values in terms of predictive value of the BMD-levels *per se*.

The incidence of osteoporosis is lower in Japanese women, even though they are subjected to the same period with menopause as other women. However, in some regions, like in Tokyo, the intake of vitamin K as MK-7 in the special fermented food of natto, soybeans together with other treatments result in higher BMD-values and lower fracture rates than age-matched woman in the United States and Europe [3].

Of other health outcome studies featuring patients with an without osteoporosis, a few warrant special attention: observational studies of subjects displaying a low long-term vitamin K intake revealed a higher incidence of osteoarthritis in the hand and knee [13], dementia [14, 15]. From population-based studies of atherosclerosis, Jie et al. revealed that, in atherosclerotic women, vitamin K status is associated with lower bone mass. All these findings support our hypothesis

that vitamin K status affects the mineralization processes in both bone and atherosclerotic plaques in a healthy manner [16]. And, from the population-based Rotterdam Study, the relation between low vitamin K status and development of coronary artery disease [17] is indisputable.

The importance of vitamin D for bone health has been known since it was used in the treatment of rachitis in the 1930s. Vitamin D and calcium supplements have been recommended as a pillar in the treatment of osteoporosis over the last three decades. Vitamin D and calcium supplementation increases spinal BMD in healthy, postmenopausal women [18, 19], and vitamin D is crucial in the process of mobilizing the Ca^{2+} ions into the bone tissue [20]. Interestingly, vitamin D and calcium alone are not able to rebuild bone tissue and infrastructure that are being lost. The synergistic effect of vitamin D and retinoic acid on osteoblast production of osteocalcin was shown in 1993 [21], where Hara et al. demonstrated that MK-4 was able to partly inhibit the bone resorption induced by inflammation, vitamin D loss and ensuing PTH induction, as seen in the calvariae and incubation medium in his mode *ex vivo* model system. This observation served later as the basis for the introduction and acknowledgement of menatetrenone (MK-4) supplementation in the clinic [22]. And finally, in 1995, Hara et al. [23] also showed that the inhibition of bone resorption was related to vitamin K2's long side chain.

2. Vitamin K-dependent proteins in the bone building process

Osteocalcin is produced by osteoblasts during bone formation, and serves as the most abundant protein in bone after collagen. Furthermore, it is crucial for bone mineralization. Activated osteocalcin is located within hydroxyapatite crystals and binds calcium strongly to facilitate mineralization of the hydroxyapatite crystal grid [24]. Osteocalcin production is regulated by a plethora of factors including retinoic acid (RA), estrogens, glucocorticoids, as well as vitamin D [25–27]. In 1995, Douglas et al. showed the percentage of carboxylated osteocalcin (cOC), as calculated from total osteocalcin, was found to be less than 60% in osteoporotic postmenopausal women compared to 70–80% in young, healthy adults [28]. The osteocalcin production is increased by vitamin D but also increased by both MK-4 and MK-7 in a synergistic fashion. From 2007, as used by Knapen et al., osteocalcin has been employed as a marker for the deficiency of vitamin K in bone. Today, the specific osteocalcin molecules are total Osteocalcin (tOC), ucOsteocalcin (ucOC), and cOsteocalcin (cOC) [28, 44]. A secondary action of MK-4 and MK-7 is the ensuing increased collagen production by cells of the osteoblastic lineage. Collagen should make the structural fundament, on which calcium and other minerals are accumulated within the bone matrix. Increased deposition of collagen makes the bone more flexible and this is very important for the attainment of "higher" or better bone quality [24]. In 2001, Yamaguchi et al. unraveled the stimulatory effect of MK-7 on osteoblastic bone formation *in vitro*, but they also discovered the suppressive effect of MK-7 on osteoclast-like cell formation and osteoclastic bone resorption in rat bone tissues *in vitro* [29]. Furthermore, in 2001, Yamaguchi and Ma [30] confirmed the dual effects of MK-7 but also a significant decrease number of osteoclasts. Finally, in 2011, Yamaguchi and Weilzmann showed

that MK-7 reinforces the synthesis of various bone-specific proteins, mediated through the pathways of calcium-dependent protein kinase C signaling, as well as cyclic AMP-dependent signaling. MK-7 also antagonizes the "receptor activator of NF-κB (RANK) ligand (RANKL)" induced NF-κB activation on osteoclast precursors. This concept now makes up the basis for the search of novel antiosteoporotic medication regimens, mimicking the plethora of effects induced by MK-7 [31].

3. Clinical-related publications featuring K1 supplementation

In relation to the loss of bone associated by deficient intake of vitamin K1, many observational studies have been conducted. However, few randomized studies have been able to reveal a significant positive rebuilding of bone mass and increased BMD. This chapter deals only with randomized clinical trials with a duration exceeding 1 year.

Schaafsma et al. from the Netherlands showed in a 1-year long randomized study from 2000 [32] (featuring four groups with 400 IU vitamin D3; vitamin K1 supplementation 80 μg daily, vitamin K1 and D3, and placebo of Dutch postmenopausal women (with a patient total, n = 141) with either normal or low bone mineral densities (BMD). It was shown that women with low BMD had a lower %cOC at baseline than the women with normal BMD. However, this difference disappeared after 1 year of supplementation with vitamin K1 [(mean ± S.D.) 68 ± 11% (95% CI = 64.5 ± 71.2%) versus 72 ± 6% (95% CI = 70.1 ± 72.9%)], respectively. On the other hand, 1 year of supplementation with vitamin D3 showed maximum increases in 25(OH)D of 33 ± 29% (95% CI = 24.8 ± 41.8%) and 68 ± 58% (95% CI = 50.1 ± 84.6%) in women with normal and low BMD, respectively. No effect was observed on BMD [32].

