

# VITAMIN K FUNCTIONS AND FUNCTIONAL MARKERS

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## ABSTRACT

The role of vitamin K extends well beyond the regulation of blood clotting to impact bone formation, and development of heart disease, and possibly cancer. The common denominator among these diverse roles is calcium. Vitamin K is required for the carboxylation of glutamate residues on specific proteins to form gamma-carboxyglutamate residues (abbreviated Gla-residues). Proteins that undergo this reaction are called Gla proteins. They either directly bind  $Ca^{++}$  or direct  $Ca^{++}$ -dependent interactions with negatively charged surfaces.<sup>1</sup> Thus, vitamin K is critical for bone formation, soft-tissue calcification, cell growth and apoptosis. It has emerged as a potential protector against osteoporosis, atherosclerosis, insulin sensitivity, and cancer.<sup>2-6</sup> In human studies the intake of vitamin K has been associated with a lower risk of coronary calcification and in animal studies vitamin K has reversed arterial calcification.<sup>2, 7, 8</sup> Vitamin K levels are used as diagnostic and therapeutic parameters in osteoporosis.<sup>3</sup> Recommendations for vitamin K intake were originally made on the basis of hepatic requirements for blood coagulation factors.<sup>7</sup> Accumulating evidence suggests that the requirements to maintain optimal bone and vessel function require higher vitamin K levels.<sup>6, 9</sup> There are two natural forms of vitamin K which differ based on their phytyl group: phylloquinone (vitamin K1) synthesized in plants, and menaquinone (vitamin K2) produced by bacteria in the gut. Both forms have been used to assess vitamin K status via direct measurement in serum, though such direct measurements are generally not accepted as a reliable index of vitamin K status.<sup>7, 8</sup> Functional markers provide a more accurate assessment of vitamin K status, they include prothrombin time, undercarboxylated prothrombin (PIVKA-II), and undercarboxylated osteocalcin. A vitamin K deficiency can lead to impairment in the carboxylation of osteocalcin, resulting in an increase in the undercarboxylated form in circulation, where it can be measured to identify vitamin K status.

# VITAMIN K FUNCTIONS AND FUNCTIONAL MARKERS

## VITAMIN K SOURCES

There are two natural forms of vitamin K which differ based on their phytol group, phylloquinone (vitamin K1) the predominant form of vitamin K in the western diet, from green leafy vegetables and some vegetable oils, and menaquinone (vitamin K2) primarily synthesized from gram-negative bacteria in the gastrointestinal tract, as well as from meat and fermented foods.<sup>10</sup> Menaquinone (MK) has several forms; MK-4 can be found in animal meat, MK-7, MK-8, and MK-9 are produced from intestinal bacterial action on fermented food products such as meat and cheese.<sup>11</sup> MK-4 is distinct from other MKs because it is not produced in significant amounts by bacteria, instead it appears to be synthesized by animals (including humans) from phylloquinone.<sup>12</sup>

Very little vitamin K is stored by the body; small amounts of this vitamin are deposited in the liver and in the bones, but the amount is only enough to supply the body's needs for a few days. The dietary intake of menaquinones was very low (27–30 µg/day) compared with that of phylloquinone (244–257 µg/day), and against the current dietary recommendations for vitamin K at 90–120 µg/day. No adverse effects have been reported for individuals consuming higher amounts of vitamin K.<sup>7</sup>

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The synthetic short chain vitamin K1 is commonly used in food supplements, though the natural, long chain MK-7 has become available as a supplement. In a comparison of absorption and efficacy between K1 and MK-7, both K1 and MK-7 were absorbed well with peak serum concentrations at 4 hours after intake. A major difference was the very long half-life time of MK-7, resulting in much more stable serum levels and accumulation of MK-7 to higher levels (7-8 fold) during prolonged intake.<sup>8</sup> These differences in half lives may be due to transport mechanisms.

