Protective Role of Vitamin K2 in Vascular Microcalcification: Clinical Implications

Mohamed Aly AbdelHafez@, PhD
Professor of Medical Biochemistry and Molecular Biology Kasr AlAiny School of Medicine, Cairo University

Correspondence to
Mohamed Aly AbdelHafez
mhafez01@kasralainy.edu.eg,
mhafez01@cu.edu.eg
Mobile phone: +20 1001025142
ORCID Id: 0000-0002-6649-1688

Kasr Al Ainy Medical Journal
2023, 29:1–7

Abstract

Vitamins K (vitamins K1and K2) are micronutrients with wide range of biological functions. Vitamin K1 (phylloquinone) is of plant origin and principally controls some blood clotting factors synthesized by the liver. Many forms of vitamin K2 (menaquinones) have various biological potencies and functions. This depends on the length of the side-chain. It is synthesized by microbiota of the large intestine besides being synthesized in tissues from phylloquinone by a specific enzyme in the liver and other tissues. Vitamin K2 is essential for preventing vascular microcalcification which affects both small and large arteries and contributes for pathological complications of renal and diabetic diseases. It has anti-osteoporotic action and maintains bone density. Its anti-inflammatory and anti-oxidant activities protect against many pathological states. It may account for improving glucose tolerance owing to its insulinotropic effect, and counter-against cancer due to both anti-apoptotic and anti-oxidant actions. It has biolological actions on the nervous system including peripheral nerves and protects against Alzheimer's disease.

1- Introduction

Vitamins K are powerful micronutrients for aging and age-related diseases [1]. They are present in nature in two biologically active forms. Vitamin K1 (phylloquinone, PK) is predominantly present in vegetables, such as lettuce, cabbage, and spinach [2]. Vitamin K2 (menaquinones, MKs), is essentially of microbial origin [3], e,g. in fermented food, and the gut microbiota [4]. Chemically, phylliquinone has phytyl side chain of four prenyl units, whereas menaquinones contain unsaturated side chain with a variable number of prenyl units (from 4 to 14).

Menaquinone-4 (MK4) is the main form in humans and animals. It can be synthesized from PK by the tissue-specific enzyme, UbiA prenyltransferase domain-containing protein 1 (UBIAD1**)** [5]. PK loses its side chain as it passes in the alimentary tract; the product is menadione. Menadione is transported to target tissues for prenylation by UBIADI [6]. MK4 is highly enriched in the liver, kidney, adipose tissue, reproductive organs, bone, and pancreas owing to its expression of **UBIAD1** [7].

Distal colon microbiota is the major source of most MKs, however, the most active site of absorption is the terminal ileum owing to the availability of bile salts needed for solubilization of MKs. Hence, dietary MKs, but not locally synthesized MKs, is the principal exogenous source of MKs [8].

Absorbed vitamin K is transported to the liver to be exported included in triglyceride-rich lipoproteins particles, VLDL or to be used for the synthesis of clotting factors. Menaquinones are released into the bloodstream, incorporation with low-density lipoproteins to target tissues for Gla-protein carboxylation [9].

2- Functions

Vitamin K2 (or menaquinones, MKs) is a cofactor of the enzyme γ-carboxylase, which drives the conversion of inactive vitamin K-dependent proteins (VKDPs) (such as osteocalcin and matrix Gla protein) from uncarboxylated to active γ-carboxyglutamate (Gla). A lack of MKs leads to pathological complications, such as vascular calcification, and osteoporosis, or is associated with other disorders, such as diabetes, and chronic degenerative conditions (such as cardiovascular diseases, Alzheimer's disease, and cancer) [10]. In addition, MKs inhibits autophagy and ferroptosis [11] to prevent degenerative disorders and cancer development.

2.1. Activation of vitamin K dependent proteins and the vitamin K cycle

Vitamin K-dependent proteins (VKDPs) are rich in glutamate residues that are acceptors of carbon dioxide in an active reaction, to form a carboxylated protein. This process requires vitamin K in its reduced form (hydroquinol). The native form of vitamin K is in the quinone form. It is reduced by vitamin K reductase

 DOI: 10.21608/kamj.2024.317803.1013

(NADPH- dependent) to the corresponding hydroquinone (or quinol)[12]. The latter is a component of γ-glutamyl carboxylase that catalyzes the carboxylation of uncarboxylated GLA proteins. The latter process is a complex and includes oxidation. The quinol is thus oxidized to epoxide. In the next step, the epoxide is reduced by epoxide reductase, to the quinone form of vitamin K, in a complex process that necessitates the presence of anti-oxidants such as vitamin C or glutathione to eliminate the generated reactive oxygen species. Therefore, vitamin K functions as an antioxidant [13]. Antioxidants play a role in protecting the bi-phospholipid cellular membranes from oxidation [14].