In the Bram et al. study from 2003, three groups were examined; group K1D: the effect of vitamin K1 (1 mg/day) and vitamin D (8 μg/day including standard mineral supplementation), group D (vitamin D and minerals) and group 3, placebo on bone loss retardation in a random-ized, double-blind, placebo-controlled 3-year intervention study. Of 181 healthy postmeno-pausal women between 50 and 60 years of age, 155 completed the observation period. The main outcomes of the study were significant changes in BMD-values of the femoral neck and lumbar spine after 3 years. The group receiving the supplement with additional vitamin K1 showed a decline in the bone loss from the femoral neck. The difference in femoral neck bone mass between the K1D group and the placebo group was 1.7% (95% CI: 0.35–3.44). The difference between the K1D group and the D group was 1.3% (95% CI: 0.10–3.41). No significant differences were observed among the three groups with respect to changes in BMD at the site of the lumbar spine. It was therefore concluded that the minerals and vitamin D, coadminis-tered with the vitamin K1 supplement, substantially contributed to a significant reduction in postmenopausal bone loss at the site of the femoral neck [33].

In a systematic review and meta-analysis of 700 patients, Cockayne et al. showed in 2006 that the MK-4 intake in Japan yielded a powerful reduction in the incident of fractures. However, the authors would not recommend vitamin K supplementation until a new randomized clinical trial confirmed the results [34].

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Boton-Smith et al. performed a 2-year, randomized, double-blind, and placebo-controlled study in 2007, scrutinizing the effect of dietary supplementation with either: (1) 200 μg vitamin K1 daily, (2) vitamin D 400 UI daily (3) and calcium 1000 mg daily, or (4) their combination on 244 healthy nonosteoporotic older women. Baseline and 6-month measurements included DXA bone mineral scans of the hip and wrist, markers of bone turnover, and vitamin status. The results reported were the following: the combined vitamin K1 with vitamin D plus calcium treatment was associated with only a modest but significant increase in BMC at the ultradistal radius, however not at other sites in the hip or radius [35].

The Booth et al.'s study was published 1 year later, in 2008. The goal of the present investigation was to pinpoint the effect of a consecutive 3-year administration of vitamin K1 on putative alterations in bone mineral density (BMD) of the femoral neck in elderly patients of both sexes, who presented upon inclusion with calcium and vitamin D repletion. In the present double-blind, controlled survey, 452 individuals (both men and women, 60–80 years of age) were evenly distributed, in a randomized fashion, each to receive a multivitamin containing either 500 μg/day of vitamin K1 or placebo, in addition to a daily, 600 mg elemental calcium with vitamin D (400 IU) supplement. Analyses of the femoral neck, spine (L2–L4), and total-body BMD, turnover of bone mass, and indigenous status of both vitamins K and D was checked every 6–12 months. Results were as follows: one could not find any distinction in BMD values localized to any pertinent body sites, when comparing the two patient groups. Furthermore, the population receiving the vitamin K1 supplement showed a markedly higher vitamin K1 level, as well as a substantially lower degree (percentage) of ucCO concentrations, when matched with the patients not ingesting K1. Neither of the additional biochemical variables measured differed between the patient treatment groups. Hence, the authors concluded that vitamin K1 supplementation, in a dose attainable in the diet, does not confer any additional benefit for bone health at the spine or hip when taken with recommended amounts of calcium and vitamin D [36].

The emerging questions to ask were then: Was the dose of vitamin K1 supplement too low, or the follow up period too short to elicit an increase of BMD, or was it possible to use other sub-family vitamin K members, such as menaquinone-4 (MK-4)? Or, could vitamin K supplementation potentially be harmful to the body?

Cheung et al. from Canada addressed the last of these questions in their 2–4-year study from 2008. In this trial, 440 postmenopausal women with osteopenia were randomized into a placebo-controlled double-blind trial, and it was conducted, mainly to determine whether daily high-dose vitamin K1 supplementation safely reduces bone loss, bone turnover, and fractures. The conclusions coming out of the study were: 5 mg of daily vitamin K1 supplementation for 2–4 years does not protect against age-related decline in BMD, but may protect against fractures and cancers in postmenopausal women with osteopenia. Overall fracture rate was reduced by 50% (9 versus 20, $P = 0.04$) versus placebo. Interestingly, cancer incidence was reduced by 75% with vitamin K1 (3 versus 12, $P = 0.02$). However, more studies are needed to further examine the effect of vitamin K on fractures and cancers [37].

Brinkley et al. from USA conducted a 1-year study in 2009 and found that low vitamin K status is associated with low BMD and an increased fracture risk. From the bulk of reports available

