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Vitamin K and metabolic syndrome: is there a link?

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Vitamin K and metabolic syndrome: is there a link?

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Dedicatória

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Vitamin K and metabolic syndrome: is there a link?

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Vitamin K and metabolic syndrome: is there a link?

Abstract: With approximately a quarter of the adult population in the world suffering from metabolic syndrome, finding strategies to delay, or even prevent, the development of diseases associated with this condition, such as cardiovascular diseases (CVD), type 2 diabetes (T2D) and obesity, is paramount. Metabolic syndrome is linked to malnutrition and micronutrients have been gaining acceptance as therapeutic tools for this condition. Low levels of the fat-soluble vitamins A, D and E seem to be associated with metabolic syndrome and, in recent years, it was discovered that vitamin K is more than just an anti-hemorrhagic vitamin. It has anti-inflammatory and antioxidant properties and, in addition to its role in the synthesis of several Gla proteins, it seems to be involved in the regulation of pathways associated with metabolism. Vitamin K may protect from several diseases, including CVD, osteoporosis, obesity and T2D. Within this work, we present an overview of vitamin K sources, metabolism and biomarkers, as well as the current available evidence of the effect of vitamins K1 and K2 on inflammation, insulin resistance, obesity, dyslipidemia and high blood pressure, and their underlying mechanisms.

Keywords: insulin resistance; inflammation; gla proteins; obesity; type 2 diabetes

Introduction

Metabolic syndrome is a well-known cluster of medical conditions that, when occur together, increase the risk of developing cardiovascular diseases, type 2 diabetes (T2D) and several cancers. According to the American Heart Association (AHA), the National Heart, Lung and Blood Institute (NHLBI) and the American Diabetes Association (ADA), it is comprised of six major components: abdominal obesity, dyslipidemia, hypertension, glucose intolerance, a pro-inflammatory state and a pro-thrombotic state (Grundy, Brewer, et al. 2004). In clinical practice, these elements need to be assessed by easily recognizable signs, such as an increased waist circumference, elevated triglycerides, reduced HDL cholesterol, high blood pressure and raised plasma glucose (Grundy, Hansen, et al. 2004; Saklayen 2018).

In the last century, due to the enormous gains made in the prevention and treatment of infectious diseases, through the development of vaccines and antibiotics, communicable diseases became no longer responsible for the majority of deaths worldwide. In fact, nowadays, both in developed and developing countries, non-communicable diseases are the biggest cause of morbidity and mortality, killing 41 million people each year, which is equivalent to 71% of all deaths globally (Non communicable diseases 2020). Most of these cases are attributed to cardiovascular diseases, which are responsible for 17.9 million deaths annually. Cancer accounts for 9.0 million deaths, whereas diabetes results in 1.6 million deaths (Non communicable diseases 2020). Since people of all ages are vulnerable to the risk factors contributing to these illnesses, battling the increase in these numbers by focusing on the prevention of metabolic syndrome may prove to be very effective.

Vitamins are fundamental micronutrients that our organism cannot produce, either at all or not in sufficient quantities, and are obtained, mainly, from the diet. The fat-soluble vitamins A, D, E and K have received significant attention in recent years, due to the discovery of new beneficial physiological properties. Vitamin K, in particular, has gained interest in the scientific community due to new research that suggests it may have a role in the prevention of cardiovascular diseases, osteoporosis, chronic kidney disease, certain neurological disorders, multiple cancers and also insulin resistance and obesity (Halder et al. 2019).

Given that, approximately, one quarter of the world population is estimated to have metabolic syndrome (Saklayen 2018), our aim was to explore the described effects of vitamin K on metabolic health and their underlying mechanisms, discussing the differences between vitamins K1 and K2.

Vitamin K – chemical structure and sources

Vitamin K is a family of structurally similar vitamins first discovered in 1935 by Henrik Dam, a Danish biochemist and physiologist, who found a fat-soluble factor comparable to vitamin E, but with distinct physiological clotting properties from any other known vitamin (H. Dam 1935). In his experiments, he noticed that chickens fed a low fat diet displayed poor coagulation abilities, which could lead to serious bleeding. When he analyzed the lipid fraction of the diet and uncovered a new anti-hemorrhagic factor, Dam named it "vitamin K", based on the Scandinavian and German word "Koagulation" (H. Dam 1935, 1946).

Vitamin K can appear in various isoforms, all containing a common polar hydrophilic 2-methyl-1,4-naphthoquinone ring structure named menadione or vitamin K3 (Simes et al. 2019) (Figure 1). Vitamin K3 can be created in a laboratory as a synthetic analogue of vitamin K, but it has been banned in the USA by the Food and Drug Administration (FDA) due to reported adverse effects of hemolytic anemia and liver toxicity. However, it is currently being researched as a potential treatment for prostate/hepatocellular cancer and for the prevention of skin toxicity secondary to kinase inhibitor anticancer therapy (Schwalfenberg 2017).

In nature, vitamin K appears as two vitamers: vitamin K1 or phylloquinone and vitamin K2 or menaquinone (Figure 1). Vitamin K1 contains a phytil side chain, whereas vitamin K2 can actually be categorized in multiple subtypes of menaquinones (MK-n), where n represents the number of prenyl units in an isoprenoid side chain (Halder et al. 2019; Simes et al 2019). [Figure 1 near here]

Long-chain menaquinones (MK-5 to MK-13) are generated by anaerobic bacteria (some of which are present in the microflora of the human gut) (Simes et al 2019), while short-chain MK-4 is obtained from the conversion of phylloquinone in certain

tissues, in mammals (Halder et al. 2019). In fact, Nakagawa et al. (2010) were the first to discover one of the enzymes responsible for the conversion of vitamin K1 to MK-4 in the human body (UBIAD1, which stands for UbiA prenyltransferase-containing domain 1), more specifically in the cerebrum, liver and pancreas. Meanwhile, phylloquinone is solely produced by plants, algae and a few species of cyanobacteria (Simes et al 2019). This environmental distribution is reflected in the dietary sources of these vitamins, since vitamin K1 appears predominantly in green leafy vegetables, but also in several fruits, such as avocado, kiwifruit, blueberries, blackberries, grapes, dried figs and prunes, nuts like cashews, pistachios and pine nuts, while vitamin K2 appears in meat, dairy products and food where fermentation by bacteria is part of the production process. Some fermented foods high in vitamin K2 include natto (a japanese dish made of fermented soy beans and the food with the highest content of vitamin K2), sauerkraut, kimchi and fermented dairy, in particular hard cheeses (Halder et al. 2019). Although it can vary with the specificities of each individual diet, in the Western world, phylloquinone accounts for the majority of the vitamin K intake, while menaquinones are associated with only 10-25% of the vitamin K content of Western diets (Shearer and Newman 2014).

In regard to the bioavailability of vitamin K from different foods, the information is scarce, but some data suggest that phylloquinone from green leafy vegetables, which represent the greatest food source of vitamin K1, is very poorly absorbed in the gut, due to its location in the membrane of chloroplasts. Simultaneously consuming vegetables with some type of dietary fat greatly improves phylloquinone absorption, but the amount absorbed is still lower compared to that from plant oils (L. Booth 2012). On the contrary, long-chain menaquinones, such as MK-5 to MK-10, are strongly lipophilic and are mainly found in the fat fraction of food products, which

results in an absorption close to 100%. Consequently, even though the dietary intake of vitamin K1 is much higher, vitamin K2 is equally important for vitamin K status, because of its better intestinal uptake (Walther and Chollet 2017).

As briefly mentioned above, menaquinones are also produced in the human gut, by the local flora, and this could be considered an alternative source of vitamin K2. The major forms of menaquinones synthesized by the gut microbiota are MK-10 and MK-11 by *Bacteroides*, MK-8 by *Enterobacteria*, MK-7 by *Veillonella* and MK-6 by *Eubacterium lentum* (Shearer and Newman 2014). The majority of menaquinones are generated in the distal part of the large intestine, but their optimum site of absorption is the terminal ileum, where the abundant bile salts emulsify fat-soluble substances. This discrepancy makes the bioavailability of this non-dietary source of vitamin K2 quite low (Walther and Chollet 2017). Therefore, diet continues to be our best source of this vitamin and a short-term reduction in the consumption of vitamin K2 is not compensated by intestinal menaquinones (Walther and Chollet 2017; Shearer and Newman 2008).

