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Vitamin K and osteoporosis

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Key words: vitamin K; bone metabolism; osteoporosis; vitamin K dependent protein.

Abstract

In this review, the function of vitamin K (VK) concerning the osteotropic process and pathophysiology of osteoporosis is analysed. VK is best known for its anticoagulant action but, recently, its other roles have been discovered, including those related to bone formation and skeletal metabolism. The review discusses how VK, especially its forms VK1 and VK2, intervenes in bone development and resolidification, as well as analysing the supporting glutamate to γ-carboxyglutamic acid (Gla) roteins such as osteocalcin. The effects on bone mineral density and the risk of fractures associated with VK deficiency are also discussed, with a focus on the therapeutic aspects of VK2 administration together with anti-osteoporotic agents. Furthermore, the review indicates possible future lines of research, emphasising the need for larger studies to establish the ideal doses and the interactions between vitamin K and nutrients and medications.

Introduction

Vitamin K overview

Vitamin K (VK) is a group of fat-soluble compounds known for their key role in coagulation processes. The name is derived from the German word 'Koagulation', meaning coagulation.[1] However, the biological roles of VKs extend beyond coagulation; several studies have highlighted its role in other physiological processes, such as maintaining bone health and regulating tissue calcification. This hypothesis is supported by the observations that VK appears to be actively implicated in the carboxylation of glutamic acid residues of proteins present in bone tissue.[2]

Four forms of VK differ in molecular structure and source of origin. All forms have a common 2-methyl.1,4-naphthoquinone (menadione) core and differ in side chain structures at the 3-position (Figure 1).

VK1 (PK: phylloquinone) is synthesised by plants and algae[3] and it is mainly absorbed in the gastrointestinal tract and transported to the liver, where it plays a crucial role in blood coagulation by activating factors II, VII, IX, and X but also participates, to a small extent, in the bone mineralisation process by activating Osteocalcin (OC).[4]

VK2 (MK: menaquinones) has a methylated naphthoquinone nucleus and a side chain composed of a variable number (n) of isoprenoid units which can vary in length from 1 to 14 units (MK-1 to MK-14). Among these MK-4 and MK-7 promote calcium deposition and increase bone strength.[5,6] Different side chain lengths influence the absorption, distribution and duration of action of VK2 in the body.

VK2 is an animal-derived vitamin found mainly in meat, eggs, butter, cheese and fermented soya products. It can also be synthesised by intestinal bacteria or formed by the metabolism of dietary PK in the liver or extra-hepatic tissues. The enzyme responsible for this modification is UbiA prenyltransferase domain-containing protein-1 which can cleave and prenylated the side chain of VK3 to produce MK-4.[7]

The simplest form of VK is VK3 (menadione); unlike the other two, it is a synthetic form (like VK4) that is also involved in bone metabolism.[8] Due to their lipophilicity, VKs must be incorporated into micelles and then chylomicrons to be absorbed by the intestine and enter the systemic circulation.[9] Compared to other fat-soluble vitamins, it is rapidly metabolised and excreted. A study conducted with radioactive isotopes demonstrated the different clearance of VK as a function of the side chain.[10] In particular, the study showed that PK has a short half-life of about 3 hours, unlike MK-7, which has a half-life of about 3 days.[10] This explains why some VKs have biological functions and MK-7 is more effective for prolonged physiological processes, such as the γ-carboxylation of OC in bone.[11]

According to the National Academy of Sciences, the amount of VK that must be taken ranges from a minimum of 2.56 mcg/day for newborns, to a maximum of 120 mcg/day in adult men and 90 mcg/day for women, in adolescents the recommended dose is 75 mcg/day.[12]
As previously indicated, VK is involved in bone metabolism. Bone metabolism is a constant and finely regulated process that maintains the structural and functional integrity of the skeletal system. This mechanism is regulated by two different cytotypes, the osteoblasts and osteoclasts. An imbalance in the functions of these cells leads to the onset of skeletal diseases such as osteoporosis.[13] This review aims to highlight the correlation between VK and osteoporosis.