Vitamin K-dependent proteins (VKDPs) include coagulation factors II, VII, IX, and X, as well as matrix GLA proteins (MGPs), osteocalcin (OC), growth arrest-specific protein 6 (Gas6), transforming growth factor beta-inducible protein (TGFβI), and protein S. Coagulation factors are synthesized in the liver and are dependent on vitamin K1. Other proteins are influenced by vitamin K2 or menaquinones (MKs) [15].

2.2. Vascular calcification

Vitamin K2-activated matrix Gla protein (MGP) is a vascular calcification inhibitor [16,17]. Arterial calcification is manifested as scattered intimal and/or medial spots or patches. Intimal calcification occurs in the coronary and small arteries as small dots of calcification, but large artery calcification present as larger patches occupying the media, and is consistent with atherosclerosis [18]. This results in arterial wall hardness and reduced arterial compliance [19]. It has been reported that adequate intake of MKs is associated with reduced coronary artery calcification and all-cause mortality [20].

In the vascular system, matrix Gla-protein (MGP) is a secretory protein of low molecular weight released by both vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) of the arterial wall [21]. MGP undergoes vitamin K-dependent carboxylation and phosphorylation to become activated with the aim of acquiring extra-negative charges to facilitate binding to matrix calcium ions; thereby inhibiting the calcification process [22]. Dephosphorylated-uncarboxylated MGP (dp-ucMGP) is unable to bind calcium in the extracellular matrix and therefore released in the circulation. The plasma concentration of dp-ucMGP is a monitor of vascular MKs status compared with other components of the MGP [23]. It is correlated with an increased risk for vascular calcification in vulnerable patients such as those on hemodialysis [24], cardiovascular and diabetic patients [25].

2.3. Mechanism and control of vascular microcalcification

Inflammation triggers microcalcification, supporting the assumption that inflammation pivots for calcification [26]. Cell necrosis, associated with atherosclerotic changes, is a potent inducer of pathological calcification. Calcifying dead macrophages were observed in the necrotic core [27].

VSMCs retain high plasticity, which allows them to modulate and switch phenotypes upon exposure to stress signals. VSMC phenotypic switching is triggered by inflammation, growth factors, and injury. Atherosclerosis, hypertension and vascular surgery are all associated with VSMC phenotypic switching[28,29]. VSMC phenotypic modulation is characterized by changes in morphology, protein expression, proliferation, and migration [30]. The contractile function of VSMCs is impaired because of downregulation of contractile proteins and acquisition of proliferation and migration properties. VSMCs are differentiated into osteo-/chondrogenic and macrophage-like phenotypes, which promote further remodeling and calcification [32].

The local tissue factors that modulate VSMC phenotype include growth factors, such as platelet-derived growth factor (PDGF) and transforming growth factor-β [33], angiotensin II [34], nitric oxide [35], growth arrest-specific 6 protein [36], reactive oxygen species [37], and oncostatin M [38]. They are secreted by macrophages M1 to modulate the osteoblastic trans-differentiation of VSMCs [39].

MGP inhibits bone morphogenic protein-2 (BMP-2), a potent pro-osteoblastic protein, which is extensively expressed in atherosclerotic lesions during inflammation and oxidative stress. This induces an osteogenic gene expression profile in VSMCs [40, 41].

Osteocalcin (OC), which is secreted by osteoblasts. It stimulates insulin and adiponectin expression, leading to improvement of glucose intolerance. On the other hand, insulin and adiponectin stimulate osteocalcin expression in osteoblasts, establishing a positive feedforward loops among bone, pancreas, and adipose tissue [42]. On the other hand, OC modulates vascular calcification by activating adiponectin which inhibits the osteoblastic differentiation of VSMCs [43]. Osteocalcin is considered as a monitor to detect the trans-diffrentiation of vascular smooth muscle cells into osteoblasts [44]. Therefore, one may suggest that the high serum level of osteocalcin in atherosclerotic patients may be a protective mechanism against vascular calcification [45].

Osteoprotegerin (OPG) is a regulatory factor produced by stromal cells of the bone marrow. It acts as a decoy receptor for the receptor activator of nuclear factor kappa-light-chain enhancer of activated B cells ligand (RANKL) system [46]. Furthermore, OPG counteracts the pro-apoptotic actions of TNF-related apoptosis-inducing ligands, so it is considered a protective factor against vascular calcification [47, 48]. MGP has an anti-calcification action on the microcirculation of the kidneys, heart, and retina, and is postulated to contribute to the microvascular integrity of these organs through its anti-calcific properties [49].

The negative charges of MGPs offer high affinity for binding with free positively charged calcium ions. They chelate matrix calcium to make hydroxyapatite crystals that are accumulated within the vessel wall as inactive complexes [50].

MGP inhibits vascular calcification through the suppression of bone morphogenetic protein-2 (BMP-2).