Vitamin K metabolism

In order to understand the multitude of roles that vitamin K has in the human body, it is fundamental to recognize how the various forms of this vitamin are metabolized. Therefore, this section will provide a brief overview of this subject.

Similarly to the other fat-soluble vitamins, vitamin K's metabolic pathway begins with the absorption of dietary vitamin K into the enterocytes and storage into chylomicrons that travel through the lymphatic vessels, before entering the blood via the thoracic duct (Shearer and Newman 2014). Once in the circulation, chylomicrons with vitamin K are transported to tissues and the reminiscent chylomicrons return to the liver. Vitamin K is then transported within the lipophilic core of the various lipoproteins

that result from the metabolism of very low density lipoproteins. This process ends with the cellular uptake of vitamin K via endocytosis (Shearer and Newman 2008). One of the most studied functions of this vitamin is as a cofactor for the microsomal enzyme gamma-glutamyl carboxylase, which transforms selective glutamate (Glu) residues to gamma-carboxyglutamate (Gla) residues on certain proteins (Gla proteins) (Shearer and Newman 2014; WHO and FAO 2001). Vitamin K from diet is generally present in a stable oxidized quinone form, but this carboxylase needs a reduced form of vitamin K, known as vitamin K hydroquinone or quinol (KH_2), as its cofactor (Figure 2). As a result, dietary vitamin K undergoes a reduction process, catalyzed by a vitamin K reductase, into vitamin K quinol, which is then converted to vitamin K 2,3-epoxide, as a by-product of the carboxylation reaction. Vitamin K 2,3-epoxide is reduced back to a quinone form by the enzyme vitamin K epoxide reductase (VKOR), completing what is known as the vitamin K epoxide cycle (Figure 2). This cycle comprises two reductions, performed by distinct reductases and, although the role of VKOR is well established in the literature, the question of which enzyme is responsible for the two-electron reduction of vitamin K to KH_2 is still under research and debate (Shearer and Newman 2014) (Figure 2). This means that vitamin K can be, at least in part, regenerated inside the organism. [Figure 2 near here]

Several coagulation proteins produced in the liver (factor II or prothrombin, factors VII, IX and X, protein S and C) and many additional proteins found in different tissues in the body are, in fact, Gla proteins, or vitamin K-dependent proteins, being activated by the aforementioned carboxylation reaction (WHO and FAO 2001).

Before the discovery of vitamin K-dependent processes in several different tissues in the body, the liver was thought to be the only significant vitamin K's storage site, since it is in the liver that the vitamin K-dependent coagulation factors are produced

(Shearer and Newman 2008). However, more recent studies revealed that this vitamin is widely distributed, with vitamin K1 being preferentially accumulated in the liver, to aid the carboxylation of clotting factors, while vitamin K2 disseminates through the circulation to extra-hepatic tissues, such as the bone, adipose tissue and vasculature (Halder et al. 2019).

Finally, when it comes to the catabolism of vitamin K, both phylloquinone and menaquinones have a common degradative pathway. Their side chain is shortened to two biologically inactive carboxylic acid metabolites, which are then excreted in the urine and, mostly in the bile, as water-soluble conjugates. There is no evidence for an entero-hepatic circulation of vitamin K (WHO and FAO 2001; Shearer, Fu and Booth 2012).

Vitamin K biomarkers

Assessing the correlation between vitamin K status and certain diseases and medical conditions requires methods of measuring the amount of this nutrient available in the body.

Many epidemiological studies have relied heavily on different types of Food Frequency Questionnaires (FFQ) in order to estimate phylloquinone and menaquinone intakes, considering they are cost and time-effective and impose minimal burden on the study participants (Shea and Booth 2016). However, similarly to most diet questionnaires, FFQ use self-report and are, thus, largely subjective and dependent on each individual's memory and perception.

These limitations can be overcome by the measurement of vitamin K status biomarkers in population and clinic-based research, as nutritional biomarkers are independent of recall, interviewer, social acceptance biases and food composition databases. They consist of biochemical compounds that are usually measured in blood,

urine, feces and adipose tissue and, unlike diet questionnaires, they account for the nutrient's bioavailability, absorption and metabolism (Shea and Booth 2016; Potischman 2003). Nevertheless, biomarkers are not perfect: they are affected by each person's health status and they can vary with the time of the day and meal pattern. Some vitamin K biomarkers commonly used in recent years include circulating phylloquinone (the primary circulating form of vitamin K) and undercarboxylated vitamin K-dependent proteins, such as osteocalcin, matrix Gla protein (MGP) and prothrombin. Circulating menaquinones are not routinely adopted as biomarkers, since they are usually not detected in the blood unless supplements have been taken or large quantities of vitamin K2 rich foods have been consumed (Shea and Booth 2016).

Although phylloquinone has been used in many studies around the world to assess vitamin K status, this compound is transported inside triglyceride-rich lipoproteins and its concentration in the blood peaks after 6 to 10 hours post-prandially, changing with dietary intake. Therefore, for a more accurate representation of each individual's vitamin K status, circulating phylloquinone should be measured in a fasting state and triglycerides levels should be taken into consideration (Shea and Booth 2016).

Osteocalcin, also known as bone Gla protein, is the most common non-collagenous protein in the bone matrix and a marker of bone turnover. It is primarily synthesized by osteoblasts during the late stage of their differentiation, where it undergoes a post-translational carboxylation process facilitated by vitamin K. Osteocalcin has three glutamic acid (Glu) residues with the potential to be carboxylated but, in fact, not all bone and serum osteocalcin is fully carboxylated: a small amount of this protein can actually contain some empty Glu residues (undercarboxylated osteocalcin). As a general rule, a higher percentage of undercarboxylated or completely uncarboxylated osteocalcin reflects a low vitamin K status, whereas more carboxylated

osteocalcin is indicative of a higher vitamin K availability (Lin et al. 2018).

Likewise, MGP is a vitamin K-dependent protein secreted by chondrocytes and vascular smooth muscle cells and it is present in the bone, heart, vessels, kidney and cartilage. It requires two post-translational modifications in order to be activated: an initial vitamin K-dependent carboxylation of its five Glu residues, followed by phosphorylation of its serine residues. Active MGP, once released into the extracellular space, acts as a potent vascular calcification inhibitor (Shioi et al. 2020; Wei et al 2019). However, it is the inactive dephosphorylated-uncarboxylated MGP, which is also commonly released into the bloodstream, that is recognized as one of the best biomarkers of vitamin K deficiency, since low vitamin K levels in the organism lead to higher levels of this form of MGP (Shioi et al. 2020).

An interesting detail to take into consideration when using osteocalcin and MGP as biomarkers in research is that the amount of both undercarboxylated/uncarboxylated osteocalcin and dephosphorylated-uncarboxylated MGP in circulation depends on the total amount of osteocalcin and MGP available, respectively. As a consequence, these uncarboxylated serum biomarkers should be expressed as a percentage or ratio of the total or carboxylated protein being measured, which is not done in many studies. This extra step helps to eliminate possible errors, as these non-coagulation vitamin K-dependent proteins can be influenced by conditions that have an impact on total protein concentrations, such as chronic kidney disease, and total MGP, in particular, also increases with age, cardiac dysfunction or any disease characterized by calcification (Shea and Booth 2016).

Undercarboxylated prothrombin, also known as protein induced in vitamin K absence or antagonist-factor II (PIVKA-II) can similarly be measured in the blood to assess vitamin K depletion. Increased PIVKA-II levels are a reflection of less vitamin K

in the body. However, this Gla protein is not particularly sensitive for detecting small variations in normal vitamin K intakes, which limits its use to patients with chronic kidney disease, due to their high prevalence of subclinical vitamin K deficiency (Shea and Booth 2016). PIVKA-II might be a superior biomarker in this context, as it does not change with renal function and dyslipidemia, both of which may confound the other vitamin K biomarkers (Elliott et al. 2014).

