The Synergy of Three Forms of Vitamin K
New Awareness for Many Important Roles of Vitamin K
by Cristiana Paul, MS

A newly defined vitamin K deficiency may impair many metabolic functions. [18]
Adequate vitamin K supplementation may help PREVENT or REVERSE osteoporosis,
arterial calcification and stiffness and optimize many other aspects of health! [23]

**Designs for Health Tri-k** provides a balance of three naturally occurring forms of vitamin K and their amounts per capsule are:
- Vitamin K1 (phytonadione or phylloquinone) 1mg (1000mcg)
- Vitamin K2(MK-4 form) (menaquinone-4) 1mg (1000mcg)
- Vitamin K2(MK-7 form) (menaquinone-7) 50mcg

One capsule of **Tri-k** is a clinically useful dose, which can be used to correct subclinical vitamin K deficiency or at therapeutically higher levels to address specific conditions.

**Soft and calcified plaque formation**

**Normal and osteoporotic bone**

There is a paradigm shift in our understanding of the physiological role of vitamin K that now goes well beyond that of blood clotting.

**Vitamin K1 and/or K2 supplementation studies have suggested that it may produce the following benefits:**
- Increased BMD (bone mineral density) (by maximizing calcium deposition in the bone)[10, 11, 22, 48]
- Reduced risk of fracture (by improving bone architecture) [5,6, 22]
- Inhibition of bone resorption (by reducing formation of osteoclasts, the cells that break down bone) [22]
- Increased peak bone mass during development [55, 56, 57]
- Enhancement of tooth mineralization and proper craniofacial development [59]
- Prevention and reversal of arterial calcification, stiffness and possibly hypertension [10, 17, 68, 73, 74].
- Reduced arterial plaque progression, arterial wall (intima) thickening and lipid peroxidation [50]
- Reduced inflammation (PGE2, COX-2 [3], IL-6 [30]) and symptom relief in rheumatoid arthritis [20]
- Reduced adipogenesis [105] (the production of new fat cells)
- Increased apoptosis (death) of cancer cells, as in myeloproliferative diseases including leukemia [34]
- Reduced risk of developing hepatocarcinoma arising from Hepatitis C [34]
- Brain and nerve myelination support [38] (maintenance of normal lipid sulfatide metabolism)
- Reduced severity of cystic fibrosis [85] (1mg vitamin K1 was used to compensate for carboxylation defects)

**Vitamin K deficiency may have the following consequences:**
- Increased risk of calcification inside arterial walls and heart valves, especially when supplementing with vitamin D (Vitamin D increases the absorption and transport of calcium, which in turn requires adequate amounts of vitamin K to activate the MGP proteins that reject calcium deposition in soft tissues, including arteries) [49],
- Increased risk of hypertension (calcification of the medial arterial muscle cells and elastic fibers [73, 74])
- Increased risk of calcification of varicose veins [37], kidney and muscle [42] and other soft tissues.
- Increased cartilage calcification [32], affects cartilage maturation (excessive growth plate mineralization) [33, 62, 65], contribution to the age-related degeneration of the intervertebral disks (increased calcified/uncalcified cartilage ratio) [61, 66]. Reduced glucosamine/glycosaminoglycans synthesis [54].
- Increased osteoarthritis disease activity [31] and CRP (C-Reactive Protein, a marker of inflammation)[104]
- Increased risk of hemorrhage (i.e., excessive menstrual, gum or nose bleeding) [92]
- Increased risk of hemorrhage in infants breastfed by vitamin K deficient mothers [81, 82, 83, 84]
- Impaired insulin secretion [21]
- Increased risk of kidney stone formation [35, 36]
- Increased skin collagen breakdown [51] and elastic fibers calcification [52] along with reduced ground substance synthesis [54]. Vitamin K dependent proteins (MGP) are found in dermis and epidermis and control collagen expression and hydrolysis as well as glycosaminoglycans biosynthesis, which constitutes a supporting layer for the skin and other epithelial surfaces, such as the gastrointestinal lining.
- Suboptimal energy production [95] 10% reduction in muscle creatine kinase, 20% reduction in intestinal mucosa alkaline phosphatase may be due to structural mitochondrial alteration when deficient in vitamin K.
Optimal dose and administration of vitamin K
Research has been accumulating for more than 10 years that suggests a need for redefining the optimal recommended intake of vitamin K to a higher level than the current Adequate Intake (AI) of 90/120mcg (women/men). [48]. The best evidence to date for an optimal intake of vitamin K suggests 1-2mg K1/day [24].

