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INVITED MEDICAL REVIEW

Regulation of bone remodeling by vitamin K2

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All living tissues require essential nutrients such as amino acids, fatty acids, carbohydrates, minerals, vitamins, and water. The skeleton requires nutrients for development, maintaining bone mass and density. If the skeletal nutritional requirements are not met, the consequences can be quite severe. In recent years, there has been growing interest in promotion of bone health and inhibition of vascular calcification by vitamin K2. This vitamin regulates bone remodeling, an important process necessary to maintain adult bone. Bone remodeling involves removal of old or damaged bone by osteoclasts and its replacement by new bone formed by osteoblasts. The remodeling process is tightly regulated, when the balance between bone resorption and bone formation shifts to a net bone loss results in the development of osteoporosis in both men and women. In this review, we focus on our current understanding of the effects of vitamin K2 on bone cells and its role in prevention and treatment of osteoporosis.

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Introduction

The skeleton in vertebrates has mechanical, hematopoietic, and endocrine functions (Rodan and Martin, 2000). To perform these functions properly, the skeleton requires essential nutrients such as amino acids, fatty acids, carbohydrates, minerals, vitamins, and water (Weaver and Gallant, 2014). Inadequate skeletal nutrition in infants and young children results in growth retardation and bony deformities. In adolescents and young adults, it results in failure of individuals to attain their genetically prescribed maximum peak bone mineral density, and in middle-aged and older adults, the consequence is rapid bone loss that can cause or aggravate osteoporosis (Holick and Nieves, 2015). The investigation of the impact of nutrients on bone strength has historically been directed to minerals, vitamin D, and proteins, while the other factors have received less attention (Eisman and Bouillon, 2014). Emerging evidence indicates that vitamin K2 also plays an important role in skeletal health. This review will examine the available evidence regarding the role of vitamin K2 in bone health in animals and in humans.

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The skeleton

The adult human skeleton has 206 bones, excluding the sesamoid bones. The skeleton is subdivided into the axial skeleton with 74 bones, the appendicular skeleton with 126 bones, plus the six auditory ossicles (Clarke, 2008). There are two major types of bones: (i) cortical bone, which is dense and compact and constitutes the outer part of the skeletal structure, comprises 80% of the skeletal weight and provides mechanical strength and protection; and (b) trabecular bone, which is found inside the ends of long bones, throughout the bodies of the vertebrae, and in the inner portion of the pelvis. Trabecular bone is more metabolically active than cortical bone and, by its breakdown, supplies minerals such as calcium, phosphorus, and magnesium, when required by the body (Feng and McDonald, 2011).

The skeleton renews itself in two ways: bone modeling and bone remodeling. Bone modeling occurs during growth and development in childhood. It involves formation of bone by osteoblasts or resorption of bone by osteoclasts on a given surface. In contrast, bone remodeling occurs after the skeleton has reached maturity during adulthood, a situation in which the activity of osteoblasts and osteoclasts occurs sequentially in a coupled manner on a given bone surface (Allen and Burr, 2014).

Bone modeling

Bone modeling begins in fetal life and continues until skeletal maturity. The primary function of bone modeling is to increase bone mass and to maintain or reshape the bone or change the position of the cortex relative to its central axis (called bone drift) in response to various

physiological stimuli (Clarke, 2008; Allen and Burr, 2014). Modeling occurs due to factors produced locally or systemically and in response to mechanical forces. Modeling can proceed via *formation modeling* by osteoblasts or *resorptive modeling* by osteoclasts. These processes must be coordinated to shape the bone correctly. Either formation or resorption modeling can occur locally on a given bone surface, or both processes can occur simultaneously throughout the skeleton. The primary signals for modeling are mechanical forces; formation modeling is activated when the local tissue strains exceed a certain threshold. Subthreshold strains activate resorptive modeling. The process of modeling occurs in two stages: (i) activation, and (ii) bone formation or resorption. During activation, the recruitment of osteoblast or osteoclast precursor cells occurs. Then, lineage-committed cells are stimulated to differentiate into more mature cells. Subsequently, during stage two, activated cells either form or remove bone to normalize local strains. In the adult skeleton, bone modeling occurs less frequently. It can be associated with disease states such as hypoparathyroidism and renal osteodystrophy, or driven by anabolic agents (Raisz, 1999; Allen and Burr, 2014).