Bone metabolism

Bone metabolism involves two mechanisms fundamental to the proper maintenance of the structural and functional integrity of the skeletal system. It is a constant and finely regulated process by two cell populations, osteoblasts and osteoclasts. Osteoblasts promote bone formation of new Extracellular Matrix (ECM), while osteoclasts degrade the ECM.[14] With advancing age, the activity of osteoclasts tends to be greater than that of osteoblasts and physiological ageing is accompanied by a certain loss of bone mass which leads to chronic diseases such as osteoporosis. Osteoporosis is a bone disease associated with progressive loss of Bone Mineral Density (BMD) and deterioration of the trabecular bone microstructure, leading to increased bone deformability and a higher fracture risk.[15]
It can be divided into primary and secondary osteoporosis. Primary osteoporosis is associated with age, gender, and early menopause. Secondary osteoporosis is often a result of endocrine disorders or systemic autoimmune diseases.[16,17]
Although this phenomenon occurs mainly in women after menopause it may be observed also in men.[18,19] There is strong evidence that estrogen deficiency in women may contribute to this bone disorder because estrogen controls calcium absorption in the intestine.[20,21] The gold standard for diagnosing osteoporosis is Dual-energy X-ray Absorptiometry (DXA), as it effectively predicts fracture risk.[22] Treatment strategies for osteoporosis include diet, hormones, drug therapy, exercise and, if necessary, pharmacological treatment.[22]
In 1984, a case-control study of subjects with femoral neck fractures and low circulating VK1 levels[23] showed that subjects with low serum VK1 concentrations had a lower Bone Mineral Density (BMD-DXA) than that measured in subjects without VK deficiency.[24] These data made it possible to initiate a series of studies that confirmed the central role that VK plays in

bone remodelling, particularly with a specific category of proteins vitamin K-dependent proteins (VDKPs).[25]

Mechanism of action of vitamin K

VK is an essential cofactor for the post-translational modifications induced during the biosynthesis of VKDPs. These modifications consist of the γ-carboxylation of specific glutamate residues to form γ-carboxyglutamic acid (Gla) (Figure 2), a process that depends on vitamin K epoxidation. This reaction confers calcium-binding properties to vitamin K–dependent proteins (VKDPs), enabling their activation and participation in calcium-dependent physiological processes,[26,27] including hemostasis, apoptosis, bone mineralization, calcium homeostasis, cell growth, and signal transduction.[28] The physiologically active form of vitamin K involved in triggering γ-glutamyl carboxylation is the reduced-form vitamin K quinol (KH2). The enzyme, in the presence of O2, CO2, and glutamate-containing substrate, catalyses the γ- carboxylation of glutamic acid residues on VKDP (Gla protein) and, at the same time, the formation of vitamin K 2,3-epoxide (Figure 2).[29]

The reaction of γ-carboxylation is facilitated by the so called “GLA domain”, which contains post-translational modifications of glutamate residues induced by vitamin K-dependent carboxylations to form gamma-carboxyglutamate (Gla).[28] The "Gla" domain present in the coagulation factors contains 45 amino acids and it is located near the N-terminal amino acid of proteins. The carboxylation reaction is triggered by the interaction between a region of the adjacent protein generally constituted by 18 to 28 amino acids and the enzyme carboxylase. In the case of coagulation proteins, the number of residues of glutamate that undergo γ-carboxylation is comprised between 9 and 13.[30] Vitamin K hydroquinone is then regenerated by a coumarin-dependent 2,3-epoxide reductase which, similarly to γ-glutamyl carboxylase, is an integral membrane protein of the endoplasmic reticulum.[31] During the process of reduction of vitamin K from epoxide to hydroquinone, the active sites of the thiol 2,3-epoxide reductase are oxidized. This results in an inactivation of the enzyme whose activity is regenerated by an unknown reductase.[32] The synthesis of reduced vitamin K is a rate-limiting factor of the carboxylation reaction in cells, while the overexpression of the 2,3-epoxide reductase appears to facilitate this process. However, because of the saturation of the reductase responsible for its activation, the increased activity of the enzyme is negligible.[25]