Aging, poor conversion of K1 to K2, genetic polymorphism affecting vitamin K activation or action [87], and severe vitamin K related conditions, may require higher doses of K1 and additional K2 [23, 44, 75]. Most patients might need only one capsule of Tri-K per day (taken with a fatty meal), if their diet and other supplements do not provide an adequate amount of vitamin K.

Tri-K may be used in conjunction with other DFH products that contain significant amounts of vitamin K: OsteoForce (vitamin K1=1mg) Vitamin D Synergy (200mcgK1), Vitamin D Supreme (500mcg K1+50mcgK2) and/or PaleoGreens (contains a variable amount of vitamin K because it is composed of extracts of vegetables). Even though vitamin K1 and K2 (MK-4) are a fat soluble vitamins, their plasma half life is relatively short (around 2-8hrs), and their effects on activating important proteins in the body may only be maximal for about 8-12 hours after supplementation [26]. Supplements that contain Vitamin K, such as OsteoForce and Vitamin D Synergy, should be administered in divided doses, as evenly as possible throughout the day. Vitamin D supreme and Tri-K, which contain K2(MK-7), may be administered once a day because K2(MK-7) has a very long plasma half life.

Tri-K may be dosed at more than one capsule per day by healthcare practitioners, based on various clinical considerations. For example, some elderly patients that have severe osteoporosis or vascular calcification may benefit from 2 or more capsules of Tri-K per day. Anything above 2 caps per day may be considered well above a normal physiological dose, similar to the15-45mg dose of K2 (MK-4) used in Japanese studies [3] and should be used with caution. At this time, it is unclear what is the optimal requirement for vitamin K is for pregnant and lactating women, but some studies have successfully used K1 at 10-20mg pre-birth [81] and 5-20mg during lactation [82, 83, 84] in order to prevent risk of hemorrhage in infants.

If fat digestion is impaired take vitamin K with DFH Phosphatidylcholine, Phosphatidylserine, LV-GB, Digestzyme or PaleoMeal (contains phosphatidylcholine).

Is it safe to take vitamin K at levels higher than AI (Adequate Intake)? Will it increase clotting?
The AI (Adequate Intake) for vitamin K (90-120mcg) is sufficient for activating the liver enzymes involved in the carboxylation of the clotting proteins. When the body receives an amount of vitamin K that is well above the level needed for clotting protein activation (90-120mcg), for example 1-3mg, the liver secures the amount of vitamin K needed for carboxylating its clotting factors and the rest of the vitamin K is distributed to other tissues in the body. Once the liver clotting factors are maximized in function (by complete carboxylation), no amount of excess vitamin K can increase clotting performance any further.[10, 11, 23, 50, 101]. Therefore, it is safe to say that clotting is not enhanced by vitamin K intakes above those necessary for optimal clotting function.

Drug Interactions
Anticoagulants designed as vitamin K antagonists (such as warfarin / Coumadin) should not be taken with Tri-K or any products containing vitamin K. However, vitamin K does not interfere with the action of blood thinners such as heparin, antiplatelet agents (such as aspirin, Plavix, clopidogrel, abciximab, tirofiban, and eptifibatide), direct thrombin inhibitors (hirudin, argatroban), or thrombolytic agents (clot dissolving proteolytic enzymes) [96, 97, 98]. Anticoagulants that interfere with vitamin K were shown to cause osteoporosis or increase risk of fracture [78], increase arterial or heart valve calcification [17] and hypertension [86]. Some researchers recommend direct thrombin inhibitors as a safer substitute to these anticoagulants [79]. It is important to note that in addition to their anti-platelet effects, aspirin (acetyl-salicylic acid) and other salicylate-containing drugs, slightly inhibit vitamin K activation and recycling, thus creating an increased demand for vitamin K. The result is reduced thrombin formation (an effect similar to warfarin / Coumadin) [90, 91, 94]. Vitamin K supplementation may overcome this effect, while not interfering with their antiplatelet (COX inhibition) action [93]. Aspirin was shown to increase bone loss and reduce fracture healing, which may be due to its effect of vitamin K metabolism, thus vitamin K supplementation may be warranted whenever aspirin or other salicylate-derived drugs are administered on a long term basis [98,99]. Antibiotics may increase the need for vitamin K supplementation because: 1) they may kill gut bacteria that normally produce vitamin K, 2) they may interfere with vitamin K activation and recycling (similar to anticoagulants), 3) they may inhibit the activation of various proteins (carboxylation) by vitamin K.[90, 77]. Examples of such antibiotics are broad spectrum cephalosporins, such as cefamandol, moxalactam and cefoperazone.