Bone remodeling

Bone remodeling is necessary to maintain structural integrity, bone volume, and calcium and phosphate homeostasis (Clarke, 2008; Feng and McDonald, 2011). Remodeling repairs bone by removing old and microdamaged bone, replacing it with strong new bone. In a year, ~5% of cortical bone and 20% of trabecular bone is remodeled. The entire adult skeleton is replaced every 10 years in humans. Cortical bone makes up 75% of all bone, but trabecular bone is ten times more metabolically active than the cortical bone (Allen and Burr, 2014; Sims and Martin, 2014). Thus, the process of bone remodeling occurs throughout life and can occur upon or within any of the periosteal, endocortical, trabecular, and intracortical bone surfaces. Endocortical remodeling and intracortical remodeling are common during growth and development, and adulthood, while periosteal remodeling is less frequent at all stages of life (Allen and Burr, 2014).

Cells involved in bone remodeling

Remodeling takes place asynchronously throughout the skeleton at distinct anatomical sites called basic multicellular units (BMUs) (Sims and Martin, 2014). The BMU contains four major types of bone cells: bone-lining cells, osteoclasts, osteoblasts, and osteocytes; the bone remodeling process results from the coordinated action of all these cells. Osteoclasts are the only cells capable of resorbing bone. They are multinucleated giant cells formed from mononuclear cells of the monocyte/macrophage lineage upon stimulation by two essential factors: monocyte/macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor κB (NFκB) ligand (RANKL). Osteoblasts are bone-forming cells derived from condensed embryonic mesenchyme that under goes either intramembranous or endochondral bone formation (Sims and Martin, 2014). During bone formation, a subpopulation of osteoblasts undergoes differentiation and becomes embedded in the bone – these cells are called osteocytes. Osteocytes are the most abundant bone cells and live longer than others. They are the primary mechanosensing cells and play a pivotal role in initiation of bone remodeling. Osteocytes reside in lacunae and have dendritic processes that interact with other osteocytes and bone-lining cells on the bone surface. Bone-lining cells, like osteocytes, are osteoblast lineage cells. They cover the bone surface in a monolayer and prevent inappropriate interactions of osteoclast precursors with the bone surface. The retraction of bone-lining cells to expose the bone surface is important. If this does not happen, the osteoclasts cannot bind to bone and resorb it. Bone-lining cells separate the bone from the marrow (Robling *et al*, 2006; Feng and McDonald, 2011; Parra-Torres *et al*, 2013).

Two types of bone remodeling are known: (i) *Targeted remodeling*, where a specific local signal directs the osteoclast to a given location to begin remodeling, occurs at sites of microdamage; and (ii) *stochastic remodeling*, a random process where osteoclasts begin remodeling without any signaling event. This type of remodeling plays a role in calcium homeostasis. At a cellular level, both targeted remodeling and stochastic remodeling proceed similarly (Allen and Burr, 2014).