at that time, it seemed that the menaquinones (menatetrenone: MK-4), might diminish the fracture risk incurred by the enrolled patients. Whether vitamin K is an important "by-player" in maintaining skeletal health in females situated in the northern part of the US remains an unsolved issue. Furthermore, different entities of K vitamins (i.e., phylloquinone (K1) and MK-4) may exert differing biological effects on the skeleton. The present study was designed to assess the efficacy of either vitamin K1 or MK-4 exposure on biomarkers of skeletal health and bone mineral density (BMD) in postmenopausal nonosteoporotic, North American women. In the present, placebo-controlled and double-blind investigation, a total of 381 postmenopausal females were given either vitamin K1 (1 mg/day), MK-4 (45 mg/day), or a placebo treatment. The whole observation period was 12 months. All enrolled patients/participants were given either Ca^{2+} or vitamin D_3 supplementation. Bone-specific BSALP (alkaline phosphatase) in blood samples, as well as the n-telopeptide of collagen, type 1 (NTX) were measured at before the onset of "medication," and subsequent to 1, 3, 6, and 12 months, respectively. Both lumbar spine and proximal femur BMD values, as well as proximal femur geometry were assessed by DXA, before the onset of the trial, and after 6 and 12 months of treatment. At the onset of the trial, all treatment groups showed identical demographic parameters. The patients' compliance rates related to the intake of either calcium, vitamin K1, or MK-4, were some 87–93%, respectively. Interestingly, K1 and MK-4 treatments both diminished the patient levels of serum ucCO, however, neither BSALP nor NTX levels were changed. Lastly, no effects of K1, or MK-4 on lumbar spine or proximal femur BMD or proximal femoral geometric parameters could be observed. This study does not support a role for vitamin K supplementation in osteoporosis prevention among healthy, postmenopausal, North American women, receiving calcium and vitamin D supplementation [38].

4. Clinical randomized controlled osteopenia/osteoporosis studies with menatetrenone-4 (MK-4) from 1 year duration, in countries with different background intake of vitamin K

In 1998, Orimo et al from Japan evaluated the effects of menatetrenone-4 (MK-4) on bone and calcium metabolism in osteoporosis patients in a 24-week double-blind placebo-controlled study, where 80 osteoporotic patients were included. Treatment was MK-4, 90 mg/day (n = 39) or placebo (n = 41). Bone density was assessed on X-ray films of the right, second metacarpal bone, using the microdensitometric method. In the MK-4 group, bone density increased by about 2.2 ± 2.5% from the baseline; in the placebo group it decreased by about 7.3 ± 3.7% (P = 0.037, K_2 treatment versus placebo). The excretion of γ-carboxyglutamic acid (Gla) to the urine was markedly enhanced (i.e., from 72.6 ± 4.1 nmol/mg of creatinine before initiation of "medication," to 88.4 ± 5.4 during the 24th week subsequent to the sustained MK-4 treatment (P = 0.008) period). In the group receiving placebo, no significant changes in the excretion of urinary Gla could be observed. However, during the 24-week long treatment period, the urinary ratio of calcium over creatinine in the K_2 treatment group was reduced from 0.14 ± 0.02 to 0.12 ± 0.02, respectively. However, in the placebo group it increased from 0.15 ± 0.02 to 0.19 ± 0.03. Accordingly, the 24-week levels shown by members of both the MK-4 and the placebo

groups turned out to be significantly different ($P = 0.03$) with unpaired test. Finally, it should be noted that there were but a few adverse effects, being attributable to the vitamin MK-4 treatment. One patients increased hepatic enzymes of GOT, GPT, al-P, and γ-GTP evaluated as probable relationship. The results suggest that MK-4, at a dosage of 90 mg/day, is effective in maintaining peripheral cortical bone density and is safe in treatment of osteoporosis. The dose was increased in order to maintaining peripheral cortical density. Interference of diet was not observed. This study is one of very few, where side effects of the treatment were observed [39].

In 2000, Shiraki et al. conducted a 2-year study in Japan, to assess whether MK-4 effectively prevented the incidence of new fractures in osteoporotic patients. Two hundred forty-one osteoporotic women were enrolled in a 24-month, randomized, and open label study. The population constituted: a control group without treatment ($n = 121$) and an MK-4 group ($n = 120$), the latter receiving 45 mg/day. All patients received follow up measurements of lumbar bone mineral density (LBMD) analyzed by DXA and the occurrence of new clinical fractures had occurred. Both serum concentrations of Glu-osteocalcin (Glu-OC) (RIA, Takara Japan), as well as MK-4 were analyzed after termination of the follow-up period, while both the level of serum-OC (RIA, CIS, France) and excretion of deoxypyridinoline (DPD) to the urine were analyzed prior to and at the end of the treatment. The demographic data of the present groups did not differ significantly, and the results obtained run as follows: the clinical vertebral fracture incidence in the control group was 30, compared with 13 in the MK-4 treated group ($P = 0.027$). Furthermore, the percentage change from the initial LBMD value at 6–24 months after the initiation of the study ranged between -1.8 ± 0.6, and $-3.3 \pm 0.8\%$ for the control group, and between 1.4 ± 0.7, and $-0.5 \pm 1.0\%$ for the MK-4-treated group, respectively. The alterations in LBMD-values around each measure point turned out to be significantly different, when comparing the control group with the treated group ($P = 0.0010$ at 6 months, $P = 0.0153$ after a year, and $P = 0.0339$ after 2 years, respectively). The blood concentrations of Glu-OC at termination of the period of observation of the controls and the group receiving active "drug" were 3.0 ± 0.30 and 1.6 ± 0.10 ng/ml, respectively ($P < 0.0001$), while blood concentrations of OC (as analyzed by standard radioimmunoassay (RIA) methodology, gave a marked and significant rise ($42.4 \pm 6.9\%$) over basal value observed in the treated group at 24 months, but only $18.2 \pm 6.1\%$ for the individuals constituting the controls ($P = 0.0081$)). However, one did not find any significant change in the amount of DPD excreted into the urine of the treatment group members. This compilation of information indicates that MK-4 treatment is effective in reducing the incidence of additional fractures, even though the MK-4 treated individuals failed to show an enhancement of LBMD. Lastly, the study was able to show that MK-4 treatment elevates the levels of γ-carboxylated OC [40].