High dose vitamin E supplementation (above 1000 IU’s) was shown to impair blood clotting by interfering with the vitamin K-dependent carboxylation reactions. [67].

Tests for Vitamin K status
Fasting plasma vitamin K1 levels are not a complete indicator of vitamin K status because they only reflect the previous day’s intake. Vitamin K is metabolized at various rates depending on the form; K1 (6-12 hrs), K2(MK-4)/(2-4hrs) or K2(MK-7) (3-6 days). Vitamin K is easily metabolized throughout several days and it does not accumulate in the body [19, 26, 27, 28]. Plasma and urine % undercarboxylated osteocalcin is the best functional measure of vitamin K status, but are not yet commercially available. Also, “apparently healthy populations” were found to have an average 30% uncarboxylated osteocalcin, and similar findings for MGP (Matrix Gla Protein), which indicate a widespread subclinical deficiency of vitamin K [66].
Vitamin K occurs in nature in two basic forms K1 and K2, which are similar in the fact that they contain the same core molecule called naphthoquinone. K1 contains an additional phytail tail. K2 occurs in a variety of forms called menaquinones that contain additional unsaturated side-chains of isoprenoid units varying in length from 1 to 14 repeats, named correspondingly K2 (MK-1) to K2 (MK-14). More than 90% of the Vitamin K2 occurring in animal and human tissue is the K2 (MK-4) form [40]. Currently there are only two commercially available forms of K2: K2(MK-4) (menaquinone-4 or menatrenone) and K2(MK-7) (or menaquinone-7), and they are both incorporated in Tri-K.

Vitamin K1 (Phylloquinone or Phytoanadione)

Vitamin K1 occurs in vegetables including algae and vegetable oils and seeds. For example 1000mcg of vitamin K1 could be provided by either of the following: about 1 cup cooked kale/collards/spinach or 2 cups of beets or 4 cups of cooked broccoli/Brussels sprouts or 4 cups raw onions or 6-7 cups of raw spinach or 6-7 cups cooked cabbage/asparagus or 8 cups iceberg lettuce or 13 cup raw broccoli or 20 cups of raw cabbage or 20 cucumbers.

Absorption of vitamin K1 from a nutritional supplement taken with fat was found to be 3-6 times better than that from foods (raw or cooked), probably because the food matrix impairs the release of naturally occurring K1.

One study showed that 1-2mg of vitamin K1 was shown to be the optimal dose for the maximal activation of a bone building protein osteocalcin [24]. Another study showed that older patients may have an increased need for vitamin K1 in order to get similar optimal activation (carboxylation) of their osteocalcin [8, 25] and it is believed that genetic polymorphisms of K1 activation may require a higher vitamin K intake [75].

Another way of estimating the modern vitamin K1 requirements might take into consideration typical K1 intake from a Paleolithic diet. This has not been evaluated yet in studies but it is plausible to have been around 1mg of K1 or higher because the Paleolithic diet was abundant in plant derived foods.

Vitamin K2 (menaquinone) is the predominant form of vitamin K found in human and animal tissues. The K2 found in the human body can be derived from various sources [11]:

1) Occurs in the liver and other tissues throughout the body from the conversion of K1 (from diet and/or supplements) to K2 in the form of K2(MK-4)
2) Absorbed from animal foods like liver (0.5-5 mcg/100g), yolk (10mcg/one egg yolk) or meats (2-3mcg/100g)
3) Absorbed from fermented foods (animal/vegetarian) rich in K2 (MK-4 to MK-12) produced by bacteria in foods like: cheese, natto (soybean and rice, mostly K2(MK-7), kimchi or sauerkraut (pickled cabbage), etc.
4) Produced by the human intestinal tract bacteria, mostly as K2 (MK-4). These bacteria were identified as Bacteroides Fragilis and a friendly E. Coli strain. Certain strains of lactobacilli were also found to produce it as well, although these are not the commonly known lactobacillus probiotics. [12].