Both γ-glutamyl carboxylase and 2,3-epoxide reductase act as a multifactorial system which also includes the protein “chaperone” calumenin. Experimental observations show that, in an *in vitro* system of γ-carboxylation, which comprises γ-glutamyl carboxylase and 2,3-epoxide

reductase, calumenin is associated with the γ-glutamyl carboxylase and inhibits the activity of this latter enzyme. The silencing of the gene encoding calumenin by siRNA results in, at least, a 5-fold increase in the activity of γ-glutamyl carboxylase.[31]

Vitamin K dependent protein

VKDPs are a family of 14 proteins commonly noted for their role in blood coagulation. Several studies have highlighted the involvement of some VKDPs in physiological bone metabolism; these proteins include Osteocalcin (OC), Matrix Gla Protein (MGP), Growth arrest-specific 6 (Gas6), periostin, and protein S.[33]All these proteins can bind calcium ions (Ca^{2+}) after carboxylation in their glutamic acid residues.

VDPK and bone metabolism

Osteocalcin

OC is the most abundant non-collagenous protein produced by osteoblasts during bone formation. It is also known as Gla protein (BGP) (Figure 3). It is encoded by the Bone Gamma-Carboxyglutamate Protein (BGLAP) gene and is composed of 49 amino acids. After synthesis, the molecule undergoes a key modification consisting of the carboxylation of the glutamic acid residues at positions 17, 21 and 24; the reaction is catalysed by the enzyme ma-glutamylcarboxylase (GGCX), which requires VK as a cofactor.[34]. The importance of this post-transcriptional modification is related to the affinity of these three domains for hydroxyapatite, which is thus incorporated into the Extracellular Matrix (ECM).[35]
The extracellular deposition of hydroxyapatite contributes to the formation of a solid bone structure. Not all circulating OC is present in its carboxylated form; approximately 40–60% of OC is undercarboxylated (uc-OC) and released into the bloodstream due to its reduced affinity for Ca^{2+}. As uc-OC levels are closely related to circulating vitamin K, uc-OC is considered a marker of both bone formation and VK status.[36] In addition to its role as a nutritional biomarker, uc-OC has also been widely used to identify reduced BMD. Several studies have demonstrated that lower serum VK levels combined with higher circulating uc-OC are predictive of increased risk of hip fracture, impaired bone status, and reduced BMD in older women.[37] In line with these findings, Xu and colleagues reported significantly higher uc-OC levels in women affected by osteoporosis.[38]

Matrix Gla protein

MGP is an 84 amino acid protein with a molecular weight of 10.6 kDa (Figure 4). The protein contains five residues with the amino acid sequence Gla in its mature form. Chondrocytes, fibroblast and vascular smooth muscle cells synthesise MGP. Studies have shown that the effect of VK involve the MGP protein. MGP can promote bone formation by regulating the Wingless/Integrated β-catenin (Wnt/β-catenin) signalling pathway. This pathway plays a key role in osteoblast differentiation by promoting the expression of osteogenic differentiation genes through *RUNX2*.[39]
MGP has a dual role in bone remodeling, promoting and inhibiting bone formation. It is upregulated in highly differentiated osteoclasts, where it acts as a negative feedback regulator that limits further osteoclast differentiation.[39]

The steroid X receptor

The Steroid X Receptor (SXR) is a nuclear receptor involved in regulating genes directly related to oxidative stress, DNA repair and the extracellular matrix. The MK-4 form of VK2 appears to be one of the compounds capable of interacting with SXR.[40,41] Upon binding to SXR, MK-4 modulates the transcription of specific target genes, some of which are involved in the formation and composition of the ECM[40.]In particular, MK-4 regulates the transcription of genes associated with extracellular matrix formation, including *Tyrosine-protein kinase* (*Tsk*), *Matrilin-2* (*Matn-2*) and *CD14*. *Tsk* is involved in the regulation of cell proliferation and differentiation of osteoblasts. *Matn-2* is engaged in the organisation of the ECM and interactions with collagen. *CD14* is a cell surface-associated receptor shown to influence the inflammatory response and osteoclast differentiation. This suggests that VK2 may influence osteoblastic and osteoclastic activity, thereby regulating bone formation and resorption (Figure 5).[40,41]