Remodeling cycle

The remodeling process involves five distinct but overlapping phases; collectively, these phases are referred to as the remodeling cycle. At any given time, there are thousands of remodeling cycles taking place throughout the body at various stages, depending on when they were initiated (Feng and McDonald, 2011; Parra-Torres *et al*, 2013; Allen and Burr, 2014; Sims and Martin, 2014). The five phases are as follows:

1 *Initiation or activation* involves detection of the remodeling site and recruitment and activation of osteoclasts to the BMU. The osteocytes sense deformed bone (caused by mechanical overloading) or detect microdamage in old bone. Both of these events transmit signals to recruit mononuclear monocyte/macrophage osteoclast precursors from the circulation to the specific site. The osteoclast precursors are recruited to the BMU either from the capillaries that penetrate the BMU or from bone marrow by crossing the bone-lining cells. In the BMU, the osteoclast precursors respond to M-CSF and RANKL. RANKL interacts with a receptor, RANK, on precursor cells of the hematopoietic lineage to initiate differentiation to multinucleated osteoclasts and maintain their resorbing activity. With the formation of osteoclasts, the remodeling process proceeds to the resorption phase (Feng and McDonald, 2011; Parra-Torres *et al*, 2013; Allen and Burr, 2014; Sims and Martin, 2014).
2 *In the resorption* phase, the predominant event is bone resorption. The attached osteoclasts form annular sealing zones and bone-resorbing compartments. The resorbing osteoclasts secrete hydrogen ions into the resorbing compartment to lower the pH to ~4.5.

The low pH facilitates dissolution of the bone mineral and exposes bone organic matrix that is digested by various proteolytic enzymes secreted by osteoclasts. The removal of bone mineral and organic matrix causes saucer-shaped Howship's lacunae in trabecular bone and Haversian canals in cortical bone. Once the resorption is finished, the osteoclasts undergo apoptosis. The size, duration, and depth of the resorptive event are controlled at least in part by RANKL, which maintains osteoclast viability. Osteoclast-mediated bone resorption takes only approximately 2–4 weeks during each remodeling cycle. During the resorptive phase, MSCs and/or osteoprogenitors are also recruited into the BMU either directly from bone marrow or from capillaries. The remodeling process enters into the formative phase as osteoblast function of osteoid synthesis begins to overtake bone resorption (Feng and McDonald, 2011; Parra-Torres *et al*, 2013; Allen and Burr, 2014; Sims and Martin, 2014).

3 *Reversal phase* is characterized by the cessation of osteoclast resorption and initiation of bone formation. After osteoclasts finish resorbing bone, the exposed bone surface in the lacuna contains remaining collagen fragments. The removal of these fragments is needed for the osteoblasts to form new bone. The resorbed surface is cleared up by lining cells and probably also by macrophages. Once the resorption pits are cleared, osteoblasts deposit a first layer of proteins (collagenous) in them to form a cement line (glycoprotein) between old and new bone that is necessary for osteoblasts to attach and begin new bone formation (Feng and McDonald, 2011; Parra-Torres *et al*, 2013; Allen and Burr, 2014; Sims and Martin, 2014).

4 The *formation phase* is the longest phase. During this phase, osteoblasts deposit an unmineralized organic matrix (osteoid), which is mineralized in two distinct phases. In the primary mineralization phase, the initial incorporation of calcium and phosphate ions into the collagen matrix occurs. Primary mineralization accounts for approximately 70% of the final mineral content. During the secondary mineralization phase, the final addition of minerals and maturation of mineral crystals occurs. The osteoblasts that participated in the new bone formation undergo one of three fates. The majority (90%) of them dies through apoptosis. Some osteoblasts are incorporated into the osteoid matrix and become osteocytes. The remaining osteoblasts form bone-lining cells (Feng and McDonald, 2011; Parra-Torres *et al*, 2013; Allen and Burr, 2014; Sims and Martin, 2014).

5 In the *quiescence or resting phase*, the bone surface is covered with lining cells. Most bone at any given time is in a state of quiescence (Allen and Burr, 2014).

Bone remodeling rate

The rate of remodeling is highly variable. At any given time in an adult human skeleton, there are 1–2 million active BMUs on cortical or trabecular surfaces. Each BMU is asynchronous with others (Parfitt, 2008). Normal bone remodeling is tightly regulated by balancing bone formation and bone resorption to ensure that there is no net change in bone mass or quality occurs after each bone remodeling event. The rate of remodeling is influenced by physical activity, nutrition, local and systemic factors, and medications. Factors that affect bone remodeling target either osteoblasts or osteoclasts and their progenitor cells resulting in changes in bone formation or resorption (Raisz, 1988; Martin *et al*, 2009; Feng and McDonald, 2011).