BMP-2 is among the factors contributing for the transformation of smooth vascular muscle cells to an osteoblastic phenotype [51].

Growth arrest-specific gene 6 (Gas6) is activated by menaquinones. Gas6 undergoes γ-carboxylation to trigger anti-apoptotic activity of Bcl-2. It also inhibits caspase 3, a pro-apoptotic protein, thus preventing the apoptosis induced by inflammation and reducing the trans-differentiation of vascular smooth muscle cells to osteoblasts [52, 53].

Vitamin K affects the gut microbiome activity through regulation of IL-1-α, IL-1-β, and tumor necrosis factor (TNF)-α release from monocyte-derived macrophages [54]. It affects gut microbial composition and the overall bacterial metabolism that may interact with local intestinal factors to prevent colon cancer development [55].

3. Menaquinones protect against glucose intolerance and vascular diabetic complications

MKs supplementation increases insulin sensitivity [56]. These compounds improve the insulin response to glucose load [57]. It is claimed that MK-4 might function as insulin secretagauge via an incretin-like mechanism , (promotion of insulin secretion via elevation of cAMP levels in insulin-producing cells prior to [58].

Osteocalcin has a role as a gluco-regulatory factor. It potentiates glucose utilization by both increasing insulin secretion and promoting adiponectin expression [59, 60]. MKs improve insulin resistance through their anti-inflammatory and anti-oxidant effect.

MKs deficiency is associated with diabetic peripheral neuritis [61], as they facilitate synthesis and repair of the myelin sheath in the peripheral nervous system. Demyelination results in deterioration of the structural and molecular features of the nerve fibers, which leads to peripheral neuropathy [62]. In addition, one of the anti-oxidant effects of MKs is through inhibition of arachidonic acid-induced oxidative damage to oligodendrocytes by indirectly blocking 12-lipoxygenase [63].

Studies on diabetic nephropathy revealed that the renal functions are also affected with vitamin K. The plasma dpucMGP level is correlated with albuminuria and inversely associated with the estimated glomerular filtration rate (eGFR) denoting that vitamin K deficiency may deteriorate renal functions.[64,65].

4. The role of menaquinones in prevention of locomotor disorders

Bone mineralization is influenced by osteocalcin because of its high affinity for hydroxyapatite, which result in the formation of a stronger skeleton and minimizes susceptibility to fracture [66]. Gene induction of osteocalcin in human mesenchymal stem cells is enhanced by MK2-7 which is initially influenced by vitamin D3. MK2-7 affects genes involved in cell growth and differentiation. Hence, supplementation of both MK2-7 and vitamin D3 potentiates the development of bone and reduces bone structure deterioration [67].

Moreover, MK2-7 upregulates osteoprotegerin, a decoy receptor for receptor activator of nuclear factor kappa-B ligand (RANKL). RANKL binds to receptor activator of NF-κB (RANK) to activate NF-κB. The latter is essential for osteoclast activity which promotes bone resorption [68]. This mechanism is, also, important for the use of MKs as anti-inflammatory agents.

Gla-rich protein (GRP) has beneficial effect on preventing osteoarthritis by inhibiting calcification of articular cartilage. In osteoarthritic cartilage undercarboxylated GRP (ucGRP) was more evident than carboxylated GRP (cGRP), as an evidence of MK inadequacy. Besides, it is associated with ectopic articular calcification. [69**]**.

Efficient vitamin K status is associated with higher physical performance score [70**]**. Muscle strength and physical performance rather than muscle mass expresses effect of vitamin K on muscle quality rather than muscle mass [71], probably due to improving mitochondrial function [72].

5. Menaquinones protection against cancer

MKs inhibit the proliferation of cancer cells through induction of cell cycle arrest. MKs interferes with the binding of NF-κB to the cyclin D1 promoter, thus inhibiting cell cycle turnover [73]. Moreover, mitochondria apoptosis is induced by vitamin K2 *via* mitogen-activated protein kinase pathways [74]. In addition, vitamin K2 can inhibit cancer cell growth by inducing autophagy [75], depending on the level of cellular expression of Bcl-2 [76].

6. Menaquinones biological effects on the nervous system

Protein S and Gas6 are among GLP that was reported to prevent apoptosis of neuronal cells[77], oligodendrocyte loss, and microglial activation [78], suggesting its protective role against Alzheimer's disease[79,80]. Disruption of microglial homeostasis leads to the activation of neurotoxic astrocytes, synaptic loss and neuronal death which induce inflammation and neurodegeneration. Overactivation of microglia trigger inflammatory cascades in the central nervous system. MKs inhibit the nuclear translocation of NF-κB in microglia, resulting in the inhibition of NF-κB signaling and the suppression of inflammatory cytokines, i.e. IL-1β, IL-6 and TNF-α [81].