Phylloquinone is predominantly transported with the triacylglycerol-rich fraction, which is very effectively cleared from circulation by the liver. Dietary phylloquinone alteration causes changes in serum osteocalcin and plasma phylloquinone levels.<sup>13</sup> Menaquinones are found in both triacylglycerol-rich lipoprotein and low-density lipoprotein, and are not cleared as quickly.<sup>14</sup> MK-7 induced more complete carboxylation of osteocalcin and clinicians should be aware that preparations supplying  $\geq 50$  microg/day of MK-7 may interfere with oral anticoagulant treatment in a clinically relevant way.<sup>8</sup>

## PHYSIOLOGIC FUNCTIONS

Vitamin K is a coenzyme for glutamate carboxylase, which mediates the conversion of glutamate to gamma-carboxyglutamate (Gla) on polypeptide chains of specific proteins. These proteins require carboxylation in order to become biologically active, leading to the binding of calcium. (Figure 1)

Much of the original research done on vitamin K was on coagulation proteins, such as prothrombin, primarily because of the ability to test clotting time.<sup>7</sup> In the past few years, research has focused away from blood coagulation proteins to new areas of vitamin K metabolism, which include bone and endovascular metabolism; cell growth, migration, apoptosis, and adhesion; insulin sensitivity.<sup>6, 9, 15</sup> It may also play a role in the risk of cancer.<sup>16, 17</sup>

## BLOOD CLOTTING

A deficiency of vitamin K results in an increase in prothrombin time. The usual clinical manifestation is a tendency to hemorrhage. Vitamin K status is especially important in the elderly and those with gastrointestinal

Vitamin K Content (ng/g) of Various Food Items			
Food Item	Phylloquinone	Menaquinone-4	Menaquinone-9
Meat, Fish	10-40	10-100	0-20
Pork Liver	2-5	3-5	10-20
Milk, yogurt	4-10	4-10	0-20
Cheese curd	20-100	20-100	400-700
Green vegetables	2000-8000	0	0
Fruit	1-30	0	0
Bread	5-30	0	9-20

conditions because of inadequate dietary intake and absorptive difficulties, frequently complicated by drug therapies. There are case reports of bleeding episodes in antibiotic-treated patients due to an acquired vitamin K deficiency resulting from a suppression of menaquinone-synthesizing organisms.<sup>7</sup> Vitamin K is also important to the millions of patients on warfarin (Coumadin) therapy, an oral anticoagulant treatment for a variety of thrombogenic conditions. The wide use of this drug is known to result in significant risk for major bleeding, which raises new clinical questions for patients who must be maintained on long-term warfarin therapy.<sup>18</sup> In humans, it has been demonstrated that oral anticoagulant treatment is associated with substantially increased heart valve calcification. Observational data has also suggested a possible association with warfarin and vascular calcification.<sup>19</sup>