In 2000, Iwamoto et al. showed in 92 postmenopausal women, aged 55–81 years, completing a 2-year randomized controlled trial in four groups receiving either menatetrenone (MK-4, 45 mg/day), 1α-hydroxyvitamin D3 (0.75 μg/day), a combination of MK-4 and D3 (same dosage as above), or calcium lactate (2 g/day). The MK-4 and vitamin D3 groups experienced significant enhancements of their BMD-values (+0.91 and 0.38%), compared to the "Calcium group" (–0.79%), while the combined MK-4 and D treatment, being synergistic, significantly

increased lumbar BMD by 1.5 %, $p < 0.001$. These findings indicate that combined administration of vitamin D3 and MK-4, compared with calcium administration alone, appears to be instrumental in increasing the BMD-values of the lumbar spine in postmenopausal women with osteoporosis [41, 42].

Ushiroyama et al. completing a randomized 2-year study in 2002, investigated the therapeutic effect of the combined use of menatetrenone MK-4 and vitamin D3 on vertebral bone mineral density in 172 postmenopausal women with low bone mass and osteoporosis. Four groups, each with 43 subjects received the following; either (a) MK-4, 45 mg/day, (b) standard vitamin D3 supplement, (c) combined MK-4 and vitamin D3 therapy, and (d) control group receiving dietary therapy alone. BMD (bone mineral density) was assessed before initiation of therapy and subsequent to 6–24 months of treatment, respectively. Analyzed biological markers of osseous metabolism constituted: serum type 1 collagen carboxyterminal propeptide (P1CP), intact or total osteocalcin, as well as urinary pyridinoline. Tests for potential of blood coagulation was done analyzing "activated-partial-thromboplastin-time" (APTT), as well as assessment of levels of antithrombin III (AT III), fibrinogen, and plasminogen, respectively. Conclusions summarized in the paper were combined therapy with MK-4 and vitamin D_3 given for 24 months significantly increased bone mineral density = BMD (4.92 ± 7.89%), $P < 0.001$, but also while MK-4 alone was significantly enhanced by 0.135 ± 5.44%, $P < 0.05$. A majority of the population (77.5%) increased their BMD values, while 22.5% experienced the opposite. In the MK-4 group, the marker of bone formation (P1CP) showed an increase by 20% after 6 months, and while thereafter returning to baseline. Urinary pyridinoline was significantly increased after 6 month, and peaked after 18 months (89.6 ± 112.3%), while slightly decreasing at 24 months to 53.4 ± 55.7%, $P < 0.05$. In the combined MK-4 and D3 group, P1PC was unchanged for the first 12 months, then it increased at 24 months to 24.2 ± 23.1%, $P < 0.05$. Urinary pyridinoline was increased throughout the 24 months to 84.5 ± 51.9%, $P < 0.01$. The MK-4 group at 24 months showed a significant positive correlation between changes of P1CP and changes of BMD, $P < 0.001$. In the MK-4 and D3 groups at 12 months, a significant positive correlation ($P < 0.03$) between changes of P1PC and changes of BMD could be seen. In the MK-4 and D3 groups, at 12 and 24 months, respectively, significant negative correlations between changes in pyridinoline and changes in BMD ($P < 0.001$ and $P < 0.004$, respectively) could be observed. The authors conclude that these findings indicate both concomitant enhancement of net bone formation, as well as some osseous resorption activities. Additionally, observed significant increments in the coagulation and fibrinolytic reaction pathways were seen. However, they were both restricted within normal physiological range, suggesting maintenance of a normalized balance within the fibrinolysis versus coagulation system, since no side effects were observed [43].

In 2007, Knapen et al. from the Netherlands presented the result of a 3-year randomized clinical intervention study of 325 healthy postmenopausal non- osteoporotic women receiving MK-4, 45 mg/day, or placebo. BMC and hip geometry were assessed by DXA, and bone strength indices were calculated from DXA-BMD, femoral neck width (FNW) and hip axis length (HAL). Computations showed that MK-4 significantly improved the hip bone strength, BMC and FNW, but in the placebo group, bone strength decreased significantly. However, MK-4 did

not affect the DXA-BMD values. It was therefore speculated that the high vitamin K2 intake prevented the postmenopausal, nonosteoporotic bone loss. The importance of K vitamins for optimal bone health has been suggested on the basis of population-based analyses, however, intervention trials performed with DXA-BMD serving as measures of clinical endpoints have given contradicting conclusions. In contrast, BMC, compared with DXA-BMD does not take into account the geometry (size, thickness) of bone, which is construed as an independent contributor to and "interpreter" of bone resilience and fracture risk [44].

Jiang et al. conducted a 1-year study in China in 2014. In this randomized, double-blinded study, it was evaluated whether the efficacy of MK-4 is inferior to α-calcidol in Chinese postmenopausal women. Two hundred and thirty-six women were randomized to two groups: Group MK-4, receiving 45 mg/day or Group α-calcidol, receiving 0.5 µg/day, respectively. Furthermore, all enrolled individuals ingested 500 mg/day of calcium. Assessment of bone mineral density (BMD) post-treatment, onset or occurrence of new fractures, as well as serum OC and ucOC levels were matched with patient baseline values in both patient groups. The information obtained was as follows: 90.3% of the patients completed the investigation. Results showed that the BMD-values in the MK-4 group significantly increased from baseline by 1.2% at the lumbar spine, and 2.7% at the trochanter, respectively ($P = 0.001$). The percentage enhancement in BMD in the α-calcidol group and the MK-4 group was 2.2 and 1.8%, respectively ($P = 0.001$). No difference could be seen when comparing either treatment groups. And one could not spot any alterations in femoral neck BMD between the populations observed. However, one tibia and one femoral neck fracture in the MK-4 group, and three lumbar spine compressive and one forearm fracture in the α-calcidol group revealed the appearance of new fractures ($P < 0.05$). Within the MK-4 group, the concentration of OC and ucOC, respectively, fell from baseline levels by some 39 and 82%, respectively ($P < 0.001$). In the α-calcidol group, OC and ucOC fell by some 26 and 35%, respectively ($P < 0.001$), and the decreases in serum OC and ucOC were more obvious in the MK-4 group than in the calcidol group ($P < 0.001$). The safety profile of menatetrenone was similar to that of α-calcidol. It was therefore concluded that MK-4 is an effective and safe choice in the treatment of postmenopausal osteoporosis in Chinese women [45].