Protein S protects neuronal system against ischemic injury; having a beneficial role for prevention of vascular-mediated cognitive impairment [82]

7. Conclusion

Menaquinones is essential in prevention of vascular calcification and injury that may lead to aortic aneurysm and cardiac valvular disorders. MKs biomedical significance may be encountered in chronic non-communicable diseases as diabetes, chronic kidney

diseases, bone, cardiovascular, and neurodegenerative disorders. Mechanism of action is reported to achieve these tasks including anti-inflammatory, anti-oxidant and/or anti-apoptotic, besides chelating calcium in the vascular tissue. Many of the findings related with menaquininos deficiency necessitate further clinical and biomolecular studies.

Abbreviations:
BMP-2: morphogenic protein-2
dp-ucMGP: dephosphorylated-uncarboxylated MGP
Gas6: growth arrest-specific gene 6
Gla: γ-carboxyglutamate
MGP: matrix Gla protein
MKs: menaquinones,
OC: osteocalcin
OPC: osteoprotegerin
PDGF: platelet-derived growth factor
PK: phylloquinone
RANK: receptor activator of NF-κB
RANKL: nuclear factor kappa-light-chain enhancer of activated B cells ligand
UBIAD1: UbiA prenyltransferase domain-containing protein 1
VKDPs: vitamin K-dependent proteins
VSMCs: vascular smooth muscle cells

Funding: This research received no funding.
Institutional Review Board Statement: Not applicable.
Informed Consent Statement: Not applicable.
Data Availability Statement: Not applicable

References

[1] Simes DC, Viegas CSB, Araújo N, Marreiros C. Vitamin K as a Powerful Micronutrient in Aging and Age-Related Diseases: Pros and Cons from Clinical Studies. Int. J. Mol. Sci. 2019; 201920:4150. doi: 10.3390/ijms20174150

[2] Booth S, Vitamin K: food composition and dietary intakes. Food Nutr Res. 2012; 56: 10.3402/fnr.v56i0.5505. doi: 10.3402/fnr.v56i0.5505

[3] Suttie JW. The importance of menaquinones in human nutrition. Annu. Rev. Nutr.1995;15:399–417.

[4] Shearer MJ. Vitamin K. Lancet. 1995;345(8944):229–234.

[5] Okano T, Shimomura Y, Suhara Y, Kamao M, Sugiura M, et al. Conversion of phylloquinone (vitamin K1) into menaquinone-4 (vitamin K2) in mice: two possible routes for menaquinone-4 accumulation in cerebra of mice. J. Biol. Chem. 2008;283(17):11270–11279

[6] Karl JP, Hernandez CJ, Mason JB, DeBose-Boyd RA, Booth SL. Multiple dietary vitamin K forms are converted to tissue menaquinone-4 in mice. J. Nutr. 2021;152(4):981-993. . doi: 10.1093/jn/nxab332

[7] Welsh JE, Bak M, Narvaez CJ. New insights into vitamin K biology with relevance to cancer. Trends. Mol. Med. 2022;28 (10): 864-88. https://doi.org/10.1016/j.molmed.2022.07.002

[8] Conly JM, Stein K. Quantitative and qualitative measurements of K vitamins in human intestinal contents. Am. J. Gastroenterol.1992; 87(3):311–316

[9] Vermeer C. Vitamin K: the effect on health beyond coagulation – an overview. Food Nutr. Res. 2012;56:5329. DOI: 10.3402/fnr.v56i0.5329.

[10] Vaidya R, Vaidya A D B, Sheth J, Jadhav S, Mahale U, Mehta D, et al. Vitamin K Insufficiency in the Indian Population: Pilot Observational Epidemiology Study. JMIR Public Health Surveill. 2022; 8, e31941. doi:10.2196/31941

[11] Jin C, Tan K, Yao Z, Lin BH, Zhang DP, Chen WK, Mao SM, Zhang W, Chen L, Lin Z et al. A novel anti-osteoporosis mechanism of VK2: Interfering with ferroptosis via AMPK/SIRT1 pathway in type 2 diabetic osteoporosis. J. Agric. Food Chem.2023; 71:2745–2761.

[12] Vervoort LM, Ronden JE, Thijssen HH. The potent antioxidant activity of the vitamin K cycle in microsomal lipid peroxidation. Biochem. Pharmacol.1997; 54:871–876. doi: 10.1016/S0006-2952(97)00254-2.