### VASCULAR DISEASE

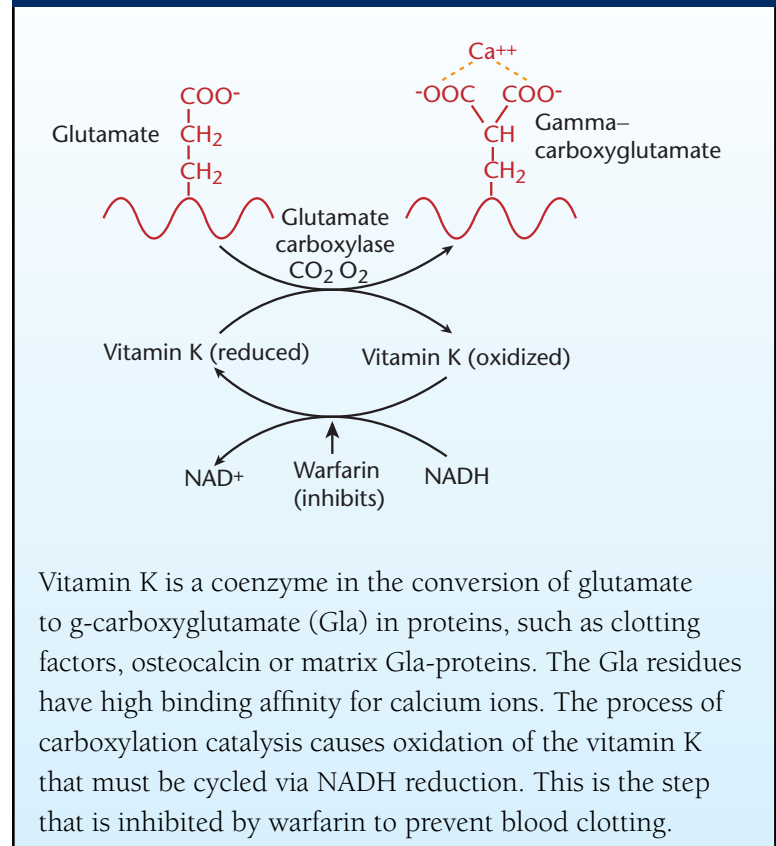
A prominent feature of vascular calcification is atherosclerosis, though the underlying mechanisms are still obscure. Vascular smooth-muscle cells and arterial intima synthesize a matrix protein that undergoes a vitamin K-dependent carboxylation to become matrix gamma-carboxyglutamic acid protein (MGP). MGP is an inhibitor of calcification. Early reports demonstrated the association between MGP and vascular calcification and put forth the hypothesis that undercarboxylation of MGP was a risk factor for vascular calcification and that the majority of dietary intakes were too low to ensure full carboxylation of MGP.<sup>6</sup> In the Rotterdam study of some 5000 participants, an adequate intake of menaquinone was clearly linked to lower rates of cardiovascular disease.<sup>2,20</sup> Since bone-associated proteins such as osteonectin, osteocalcin, and MGP have been detected in calcified vascular tissues, calcification is now considered to be an organized, regulated process similar to mineralization in bone tissue.<sup>21</sup> Concurrent arterial calcification and osteoporosis have been called the “calcification paradox” and occur frequently in postmenopausal women.<sup>9</sup> In a population-based study of postmenopausal women, subjects with aortic atherosclerosis had low vitamin K intake and increased ucOC.<sup>22</sup>

Known mutations in the MGP have been characterized by calcification of the arteries. Targeted deletion of the MGP gene in mice caused rapid calcification of the arteries; vitamin K supplementation has been shown to reverse

arterial calcification in animal studies. While early human studies were inconsistent between dietary vitamin K intake and coronary artery calcification they looked primarily at K1 or phyloquinone. Menaquinone or vitamin K2 intake has been associated with a lower risk of coronary heart disease mortality.<sup>4, 6, 23</sup> The metabolism of menaquinone versus phyloquinone has been cited as a probable reason as to why the different forms of vitamin K showed different results with respect to calcification. The gamma-carboxylation of MGP is also regulated by several other factors including retinoic acid, vitamin D, and extracellular calcium ions.<sup>24</sup>

Though dietary intake of phyloquinone has not been found to be associated with coronary calcification, intravenous phyloquinone has been widely used therapeutically to induce a dose-dependent hypotension.<sup>2</sup> In animal studies it results in an acute increase followed by a more sustained decrease.<sup>25</sup> This decrease in blood pressure is believed to be due to the activation of the nitric oxide (NO) pathway and the release of vasodilator prostanoid(s).<sup>19,25</sup> Current dietary recommendations of vitamin K are based on saturation of the coagulation system and may be insufficient to maintain vascular and bone health since individual functions are

Figure 1. Vitamin K Carboxylation



Vitamin K is a coenzyme in the conversion of glutamate to g-carboxyglutamate (Gla) in proteins, such as clotting factors, osteocalcin or matrix Gla-proteins. The Gla residues have high binding affinity for calcium ions. The process of carboxylation catalysis causes oxidation of the vitamin K that must be cycled via NADH reduction. This is the step that is inhibited by warfarin to prevent blood clotting.

independent of each other. Researchers have noted that the present RDA values are too low to ensure full carboxylation of MGP.<sup>4,6,23</sup>