Vitamin K deficiency and requirements

Clinically, vitamin K deficiency presents itself as an excessive bleeding susceptibility secondary to the inactivity of the vitamin K-dependent coagulation proteins (Simes et al. 2020). It is much more prevalent in newborns during the first few weeks of life, due to the poor placental transfer of vitamin K, the reduced content of vitamin K in breast milk in comparison to cow's milk-based formula, the liver immaturity that results in an inefficient use and recycling of the vitamin and the low production of menaquinones by the immature gut flora (Schulte et al. 2014). This condition is called vitamin K deficiency bleeding (VKDB) and exclusively breastfed infants have a higher risk of developing the deficiency, since dietary intake is the newborn's major source of vitamin K (Shearer 2009). To diminish its incidence, the World Health Organization (WHO) recommends the administration of a single intramuscular dose of 1 mg of vitamin K to all infants, within the first 6 hours after birth (WHO 2012).

In the rest of the population, vitamin K deficiency is not as common, at least in part due to its recycling, as described above in the metabolism section, but it can appear in certain scenarios: in patients taking antibiotics or other medications that interact with vitamin K, in anyone with a particularly poor vitamin K-content diet and in individuals suffering from gastrointestinal disorders that might compromise the pancreatic and biliary functions, fat and vitamin K absorption, such as celiac disease, ulcerative colitis,

Crohn's disease, cystic fibrosis, cholestasis, short bowel syndrome and even bariatric surgery (Simes et al. 2020). Antibiotics may, sporadically, lead to vitamin K deficiency as they reduce the growth of vitamin K-producing intestinal bacteria and, cephalosporins in particular, can also inhibit the vitamin K-epoxide reductase and vitamin K-dependent carboxylase, increasing the risk of hemorrhagic events (Chen et al. 2016). Treatment with vitamin K antagonists, such as warfarin, a famous anticoagulant widely used to prevent thromboembolic events, also inhibits the enzyme VKOR and, thus, the recycling of vitamin K and the synthesis of the active vitamin K-dependent clotting factors, which can result in hemorrhagic complications. Medications that hinder fat absorption, such as bile acid sequestrants, drugs that interact with lipases activity or cholesterol absorption inhibitors will, equally, affect the absorption of fat-soluble vitamins and vitamin K levels (Simes et al. 2020). Hence, it is critical to pay closer attention to the elderly, especially those who are institutionalized, as poly-pharmacy and malnutrition put them at a greater risk of developing vitamin K deficiency, as well as other vitamin and mineral deficiencies.

Currently, the daily recommended intake for vitamin K is exclusively based on vitamin K1 and it is termed adequate intake (AI), which can be defined as the median of daily intake in healthy individuals (Halder et al. 2019). Since dietary habits and the consumption of vitamin K-rich foods differ by age and region in the world, determining precise dietary guidelines is complicated and has generated controversies regarding the existence of subclinical vitamin K deficiency and the true requirements of this vitamin (Shearer and Newman 2014). As a result, different institutions and organizations have distinct recommendations. In the United States, the National Academy of Medicine (NAM) concluded, in 2001, that the adequate intake of vitamin K is 90 and 120 μg/day for women and men, respectively (Food and Nutrition Board, Institute of Medicine

2001). The WHO and FAO recommend a dosage of 1 µg/day/kg of vitamin K, or approximately 65 µg/day for men and 55 µg/day for women (WHO and FAO 2004). The European Food Safety Authority, following a request from the European Commission, set an AI of 1 µg of phylloquinone per kg per day for all population groups, including pregnant and lactating women, leading to an estimate of 70 µg/day for adults (Turck et al. 2017). Additionally, NAM suggests that the recommended intake of vitamin K1 can be easily achieved by the Western diet, while the dietary intake of vitamin K2 seems to be insufficient and may require supplementation, although this is not specified (Halder et al. 2019).

Vitamin K functions

The most studied function of vitamin K is its role as a cofactor of the microsomal enzyme gamma-glutamyl carboxylase and thus its involvement in the synthesis of Gla proteins.

Up to this point, at least 20 distinct Gla proteins have been identified in humans (Azuma and Inoue 2019). The vitamin K-dependent proteins formed in the liver comprise the coagulations factors II, VII, IX and X and the anti-coagulation proteins C, S and Z. By contrast, the extra-hepatic Gla proteins are widely distributed in the body and are, thus, involved in a broad range of biological functions (Simes et al. 2020). Apart from osteocalcin and MGP, which were already mentioned in the biomarkers chapter, other extra-hepatic vitamin K dependent proteins include growth arrest-specific protein (Gas6), Gla-rich protein (GRP), proline-rich Gla proteins (PRGP1 and 2), transmembrane Gla proteins (TMG3 and 4), periostin, TGFβ induced (TGFβI), androgen receptor and, of course, gamma-glutamyl carboxylase (Simes et al. 2020; Azuma and Inoue 2019).

Osteocalcin is one of the most studied vitamin K-dependent proteins, since it

participates in the pathophysiology of a number of chronic illnesses. It is synthesized by osteoblasts and its gamma-carboxylated Glu residues have a calcium binding site that attracts calcium ions, which are later incorporated in the hydroxyapatite crystals that form the bone matrix (Wen et al. 2018). Therefore, osteocalcin is implicated in bone mineralization and it seems to have a bone protective effect (Azuma and Inoue 2019), but it is also known for its metabolic and cardiovascular functions, improving insulin sensitivity, protecting against atherosclerosis and lowering adipose mass (Silaghi et al. 2019), reinforcing the recent concept of the bone as an endocrine organ.

MGP, as it was previously discussed, acts mostly as a calcification regulator all over the body (Azuma and Inoue 2019). Since hemodialysis patients are prone to early and accelerated vascular calcification and this is a prediction of their all-cause mortality, the potential benefits of vitamin K supplementation in this population have been evaluated in multiple studies (Caluwe et al. 2014; Westenfeld et al. 2012), suggesting that individuals suffering from later-stage chronic kidney disease have higher circulating levels of dephosphorylated-uncarboxylated-MGP and increased renal calcification, which may be prevented with vitamin K administration (Halder et al. 2019).

Gas6, a structurally and functionally similar protein to the vitamin K-dependent protein S, acts as a ligand for the TAM (Tyro3, Axl and Mer) receptor family and is expressed in the lung, heart, kidney, intestine, endothelium, vascular smooth cells, bone marrow, osteoblasts, osteoclasts, monocytes, etc. (Azuma and Inoue 2019). It is involved in a vast range of physiological activities, stabilizing thrombus, having anti-inflammatory properties and being a regulator of proliferation, migration, differentiation, adhesion and apoptosis in different tissues (Simes et al. 2020). Consequently, many studies have been examining the Gas6/TAM pathway in

oncogenesis and cancer therapy, insulin resistance and inflammation (Silaghi et al. 2019; Dihingia et al. 2019).

GRP was, equally, discovered to be a vitamin K-dependent protein and it is mainly found in the skin, cartilage, bone and vascular smooth cells. It is predominantly responsible for suppressing calcification in blood vessels and cartilage, by binding to large amounts of calcium ions due to an exceptionally high number of Gla residues (Azuma and Inoue 2019; Silaghi et al. 2019).

Periostin and TGFβI are homologous molecules that are mostly involved in bone and ligament maintenance and development (Azuma and Inoue 2019). PRGP1, PRGP2, TMG3 and TMG4 are, so far, simply recognized as trans-membrane proteins responsible for signal transduction (Simes et al. 2020). The androgen receptors participate, naturally, in the induction of male sex characteristics, the enhancement of skeletal muscle mass and bone protection (Azuma and Inoue 2019).

Nonetheless, it was more recently found that vitamin K has other modes of function that are independent from the gamma-carboxylation reaction and Gla proteins. For example, some subtypes of vitamin K2 (MK-2, 3 and 4) are ligands for the nuclear steroid and xenobiotic receptor (SXR) and its murine homolog pregnane X receptor (PXR), up-regulating genes associated with detoxification and drug excretion, such as *CYP3A4* and genes that participate in extracellular matrix formation, osteoblastogenesis and osteoclastogenesis (Azuma and Inoue 2019). These findings reveal that vitamin K2 may stimulate bone formation and turnover by altering gene expression and not just via osteocalcin and other vitamin K-dependent proteins, as it was previously expected (Ichikawa et al. 2006). MK-4, in particular, can also induce the phosphorylation of protein kinase A (PKA), leading to increased expression of the *GDF15* and *STC2* genes in osteoblastic cells, affecting osteogenesis and chondrogenesis through an additional

mechanism (Ichikawa et al. 2007). Covalent binding between the epoxide form of MK-4 and the pro-apoptotic protein BCL-2 antagonist killer 1 (BAK) is also able to regulate apoptosis in human promyelocytic cells and osteoblasts (Azuma and Inoue 2019). Furthermore, it was found that vitamin K2 activates 17β-hydroxysteroid dehydrogenase type 4, the enzyme converting estradiol to estrone. Since estrogen has been shown to protect bone tissue, this may be yet another way to explain the apparent beneficial role of vitamin K on the skeleton (Azuma and Inoue 2019).