Bisphosphonates, combined with vitamin D and calcium are widely used around the world in the treatment of osteoporotic patients. Vitamin K apparently sustains the lumbar BMD, thus reducing the incidence of osteoporotic fractures it was natural to investigate and compare bisphosphonates and MK-4.

In 2001, Iwamoto et al. presented a 2-year preliminary randomized study, comparing the effects of etidronate (E) and MK-4 on forearm BMD-values and fracture incidence. Seventy-two osteoporotic women, all 5 years after menopause were enrolled. The E-group received 200 mg/day, 14 days per 3 months; $n = 25$, the MK-4 group received 45 mg/day; $n = 23$, and the placebo (C = control group) was given calcium lactate 2 g/day; $n = 24$. At baseline, there was no difference between the three groups. Significant results obtained were summarized as follows: mean percentage change in BMD in the E-group was a significant increase of 2.1%; $P < 0.01$. In the MK-4 group, it was −0.1%, which was not significant. In the C-group, there was a significant decrease in BMD; −1.7%; $P < 0.0001$. Incidence of new fractures was in the E-group; total 13

(nine vertebral and four lumbar), in the MK-4 group total 10 (seven vertebral and three lumbar), and in the C-group; total 12 (seven thoracal and five lumbar). No significant differences could be spotted between groups. It was therefore concluded that a maintained BMD seen in the MK-4 group could be construed as a positive effect, which should be evaluated together with etidronate treatment [46].

In 2003, Iwamoto et al. examined 98 postmenopausal osteoporotic women in a randomized, controlled, 2 years study, comprising four groups: (1) MK-4, 45 mg/day of vitamin K; (2) etidronate 200 mg/day for 14 days per 3 months; (3) etidronate and MK-4 combined; and (4) calcium supplement. End points assessments were: Forearm (distal radius) BMD measured by DXA using DTX-200 (Osteometer®), and incidence of vertebral fractures (level T4-L4). Results reported are summarized as follows: the forearm BMD in the calcium group was reduced from baseline, sustained by MK-4, increased significantly by etidronate, and increased even more in the MK-4 and etidronate group. The incidence of vertebral fractures in the MK-4 group was 8.0%, in the etidronate group 8.7%, in the MK-4 and etidronate group 3.8%, and in the calcium group 20.8%, respectively. The compelling conclusion is combined treatment with MK-4 and bisphosphonates showed significant different ($P < 0.01$) between other groups alone. The author concluded that combined treatment seems to have the greatest efficacy in prevention of new osteoporotic fractures, and this treatment should be recommended to treat osteoporotic women [47, 48].

Ishida and Kawai published in 2004 a 2-year long study, examining 396 postmenopausal women with osteoporosis, randomized to six equally sized groups: (1) hormone replacement therapy (conjugated estrogen 0.625 mg/day plus medroxyprogesterone 2.5 mg/day), (2) etidronate (2 weeks of treatment with 200 mg/day followed by a 10-week medication-free period), (3) ell calcitonin (20 IU/week), (4) α-calcidol 1 μg/day, (5) MK-4 45 mg/day, and (6) control group (i.e., no treatment). End point BMD, bone markers and incidence of vertebral fractures served as clinically relevant end point measures. The results (2-year mean changes in BMD) were 2% for the hormone replacement therapy, −0.5% for the etidronate treatment, 1.6% for the calcitonin treatment, −3.6% for the α-calcidol treatment, −1.9 for the MK-4 treatment, and −3.3 for the controls, respectively. Seventeen (26%) of the 66 control patients developed new vertebral fractures. Compared with controls, the relative risk of incurring vertebral fractures was 0.35 (95% CI: 0.14–0.83) in the hormone replacement therapy, 0.40 (95% CI: 0.17–0.92) in the etidronate group, 0.41 (95% CI: 0.17–0.93) in the calcitonin group, 0.56 (95% CI: 0.26–1.12) in the α-calcidol group, and 0.44 (95% CI: 0.20–0.99) in the MK-4 group, respectively. Hence, the conclusion: a substantial and significant reduction in the incidence of vertebral fractures was achieved due to either: (a) hormone replacement therapy, (b) etidronate, or (c) calcitonin medication. Significant improvements in BMD were obtained by the patients enrolled in the hormone replacement therapy group and in the calcitonin group [49].

Hirao et al. conducted a 1-year study in Japan in 2008. He enrolled and examined 48 postmenopausal women, but only 44 were followed up after 1 year. This randomized controlled study consisted of the following groups A, monotherapy of alendronate (5 mg/day) and group AK, alendronate plus MK-4 (5 mg/day an 45 mg/day, respectively). The take home message was clear: MK-4 decreased undercarboxylated osteocalcin significantly more than alendronate,

which is known not to influence the degree of carboxylated osteocalcin. In the AK group, the femoral neck BMD was significantly increased. The small number of patients enrolled, and the short observation time undermines any conclusion drawn from this study. Further investigations using this combination therapy were recommended [50].