## GLUCOSE CONTROL

The pancreas, which makes insulin, is a site of synthesis for certain vitamin K-dependent proteins, and may play a role in blood sugar regulation, which may exacerbate any increase in coronary calcification.<sup>16, 26</sup> Research has also shown that osteocalcin, a bone protein whose mineralization function is vitamin K dependent acts as a hormone, causing beta cells to release more insulin, and at the same time directing fat cells to release the hormone adiponectin, which increases sensitivity to insulin.<sup>27</sup> In a 36-month, randomized, double-blind, controlled trial designed to assess the impact of supplementation with 500 microg/d of phylloquinone on bone loss, the primary outcome of the study was reduced insulin resistance in men, but not women.<sup>28</sup> The Framingham Offspring Cohort found higher reported phylloquinone intake was associated with greater insulin sensitivity and glycemic status.<sup>29</sup>

## BONE ACTIVITY

Vitamin K has been shown to be a valuable diagnostic as well as therapeutic parameter in osteoporosis and bone fractures.<sup>30,31</sup> Higher vitamin K status has been associated with lower fracture risks.<sup>32</sup> The Nurses' Health Study followed more than 72,000 women for ten years and found

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that those women with the lowest vitamin K intakes had a 30% higher risk of hip fracture than those with the highest intakes.<sup>33</sup> In other studies, calcium loss in those with low vitamin K levels was found to be reduced by up to 50% with vitamin K supplementation.<sup>3, 34</sup>

A number of reports have correlated decreased bone mineral density or increased fracture rates with a five to eight fold increase in undercarboxylated osteocalcin (ucOC), a vitamin K-dependent bone protein. Research

has found that vitamin K intakes similar to those reported for the general population did not ensure complete carboxylation of osteocalcin and that ucOC could be decreased by increasing vitamin K intake.<sup>7</sup> Vitamin K supplementation has also been shown to decrease the level of circulating ucOC, as well as improve bone turnover. These reports have led to the suggestion that vitamin K requirements for bone function are probably much higher than those needed to maintain normal hemostasis and that the recommendation should be higher than currently set.<sup>7</sup> The mechanism by which vitamin K may promote mineralization of bone, while inhibiting mineralization (calcification) of vessels, is not entirely clear. Animal studies have demonstrated that osteoblasts are involved in postprandial lipoprotein metabolism which may also be vitamin K mediated.<sup>35</sup>

## CANCER

Cancer induced hypercoagulable is a well-established feature, and venous thromboembolism occurs in 4% to 20% of cancer patients and is the second cause of mortality in cancer.<sup>36</sup> Anticarcinogenic activities of vitamin K have been observed in various cancer cell lines, including prostate cancer cells. Research has suggested an inverse association between the intake of menaquinones, but not that of phylloquinone and prostate cancer.<sup>5</sup> Research has also found that vitamin K2 administration significantly suppressed hepatocellular carcinoma recurrence.<sup>37</sup>

## ASSESSMENT OF STATUS

### DIRECT MEASUREMENTS

Methods for direct measurement of vitamin K concentration in blood or urine have been reported.<sup>38</sup> Both phylloquinones and the menaquinones have been used to assess status, phylloquinone is primarily studied because it is the predominate source of vitamin K in western countries. Human subjects placed on a low vitamin K diet for several weeks show declines in body pools of vitamin K from normal values of ~1.0 g/kg body weight. Positive correlations between circulating phylloquinone concentration and dietary intake have been reported, but the strength of this association has varied according to studies.<sup>7</sup> During dietary restriction, fecal excretion of an administered dose of vitamin K fell, but urinary

excretion rose, indicating activation of intestinal absorption mechanisms.<sup>39</sup> Phylloquinone concentrations in plasma are decreased in acute-phase response and, unless corrected for plasma triglyceride concentration, may not be a reliable index of vitamin K status.<sup>40</sup> The more clinically relevant question of body pool size calls for measures of functional status.