Antibiotic therapy or the antibiotics found in the animal food supply may diminish the vitamin K producing bacteria in the human gut. Also, poor conversion of K1 to K2 in some patients due to age or various metabolic challenges [23] may make supplementation with K2 very beneficial.

Vitamin K2 in the MK-4 form, K2(MK-4) was used in many Japanese studies in doses of 15mg-45mg/day with success for the treatment of osteoporosis. Increases in BMD (Bone Density Markers) were as high as 1.1%, 5.2% and 7.5% after 6, 12, 24 months, respectively, using a dose of 45mg/day of K2-MK-4. Other studies have shown a reduction in the rate of bone loss in post-menopausal women or bone fracture risk was reduced even when the BMD did not show an impressive change [3]. K2-MK-4 is used in Japan as a pharmaceutical because the dose of 15-45mg is well above that which can be derived naturally. No side-effects have been observed so far in studies using high dose K2(MK_4) [3] for the last 10 years.

DFH Tri-K was designed to include only 1mg of K2-MK-4 per dose, which is in physiological range, because its purpose is primarily to correct nutritional deficiencies and to maintain maximum safety of supplementation.

<table>
<thead>
<tr>
<th>Foods with high vitamin K1 content</th>
<th>weight in grams</th>
<th>measure</th>
<th>mcg of K1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kale, cooked drained</td>
<td>130</td>
<td>1 cup</td>
<td>1062</td>
</tr>
<tr>
<td>Collards, cooked drained</td>
<td>170</td>
<td>1 cup</td>
<td>1059</td>
</tr>
<tr>
<td>Spinach, cooked drained</td>
<td>180</td>
<td>1 cup</td>
<td>889</td>
</tr>
<tr>
<td>Beets, cooked drained</td>
<td>144</td>
<td>1 cup</td>
<td>697</td>
</tr>
<tr>
<td>Broccoli, cooked drained</td>
<td>156</td>
<td>1 cup</td>
<td>220</td>
</tr>
<tr>
<td>Brussels, cooked drained</td>
<td>156</td>
<td>1 cup</td>
<td>219</td>
</tr>
<tr>
<td>Onions, raw</td>
<td>100</td>
<td>1 cup</td>
<td>207</td>
</tr>
<tr>
<td>Parsley, raw</td>
<td>10</td>
<td>10 sprigs</td>
<td>164</td>
</tr>
<tr>
<td>Cabbage, cooked drained</td>
<td>150</td>
<td>1 cup</td>
<td>163</td>
</tr>
<tr>
<td>Spinach, raw</td>
<td>30</td>
<td>1 cup</td>
<td>145</td>
</tr>
<tr>
<td>Asparagus, cooked drained</td>
<td>180</td>
<td>1 cup</td>
<td>144</td>
</tr>
<tr>
<td>Lettuce, iceberg</td>
<td>129</td>
<td>1 head</td>
<td>129</td>
</tr>
<tr>
<td>Broccoli, raw</td>
<td>88</td>
<td>1 cup</td>
<td>89</td>
</tr>
<tr>
<td>Cabbage, raw</td>
<td>70</td>
<td>2 cup</td>
<td>53</td>
</tr>
<tr>
<td>Cucumber, raw</td>
<td>301</td>
<td>1 large</td>
<td>49</td>
</tr>
</tbody>
</table>
The practitioner has the liberty to review the available studies [3] and use DFH Tri-K at higher dosage based on the individual clinical need, while monitoring the patient closely. To put the vitamin K2 dose in perspective, keep in mind that while it is possible to derive 1-2mg of vitamin K1 from a Paleolithic diet, we can also consider that K1 converts to a similar amount (or less) of vitamin K2-MK-4 in the body.

1mg of K2-MK-4 may be a reasonable physiological daily dose that humans may have obtained in Paleolithic conditions coming from two sources: one is K2 converted from dietary K1 and the other is K2 from animal foods and bacteria in the intestinal tract. One study showed that in modern humans the GI bacteria can provide, at times about 50% of the total Vitamin K in the body [12], but this varies based on dietary vitamin K intake.