Je et al. from Korea published in 2011, a study evaluating the effects of MK-4 supplementation on bone mineral density and undercarboxylated osteocalcin (ucOC) in 78 postmenopausal Korean women 60-years-old plus. These women, not receiving any medical treatment, were randomized into two groups: (1) calcium and vitamin D, $n = 40$) or (2) vitamin K supplementation, $n = 38$, receiving 15 mg of MK-4 three times a day after every meal, calcium carbonate 315 mg twice daily, and active vitamin D3, 400 UI once a day. The dosage of calcium and vitamin D3 was the same in both groups. After 6 months of treatment, the members of the MK-4 group showed a decrease in ucOC (-1.6 ± 1.6 ng/dl versus -0.4 ± 1.1 ng/dl) with a P-value of 0.008. The vitamin K (MK-4) group members showed a significant improvement of L3 BMD-values, however, no significant enhancement of the BMD-values in L1, L2, and L4. Similarly, no significant increase was observed for femoral BMD, which remained unchanged in the women receiving vitamin D and calcium [51].

In 2012, Fang et al. presented a meta-analysis of randomized controlled trials published during the period of 1999–2009. The analysis examined the role of vitamin K on bone mineral density (BMD). The study revealed that vitamin MK-4 supplementation was associated with increased BMD at the lumbar spine, however not necessarily at the femoral neck. This untoward heterogeneity may be the result, may reside within different participant groups, different regions of the skeleton, gender, and type of vitamin K1 and MK-4 supplementation. Hence, further studies are deemed required to investigate and unravel the detailed effects of vitamin K2 sub"populations" or metabolites on BMD [52].

5. Clinical randomized controlled studies with menaquinone-7 (MK-7)

Japanese menopausal women have traditionally a lower fracture risk than women from the western world [3]. An association with the ingestion of natto (a processed food containing fermented beans) was evaluated by Katsuyama et al. [53] and Ikeda et al. [54] in the so-called Japanese population-based osteoporosis (JPOS) study. The dietary natto intake over 3 years was shown to significantly increase the changes of total hip BMD $P < 0.003$. The alleged prevention of postmenopausal bone loss through the effects of MK-7 is more abundant with natto than other soybean products. More randomized controlled studies are clearly warranted to substantiate this contention [54].

The Norwegian study published by Emaus et al. in 2010. features a 1-year randomized double-blind placebo-controlled study with 334 early menopausal, but otherwise healthy, women. The patient groups received either MK-7 (360 µg/day) or placebo. The summary of the present trial disclosed the following: in the MK-7 group, serum uncarboxylated Osteocalcin (uc-OC) was significantly reduced (from 4.14 to 2.22 ng/ml, respectively). In contrast, carboxylated Osteo-calcin (c-OC) was significantly increased (from 13.5 to 19.1 ng/ml, respectively). However, there was no statistical difference in bone loss rate between the groups at the "total hip site," nor at

any other measurements sites. This was most probably due to the shorter follow-up time, i.e., after only 1 year of MK-7 exposure [55].

After solid organ transplantations, loss of bone mass often occurs and may cause substantial health problems. In a study (published in 2010) on such a patients group, Forli et al. from Norway looked at the effect of MK-7 on bone mass, measured as BMD of the lumbar spine. Despite the fact that the impact of MK-7 on the measured BMD was not conclusive, it was recommended that further studies over an extended period of time should be conducted. Here, we refer to the main findings in the study.

This study was the first in organ transplantation, featuring the effect of MK-7 on bone mass, 1 year after lung and heart transplantation. Postoperatively, 35 lung and 59 heart recipients were actively treated with MK-7 in a prospective and longitudinal study, receiving MK-7 supplement, 180 µg/day or placebo. The results reported were the following: 1 year after solid organ transplantation, the difference between MK-7 and placebo for the lumbar spine (L2–L4) BMD was 0.028 (SE 0.014) g/cm^2, $P = 0.055$, and for L2 to L4, BMC emerged as 1.33 (SE = 1.91) g/cm^2, $P = 0.5$. Scrutinizing the lung recipients separately, the difference for BMC was 3.39 g (SE = 1.65), $P = 0.048$. In the heart recipients, however, observed values were 0.45 (SE = 0.02) g, $P = 0.9$ subsequent to correcting for measures of baseline values.

In a stepwise linear regression analysis, alterations in the L2-L4 BMD, controlled for alleged confounding variables (which include the use of bisphosphonates), significant predictors turned out to be: (a) organ (if heart = 1, BMD = −0.065 g/cm^2 , $P = 0.001$) and (b) MK-7 versus placebo (BMD = 0.034 g/cm^2, $P = 0.019$). It so happened, that insufficient vitamin D status was frequent, and that PTH (parathyroid hormone) levels were augmented in the MK-7 group, indicating a more imminent need for ingestion of vitamin D. In conclusion, it turned out that 12 months of MK-7 ingestion generally suggests a positive effect on BMD of the lumbar spine, but with diverging responses in "cardio-pulmonary" recipients. Thus, the patients' vitamin D status would benefit from a closer monitoring during vitamin K supplementation [56].