Prostaglandins

Prostaglandins E (PGE) are molecules that play an important role in regulating inflammation, pain and tissue repair. PGE2 normally affects bone resorption through increased Receptor Activator of Nuclear Factor κ-B (NFκB) Ligand (RANKL) expression and inhibition of Osteoprotegerin (OPG) expression in osteoblastic cells.[42] However, these molecules appear to be associated with VK and bone health. In particular, studies show that VK2 modulates PGE2 activity, acting mainly through its receptors (EP1-EP4) on osteoblasts and osteoclasts, thereby influencing bone formation and resorption.[43]

In conclusion, Table 1 summarizes the main roles of Vitamin K, which acts not only as a cofactor for carboxylation (OC and MGP) but also as a nuclear ligand (SXR) and a PGE, influencing bone homeostasis in multiple ways

Animal model and therapeutic approach

Animal models are crucial in the study of osteoporosis, as they provide the opportunity to examine pathogenetic mechanisms and test new treatments in well-defined populations. Among the most commonly used models is the ovariectomised rat, in which surgical removal of the ovaries induces oestrogen deficiency and mimics bone loss associated with post-menopausal osteoporosis.[44] This model accurately reproduces the increase in bone remodelling driven by low oestrogen levels, making it suitable for studying bone metabolism and testing anti-resorptive therapies. Mice are also widely used, particularly for investigating genetic effects on bone metabolism, as genetic differences can be directly manipulated to examine the influence of specific genes on bone health and osteoporosis pathogenesis.[45] Despite their limitations, these animal models provide a unique opportunity to better understand the disease and to devise new therapeutic strategies. Osteoporosis is currently treated by three categories of drugs. Two of these selectively target osteoclast activity, resulting in an anti-resorptive effect, or stimulating osteoblast activity, inducing the formation of new bone tissue. The third category includes drugs that have a dual action, i.e. both anti-resorptive and new tissue formation.[46]

The category of anti-resorptive drugs includes bisphosphonates, antibody-activating RANKL and Selective Oestrogen Receptor Modulators (SERMs). Bisphosphonates are inorganic compounds that inhibit the dissolution of Hydroxyapatite (HA) crystals and hinder their calcification and degradation.[47] Although the use of bisphosphonates may reduce the incidence of osteoporosis, its use is associated with side effects such as osteonecrosis of the jaw, flu-like symptoms (such as headache, fever, myalgia), gastric ulcers and upper gastrointestinal intolerance, and coronary artery disease.[48,49]

Denosumab is a monoclonal antibody belonging to the IG2 category that binds to the RANKL receptor, neutralising it and preventing osteoclast recruitment and activation;[50] like bisphosphonates, it is associated with rare side effects such as osteonecrosis of the jaw.[51]

SERMs act similarly to oestrogens. They bind to specific receptors and have an effect similar to oestrogen on bone tissue, but at the same time do not affect other tissues.[52]

Given the limitations of these drugs and the role that VK2 plays in the context of bone metabolism, studies in rats have shown that combining them with VK2 administration may be

a valuable therapeutic approach. A study showed how the intake of MK-7 combined with other nutrients (such as vitamin D, Ca2 and Mg2+) inhibited bone loss in postmenopausal women.[53] The Ovariectomized (OVX) studies showed that administration of MK-7 after 12 weeks shows an increase in trabecular bone tissue compared to the control groups as well as mechanical recovery of the skeletal system itself.[53]

The effectiveness of administering VK2 to other drugs already in circulation was also evaluated in a study that focused on assessing the possible beneficial effects of treatment between VK2 and bisphosphonates.

The results showed that although zoledronic acid alone produced positive effects, the addition of VK2 enhanced BMD, thus highlighting the benefit of the combination for the treatment of osteoporosis.[54]

Conclusions

VK2 proves to be an important piece in the bone health puzzle. According to some scientific research, the administration of V K2, and in particular MK-7, can lead to a significant decrease in the risk of osteoporosis and bone fractures. Currently, treatments for this disease are bisphosphonates, or monoclonal antibodies, while the inclusion of VK2 in these therapies could be even more effective.