In healthy individuals, bone remodeling at the BMU level is always coupled, but not always balanced. *Coupling* refers to sequential osteoclast and osteoblast activity; that is, if there is less bone resorption, there will be less bone formation, which occurs in conditions of low bone resorption or treatment with antiresorptive drugs. *Balance* refers to the amount of bone formed relative to the amount of bone resorbed at each BMU. Bone balance can be positive or negative. Positive bone balance is where more bone is formed than is resorbed at the individual BMU. This occurs with intermittent rhPTH treatment (Allen and Burr, 2014). Negative bone balance is where less bone is formed than resorbed. In healthy individuals, bone balance is slightly more negative in cortical bone than trabecular bone to accommodate the central canal in the osteon. This negative balance is magnified in diseases such as postmenopausal osteoporosis, where initially, the number of BMUs is increased resulting in an overall significant bone loss (Allen and Burr, 2014; Sims and Martin, 2014).

The most common bone remodeling disease is osteoporosis. Osteoporosis is characterized by low bone mass and defects in bone microarchitecture. Osteoporosis causes bone fragility, and people suffering from the problem are prone to fractures. Osteoporosis represents a group of pathological conditions, rather than a single disease, and there are primary or secondary types. The primary type can be subdivided into type I also called postmenopausal osteoporosis, which occurs primarily in women due to estrogen deficiency, and type II or age-related osteoporosis, which occurs in both males and females due to age. In contrast, secondary osteoporosis refers to osteoporosis caused by complications of other medical conditions, adverse effects of drugs, or reduced physical activity (Feng and McDonald, 2011). More than 25 million Americans and untold millions of people all over the world are at risk of osteoporosis and its consequences. In the USA alone, there are more than 2 million fractures caused by osteoporosis per year, costing $19 billion (McGowen *et al*, 2004). Therefore, prevention of osteoporosis and reducing osteoporosis-related bone fractures is very important, and nutrition is one of the cornerstones for prevention of the problem at the community level. This is a realistic and cost-effective option. Vitamin K2 supplements have been shown to have important effects on bone health (Feskanich *et al*, 1999; Booth *et al*, 2000) and the vitamin appears to prevent osteoporosis and its consequences.

Vitamin K

Vitamin K is a fat-soluble vitamin. It was discovered in 1929 by the Danish scientist, Henrik Dam. Three vitamin

K isoforms are known: vitamin K1 (phylloquinone), vitamin K2 (menaquinones), and vitamin K3 (menadione). Vitamin K1 is synthesized by plants and is the predominant form of vitamin K in the human diet. Vitamin K2 is a bacterial by-product and is mainly found in fermented products or in food with animal origins (Booth and Suttie, 1998). Vitamin K2 has different chemical variants (vitamers), abbreviated as MK-n, where 'n' specifies the number of isoprenyl units in the side chain. In humans, the most common MK is the short-chain MK-4, which is primarily produced endogenously via systemic conversion of K1 to MK-4. The long-chain forms of MKs, MK-7 through MK-10, are synthesized by intestinal bacteria in all mammals (Booth and Suttie, 1998; Booth, 2012; Beulens *et al*, 2013). When the number of isoprenyl units in the side chain length is 0, it is called vitamin K3. Vitamin K3 is a synthetic compound found only in supplements and is used for animal feed. K3 is a provitamin that needs to be converted to MK-4 to be active (Bentley and Meganathan, 1982; Bugel, 2008).