Interestingly, vitamin K2 has also been studied as a potential anticancer agent, by acting in a number of signaling pathways involving protein kinase A, (PKA), protein kinase C (PKC), nuclear factor kappa B (NF-kB) and SXR (Halder et al. 2019). On a similar note, vitamin K2 may also possess immune-modulatory properties, by regulating the expression of several cytokines and the proliferation of T-cells, as well as a possible protective role in neurological development and diseases (Halder et al. 2019). Vitamin K is also involved in metabolic regulation independently of Gla proteins, as it will be more thoroughly discussed below.

Vitamin K and metabolic syndrome

One of the highlights of vitamin K research in recent years has been the discovery of a beneficial role of this vitamin on cardiovascular diseases and metabolic disorders, namely insulin resistance, T2D and obesity. Since metabolic syndrome is intimately connected with an increased risk of all these conditions, understanding the health properties of this micronutrient may bring a new perspective on potential nutritional treatments in the future. As with any other vitamin K function, its effect on glucose tolerance, blood pressure, adipogenesis and dyslipidemia is determined, not only, by the formation of Gla proteins by a vitamin K-dependent gamma-glutamyl carboxylase, but also by the activation of several pathways that control the homeostasis of the human

body. Metabolic syndrome is equally characterized by a chronic systemic inflammatory state, so the impact of vitamin K on inflammation will also be explored more in-depth below.

Vitamin K, inflammation and oxidative stress

Vitamin K deficiency has long been associated with the development of inflammatory conditions, whether we are talking about cardiovascular diseases, osteoarthritis, osteoporosis, chronic kidney disease or T2D (Simes et al. 2019). Some of these connections have been linked to the vitamin K-dependent proteins Gas6 and GRP.

Gas6 and its receptor, the TAM receptor family, are recognized to be important mechanisms of defense by regulating systemic inflammation and apoptotic cell clearance (Hurtado and de Frutos 2010). Specifically when Gas6 binds to the AXL receptor, they interact with the interferon α and β receptor (IFNAR1), leading to the activation of signal transducer and activator of transcription (STAT) 1, which up-regulates the expression of the intracellular proteins SOCS (supressor of cytokine signaling). The SOCS family is responsible for inhibiting cytokines and Toll-like receptors (TLR). TLR are usually known to induce inflammation by activating NF-kB (Dihingia, Kalita and Manna 2017), a well-known transcript factor that regulates pro-inflammatory pathways that culminate in the expression of cytokines, chemokines and adhesion molecules. Accordingly, some studies have observed that plasma Gas6 concentrations are negatively correlated with tumor necrosis factor (TNF)-α, interleukin (IL)-6 and vascular cell adhesion molecule (VCAM)-1 (Hung et al. 2010; Kuo et al. 2014).

GRP, also known as Ucma (unique cartilage matrix-associated protein), has been especially linked to calcification-induced inflammation. After a vitamin K-dependent carboxylation reaction, active GRP inhibits the osteochondrogenic differentiation of

smooth muscle cells and consequent extracellular matrix calcification, leading to a subsequent reduced expression of cyclooxygenase 2 (COX2) and lower inflammatory markers like TNF-α and prostaglandin E2 (PGE2) (Bordoloi et al. 2018).

However, vitamin K is also associated with anti-inflammatory and antioxidant benefits independently from its action as a cofactor of the gamma-glutamyl carboxylase and the production of Gla proteins (Simes et al. 2019; Manna and Kalita 2016). Ohsaki et al. (2010) suggested that the 2-methyl-1,4-naphtoquinone ring was the structure responsible for this anti-inflammatory activity, directly leading to the inhibition of the NF-kB signaling pathways and, thus, reducing the levels of TNF-α, IL-1, IL-6 and monocyte chemotactic protein (MCP)-1 (Lawrence 2009). This discovery helps to explain the reason why Reddi et al. (1995) found that either vitamin K1, K2 or K3 inhibited IL-6 production in lipopolysaccharide-stimulated human fibroblasts. Although more research is necessary to explain the processes behind the vitamin K inhibition of NF-kB, it appears that this happens through the inactivation of IKK (IkB kinase) and PKC activities, proteins responsible for inhibiting the NF-kB signal transduction cascade (Hodges et al. 2017; Li et al. 2018).

As it will be discussed next, vitamin K seems to be able to suppress hyperglycemia, which is known to be associated with an inflammatory state, making this another anti-inflammatory side-effect from this micronutrient. Therefore, by helping to prevent hyperglycemia, vitamin K inhibits the production of sorbitol and fructose from excess glucose and the synthesis of advanced glycation end-products (AGEs), which usually lead to the production of reactive oxygen species (ROS), oxidative stress and activation of NF-kB (Karamzad et al. 2020). Additional vitamin K antioxidant properties include increasing the activity of superoxide dismutase (SOD) and the levels of reduced glutathione, resulting in lower levels of ROS (Karamzad et al.

2020).

Vitamin K and insulin resistance

Insulin resistance is a metabolic syndrome factor that may predispose to T2D. Several studies have been done regarding insulin resistance and dietary intake or supplementation with both vitamin K1 and K2, and the mechanisms behind this association.

On one hand, dietary and supplemental phylloquinone has revealed mixed results on insulin sensitivity and glucose metabolism. Yoshida et al. (2008) found that daily supplementation with 500 μg of vitamin K1 for 36 months was beneficial for the progression of insulin resistance in older men, but no significant differences in outcome were observed in the female population. Similarly, Kumar, Binkley and Vella (2010) showed that phylloquinone supplementation in healthy postmenopausal women did not change their fasting serum glucose or insulin concentrations. By contrast, Beulens et al. (2010) published a prospective study of a large cohort of 38 094 Dutch men and women, where a significant T2D risk reduction was observed at higher intakes of phylloquinone when compared to menaquinone. This finding was later confirmed in a group of 1925 elderly subjects with a high cardiovascular risk, followed for a median of 5.5 years, that demonstrated a 17% lower risk of T2D for each additional intake of 100 μg of vitamins K1 per day. Furthermore, when compared with subjects who diminished or did not change their phylloquinone intake during follow-up, the subjects who increased their intake had 51% less risk of developing T2D (Ibarrola-Jurado et al. 2012). More recently, a double blind randomized controlled clinical trial by Rasekhi et al. (2015) observed, after a 4-week phylloquinone supplementation period, a decrease in glucose and insulin concentrations and an increase in insulin sensitivity after 2 hours of an oral glucose tolerance test.

On the other hand, research on the effects of vitamin K2 on insulin resistance and T2D seems to conduct to more consistent results. During the prospective cohort study by Beulens et al. (2010) mentioned above, dietary menaquinone intake was also analyzed, and it was observed that it tended to be inversely associated with T2D risk. Choi et al. (2011) reported a higher insulin sensitivity index after vitamin K2 supplementation of young healthy men during 2 weeks.

Both animal and human studies on the underlying mechanisms concerning vitamin K deficiency and insulin resistance suggest that multiple factors might be involved, including osteocalcin, adipokines and the activation of different signaling pathways connected to glucose metabolism, inflammation and oxidative stress (Al-Suhaimi and Al-Jafary 2019; Karamzad et al. 2020).

Osteocalcin, after vitamin K-dependent carboxylation, improves β-cell proliferation, insulin secretion and insulin sensitivity. It is speculated that this stems from osteocalcin directly stimulating β-cells, as well as up-regulating the expression of adiponectin in adipocytes, independently of its effects on β-cells (N. Lee et al. 2007). Subsequently, adiponectin, a well-known adipokine, acts on several tissues.