Knapen et al., the Netherlands, published in 2013 the results of their 3 year study on the effect of low-dose MK-7 supplementation on bone loss in 244 healthy postmenopausal women. The study was a double-blind, randomized placebo controlled study, with two groups: (a) active low-dose vitamin K2 (MK-7, 180 µg/day), and placebo. Their main task was to investigate whether low-dose MK-7 supplements beneficially could affect bone health in general.

Secondary to an improved and favorable vitamin K status, MK-7 ingestion from supplements should have the possibility to significantly reduced age-related loss of bone mineral density and ensuing bone mechanical properties. Hence, low-dose MK-7 supplements should consequently result in preventing bone loss in postmenopausal women. In spite of contradictory data emanating from trials with vitamin K supplementation on the status of bone health, the European Food Safety Authorities (EFSA) has accepted the health claim on vitamin K's role in the maintenance of normal and healthy bone structure. In accordance with EFSA's opinion, it was clearly demonstrated that a 3-year high-dose of vitamin K1 and MK-4 supplementation improved bone health after menopause.

Because of the longer half-life, bioavailability, and greater potency of the long-chain MK-7, they also measured the effect of low-dose MK-7 supplementation on bone health, as reflected by bone mineral density (BMD) of lumbar spine, total hip, and femoral neck. The assessment of vertebral fractures was performed using DXA. Furthermore, blood levels of ucOC and cOC were also analyzed, and the ucOC/cOC ratio functions as an indicator of vitamin K "health" status. All analyses were performed at baseline, and subsequent to 1, 2, and 3 years of supplementation, respectively. A carboxylation rate of >50% was achieved during the first year of treatment, and it was maintained throughout the study period.

The main results obtained were as follows: MK-7 ingestion significantly enhanced vitamin K status and decreased the age-related reduction in bone mass, as well as both BMC and BMD at the level of lumbar spine and femoral neck. However, total hip BMC and BMD decline could not be "rescued." Bone strength also seemed to be favorably affected by MK-7 ingestion, significantly decreasing the loss of vertebral height of the lower thoracic region at the mid-site of the vertebrae. These results confirm the hypothesis that long term supplementation with MK-7 beneficially affects bone health. Whether these results can be extrapolated to other populations with osteoporosis, needs further investigation [57].

6. Anticoagulation and vitamin K-antagonist association with loss of bone mass

The need of vitamin K for the activation of clotting factors is lower than for the activation of extrahepatic vitamin K-dependent proteins. This is the plausible reason for deficient rebuilding of bone mass and increased calcification process in blood vessels. Long-term use of anticoagulants, likes warfarin, may potentially lead to the loss of bone, and/or an increased incidence of osteopenia or osteoporosis with and without fractures. The crucial question then is: What time span for patients on warfarin medication will suffice for the detection of bone loss?

In an observational study conducted in Japan by Namba et al. 2015, the biomarkers during warfarin use in a 1-year follow-up on 42 patients treated for atrial fibrillation were described. Twenty-four patients received warfarin (WF group) and 18 patients received non-warfarin treatment (Non-WF group). Results revealed an increased significant difference in ucOC in WF the group 10.3 ± 0.8 ng/ml, versus non-WF group 3.4 ± 0.9 ng/ml, $P < 0.01$. In cytokines, RANKL in WF group 0.6 ± 0.1 ng/ml versus non-WF group 0.4 ± 0.1 ng/ml, $P < 0.01$. After 1 year, DEXA scan showed no significant different between groups. It was concluded long-term use of warfarin might be associated with high risk of osteoporosis but also risk of ectopic calcification in blood vessels. Further randomized studies are needed to evaluate these patients [58].

Twenty years ago, clinical observations and research demonstrated that women, taking warfarin during the first trimester of their pregnancy, gave birth to children with punctate calcifications in the axial skeleton, proximal femurs, and calcanei. The presumed reason has since long been that prenatal vitamin K deficiency, induced by warfarin, was the reason for these calcifications [59].

Large clinical studies on bone mass have given different results, and in early observation studies, the evaluation of duration of warfarin use and other patients receiving treatment for osteoporosis was not included. However, two newer studies showed no further risk on bone mass of warfarin use in elderly patients: In the first one published by Woo et al. in 2008, in a large cohort of elderly community-dwelling men, no association was observed between current warfarin use and bone mass, bone loss or fracture risk. Although warfarin use was based upon a single assessment, the findings suggest that current warfarin use in older men does not appear to have clinically important effects on the skeleton [5, 60].

The second experience was summarized by Misra et al. in 2014, featuring long-term treatment of incident atrial fibrillation without prior history of fractures. Long-term warfarin use was defined in two ways: (1) warfarin use ≥1 year; (2) warfarin use ≥3 years. Event-score on warfarin users and nonusers were created to evaluate the association between long-term warfarin use and risk of hip, spine, and wrist fractures separately, as well as combined, using Cox-proportional hazards regression models. Among more than 20,000 participants with incident atrial fibrillation, the hazard ratios (HR) for hip fracture with warfarin use ≥1 and ≥3 years, respectively, were 1.08 (95% CI 0.87, 1.35) and 1.13 (95% CI 0.84, 1.50).

The conclusion of the present trial was as follows: long-term warfarin use among elders (i.e., >65 years of age) with atrial fibrillation was not associated with any increased risk of osteoporotic fractures and therefore does not appear to necessitate additional surveillance or prophylaxis [61]. These observational studies have focused on clinical fractures as endpoints below follow-up time at 5–10 years, but the thesis that warfarin-induced clinical fractures was not confirmed. This may be due to the beneficial effect of MK-7 on bone mass, which appears to stay unaffected by the impact of warfarin on vitamin K1, which again reinforces the notion that vitamin K2 status (measured as ucOC) per se is a good marker of bone homeostasis [58].