Dietary source of vitamin K

Vitamin K1 and K2 intake varies among different populations around the world. Vitamin K1 is the primary source of vitamin K in most Western countries. Vitamin K2 is the predominate source of vitamin K in some Asian countries where the fermented soy product, natto, is consumed. The main dietary sources of vitamin K1 are green leafy vegetables, which contributes up to 60% of the total. Other sources are vegetable oils and margarines (Booth *et al*, 1996). In the USA, poultry products are the primary dietary sources of MK-4. MK-7 is primarily found in natto. MK-8 and MK-9 are found in certain types of cheeses. Both MK-4 and MKs 7-10 are present in the diet in microgram (μg) amounts. Various vitamin K2 forms have different bioavailability and plasma half-lives; MK-7 has a greater bioavailability and longer half-life than vitamins K1 and MK-4. Longer-chain menaquinones (MK-10 to MK-13) are produced by colonic anaerobic bacteria, but they are poorly absorbed and have little vitamin K activity (Booth and Suttie, 1998; Shearer and Newman, 2008; Booth, 2012; Beulens *et al*, 2013).

Absorption and excretion

Dietary vitamin K is absorbed in the small intestine. After intestinal absorption, K1 and K2 are transported in triacylglycerol-rich lipoproteins (chylomicrons) through the lymphatic circulation to the liver and other tissues. Most of the K1 taken up by the liver is metabolized and excreted (Barkhan and Shearer, 1977). Only a small amount of vitamin K1 reenters the systemic circulation in very low-density lipoprotein (VLDL) particles secreted by the liver. It is then transported to extrahepatic tissues. Vitamin K2 is transported by low-density lipoproteins from the liver to extrahepatic tissues such as bone. MK-4 is transported by both low-density and high-density lipoproteins (Schurgers and Vermeer, 2002). Vitamin K1 and very long-chain vitamin K2 are mainly stored in the liver, whereas MK-4 is predominantly stored in the brain, reproductive organs, pancreas, and other glands (Booth and Suttie, 1998; Sato *et al*, 2002).

Function of vitamin K

Vitamin K functions as a cofactor for the enzyme, γ-glutamylcarboxylase, which catalyzes the carboxylation of glutamic acid (Glu) to γ-carboxyglutamic acid (Gla). This γ-carboxylation occurs only on specific glutamic acid residues in vitamin K-dependent proteins. There are 14 different vitamin K-dependent proteins found in blood, bone, dentin, renal stones, atherosclerotic plaques, semen, lung surfactant, neural tissue, and urine. Vitamin K-dependent proteins synthesized primarily in the liver are coagulation factors (VII, IX, X, prothrombin) protein C, protein S, and protein Z. There are four proteins of the transmembrane Gla family, PRGP1, PRGP2, TMG3, and TMG4, whose functions have not yet been defined. There are three vitamin K-dependent proteins in bone: osteocalcin, matrix Gla protein, and protein S (Booth, 1997; Ferland, 1998). Vitamin K2, the most abundant form of vitamin K in non-hepatic tissue, participates in the carboxylation of bone-associated vitamin K-dependent proteins.

Vitamin K-dependent proteins in bone

Osteocalcin is one of the most abundant non-collagenous proteins in bone. Osteoblasts synthesize osteocalcin, which is secreted into the bone extracellular matrix where it binds to hydroxyapatite crystals. Binding of osteocalcin to hydroxyapatite is dependent on the carboxylation of three Glu residues by vitamin K (Cairns and Price, 1994). Carboxylation of the Glu residue at position 17 is essential for the selective binding of osteocalcin to hydroxyapatite (Nakao *et al*, 1994). Most osteocalcin is bound to bone minerals and only small amounts are detected in the circulation, where it is a useful biomarker for bone formation. Forty percent of the circulating osteocalcin is in the undercarboxylated form. MK-7 is more effective at carboxylating osteocalcin than vitamin K1. In addition to γ-carboxylation of osteocalcin, vitamin K may also affect the transcription of genes required for the expression of osteoblastic markers and collagen assembly. Although there are multiple vitamin K-dependent proteins in bone, circulating undercarboxylated osteocalcin is used as a marker of vitamin K status (Gundberg *et al*, 1998; Beulens *et al*, 2013).