In skeletal muscle cells, once it binds to adiponectin receptor (AdipoR) 1, adiponectin activates AMP-activated protein kinase (AMPK) and sirtuin (SIRT)-1, important signaling molecules that increase peroxisome proliferator-activated receptor (PPAR)-α and uncoupling protein (UCP)-1 gene expression, leading to an increase in fatty acid oxidation and glucose uptake by glucose transporter type (GLUT)-4 (Karamzad et al. 2020; Li et al. 2018). AMPK also improves glucose uptake and insulin sensitivity by activating insulin receptor substrate (IRS) and the phosphoinositide 3-kinase (PI3K) pathway (Boucher, Kleinridders and Kahn 2014; Karamzad et al. 2020).

Similarly, in hepatocytes, adiponectin connects to AdipoR2, activating the AMPK

and SIRT-1 signaling pathway, which leads, in this case, to an increase in the expression of peroxisome proliferator-activated receptor-gamma co-activator (PGC-1α), up-regulating PPAR-α and down-regulating PTEN and GLUT-2. This process results in the reduction of hepatic glucose output, by increasing glycogenesis and decreasing gluconeogenesis (Karamzad et al. 2020; J. Lee et al. 2018; Udomsinprasert, Honsawek and Poovorawan 2018). Interestingly, vitamin K can directly initiate the SIRT1 and AMPK cascade, independently from adiponectin (Dihingia et al. 2018).

Since higher levels of oxidative stress and inflammation are critical factors in insulin resistance, the anti-inflammatory and antioxidant properties of vitamin K discussed previously should be also taken into consideration when understanding the mechanisms behind vitamin K and glucose metabolism. The effects of dyslipidemia on insulin resistance and their relationship with vitamin K will be explored, in more detail, further below.

Vitamin K and obesity

There are fewer studies analyzing the direct impact of vitamin K on obesity, but since obesity is often associated with a chronic pro-inflammatory state, and adipose tissue is responsible for secreting many inflammatory factors (Knapen, Jardon and Vermeer 2017), this vitamin could be another helpful tool to fight this 21st century epidemic.

Knapen et al. (2011) demonstrated that, in a cohort of 244 healthy postmenopausal women, adiponectin and carboxylated osteocalcin were inversely and independently associated with body weight, body mass index (BMI), waist circumference, waist:hip ratio, fat mass and fat mass of the trunk (FMT). They also observed that body weight and BMI remained unchanged in postmenopausal women who received a 45 mg MK-4 supplement for 3 years, while these parameters increased significantly in the placebo group. More recently, Knapen, Jardon and Vermeer (2017)

conducted a randomized human intervention trial, where a group of middle-aged women received, either, 180 μg of MK-7 per day or placebo, for a 3-year period. Only the women with an above-median response to supplementation saw a significant increase in adiponectin and a reduction of abdominal fat mass, when compared with the placebo group and the poor responders. At baseline, the women with low plasma concentrations of carboxylated osteocalcin (suggesting a poor vitamin K status) had a higher waist circumference and a higher fat mass in the android region. Similarly, a prospective assessment of morbidly obese patients before bariatric surgery showed that vitamin K insufficiency was present in 40% of them, despite a clear calorie excess intake (Ewang-Emukowhate et al. 2014).

In contrast, a 3-year randomized controlled trial by Shea, Dawson-Hughes et al. (2016) did not notice any changes in fat mass between the vitamin K supplementation group and controls, both in men and women, even though the supplemented subjects had a substantial reduction in uncarboxylated osteocalcin over the years. However, the supplement used in this trial was phylloquinone, and not menaquinone, like the previous studies mentioned.

Despite the evidence suggesting a beneficial role of vitamin K2 on body weight and fat mass, there is less research exploring the underlying mechanisms of this association. One of the proposed mechanisms is that vitamin K increases carboxylated osteocalcin and adiponectin levels, activating the AMPK/SIRT-1/PGC-1α pathway in the liver. PGC1α is a known regulator of energy metabolism, promoting fatty acid oxidation, and its expression is reduced in obesity (Crunkhorn et al. 2007; Liang and Ward 2006). A few studies also suggest a connection between obesity and Gas6 and its Axl receptor (Dihingia, Kalita and Manna 2017; Wu et al. 2015). Since obesity is considered to be a chronic inflammatory disease, the anti-inflammatory properties of

vitamin K are equally beneficial for this condition (Wu et al. 2015).

Vitamin K and dyslipidemia

Reduced HDL cholesterol and elevated triglycerides are markers of dyslipidemia and two of the five signs of metabolic syndrome. Several studies have shown a favorable impact of vitamin K2 supplementation on lipids metabolism.

Kawashima et al. (1997) found that daily vitamin K2 supplementation of hypercholesterolemic rabbits significantly decreases their plasma total cholesterol levels and suppresses the progression of atherosclerotic plaque, intimal-thickening, pulmonary atherosclerosis and ester-cholesterol deposition in the aorta. These findings were further consolidated in another animal study, by Sogabe et al. (2011), that reveals a major reduction in serum triglycerides and total fat accumulation in a group of rats fed a diet supplemented with both phylloquinone and MK-4, when compared with controls. In a 10-year follow-up study with 625 adult participants, V. Dam et al. (2015) managed to extrapolate the previous outcomes in animals to the human population, showing that high intakes of menaquinone and a high vitamin K status were associated with a lower occurrence of metabolic syndrome. These correlations were mainly driven by the effects of vitamin K2 on triacylglycerol and waist circumference.

While the effects of vitamin K2 on dyslipidemia have been consistent so far, conclusions on vitamin K1 remain unclear. In 2015, a double blind placebo controlled trial in women with rheumatoid arthritis found no significant differences on the lipid profile of those who took a phylloquinone supplement (Kolahi et al. 2015), but the subject requires further investigation.

PPAR-α is a major regulator of lipid metabolism in the liver, being the target of fibrates. These pharmaceutical drugs are used to treat dyslipidemia, since they tend to lower plasma triglyceride and elevate HDL cholesterol levels. Vitamin K might be

implicated in this process since this vitamin stimulates the hepatic AMPK/SIRT-1 pathway, which leads to an increase of PPAR-α expression and a decrease in lipid accumulation (Yoon 2009). Nevertheless, more research is necessary regarding the processes by which vitamin K improves dyslipidemia.

Vitamin K and hypertension

Beyond its role on inflammation and metabolic disorders, vitamin K is also involved in vascular calcification. And, although there have been numerous studies addressing the beneficial effects of this micronutrient on cardiovascular health, not many have focused, specifically, on blood pressure. However, there is clear evidence demonstrating that mineral deposition in the vasculature, which leads to calcification in valves and vessels, is linked with vascular stiffness and increased blood pressure (Jensky et al. 2010; van Ballegooijen et al. 2017).

One of the few population-based studies devoted to this particular issue analyzed the impact of both vitamin D and vitamin K on hypertension and found that the combination of low amounts of those two vitamins in the body was associated with significantly higher systolic and diastolic pressure and increased hypertension risk (van Ballegooijen et al. 2017). In 2017, another study highlighted that low plasma phylloquinone was connected to an increased risk of cardiovascular disease among individuals treated for hypertension (Shea, Booth et al. 2017).

Although we still need further clinical trials and observational studies regarding the putative influence of vitamin K on blood pressure, the mechanism underlying this correlation is somewhat understood. Vascular calcification can occur due to intimal atheromatosis, or in the medial layer of vessels by a gradual phenotypic change of the vascular smooth muscle cells into osteoblasts (Roumeliotis 2019). MGP, one of the aforementioned vitamin K-dependent proteins, prevents this osteoblast transformation

of smooth muscle cells within the arterial wall, being considered the most powerful calcification inhibitor in the human body, not only inhibiting, but also reversing the calcification process (Roumeliotis 2019). In order to function, MGP must be activated by going through gamma-carboxylation, which is dependent on vitamin K as an enzymatic substrate. Therefore, vitamin K deficiency leads to higher amounts of inactive MGP in the body, accelerating vascular calcification (Chirinos et al. 2018; Roumeliotis 2019). Besides MGP, other Gla proteins, such as osteocalcin and GRP, are also beneficial against vascular calcification and atherosclerosis, but to a lesser extent (Azuma and Inoue 2019; Silaghi et al. 2019). As it was briefly mentioned before, all these molecules are able to inhibit calcification through their inner ability to bind to calcium ions to their Gla residues.