[13] Nowicka B, Kruk J. Occurrence, biosynthesis and function of isoprenoid quinones. Biochimica et Biophysica Acta (BBA). Bioenergetics. 2010; 1797(9):1587–1605. DOI: 10.1016/j.bbabio.2010.06.007

[14] Li J, Lin JC, , Wang H, Peterson JW, Furie BC, Furie B, et al. Novel Role of Vitamin K in Preventing Oxidative Injury to Developing Oligodendrocytes and Neurons. J. Neurosci.. 2003; 23(13):5816–5826.. doi: 10.1523/JNEUROSCI.23-13-05816.2003

[15] Simes DC, Viegas CSB, Araújo N, Marreiros C. Vitamin K as a Powerful Micronutrient in Aging and Age-Related Diseases. Pros and Cons from Clinical Studies. 2019; Int. J. Mol. Sci. 20(17): 4150 doi: 10.3390/ijms20174150

[16] Kidd PM. Vitamins D and K as pleiotropic nutrients: clinical importance to the skeletal and cardiovascular systems and preliminary evidence for synergy. Altern. Med. Rev.2010; 15(3):.199–222.

[17] Shea MK, Berkner KL, Ferland G, Fu X, Holden RM, Booth SL. Perspective: Evidence before Enthusiasm—A Critical Review of the Potential Cardiovascular Benefits of Vitamin K. Adv. Nutr. 2021; 12(3): 632–646. doi:10.1093/advances/nmab004.

[18] Andrews L, Psaltis PJ, Bartolo BAD, Nicholls SJ, Puri R. Coronary arterial calcification: a review of mechanisms, promoters and imaging. Trends Cardiovasc. Med. 2018; 28(8):491–501.

[19] Zettervall S.L Marshall A, Fleser P, Guzman RJ. Association of arterial calcification with chronic limb ischemia in patients with peripheral artery disease. J. Vasc. Surg. 2018; 67:507–513. doi: 10.1016/j.jvs.2017.06.086

[20] Beulens JW, Bots ML, Atsma F, Bartelink, ML, Prokop M, Geleijnse JM, et al High dietary menaquinone intake is associated with reduced coronary. **Atherosclerosis**. 2009**;** 203:489–493. doi:10.1016/j.atherosclerosis.2008.07.010

[21] Barrett H, O'Keeffe M, Kavanagh E, Walsh M, O'Connor EM. Is Matrix Gla Protein Associated with Vascular Calcification? A Systematic Review. Nutrients 2018; 10:415.

[22] Jaminon AMG, Dai L, Qureshi A, Evenepoel P, Ripsweden J Söderberg M, et al. Matrix Gla protein is an independent predictor of both intimal and medial vascular calcification in chronic kidney disease. Sci. Rep. 2020; 10: 6586.

[23] Schurgers J, Barreto DV, Barreto FC, Liabeuf S, Renard C, Magdeleyns J. et al. The circulating inactive form of matrix gla protein is a surrogate marker for vascular calcification in c0hronic kidney disease: A preliminary report. Clin. J. Am. Soc. Nephrol. 2010; 5: 568–575.

[24] Delanaye P, Krzesinski J-M, Warling X, Moonen M, Smelten N, Médart L, et al. Dephosphorylated-uncarboxylated Matrix Gla protein concentration is predictive of vitamin K status and is correlated with vascular calcification in a cohort of hemodialysis patients.BMC Nephrol. 2014; 15: 145.

[25] Roumeliotis S, Dounousi E, Eleftheriadis T, Liakopoulos V. Association of the Inactive Circulating Matrix Gla Protein with Vitamin K Intake, Calcification, Mortality, and Cardiovascular Disease. Int. J. Mol. Sci. 2019; 20: 628.

[26] Van der Heiden K, Barrett HE, Meester EJ, van Gaalen K, Krenning B, Beekman FJ. SPECT/CT imaging of inflammation and calcification in human carotid atherosclerosis to identify the plaque at risk of rupture. J. Nucl. Cardiol. 2022 Oct; 29(5):2487-2496.doi: 10.1007/s12350-021-02745-0

[27] Jinnouchi H, Sato Y, Sakamoto A, Cornelissen A, Mori M, Kawakami R. Calcium deposition within coronary atherosclerotic lesion: implications for plaque stability. Atherosclerosis. 2020 Jun; 306:85–95.

[28] Rzucidlo EM, Martin KA, Powell RJ. Regulation of vascular smooth muscle cell differentiation. **J. Vasc. Surg.** 2007; 45(suppl A):A25–A32. doi: 10.1016/j.jvs.2007.03.001,

[29] Branchetti E, Poggio, Sainger R, Shang E, Grau JB, Jackson BM, et al. Oxidative stress modulates vascu,r smooth muscle cell phenotype via CTGF in thoracic aortic aneurysm**. Cardiovasc. Res.** 2013; 100:316–324. doi: 10.1093/cvr/cvt205

[30] Hao H, Gabbiani G, Bochaton-Piallat ML. Arterial smooth muscle cell heterogeneity: implications for atherosclerosis and restenosis development. **Arterioscler Thromb. Vasc. Biol**. 2003; 23:1510–1520. doi: 10.1161/01.ATV.0000090130.85752.ED

[31] Petsophonsakul P, Furmanik M, Forsythe R, et al. Role of Vascular Smooth Muscle Cell Phenotypic Switching and Calcification in Aortic Aneurysm Formation. Arterioscler. Thromb. Vasc. Biol. 2019; 39:1351–1368 https://doi.org/10.1161/ATVBAHA.119.312787.