Matrix Gla protein (MGP) is found in cartilage, bone, and soft tissues, including blood vessel walls. MGP appears earlier than osteocalcin and binds to both organic and hydroxyapatite crystals of bone. MGP prevents calcification at various sites, including cartilage, vessel walls, skin elastic fibers, and the trabecular meshwork of the human eye. It inhibits vascular calcification (Luo *et al*, 1997; Murshed *et al*, 2004).

Protein S, a protein cofactor for the anticoagulant activities of protein C, is also synthesized by osteogenic cells. Protein S has significant homology to a vitamin K-dependent growth arrest-specific gene product (gas6), which has been identified in chondrocytes and also regulates osteoclast activity (Pan *et al*, 1990; Nakamura *et al*, 1998).

The vitamin K cycle

Vitamin K is stored in very low amounts compared to other fat-soluble vitamins and is rapidly depleted without regular dietary intake. Consequently, the body recycles vitamin K through a process called the vitamin K cycle, which allows the vitamin to be reused many times, decreasing the dietary requirement for it. In brief, the reduced form of vitamin K, hydroquinone, is converted to an epoxide (the oxidized form), which promotes carboxylation of Glu residues on vitamin K-dependent proteins. After carboxylation, the epoxide is recycled back to hydroquinone. The anticoagulant, warfarin, acts as a vitamin K antagonist by preventing vitamin K recycling (Oldenburg *et al*, 2008).

Vitamin K2 and bone health

In this section, we review the role of vitamin K2 on bone health because studies in cultured cells, animal models, and humans have shown that vitamin K2 is more effective than vitamin K1 in improving bone health.

In vitro studies on bone cells

Vitamin K2 promotes bone marrow stem cell (BMSC) proliferation, stimulates osteoblast differentiation, and inhibits adipocyte differentiation. This was not seen with vitamin K1. Vitamin K2 protects osteoblasts from apoptosis (Urayama *et al*, 2000). The increase in osteoblast numbers results in the formation of more osteocytes, greater lacunar occupancy by these cells, and reduced cortical porosity (Iwamoto *et al*, 2008). On the other hand, vitamin K2 inhibits osteoclast formation by inhibiting expression of RANKL and promotes osteoclast apoptosis (Kameda *et al*, 1996). Vitamin K2 also inhibits osteoclast formation indirectly by decreasing the expression of RANKL and increasing the expression of osteoprotegerin (osteoclast inhibitory factor) in human stromal cells (Koshihara *et al*, 2003). These studies *in vitro* suggest that vitamin K2 regulates bone cells either directly or indirectly (Figure 1). Overall, it has an anabolic effect on bone.

Rodent models

Evidence from animal studies supports the role of vitamin K2 in bone health. Vitamin K2 has been used as a treatment for osteoporosis induced by ovariectomy, orchidectomy (Iwamoto *et al*, 2003b), glucocorticoids (Iwamoto *et al*, 2009), removal of the sciatic nerve (Iwamoto *et al*, 2010), and calcium- or magnesium-deficient diets in rats (Kobayashi *et al*, 2002). The results of these studies show that vitamin K2 has the potential to suppress bone resorption or turnover by inhibiting osteoclast-induced bone resorption and/or increasing osteoblast activity. Overall, vitamin K2 prevented decreases in femoral bone mineral density (BMD), loss of trabecular and cortical bone mass, and reductions in bone mineral content of lumbar vertebrae. Combining vitamin K2 with vitamin D3 (Gigante *et al*, 2008) or bisphosphonates (Sasaki *et al*, 2010) showed an additional protective effect on osteoporosis *vs* vitamin K2 treatment alone. That said, it should be mentioned that not all vitamin K2 studies showed positive effects. It did not seem to have a protective effect in ovariectomy-induced osteoporosis. The reason for this is not clear.