Future directions of research on VK2 include larger and longer future studies to establish the observed benefits, as well as define the necessary doses of the vitamin and its forms. This requires investigating possible interactions of VK2 with other nutrients and drugs to achieve the best possible treatment of osteoporosis. A more responsive analysis of how V K2 releases its signal of action may lay the foundation for new lines of treatment that would increase bone health and, consequently, the quality of life of people with osteoporosis.[55,56]

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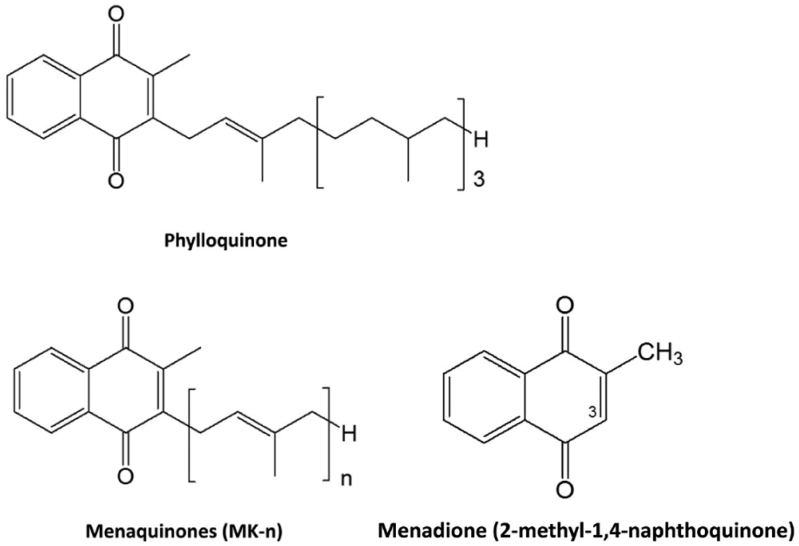

Figure 1. Chemical structures of vitamin K compounds. From left to right: phylloquinone (vitamin K_1), characterized by a phytyl side chain; menaquinones (MK-n, vitamin K_2), defined by isoprenoid side chains containing n repeating units and menadione (vitamin K_3), the synthetic form lacking a side chain. These structural differences determine variations in biological properties and tissue distribution. Created with MarvinSketch.com.

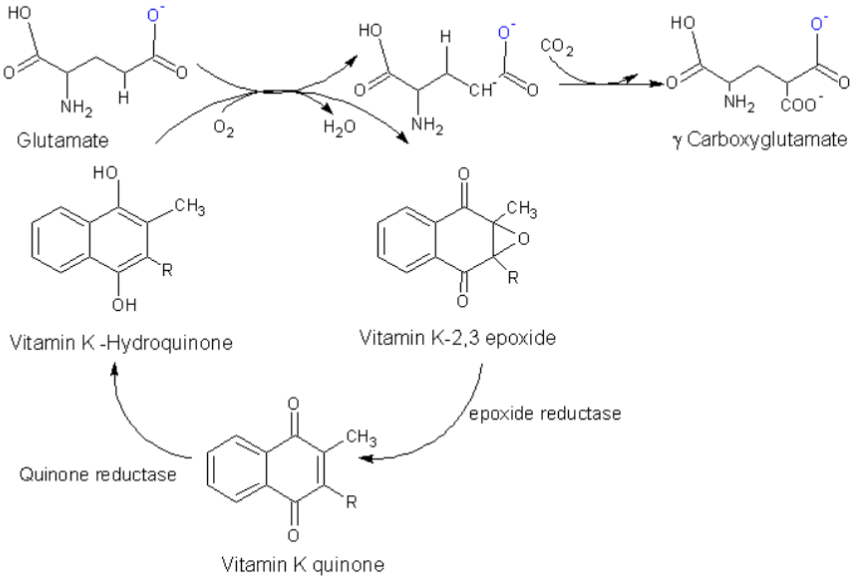

Figure 2. The vitamin K cycle and γ-carboxylation of glutamate residues. The diagram illustrates the vitamin K–dependent conversion of glutamate (Glu) to γ-carboxyglutamate (Gla) mediated by γ-glutamyl carboxylase, a reaction that requires vitamin K hydroquinone