**Vitamin K2 in the MK-7 form, K2(MK-7 or menaquinone-7)**

K2 (MK-7) is a product of bacterial food fermentation found in foods such as cheeses, cabbage, fermented soy or natto, but it is most economically derived from natto (a traditional soy and rice fermented mixture).

The supplemental form of K2 (MK-7) is purified and free of soy allergens by removing the soy protein.

The range of vitamin K intake in Japanese women consuming a diet rich in soy and natto was found to be 35-247mcg [39] out of which K2 (MK-7) is typically contributing 50-100mcg [39]. K2 (MK-7) is thought to convert in the body to K2(MK-4) very slowly [18], which is an advantage because it provides a continuous plasma reservoir of vitamin K2 between supplementation times. This is the main reason K2-MK-7 was added in the Tri-K formula, as it complements the metabolism of K1 and K2 (MK-4).

**How does vitamin K perform its various functions?**

The vitamin K function of supporting blood clotting is well known. However Vitamin K is essential in activating a large number of vitamin K-dependent proteins (VKD) throughout the body, by carboxylating certain glutamate residues and changing them to gamma-carboxyglutamate residues, abbreviated Gla. Gla has the property of binding calcium, and so as soon as VKD proteins get their glutamate residues carboxylated by vitamin K, they become able to bind calcium as well.

The more vitamin K is available in the body, the better the chance that all the VKDs needing gamma-carboxylation will end up with a maximum amount of Gla residues in them, or close to a 100% carboxylation. The activity of these proteins is proportional to how many of their glutamate residues are carboxylated.

Until now, vitamin K adequate intake has been defined as the amount needed to completely carboxylate thrombin (a clotting protein) but with the discovery new roles for various VKDs throughout the body, many researchers propose that vitamin K status be considered adequate only when all VKDs are maximally carboxylated.

The following vitamin K dependent proteins are currently known:

- **blood coagulation factors**: factors II (prothrombin), VII, IX, and X, the anticoagulant proteins C, S and Z [92].

- **osteocalcin** is a protein produced by osteoblasts (bone building cells) and fixes calcium in the mineral structure of bone [10, 22, 103]. and tooth dentin [59] It also produces collagen type I, which is enhanced by vitamin K. [102]

- **Matrix Gla Protein or MGP** is found mostly in the arterial walls/veins and cartilage (associated with chondrocytes) but also associated with cells of other soft tissues: brain, kidney, lung, skin, testes, sperm, salivary glands. One identified role of MGP is to reject calcium deposition in these tissues.

Activated vitamin D, 1,25 (OH)D3 stimulates the synthesis of osteocalcin and MGP [63].

Vitamin A (retinoic acid) stimulates the synthesis of osteocalcin but inhibits that of MGP [60,64].

**Vitamin K works in synergy with vitamin D and vitamin A, and in order for vitamin K to perform its functions efficiently, vitamin D and vitamin A statuses have to be optimized as well** [60].

- **Gas-6 protein (Growth Arrest Specific)** is believed to regulate cell growth and apoptosis, and was shown to support the survival of cells in various tissues such as arterial muscle, epithelial eye lens, brain and possibly others.

- **GST (Galactocerebrosidase Sulfotransferase)** enzyme is involved in sulfatide synthesis, which is an important component of myelin. This is important for the health of nerves and the brain and may be helpful to maximize this process for patients suffering from in Multiple Sclerosis, ALS or CIDP. [38]

- Four **transmembrane Gla proteins (TMGs)** have been identified and their function is at presently unknown. [11]

In addition to being an enzyme cofactor for the carboxylation of all above mentioned proteins, **Vitamin K2 has a transcription regulation activity**:  
- It stimulates the SXR (Steroid and Xenobiotic Receptor), which upregulates detoxification pathways and the expression of various bone osteoblastic markers [58].  
- inhibits the expression of osteoclast differentiation factor (ODF)/RANKL [105, 106] through its geranylgeraniol component  
- inhibits adipogenesis [105] (the production of new fat cells)

**Vitamin K2 was shown to reduce inflammation, autoimmune disease activity and may be able to reduce CRP.**

This may be due to the inhibition of PGE2, COX-2 and IL-6) [3, 30]. Vitamin K2 was shown to reduce the activity of rheumatoid arthritis (by inhibiting the proliferation of the rheumatoid synovial cells) [20].