## FUNCTIONAL MARKERS OF VITAMIN K

### UNDERCARBOXYLATED OSTEOCALCIN (ucOC)

Osteocalcin (OC) or BGP (Bone Gla protein) is secreted by osteoblasts and plays a role in mineralization and calcium ion homeostasis. It accounts for 10-20% of the non-collagenous protein in bone. OC is a product almost exclusively of mature, active osteoblasts and is a vitamin K-dependent Ca<sup>+</sup> binding protein. Transcription of the OC gene, located on chromosome 1, is regulated by 1,25-dihydroxyvitamin D, estrogens, glucocorticoids, and other molecules. Posttranslational modification of OC occurs through the vitamin K-dependent gamma-carboxylation of three glutamate molecules in positions 17, 21, and 24 of the protein. This gamma-carboxylation is largely responsible for its calcium binding properties, which is known to mediate strong binding of OC to hydroxyapatite. Undercarboxylated OC decreases binding to the bone and increases in circulating blood and urine where it can be measured. Undercarboxylated osteocalcin is an assay based on the amount of osteocalcin that is undercarboxylated.

The serum concentration of ucOC is a sensitive indicator of vitamin K status, as high serum levels of ucOC are indicative of low vitamin K status and vice versa.<sup>13, 41</sup> Research has shown it to correlate with plasma PIVKA-II concentrations ( $r = 0.27$ ,  $P < 0.001$ ) and with plasma phylloquinone concentrations ( $r = -0.35$ ,  $P < 0.001$ ), whereas the agreement between plasma phylloquinone and PIVKA-II concentrations was not as strong ( $r = -0.15$ ,  $P < 0.05$ ). In addition, the circulating concentration of ucOC has been reported to be a predictor of low BMD<sup>42, 43</sup> and hip fracture risk.<sup>43</sup>

This undercarboxylation is significantly increased in elderly women, and reflects not only some degree of vitamin K deficiency but also their poor vitamin D status, suggesting that vitamin D may be important, either directly or

indirectly through its effect on bone turnover, for achieving a normal gamma-carboxylation of OC.<sup>43,44</sup> This may be of concern for those supplementing with calcium, but without adequate vitamin K, possibly leading to an increase in free calcium. Though low vitamin K status is associated with low bone density and increased risk of osteoporotic fractures in adults, little is known about vitamin K status and bone health in children. Research has found a marked correlation between the markers for bone metabolism and ucOC in children, as well as a pronounced low vitamin K status of bone during growth. The greatest need was from ages 8-13 and in Tanner stages II – IV. Research has not yet shown if children would benefit, by improved bone health or stronger bones, from higher vitamin K levels.<sup>45, 46</sup>

Until now, measurement of ucOC in serum sample was performed by a hydroxyapatite combination radio immunoassay, through a monoclonal antibody specific to

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undercarboxylated osteocalcin. A constructed ELISA system is now available.<sup>47</sup> Undercarboxylated OC does not bind to hydroxyapatite. The ucOC assessment utilizes monoclonal antibodies highly reactive to the ucOC. This measurement provides useful clinical information of bone metabolism, and thus vitamin K status.<sup>48, 49</sup>

### UNDERCARBOXYLATED PROTHROMBIN (PIVKA-II)

Insufficient vitamin K leads to increased plasma levels of biologically inactive, under-gamma-carboxylated forms of vitamin K-dependent clotting factors. These proteins are referred to as protein induced by vitamin K absence or antagonism (PIVKA). In reference to prothrombin, also called factor II, the term used is PIVKA-II.<sup>50-52</sup> Binding of calcium is critical for the sequence of reactions leading to blood clotting and requires vitamin K. Multiple proteins of the blood clotting cascade have sites for the carboxylation. The progression of PIVKA-II resulted in elevated values with each trimester of pregnancy and has led to the suggestion that some women may develop

a sub-clinical vitamin K deficiency during gestation.<sup>53</sup> Some tumors produce PIVKA-II, including gastric cancer and hepatocellular carcinoma.<sup>54, 55</sup> Undercarboxylation of coagulation factors due to dietary vitamin K deficiency is rarely seen in healthy subjects, though undercarboxylated osteocalcin, is found in a substantial part of the population, notably in elderly women.<sup>56</sup>

### PROTHROMBIN

The measurement of prothrombin time in plasma reveals the vitamin K-dependent activation of prothrombin and other clotting factors. Prothrombin times longer than 12 seconds can indicate vitamin K deficiency. Prothrombin