as a cofactor. During carboxylation, vitamin K hydroquinone is oxidized to vitamin K 2,3-epoxide, which is subsequently recycled back to vitamin K quinone and then to hydroquinone through the actions of epoxide reductase and quinone reductase. This cycle enables the continuous activation of vitamin K–dependent proteins. Created with MarvinSketch.com.

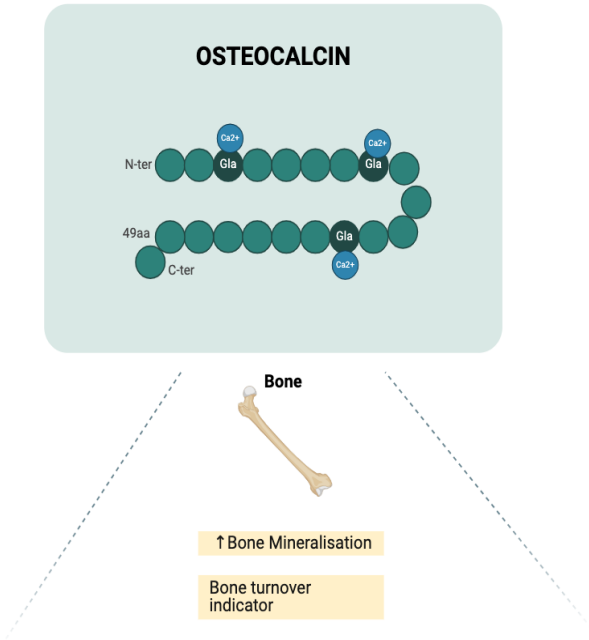

Figure 3. Osteocalcin (BGP): structure and role in bone mineralization.

The figure illustrates the structure and main functions of Osteocalcin (Bone γ-Carboxyglutamic Acid Protein, or BGP), an abundant, non-collagenous protein produced by osteoblasts. Structure: The protein is a 49-amino acid peptide (N-ter to C-ter) characterized by the presence γ-carboxyglutamic acid (Gla) residues, which result from the Vitamin K-dependent post-translational carboxylation of glutamic acid residues.

The Gla residues enable Osteocalcin to bind Ca^{2+} ions (indicated in blue), which is essential for its interaction with hydroxyapatite. Osteocalcin is involved in the process of Bone Mineralization and is used as an important Bone Turnover Indicator, particularly for the formation phase. Created with BioRender.com