Conclusion

Both phylloquinone and menaquinone, the two naturally occurring subtypes of vitamin K, seem to be beneficial against metabolic syndrome and its components, whether through dietary intake or supplementation. This results from the actions of the various vitamin K-dependent proteins and intracellular signaling pathways associated with vascular health, fat and glucose metabolism, inflammation and oxidative stress, which are all interconnected. However, there is still plenty of room for research in this area, especially regarding the repercussions of the current body of evidence in clinical practice, since the major health organizations in the world established an adequate intake of vitamin K for the general population only based on vitamin K1 and its role on coagulation. The present overview of the effects of vitamin K on metabolic syndrome will, hopefully, provide helpful information for the development of future adjuvant therapies for those who suffer from T2D, obesity, dyslipidemia and hypertension.

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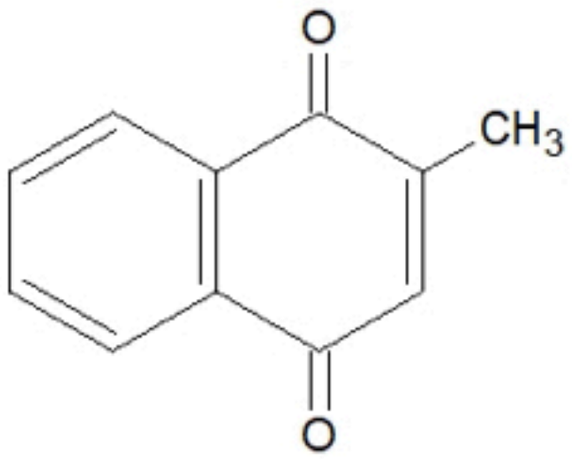
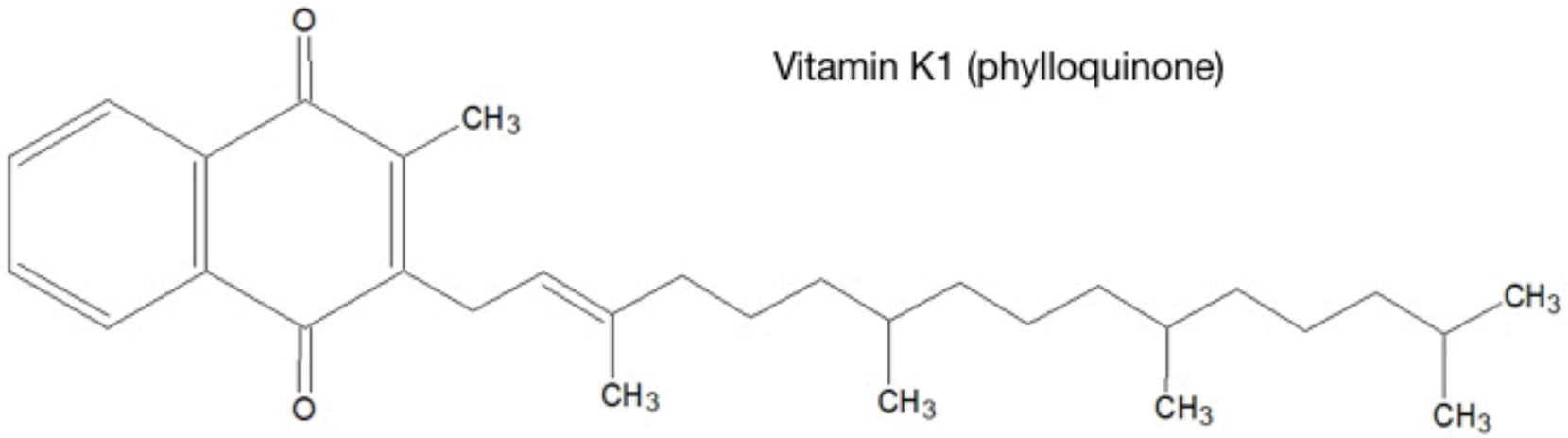
Figure 1. Chemical structure of vitamins K1, K2 and K3.

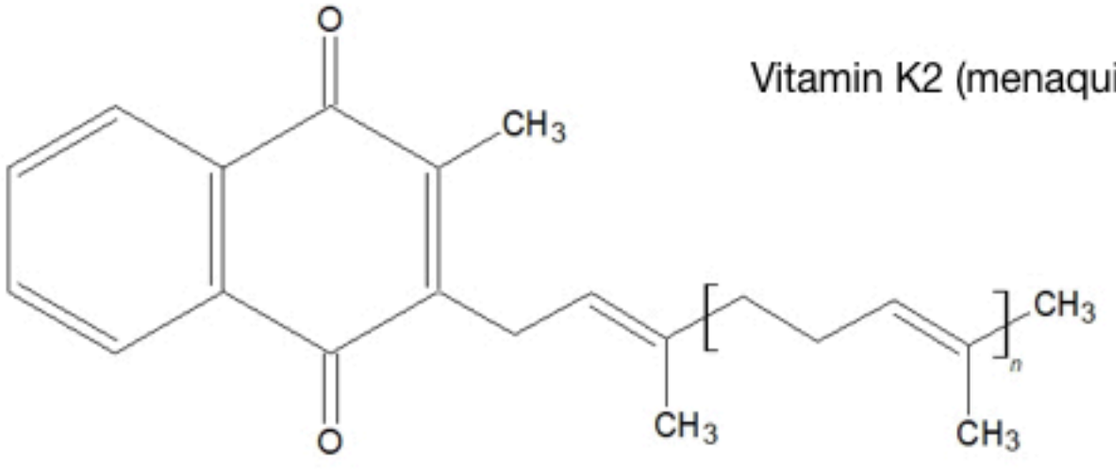
Figure 2. Vitamin K epoxide cycle.



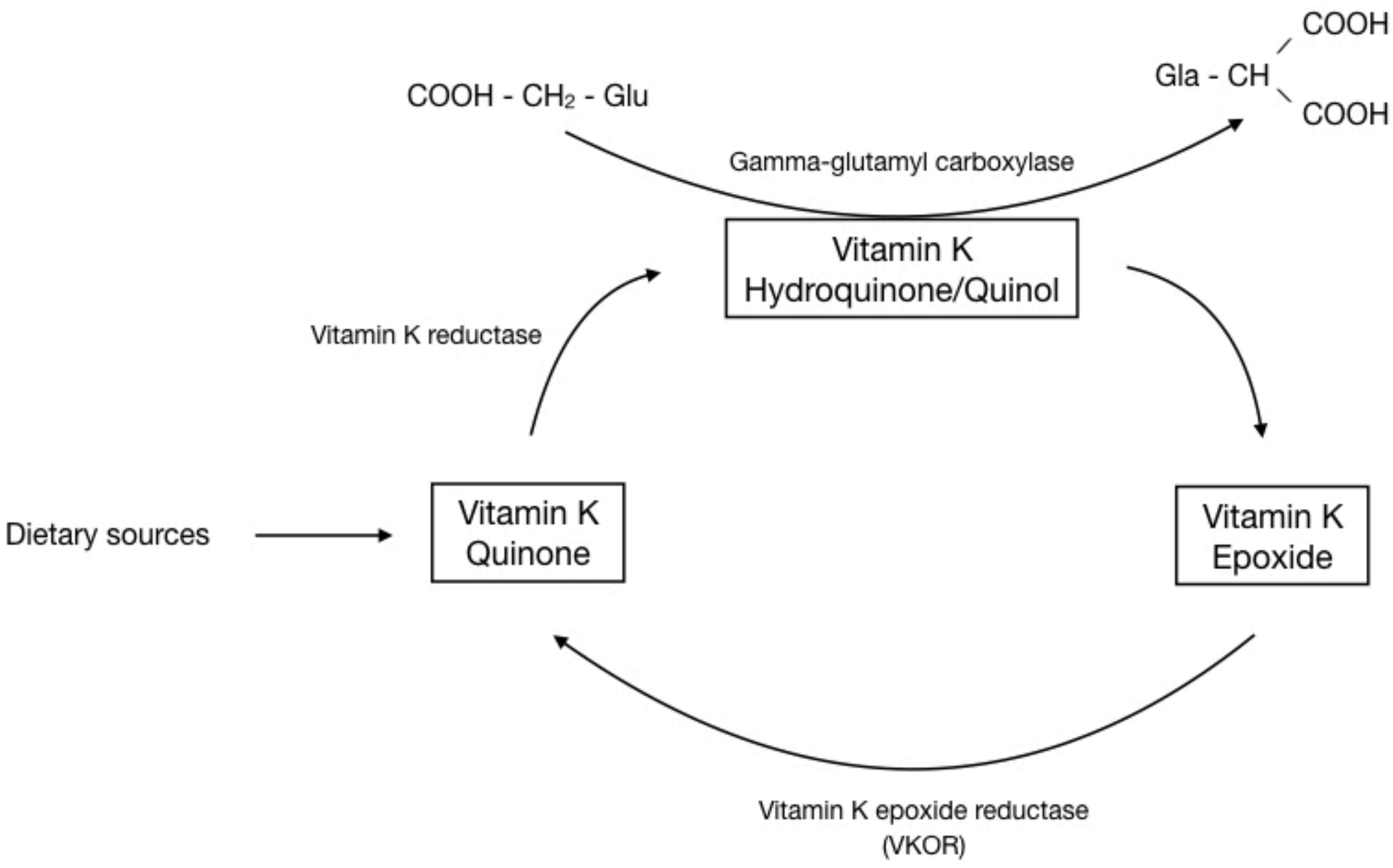
Vitamin K3 (menadione)