Human studies

Observational studies. In humans, few studies have examined the association between MK-4 and bone health. These trials were based on the association between either dietary intake or serum concentration of markers of vitamin K status and fracture rates as an indicator of age-related bone loss. In these studies, the limiting factor was that MK-4 obtained exclusively from the diet is undetectable in serum (Tsugawa *et al*, 2006). Studies with MK-7, which is high in natto, showed that in Japanese postmenopausal women, low dietary intake of the vitamin was associated with an increased risk of hip fracture (Kaneki *et al*, 2001). Other studies showed no differences in serum concentrations of MK-7 between women with osteoporosis and no osteoporosis, and women with fracture history and healthy postmenopausal women (Horiuchi *et al*, 2004). Based on these observational studies, it is difficult to conclude that vitamin K2 is a

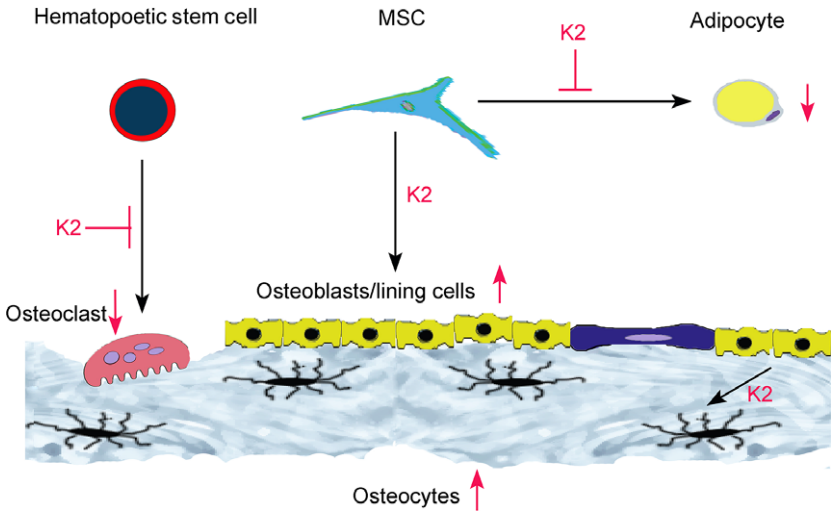

Figure 1 Cellular targets of vitamin K2 in bone remodeling

protective factor for bone health because they do not take into account other dietary compounds that have potential benefits (Theuwissen *et al*, 2012).

Intervention studies. Several clinical trials have tested the effect of vitamin K2 supplements on bone health. The majority were done using MK-4 as it has been reported to have protective effect on bone loss and fracture risk in both healthy and osteoporotic postmenopausal women. A dose of 45 mg day^{-1} was used in these studies, which is about 150–180 times greater than the recommended daily dietary intake of vitamin K (250–300 μg) (Orimo *et al*, 2012; Iwamoto, 2014). This dosage was determined as an optimal dose in a dose-finding study of MK-4 in Japan, where the patients were administered daily doses of 15, 45, 90, and 135 mg (Iwamoto, 2014). Forty-five milligram was the minimum effective dose for improving bone mass parameters evaluated by microdensitometry and/or single photon absorptiometry in postmenopausal women with osteoporosis. No toxic effects of MK-4 (45 mg day^{-1}) have been reported (Iwamoto, 2014).