A correlation was found between vitamin K status (plasma Vitamin K1) and osteoarthritis disease activity in hands and knees [31]. Plasma Vitamin K1 was shown in the Framingham study to correlate with CRP (a marker of inflammation). [104]
Vitamin K1 or K2 supplementation was shown to increase or prevent a decline in BMD and reduce the risk of osteoporosis (at this time) because their deficiency has been shown to cause osteoporosis. Vitamin K2 status was shown to reduce inflammation and vitamin K1 was found to correlate with CRP. MGP proteins, found in the arterial wall and inside the arterial plaque, may support arterial cell survival while preventing cell death. Decreasing bone resorption by inhibiting osteoclast cells formation (inhibits the expression of osteoclast differentiation factor (ODF)/RANKL), as well preventing osteolast activation by inflammation (reduced IL-6, COX-2, PGE2, ODF/RANKL) is increased by Vitamin K (through upregulation of collagen type I genetic expression). Vitamin K2 supplementation right after warfarin discontinuation was able to completely carboxylate osteocalcin (1mg-2mg K1 with MK-4). However, none of these studies maximized the patient’s status of vitamin D, used an adequate combination of minerals in their most absorbable forms (calcium, magnesium, boron etc) or optimized acid/alkaline balance. Vitamin K may need to be supplemented to children in order to achieve maximum bone mass during development. Osteocalcin is also involved in tooth mineralization and dental bone metabolism which means that vitamin K plays a role in these functions as well. Bone ultrasound test are now available and can be performed on the heel in order to evaluate the elastic properties of the bone and the results correlate well with the risk of bone fracture. Vit K status and supplementation is more likely to corelate with a bone ultrasound than a BMD test.

What have the studies shown in regards to arterial calcification or stiffness prevention and reversal?

Arterial calcification is thought to be initiated by inflammation (through TNF-alpha), oxidized or glycated LDL, hyperglycemia or arterial cell death (due to injuries from hypertension for example). Gas-6 vitamin K dependent proteins may support arterial cell survival while MGP proteins, found in the arterial wall and inside the arterial plaque (right along where calcification occurs), have the role of preventing calcium deposition in those tissues. Vitamin K2 status was shown to reduce inflammation and vitamin K1 was found to correlate with CRP.

In a study from 2007, rats developed arterial calcification within a few months of taking Warfarin (a vitamin K deficient state). Vitamin K1 or K2(MK-4) supplementation right after warfarin discontinuation was able to reverse the arterial calcification by 35% in 6 weeks. The effective dose for a similar effect in humans in not known, but some researchers hypothesize that it be the vitamin K dose able to completely carboxylate the MGP proteins. This is because human studies have observed a correlation between uncarboxylated MGP and arterial calcification. It is not known at the present time what is the ideal dose of vitamin K that is able to completely carboxylate all the MGP proteins found throughout the body, but it may be similar to the dose shown to completely carboxylate osteocalcin (1mg-2mg K1 with additional K2 needed for patients that do not convert well K1 to K2).

One study, that gave women 1mg K1 along with vitamin D and minerals, has shown that vitamin K1 was able to prevent an increase in arterial stiffness (it maintained the elastic properties of the arteries by measuring compliance) observed in the group of women taking vitamin D and minerals without vitamin K. Arterial calcification was thought to be the cause of the decreased arterial elasticity. One animal study showed that high dose vitamin K2 (1mg or 10mg/kg) “suppressed progression of arterial plaque, intima thickening, pulmonary atherosclerosis, reduced total cholesterol and lipid peroxidation and did not promote coagulative tendencies”.

Vitamin K deficiency may cause hypertension through increased arterial stiffness, which may be due calcifications in the arterial wall either within medial muscle cells and/or around the elastic fibers. Vitamin D supplementation can cause increased arterial calcification and stiffness when vitamin K is deficient (due to deficient intake of vitamin K or warfarin/coumadin treatment, which creates a vitamin K deficiency). This may be due to vitamin D increasing calcium absorption and transport, and upregulating MGP and osteocalcin expression. Thyroid hormones influence the synthesis of MGP proteins such that arterial calcification is increased and MGP expression is decreased in hypothyroidism.