[32] Fusaro M, Gallieni M, Porta C, et al. Vitamin K effects in human health: new insights beyond bone and cardiovascular *health.J. Nephrol.* 2020*;* 33*:* 239–49.doi:10.1007/s40620-019-00685-0

[33] Hao H, Ropraz P, Verin V, Camenzind E, Geinoz A, Pepper M.S, et al. Heterogeneity of smooth muscle cell populations cultured from pig coronary artery**. Arterioscler Thromb. Vasc. Biol.** 2002; 22:1093–1099

[34] Bascands JL, Girolami JP, Troly M, Escargueil-Blanc I, Nazzal D, Salvayre R, Blaes N. Angiotensin II induces phenotype-dependent apoptosis in vascular smooth muscle cells**. Hypertension,** 2001; 38,1294–1299

[35] Hariri E, Kassis N, Iskandar J-N, Schurgers L, Saad A, Omar Abdelfattah O, et al. Vitamin K_2 a neglected player in cardiovascular health: a narrative review. Open Heart 2021; 8**:**e001715. doi: 10.1136/openhrt-2021-001715

[36] Hasanbasic I, Rajotte I, Blostein M. The role of gamma-carboxylation in the anti-apoptotic function of Gas6. *J. Thromb. Haemost.* 2005*;* 3*:* 2790–7.doi:10.1111/j.1538-7836.2005.01662.x,

[37] Clempus RE, Griendling KK. Reactive oxygen species signaling in vascular smooth muscle cells. **Cardiovasc. Res.** 2006; 71:216–225. doi: 10.1016/j.cardiores.2006.02.033.

[38] Zhang X, Li J, Qin JJ, et al. Oncostatin M receptor β deficiency attenuates atherogenesis by inhibiting JAK2/STAT3 signaling in macrophages. J. Lipid Res. 2017; 58:895–906

[39] Tintut Y, Patel J, Parhami F, Demer LL. Tumor necrosis factor-alpha promotes in vitro calcification of vascular cells via the cAMP pathway. Circulation, 2000; 102:2636–2642.

[40] Sweatt A, Sane DC, Hutson SM, et al. Matrix Gla protein (MGP) and bone morphogenetic protein-2 in aortic calcified lesions of aging rats. *J. Thromb. Haemost.* 2003*;* 1:178–85.doi:10.1046/j.1538-7836.2003.00023.x.

[41] Shioi A, Morioka T, Shoji T, Emoto M. The inhibitory roles of vitamin K in progression of vascular calcification. Nutrients. 2020; 12:583

[42] Kanazawa I. Osteocalcin as a hormone regulating glucose metabolism World J. Diabetes. 2015; 6(18): 1345–1354.doi: 10.4239/wjd.v6.i18.1345.

[43] Luo, X.H.; Zhao, L.L.; Yuan, L.Q.; et al. Development of arterial calcification in adiponectin-deficient mice: adiponectin regulates arterial calcification. *J. Bone Miner. Res.* 2009, 24,1461–8. doi:10.1359/jbmr.090227

[44] Millar SA, John SG, McIntyre CW, Ralevic V, Anderson SI, O'Sullivan SE. An investigation into the role of osteocalcin in human arterial smooth

muscle cell calcification. Front Endocrinol. (Lausanne) 2020; 11:369

[45] Zhang M, Zhang Q, Du P, Chen X. Zhang Y. Roles of vitamin K-dependent protein in biomineralization. Int. J. Mol. Med. 2024; 53(1): 6.2023 Nov 24. doi: 10.3892/ijmm.2023.5330

[46] Jadhav N, Ajgaonkar S, Saha P, Gurav P, Pandey A, Basudkar V, et al. Molecular pathways and roles for vitamin K2-7 as a health-beneficial nutraceutical: Challenges and opportunities. Front. Pharmacol. 2022; 13; 896920.

[47] Wu W-J, Kim MS, Ahn B-Y. The inhibitory effect of Vitamin K on RANKL-induced osteoclast differentiation and bone resorption. Food & Funct. 2015; 6(10). DOI: 10.1039/C5FO00544B

[48] EomYJ, Kim JW, RimYA, Lim J, Jung SI, Ju JH. Effects of stepwise administration of osteoprotegerin and parathyroid hormone-related peptide DNA vectors on bone formation in ovariectomized rat model. Scientific Reports. 2024; 14, Article number: 2477. https://doi.org/10.1038/s41598-024-51957.