A meta-analysis of 13 studies of MK-4 at 45 mg day^{-1} was published in 2006. It revealed that MK-4 has a protective effect on BMD and reduced risk of hip, vertebral, and non-vertebral fractures (Cockayne *et al*, 2006). A 3-year placebo-controlled interventional trial with 325 healthy postmenopausal women found that MK-4 supplements improved bone strength compared to placebo (Knapen *et al*, 2007). In contrast, a non-placebo-controlled study of 4000 postmenopausal Japanese women who received only calcium or a combination of MK-4 and calcium for 3 years showed no differences between the groups regarding incidence of vertebral fractures (Inoue *et al*, 2009). A 1-year, randomized, double-blinded, placebo-controlled trial in USA with 365 healthy postmenopausal women with vitamin K inadequacy found that giving vitamin K1 or MK-4 (45 mg day^{-1}) had no effect on BMD or serum bone turnover markers (Binkley *et al*, 2009). There are controversial results regarding MK-4 in improving bone health. Studies with small sample sized randomized controlled trials showed beneficial effect of MK-4. However, the results of the largest and longest MK-4 trial did not find any beneficial effect on vertebral fracture, except in women with advanced osteoporosis (Huang *et al*, 2015). In contrast, studies in which MK-4 and vitamin D were combined always showed a positive effect on bone health. Combined administration increased BMD of lumbar spine in primary osteoporosis and postmenopausal women with osteoporosis (Iwamoto *et al*, 2003a). This combination also protected against bone loss in patients with chronic glomerulonephritis receiving steroid treatments (Yonemura *et al*, 2000; Tanaka and Oshima, 2007). Studies in women with osteoporosis also showed that combined administration markedly increased BMD compared to vitamin K2 treatment alone (Ushiroyama *et al*, 2002).

Recent clinical trials with MK-7 have increased in number due to its longer circulating half-life and greater potency compared to MK-4. In a randomized double-blind placebo-controlled trial of 334 healthy postmenopausal Norwegian women given 360 μg day^{-1} of vitamin K2 in the form of Natto capsules (rich in MK-7) for 1 year, it showed no effect on bone loss (Emaus *et al*, 2010). In contrast, another placebo-controlled trial in 244 healthy Dutch postmenopausal women supplemented with 180 μg day^{-1} of MK-7 for three years showed a small but significant reduction in femoral neck and lumbar spine BMD and BMC (bone mineral content) loss compared to placebo (Knapen *et al*, 2013).

Recent meta-analysis of randomized controlled trials encompassing 6759 participants concluded that vitamin K2 does play a role in the maintenance and improvement of vertebral BMD, and in the prevention of fractures in postmenopausal women with osteoporosis. However, vitamin K2 did not show any effect in postmenopausal women without osteoporosis (Huang *et al*, 2015).

Vitamin K2 and oral health

Periodontal disease, like osteoporosis, is associated with negative bone balance. Alveolar bone loss is a prominent feature of periodontal disease. Osteoporosis has been hypothesized to be an aggravating factor in periodontal bone loss. Cross-sectional and case–control studies report reduced bone mineral density is a shared risk factor between osteoporosis and periodontal bone loss, but osteoporosis is not the causative factor (Megson *et al*, 2010).

One of the approved treatments of osteoporosis is antiresorptive therapy with bisphosphonates (Greenspan *et al*, 2000), which has proven to be effective in reducing bone loss. However, the incidence of osteonecrosis of the jaw is a serious clinical concern after invasive dental treatment procedures (Marx, 2003; Ficarra *et al*, 2005). Given the specific effects of vitamin K2 on osteoclasts and in bone remodeling, vitamin K2 appears to be a safe alternative to use instead of bisphosphonates, or use along with bisphosphonates to reduce the side effects. Vitamin K2 randomized controlled trials need to be done to see whether K2 can prevent periodontal bone loss.

Conclusion

In conclusion, *in vitro* and whole animal data on vitamin K2 are promising, but the clinical trial data with vitamin K2 treatment alone are inconclusive. On the other hand, combined administration of vitamin K2 and vitamin D3 shows encouraging results. It would be prudent to conduct multiple clinical trials of MK-7 plus vitamin D3 to determine the protective effect on bone health in various at-risk populations.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

EM conceptualized the review, generated the figure, and edited the manuscript. VM outlined the review and wrote the manuscript.

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