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time in plasma is not a sensitive indicator of vitamin K status because plasma prothrombin concentration must be decreased by approximately 50 percent before a value is outside of the “normal” range. In addition, prothrombin may not be reflective of changes in extrahepatic vitamin K-dependent proteins.<sup>52</sup>

### MATRIX GLA PROTEIN (MGP)

MGP is a vitamin K-dependent protein which is synthesized in a variety of tissues such as lung, heart, kidney, cartilage, and bone. The function of MGP in these tissues is unclear. It is present in bone together with the related vitamin K-dependent protein OC. Keutel syndrome, a rare condition characterized by abnormal calcium deposition in cartilage leading to artery stenosis is due to an impaired MGP gene.<sup>57</sup> Mice that lack MGP develop arterial calcification which leads to blood-vessel rupture. MGP mRNA transcription is substantially up regulated in atherosclerotic lesions.<sup>58</sup> Researchers have indicated that the loss of MGP expression may be associated with tumor progression and metastasis.<sup>59</sup>

### URINARY GAMMA-CARBOXYGLUTAMYL (GLA) RESIDUES

After protein catabolism, Gla residues contained in the

vitamin K-dependent proteins are excreted in the urine and have been used as an indicator of vitamin K status. Urinary Gla responds to alterations in dietary intake, but periods of several days are needed before any change can be observed, and significant changes take longer. Increases in vitamin K intake have not been shown to induce significant changes in urinary Gla. Response of urinary Gla to vitamin K intake also appears to be age-specific. Depletion periods result in significant decreases in urinary Gla excretion in younger, but not the older, subjects. There is insufficient data for using urinary Gla excretion for estimating vitamin K intake.<sup>7</sup>

### SERUM OSTEOCALCIN

Serum osteocalcin (OC) is associated with bone turnover, which is a determinant of osteoporosis. The serum concentrations of OC increase with advancing age in both men and women.<sup>30, 31</sup> Results concerning the relationship between OC and the occurrence of fracture are contradictory. Some authors suggest that there is a positive association, whereas others suggest that there is no association. Assay designs and antibody specificity for different forms of circulating OC in different studies are highly variable, complicating clinical interpretation.

### RATIO OF UNDERCARBOXYLATED OSTEOCALCIN/OSTEOCALCIN - UCR

Both the undercarboxylated osteocalcin (ucOC) fraction and the ucOC/carboxylated osteocalcin (cOC) ratio, also noted as the UCR, are sensitive indicators for vitamin K status.<sup>46</sup> Previous studies have indicated that a low serum cOC level or a high ucOC level are both risk factors for femoral neck fracture. In addition, it has been shown that serum ucOC decreases and cOC increases rapidly during vitamin K2 treatment.

### SERUM PERCENT ucOC

Serum Percent ucOC is determined by assessment of both serum total OC and ucOC. Undercarboxylated OC is then expressed as the percentage of total OC (Percent ucOC). Though research has found that changes in the total percentage are generally due to a change in ucOC, while cOC were not generally affected by dietary intake, thus ucOC is the primary marker.<sup>13</sup>