[49] Mandatori D, Pelusi L, Schiavone V, Di Pietro C P-N, Pandolf A. Crosstalk: Opposite Effects on Bone Loss and Vascular Calcification. *Nutrients* 2021; *13*(4): 1222; https://doi.org/10.3390/nu13041222

[50] Shanahan CM. Mechanisms of vascular calcification in renal disease. Clin. Nephrol. 2005; 63:146–57. doi: 10.5414/CNP63146

[51] Bostrom K, Watson KE, Horn S, Wortham C, Herman IM, Demer LL. Bone morphogenetic protein expression in human atherosclerotic lesions. J.,Clin. Invest. 1993; 91:1800–9. doi: 10.1172/JCI116391

[52] Roumeliotis S, Dounousi E, Salmas M, Eleftheriadis T, Liakopoulos V. Vascular Calcification in Chronic Kidney Disease: The Role of Vitamin K- Dependent Matrix Gla Protein. Front. Med. (Lausanne) 2020; 7: 154. doi:10.3389/fmed.2020.00154

[53] Villa J K D, Diaz M A N, Pizziolo V R, Martino H S D. Effect of Vitamin K in Bone Metabolism and Vascular Calcification: A Review of Mechanisms of Action and Evidences. Crit. Rev. Food Sci. Nutr. 2017; 57: 3959–3970. doi:10.1080/10408398.2016.1211616

[54] Pan M-H, Maresz K, Lee P-S, Wu J-C, Ho C-T, Popko J, Mehta DS, Stohs SJ Badmaev11 V i. J. Med. Food. 2016; 19(7):663-9. doi: 10.1089/jmf.2016.0030. Epub 2016 May 20.

[55] Zheng X, Huang F, Zhao A, Lei S, Zhang Y, Xie G, Chen T, Qu C, Rajani C, Dong B. Bile acid is a significant host factor shaping the gut microbiome of diet-induced obese mice. BMC Biol. 2017; 15:1–15

[56] Choi HJ, Yu J, Choi H, An JH, Kim SW, Park KS.; et al. Vitamin K2 supplementation improves insulin sensitivity via osteocalcin metabolism: A placebo-controlled trial. Diabetes Care 2011; 34: e147.

[57] Ho H-J.; *Komai M, Shirakawa* H. Beneficial Effects of Vitamin K Status on Glycemic Regulation and Diabetes Mellitus. Nutrients. 2020; 12(8): 2485;https://doi.org/10.3390/nu12082485.

[58] Ho HJ, Shirakawa H.; Hirahara K, Sone H, Kamiyama S, Komai, M. Menaquinone-4 Amplified Glucose-Stimulated Insulin Secretion in Isolated Mouse Pancreatic Islets and INS-1 Rat Insulinoma Cells. Int. J. Mol. Sci. 2019; 20: 1995

[59] Rasekhi H, Karandish M, Jalali MT, Mohammadshahi M, Zarei M, Saki A, Shahbazian H. Phylloquinone supplementation improves glycemic status independent of the effects of adiponectin levels in premonopause women with prediabetes: A double-blind randomized controlled clinical trial. J. Diabetes Metab. Disord. 2015; 14(1): 1. doi: 10.1186/s40200-014-0127-9

[60] Al-Suhaimi E A, Al-Jafary M A. Endocrine Roles of Vitamin K-dependent- Osteocalcin in the Relation between Bone Metabolism and Metabolic Disorders. Rev. Endocr. Metab. Disord. 2020; 21: 117–125. doi:10.1007/s11154-019-09517-9.

[61] Jeannin AC, Salem JE, Massy Z, Aubert CE, Vemeer C, Amouyal C, et al. Inactive matrix gla protein plasma levels are associated with peripheral neuropathy in Type 2 diabetes. PLoS ONE, 2020;15: e0229145.

[62] Vaidya R, Godse C, Jadhav S, Saha P, Ajgaonkar S, Pandey A, et al. An Intrinsic Need for K2-7 Supplementation: A Narrative Review of K2-7 and Peripheral Neuropathy. Communicated: Biomed. J. Sci. Tech. Res. 2022; 42: 33679–33687. 10.26717/BJSTR.2022.42.006761

[63] Li J, Wang H, Rosenberg PA. Vitamin K prevents oxidative cell death by inhibiting activation of 12-lipoxygenase in developing oligodendrocytes. J. Neurosci. Res. 2009; 87:1997–2005. doi: 10.1002/jnr.22029

[64] Wei FF, Trenson S, Thijs L, Huang QF, Zhang ZY, Yang W, et al. Desphospho-uncarboxylated matrix Gla protein is a novel circulating biomarker predicting deterioration of renal function in the general population. Nephrol. Dial. Transpl. 2018; 33: 1122–1128

[65] Jaminon AMG, Dai L, Qureshi AR, Evenepoel P, Ripsweden J, Soderberg M. et al. Matrix Gla protein is an independent predictor of both intimal and medial vascular calcification in chronic kidney disease. Sci. Rep. 2020; 10: 6586.