Vitamin K1 (phylloquinone)



Vitamin K2 (menaquinone-n, MK-n)


COOH - CH2 - Glu
Gla - CH
COOH
COOH
Gamma-glutamyl carboxylase
Vitamin K
Hydroquinone/Quinol
Vitamin K reductase
Dietary sources
Vitamin K
Quinone
Vitamin K
Epoxide
Vitamin K epoxide reductase
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Book with titled volume of multivolume work and edition model	Format Author, A., B. Author, C. Author, and D. Author. yyyy. *Volume title*. Vol. # of *Title of the multivolume work*. # ed. City, State Abbr./Country [optional]: Publisher Name. Example Bowlby, J. 1982. *Loss: Sadness and depression.* Vol. 3 of *Attachment and loss*. 3rd ed. New York: Basic Books.

Book with translator and edition model	Format Author, A., B. Author, C. Author, and D. Author. yyyy. *Title of the book*. Trans. A. Name and B. Name. City, State Abbr./Country [optional]: Publisher Name. Example Wittgenstein, L. 1968. *Philosophical investigations.* Trans. G. E. M. Anscombe. 3rd. ed. London: Macmillan.
Book with non-English title model	Format Author, A., B. Author, C. Author, and D. Author. yyyy. *Original title of the book* [Author-supplied English translation of title]. City, State Abbr./Country [optional]: Publisher Name. Example Hupfer, P. 1996. *Unsere Umwelt: Das Klima—Globale und lokale Aspekte* [Our environment: The climate—Global and local aspects]. Stuttgart: Teubner Verlag.
Introduction, preface, or foreword in book model	Format Introducer, A., and B. Introducer. Yyyy. Introduction | Preface | Foreword. In *Title of book*, by A. Author and B. Author, ###–###. City, State Abbr./Country [optional]: Publisher Name. Example Yurick, S. 1972. Introduction. In *Studies and further studies in a dying culture*, by C. Caudwell, 5–29. New York: Monthly Review Press.
Edited book, cited in full, model	Format Editor, A., B. Editor, C. Editor, and D. Editor, ed(s). yyyy. *Title of the book.* City, State Abbr./Country [optional]: Publisher Name. Example Nagle, S. J., and S. L. Sanders, eds. 2003. *English in the Southern United States*. Cambridge, UK: Cambridge University Press.

Chapter in edited book model	Format Author, A., B. Author, C. Author, and D. Author. yyyy. Title of book chapter. In *Title of the book*, ed. A. Editor and B. Editor, ###–###. City, State Abbr./Country [optional]: Publisher Name. Example Gordon, S., and D. Lavallee. 2004. Career transitions in competitive sport. In *Sport psychology: Theory, applications and issues,* ed. T. Morris and J. Summers, 584–610. 2nd ed. Brisbane: Wiley.
Chapter in edited book with volume and edition model	Format Author, A., B. Author, C. Author, and D. Author. yyyy. Title of book chapter. In *Title of the multivolume work,* ed. A. Editor and B. Editor, vol. #, # ed., ###–###. City, State Abbr./Country [optional]: Publisher Name. Example Remael, A. 2012. Audiovisual translation. In *Handbook of translation studies,* ed. Y. Gambier and L. van Dooslaer, vol. 1, 2nd ed., 12–17. Amsterdam: John Benjamins.
Chapter in edited book with translator model	Format Author, A., B. Author, C. Author, and D. Author. yyyy. Title of the chapter, trans. A. Translator and B. Translator. In *Title of the book*, ed. A. Editor and B. Editor, ###–###. City, State Abbr./Country: Publisher Name. Example Piaget, J. 1995. Logical operators and social life, trans. W. Mays. In *Sociological studies*, ed. L. Smith, 134–57. London: Routledge.
Encyclopedia, entry without author listing, model	Format Title of the entry. In *Title of the book*, ed. A. Editor, B. Editor, C. Editor, and D. Editor, eds. yyyy. City, State Abbr./Country [optional]: Publisher Name. Example Mexico. In *Encyclopedia of Latin American theater,* ed. Cortés, E., and M. Barrea-Marlys, 278–327. 2003. Westport, CT: Greenwood Press.

Journal	
Journal article model	Format Author, A., B. Author, and C. Author. yyyy. Title of the article. *Journal Title* ## (#):####–####. doi: ############## [optional]. Example Xie, R.-F., B.-R. Yang, P.-P. Cheng, S. Wu, Z.C-. Li, J.-Y. Tang, S. Li, N. Tang, S. M. Y. Lee, Y.-H. Wang, et al. 2015. Study on the HPLC chromatograms and pro-angiogenesis activities of the flowers of *Panax notoginseng*. *Journal of Liquid Chromatography & Related Technologies* 38:1286–95. doi: 10.1080/10826076.2015.1037451.
Online first publication model	Format Author, A., B. Author, and C. Author. yyyy. Title of the article. *Journal Title*. Advance online publication. doi: ############## [optional]. Example Ohba, Y., T. Nakajima, M. Kanda, H. Hayashi, Y. Matsushima, Y. Nakagawa, H. Koike, C. Nagano, K. Sekimura, K. Otsuka, et al. 2018. Simultaneous determination of nine acaricides and two metabolites in comb honey by LC/MS/MS. *Food Additives & Contaminants: Part A*. Advance online publication. doi: 10.1080/19440049.2018.1539252.

Journal article with non-English title and e-location model	Format Author, A., B. Author, C. Author, and D. Author. yyyy. Original title of the article [Author-supplied English translation of title]. *Journal Title* ## (#):####–####. doi: ########### [optional]. Example Hadeid, M., S. A. Bellal, T. Ghodbani, and O. Dari. 2018. L'agriculture au Sahara du sud-ouest algérien: entre développement agricole moderne et permanences de l'agriculture oasienne traditionnelle [Agriculture in the Algerian south-west Sahara: Between modern agricultural development and traditional oasis agriculture permanencies]. *Cahiers Agricultures* 27 (1):15005. doi: 10.1051/cagri/2017060.
In-press journal article model	Format Author, A., B. Author, C. Author, and D. Author. Forthcoming. Title of the article. *Journal Title*. Example Bustamante, D. E., B. Y. Won, S. C. Lindstrom, and T. O. Cho. Forthcoming. The new genus *Symphyocladiella gen. nov.* (Ceramiales, Rhodophyta) based on *S. bartlingiana comb. nov.* from the Pacific Ocean. *Phycologia*.
Conference	
Serial proceedings model	Format Author, A., B. Author, and C. Author. yyyy. Title of paper/presentation. *Serial Proceedings/Journal Title* ## (#):####–####. doi: ########### [optional]. Example Svit, K., D. Protasov, S. Teys, L. Sveshnikova, Y. Maksim, and K. Zhuravlev. 2016. Peculiarities of CdS nanocrystal formation at annealing of a Langmuir-Blodgett matrix. *Physica Status Solidi C* 13:417–20. doi: 10.1002/pssc.201510285.