## REFERENCES

1. Furie B, Bouchard BA, Furie BC. Vitamin K-dependent biosynthesis of gamma-carboxyglutamic acid. *Blood*. Mar 15 1999;93(6):1798-1808.
2. Beulens JW, Bots ML, Atsma F, et al. High dietary menaquinone intake is associated with reduced coronary calcification. *Atherosclerosis*. Jul 19 2008.
3. Binkley NC, Suttie JW. Vitamin K nutrition and osteoporosis. *J Nutr*. Jul 1995;125(7):1812-1821.
4. Erkkila AT, Booth SL. Vitamin K intake and atherosclerosis. *Curr Opin Lipidol*. Feb 2008;19(1):39-42.
5. Nimptsch K, Rohrmann S, Linseisen J. Dietary intake of vitamin K and risk of prostate cancer in the Heidelberg cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Heidelberg). *Am J Clin Nutr*. Apr 2008;87(4):985-992.
6. Schurgers LJ, Dissel PE, Spronk HM, et al. Role of vitamin K and vitamin K-dependent proteins in vascular calcification. *Z Kardiol*. 2001;90 Suppl 3:57-63.
7. Medicine Io. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. In: *Intakes SCotSEoDR*, ed: National Academies Press; 2000.
8. Schurgers LJ, Teunissen KJ, Hamulyak K, Knapen MH, Vik H, Vermeer C. Vitamin K-containing dietary supplements: comparison of synthetic vitamin K1 and natto-derived menaquinone-7. *Blood*. Apr 15 2007;109(8):3279-3283.
9. Adams J, Pepping J. Vitamin K in the treatment and prevention of osteoporosis and arterial calcification. *Am J Health Syst Pharm*. Aug 1 2005;62(15):1574-1581.
10. Shearer MJ. Vitamin K. *Lancet*. Jan 28 1995;345(8944):229-234.
11. Morishita T. TN. Production of menaquinones by lactic acid bacteria. *Journal of Dairy Science* 1999;82(9):1897-1903.
12. Okano T, Shimomura Y, Yamane 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*. Apr 25 2008;283(17):11270-11279.
13. Sokoll LJ, Booth SL, O'Brien ME, Davidson KW, Tsaion KI, Sadowski JA. Changes in serum osteocalcin, plasma phylloquinone, and urinary gamma-carboxyglutamic acid in response to altered intakes of dietary phylloquinone in human subjects. *Am J Clin Nutr*. Mar 1997;65(3):779-784.
14. Beavan SR, Prentice A, Stirling DM, et al. Ethnic differences in osteocalcin gamma-carboxylation, plasma phylloquinone (vitamin K1) and apolipoprotein E genotype. *Eur J Clin Nutr*. Jan 2005;59(1):72-81.
15. Merli GJ, Fink J. Vitamin K and thrombosis. *Vitam Horm*. 2008;78:265-279.
16. Sakamoto N, Wakabayashi I, Sakamoto K. Low vitamin K intake effects on glucose tolerance in rats. *Int J Vitam Nutr Res*. Jan 1999;69(1):27-31.
17. Sakamoto N, Nishiike T, Iguchi H, Sakamoto K. Possible effects of one week vitamin K (menaquinone-4) tablets intake on glucose tolerance in healthy young male volunteers with different descarboxy prothrombin levels. *Clin Nutr*. Aug 2000;19(4):259-263.
18. Shibayama-Imazu T, Aiuchi T, Nakaya K. Vitamin K2-mediated apoptosis in cancer cells: role of mitochondrial transmembrane potential. *Vitam Horm*. 2008;78:211-226.
19. Danziger J. Vitamin K-dependent Proteins, Warfarin, and Vascular Calcification. *Clin J Am Soc Nephrol*. May 21 2008.
20. Geleijnse JM, Vermeer C, Grobbee DE, et al. Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: the Rotterdam Study. *J Nutr*. Nov 2004;134(11):3100-3105.
21. Trion A, van der Laarse A. Vascular smooth muscle cells and calcification in atherosclerosis. *Am Heart J*. May 2004;147(5):808-814.
22. Jie KS, Bots ML, Vermeer C, Witteman JC, Grobbee DE. Vitamin K intake and osteocalcin levels in women with and without aortic atherosclerosis: a population-based study. *Atherosclerosis*. Jul 1995;116(1):117-123.
23. Seyama Y, Wachi H. Atherosclerosis and matrix dystrophy. *J Atheroscler Thromb*. 2004;11(5):236-245.
24. Kaneki M HT, Ouchi Y, Orimo H. Pleiotropic actions of vitamin K: protector of bone health and beyond? *Nutrition*. 2006; Jul-Aug; 22 (7-8):845-852.
25. Tirapelli CR, Resstel LB, de Oliveira AM, Correa FM. Mechanisms underlying the biphasic effect of vitamin K(1) (phylloquinone) on arterial blood pressure. *J Pharm Pharmacol*. Jul 2008;60(7):889-893.
26. Stenberg LM, Nilsson E, Ljungberg O, Stenflo J, Brown MA. Synthesis of gamma-carboxylated polypeptides by alpha-cells of the pancreatic islets. *Biochem Biophys Res Commun*. May 4 2001;283(2):454-459.
27. Lee NK, Sowa H, Hinoi E, et al. Endocrine regulation of energy metabolism by the skeleton. *Cell*. Aug 10 2007;130(3):456-469.
28. Yoshida M JP, Meigs JB, Saltzman E, Shea MK, Gundberg C, Dawson-Hughes B, Dallal G, Booth SL. Effect of vitamin K supplementation on insulin resistance in older men and women. *Diabetes Care*. 2008; Aug 12.
29. Yoshida M, Booth SL, Meigs JB, Saltzman E, Jacques PF. Phylloquinone intake, insulin sensitivity, and glycemic status in men and women. *Am J Clin Nutr*. Jul 2008;88(1):210-215.
30. Hodges SJ, Akesson K, Vergnaud P, Obrant K, Delmas PD. Circulating levels of vitamins K1 and K2 decreased in elderly women with hip fracture. *J Bone Miner Res*. Oct 1993;8(10):1241-1245.
31. Weber P. The role of vitamins in the prevention of osteoporosis--a brief status report. *Int J Vitam Nutr Res*. May 1999;69(3):194-197.
32. Heiss C, Hoesel LM, Wehr U, et al. Diagnosis of osteoporosis with vitamin k as a new biochemical marker. *Vitam Horm*. 2008;78:417-434.
33. Feskanich D, Weber P, Willett WC, Rockett H, Booth SL, Colditz GA. Vitamin K intake and hip fractures in women: a prospective study. *Am J Clin Nutr*. Jan 1999;69(1):74-79.
34. Bugel S. Vitamin K and bone health in adult humans. *Vitam Horm*. 2008;78:393-416.
35. Niemeier A, Niedzielska D, Secer R, et al. Uptake of postprandial lipoproteins into bone in vivo: Impact on osteoblast function. *Bone*. Apr 10 2008.
36. Debourdeau P El, de Raignac A, Meria P, Gornet JM, Amah Y, Korte W, Marty M, Farge D. Long-term use of daily subcutaneous low molecular weight heparin in cancer patients with venous thromboembolism: why hesitate any longer? *Support Care Cancer*. 2008;Aug 15.
37. Mizuta T OI. Hepatocellular carcinoma and vitamin K. *Vitam Horm*. 2008;78:435-442.