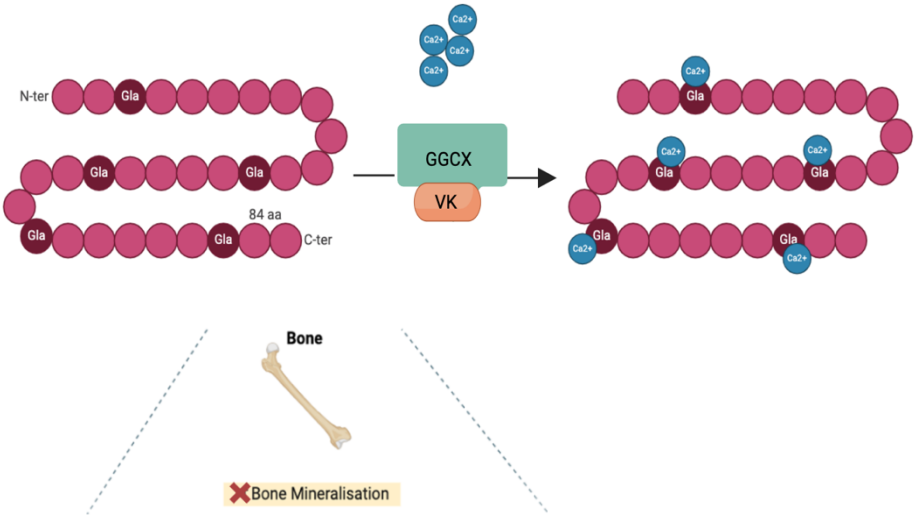

Figure 4. Post-Translational Modification of Gla Proteins. This figure illustrates the mechanism of carboxylation involving Gla proteins γ-carboxyglutamic acid-containing proteins), such as Osteocalcin or coagulation factors, a process crucial for their biological function. A precursor protein (in this case, an 84-amino acid protein, 84 aa) is shown, featuring Glutamic Acid (Glu) residues that require modification. The enzyme γ -Glutamyl Carboxylase (GGCX) catalyzes the reaction, converting Glu residues into γ -carboxyglutamic acid (Gla) residues. This reaction is strictly dependent on VK as a cofactor. The modified protein (mature Gla protein) now contains the additional Gla residues. The presence of two carboxyl groups on each Gla residue gives the protein a high affinity for Calcium (Ca^{2+}) ions. The binding of Ca^{2+} (indicated in blue) is essential for the activation and biological function of these proteins, for example, by binding to hydroxyapatite in the bone or interacting with platelet membranes in coagulation. Created with BioRender.com

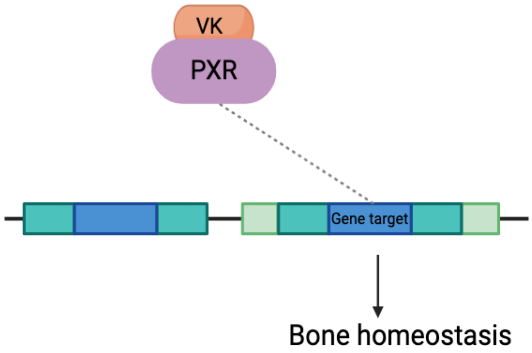

Figure 5. Transcriptional Action Mechanism of the Pregnane X Receptor (PXR)
This figure illustrates a genomic mechanism through which lipophilic ligands, such as VK or its metabolites, can influence bone homeostasis. Receptor Complex: VK binds to the PXR (SXR in mice), acting as a ligand. Transcriptional Activation: The VK-PXR complex binds to specific nucleotide sequences (response elements) located in the regulatory region (promoter or enhancer) of a target gene. Gene Regulation: The binding of the PXR complex activates or represses the transcription of the target gene, leading to the modulation of proteins involved in maintaining Bone Homeostasis. This mechanism suggests a role for VK that goes beyond its well-known role as a cofactor for γ-glutamyl carboxylase, also acting as a signaling molecule that regulates gene expression in the bone. Created with BioRender.com

Table 1. A summary of the key interactions between Vitamin K and the receptors/proteins involved in regulating bone metabolism.

Protein/Receptor	Description	Relationship with Vitamin K
Osteocalcin (OC)	Most abundant non-collagenous protein produced by osteoblasts during bone formation.	Vitamin K activates osteocalcin through the carboxylation of glutamic acid residues, allowing it to bind calcium and contribute to bone mineralization.
Matrix Gla Protein (MGP)	84-amino acid protein involved in bone formation regulation.	Vitamin K activates MGP, which promotes bone formation by regulating the Wnt/β -catenin signaling pathway and can also inhibit osteoclastogenesis through a negative feedback mechanism.
Steroid X Receptor (SXR)	Nuclear receptor involved in regulating genes related to oxidative stress, DNA repair, and extracellular matrix.	The MK-4 form of vitamin K2 interacts with the SXR receptor, activating or blocking the transcription of genes that influence extracellular matrix formation and composition, thereby regulating osteoblastic and osteoclastic activity.
Prostaglandins (PGE)	Molecules that regulate inflammation, pain, and tissue repair.	Vitamin K2 modulates prostaglandin E2 (PGE2) activity through its receptors (EP1-EP4) on osteoblasts and osteoclasts, influencing bone formation and resorption.

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Availability of data and materials: all data generated or analyzed during this study are included in this published article.