[66] Hoang Q Q, Sicheri F, Howard A J, Yang D S. Bone Recognition Mechanism of Porcine Osteocalcin from Crystal Structure. Nature, 2003; 425: 977–980. doi:10.1038/nature02079

[67] Jadhav N, AjgaonkarS, Saha P. et al. Molecular Pathways and Roles for Vitamin K2-7 as a Health-Beneficial Nutraceutical: Challenges and Opportunities, Front. Pharmacol., Sec. Gastrointestinal and Hepatic Pharmacology, 2022; 13 https://doi.org/10.3389/fphar.2022.896920

[68] Badmaev V, Mehta D, Jonas R, Rosenbush S, Hulse S. Evolving Story of Bone Health and the Nutritional Support. Nutra.Cos, 2011; 2–5.

[69] Rafael MS, Cavaco S, Viegas CS. Insights into the association of Gla-rich protein and osteoarthritis, novel splice variants and γ-carboxylation status. Mol. Nutr. Food Res. 2014;58:1636–1646. DOI: 10.1002/mnfr.201300941

[70] van Ballegooijen AJ, van Putten SR, Visser M et al. Vitamin K status and physical decline in older adults—the longitudinal aging study Amsterdam. Maturitas 2018;113:73–79.

[71] Azuma K Osuka Y, Kojima N, et al. Vitamin K insufficiency predicts incidence of frailty in community-dwelling older adults: the Otassha Study. J. Bone Miner. Metab. 2023; 41:817– -821 85.

[72] Su X, Zhou J, Wang W et al. VK2 regulates slow-twitch muscle fibers expression and mitochondrial function via SIRT1/SIRT3 signaling. Nutrition. 2022;93:111412.

[73] Xia J, Matsuhashi S, Hamajima H, Iwane S, Takahashi H, Eguchi Y, et al. The Role of PKC Isoforms in the Inhibition of NF-Kb Activation by Vitamin K2 in Human Hepatocellular Carcinoma Cells. J. Nutr. Biochem. 2012; 23: 1668–1675. doi:10.1016/j.jnutbio.2011.11.010

[74] Showalter S L, Wang Z, Costantino C L, Witkiewicz A K, Yeo C J, Brody J R. et al. Naturally Occurring K Vitamins Inhibit Pancreatic Cancer Cell Survival through a Caspase-dependent Pathway. J. Gastroenterol. Hepatol. 2010; 25: 738–744. doi:10.1111/j.1440-1746.2009.06085.x

[75] Yokoyama T, Miyazawa K, Naito M, Toyotake J, Tauchi, T, Itoh M. et al. Vitamin K2 Induces Autophagy and Apoptosis Simultaneously in Leukemia Cells. Autophagy, 2008; 4: 629–640. doi:10.4161/auto.5941

[76] Miyazawa K, Yokoyama T, Naito M. Vitamin K2 Induces Autophagy and Apoptosis Simultaneously in Leukemia Cells and Bcl-2 Expression Level Determines the Phenotype of Their Cell Death. Blood, 2007; 110(11):3478-3478, DOI: 10.1182/blood.V110.11.3478.3478

[77] Funakoshi H, Yonemasu T, Nakano T et al. Identification of Gas6, a putative ligand for Sky and Axl receptor tyrosine kinases, as a novel neurotrophic factor for hippocampal neurons. J Neurosci Res 2002; 68:150–160. doi: 10.1002/jnr.10211.

[78] Binder MD, Cate HS, Prieto AL, et al. Gas6 deficiency increases oligodendrocyte loss and microglial activation in response to cuprizone-induced demyelination. J Neurosci. 2008; 28:5195–5206

[79] Hadipour E, Tayarani-Najaran Z, Fereidoni M. Vitamin K2 Protects PC12 Cells against Aβ (1-42) and H2O2-Induced Apoptosis via P38 MAP Kinase Pathway. Nutr. Neurosci. 2020; 23: 343–352. doi:10.1080/1028415X.2018.1504428

[80] Huang S H, Fang S T, Chen Y C. Molecular Mechanism of Vitamin K2 Protection against Amyloid-β-Induced Cytotoxicity. Biomolecules 2021; 11: 423. doi:10.3390/biom11030423.

[81] Saputra W D, Aoyama N, Komai M, Shirakawa H. Menaquinone-4 Suppresses Lipopolysaccharide-Induced Inflammation in MG6 Mouse Microglia-Derived Cells by Inhibiting the NF-Kb Signaling Pathway. Int. J. Mol. Sci. 2019; 20: 2317. doi:10.3390/ijms20092317

[82] Liu D, Guo H, Griffin JH et al. Protein S confers neuronal protection during ischemic/ hypoxic injury in mice. Circulation 2003; 107:1791–1796.