Non-serial proceedings model	Format Author, A., B. Author, C. Author, and D. Author. yyyy. Title of paper/presentation. In *Title of the Proceedings*, ed. A. Editor and B. Editor, ###–###. City, State Abbr./Country [optional]: Publisher Name. Example Mazzone, A., C. Spagno, and A. Kunz. 2004. The HoverMesh: A deformable structure based on vacuum cells. In *ACE '04: Proceedings of the 2004 ACM SIGCHI International Conference on Advances in Computer Entertainment Technology*, ed. R. Nakatsu, M. Billinghurst, and G. Yu, 187–93. New York: ACM Press.
Paper presentation model	Format Author, A., B. Author, C. Author, and D. Author. yyyy. Title of the presentation. Paper presented at Conference Name, Conference City, State Abbr./Country [optional], Month dd. Example Alfermann, D., and A. Gross. 1997. Coping with career termination: It all depends on freedom of choice. Paper presented at the 9th annual World Congress on Sport Psychology, Netanya, Israel, January 23.
Poster model	Format Author, A., B. Author, C. Author, and D. Author. yyyy. Title of the poster. Poster presented at Conference Name, Conference City, State Abbr./Country [optional], Month dd. Example Mack, L. 2004. Beyond BI: Interdisciplinary campus connections that market and strengthen the small music library. Poster presented at the Music Library Association Annual Meeting, Arlington, VA, February 13.
Thesis	

Thesis model	Format Author, A. yyyy. Title of dissertation or thesis. Diss. or thesis type, Institution Name. Example Allison, N. 1981. Bacterial degradation of halogenated aliphatic acids. PhD diss., Trent Polytechnic.
Internet	
Webpage model	Format Author, A. yyyy. Online site or webpage title. Last Modified Month dd, yyyy [Optional]. Accessed Month dd, yyyy. http://XXXXXX.XXX|https://XXXXXX.XXX. Example Harris, B., and S. Zucker. Haussmann the demolisher and the creation of modern Paris. Last Modified August 9, 2015. Accessed November 16, 2018. https://smarthistory.org/haussmann-the-demolisher-and-the-creation-of-modern-paris.
Electronic mailing list	Do not include in reference list but cite in text Format ("Name of List," Month dd, yyyy, e-mail) Example ("OCE Informational Listserv," December 1, 2015, OCENEWSLETTER@listserv.nsf.gov)
Blog	Do not include in reference list but cite in text: Format (A. Blogger, "Title of Blogpost," Month dd, yyyy, http://XXXXXX.XXX|https://XXXXXX.XXX) Example (Mark Lorch, "What Links Self-Heating Drinks and the D-Day Landings?", June 22, 2018, http://www.chemistry-blog.com/2018/06/22/what-links-self-heating-drinks-and-the-d-day-landings/)

Newspaper or magazine	
Print publication model	Format Author, A., B. Author, and C. Author. yyyy. Title of article. *Name of Newspaper,* Month dd. Example Protzman, F. 1989. Clamor in the East: East Berliners explore land long forbidden. *New York Times*, November 10.
Online publication model	Format Author, A., B. Author, C. Author, and D. Author. yyyy. Title of article. *Name of Newspaper,* Month dd. Accessed Month dd, yyyy. http://XXXXXX.XXX|https://XXXXXX.XXX. Example Protzman, F. 1989. Clamor in the East: East Berliners explore land long forbidden. *New York Times*, November 10. Accessed November 12, 2018. https://www.nytimes.com/1989/11/10/world/clamor-in-the-east-east-berliners-explore-land-long-forbidden.html.
Report	
Print model	Format Author, A., B. Author, C. Author, and D. Author. yyyy. Title of paper or report. Report /Paper No. ###, Agency Name, City, State Abbr./Country [optional]. Example Lempert, R. J., P. Norling, C. G. Pernin, S. A. Resetar, and S. Mahnovski. 2003. Next generation environmental technologies: Benefits and barriers. MR-1682-OSTP, Science and Technology Policy Institute, RAND, Arlington, VA.

Online model	Format Author, A., B. Author, C. Author, and D. Author. yyyy. Title of paper or report. Report /Paper No. ###, Agency Name, City, State Abbr./Country [optional]. Accessed Month dd, yyyy. http://XXXXXX.XXX|https://XXXXXX.XXX. Example Warner-Griffin, C., H. Liu, C. Tadler, D. Herget, and B. Dalton. 2017. Reading achievement of U.S. fourth-grade students in an international context: First look at the Progress in International Reading Literacy Study (PIRLS) and ePIRLS 2016. NCES 2018-017, Institute of Education Sciences, National Center for Education Statistics, Washington, DC. Accessed September 8, 2018. https://nces.ed.gov/pubs2018/2018017.pdf.
Personal communication	
Personal Letter, telephone conversation, or email	Do not add personal communications to the reference list. Instead, cite personal communication in the running text: … as mentioned in an e-mail sent to me by Geoffrey Harpham on August 3, 2001, … The personal communication can also be cited parenthetically Format (A. Contact, type of communication, Month dd, yyyy) Example (Geoffrey Harpham, e-mail to author, August 3, 2001)
Other reference types	

Document in archive model	Format Author, A., and B. Author. yyyy. Title of document | Descriptive label for document. Collection Name. ###### [accession details]. Archive/Library Name. Example Long, H. P. 1935. Our blundering government. William B. Wisdom Collection on Huey P. Long, 1924–1975. LaRC/Manuscripts Collection 282, box 1, folder 1. Louisiana Research Collection, Tulane University.
Personal letter in archive model	Format Sender, A. yyyy. Letter to A. Recipient and B. Recipient, dd Month. Collection Name. ###### [accession details]. Archive/Library Name. Example Toole, J. K. 1962. Letter to J. D. Toole and T. Toole, 6 August. John Kennedy Toole Papers, 1930–1999. LaRC/Manuscripts Collection 740, box 1, folder 2. Louisiana Research Collection, Tulane University.
Patent model	Format Inventor, A., B. Inventor, C. Inventor, and D. Inventor. ####. Title of patent. Patent Code ########, filed Month ##, ####, and issued Month ##, ####. Example Pfeifer, A., A. Muhs, M. Pihlgren, O. Adolfsson, and F. Van Leuven. 2012. Humanized tau antibody. US Patent 9,657,091, filed April 5, 2012, and issued May 23, 2017.
Computer software with developer model	Format Developer, A., B. Developer, C. Developer, and D. Developer. yyyy. *Title of Program* (version #.#). City, State Abbr./Country [optional]: Producer Name. Example Noguera, J., and C. Cumby. 2017. *SigmaXL* (version 8.0). Kitchener, Canada: SigmaXL, Inc.
Computer software without developer model	Format *Title of Program* (version #.#). yyyy. City, State Abbr./Country [optional]: Producer Name. Example *SPSS Amos* (version 22.0). 2013. Armonk, NY: IBM.

Film (DVD) model	Format *Title of film.* [yyyy] yyyy. Medium [optional]. Dir. by A. Director. City, State Abbr./Country [optional]: Studio/Distributor Name. Example *Citizen Kane.* [1941] 2016. DVD. Dir. O. Welles. Burbank: Warner Bros. Home Entertainment.
Television episode (recorded) model	Format Title of episode. [Month dd, yyyy] yyyy. Dir. A. Director. Writ. A. Writer and B. Writer. In *Title of series/collection.* Medium [optional]. City, State Abbr./Country [optional]: Studio/Distributor Name. Example Eye of the beholder. [November 11, 1960] 2016. Dir. D. Hayes. Writ. R. Serling. In *The Twilight Zone: The complete series.* DVD. Los Angeles: Paramount Home Media.
Song (album recording) model	Format Composer, A., and B. Composer. yyyy. Title of song. In *Title of album.* Medium [optional]. Rec. A. Artist. City, State Abbr./Country [optional]: Recording Manufacturer Name. Example Cohen, L., and S. Robinson. 1988. Everybody knows. In *I'm your man.* CD. Rec. L. Cohen. New York: Columbia.
Dataset model	Format Researcher, A., B. Researcher, and C. Researcher. yyyy. Title of dataset: Subtitle [dataset]. Name of Archive/Repository/Database. Accessed Month dd, yyyy. http://dx.doi.org/XXXXXXXXXX OR https://doi.org/XXXXXXXXXX OR https://[non-DOI URL]. Example Wang, Guang-Yan, Zhao-Ming Zhu, Shan Cui, and Jin-Hui Wang. 2017. Data from: Glucocorticoid induces incoordination between glutamatergic and GABAergic neurons in the amygdala (dataset). Dryad Digital Repository. Accessed December 22, 2017. https://doi.org/10.5061/dryad.k9q7h.

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