7. Chronic kidney disease and loss of bone mass (CKD-MBD)

The link between increased calcification of vessels and bone complications changes the definition of CKD-MBD to better describe the complexity of the syndrome [62]. The link between osteoporosis and cardiovascular morbidity is well described in postmenopausal women with intact renal function [63]. In chronic hemodialysis patients, a lower bone volume is associated with higher coronary calcification scores measured by multislice computed tomography, reflecting a higher risk of cardiovascular events [64]. This association between vascular calcification, arterial stiffness and bone mineral density in chronic kidney disease was also described in 2008 by Toussaint et al. [65]. Furthermore, the mortality of hemodialysis patients was evaluated in 2003 by Taal et al. among 88 hemodialysis patients over a 3.5 years follow-up period. Here, it appeared that mortality was associated with age, Ca-P product, lack of transplantation and a low bone mineral density measured at the hip. The leading cause of death (42.5%) appeared to be related to cardiovascular events [66].

The therapeutic options are few, since many women on renal replacement treatment did not accept reinstigation of hormone replacement therapy. However, the efficacy of hormone

replacement was confirmed in a randomized trial in women on continuous dialysis, receiving hormone therapy (estradiol and cyclic norethisterone) for 1 year. At the end of the study, the active group showed an increase in bone mineral density at the lumbar spine. This difference between the active and control group (receiving cinacalcet) was significant at all measurement sites [67]. Active vitamin D analogs, calcimimetics administration and phosphate-binders are widely used to suppress iPTH and thus bone specific alkaline phosphatase, as a marker of enhanced bone turnover.

Kohlmeier et al. were the first to show an independent association between serum concentration of phylloquinone <1.2 nmol/l or less (poor vitamin K status) and an increased risk of bone fracture in patients with end-stage renal disease [68]. This observation was confirmed by Fusaro et al. [69] in 2013, showing that hemodialysis patients treated by warfarin for longer than 1 year had an increased risk of vertebral fractures, compared with patients not on warfarin. McCabe et al. enrolling 172, stage 3–5 CKD patients without dialysis treatment, showed that intake of vitamin K was insufficient in more than 50–60% of individuals on a given diet, if measures of ucOC were conducted (>20% ucOC), and 97% if evaluation was done by the prothrombin induced by vitamin K absence-II (PIVKA-II) assessment (>2 nmol/l) [70, 71]. After establishment of dialysis as a therapeutic intervention, Cranenburg et al. showed in a study of 40 chronic hemodialysis patients from 2012 that the dietary intake of vitamin K1 and K2, in general, was insufficient. This was reflected by analyses of plasma levels of desphospho-undercarboxylated (dp-uc) MGP (matrix-GLA protein), which was increased over the normal range by some 82.5% with elevated PIVKA-II values 3.81.4–12.4 ng/ml, reference value <2 ng/ml. [72]. Elevated dp-ucMGP levels suggest insufficient vitamin K2 levels on the vascular site, while high ucOC reflects insufficient vitamin K2 on bone or osseous sites.

A 6 weeks randomized controlled trial on hemodialysis patients evaluated the response of biomarkers of vitamin K status (dp-ucMGP, PIVKA-II and ucOC) to the ingestion of 45, 135, 360 μg/day of MK-7. The study confirmed that most patients displayed a functional deficiency at baseline, and that MK-7 supplementation decreased dp-ucMGP and PIVKA-II. However, only the highest doses brought about a significant decrease in ucOC [73].

In osteoporosis, the main treatment aims at inhibiting osteoclastic bone resorption. The osteoclast and osteoblast are functionally tightly coupled, and the mechanism of this reciprocal link is now very well known. By the discovery of MK-7, which is able to play a role in the prevention of bone loss from most sites of the skeleton, there is hope for efficient treatment. MK-7 has been shown to stimulate osteoblastic bone formation, as well as suppressing osteoclastic bone resorption *in vitro* and in humans, as showed by Knapen et al. [57]. MK-7 suppresses the activation of NF-κB signaling pathways in both osteoblasts and osteoclasts. These treatments have not yet been enrolled side by side with vitamin D analogs in CKD patients. Unfortunately, vitamin K2 is rare in Western diets, but in CKD patients, vitamin K2 levels are very low due to recommended restriction of potassium and phosphate in the diet.

New trials enrolling CKD and chronic dialysis patients treated with MK-7 supplementation are presently being conducted to fully evaluate the effect of MK-7 on atherosclerosis and bone mineral density.

8. Conclusion

In the near future, the dose, bioavailability and potency of the vitamin K2 subfamily member menaquinone MK-7, will most probably make it possible to improve on the bone building process, yielding enhanced bone strength and resilience in several bone-losing patient categories, such as those suffering from osteoporosis of different etiologies, and patients presenting with low bone mass (osteopenia).

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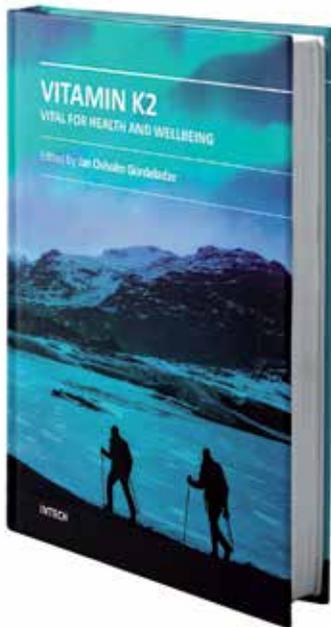
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