38. Dolnikowski GG, Sun Z, Grusak MA, Peterson JW, Booth SL. HPLC and GC/MS determination of deuterated vitamin K (phylloquinone) in human serum after ingestion of deuterium-labeled broccoli. *J Nutr Biochem.* Mar 2002;13(3):168-174.
39. Olson RE, Chao J, Graham D, Bates MW, Lewis JH. Total body phylloquinone and its turnover in human subjects at two levels of vitamin K intake. *Br J Nutr.* Jun 2002;87(6):543-553.
40. Azharuddin MK, O'Reilly DS, Gray A, Talwar D. HPLC method for plasma vitamin K1: effect of plasma triglyceride and acute-phase response on circulating concentrations. *Clin Chem.* Sep 2007;53(9):1706-1713.
41. Vermeer C, Jie KS, Knapen MH. Role of vitamin K in bone metabolism. *Annu Rev Nutr.* 1995;15:1-22.
42. Jie KG, Bots ML, Vermeer C, Witteman JC, Grobbee DE. Vitamin K status and bone mass in women with and without aortic atherosclerosis: a population-based study. *Calcif Tissue Int.* Nov 1996;59(5):352-356.
43. Szulc P, Chapuy MC, Meunier PJ, Delmas PD. Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture in elderly women. *J Clin Invest.* Apr 1993;91(4):1769-1774.
44. Einhorn TA, Gundberg CM, Devlin VJ, Warman J. Fracture healing and osteocalcin metabolism in vitamin K deficiency. *Clin Orthop Relat Res.* Dec 1988(237):219-225.
45. van Summeren M, Braam L, Noirt F, Kuis W, Vermeer C. Pronounced elevation of undercarboxylated osteocalcin in healthy children. *Pediatr Res.* Mar 2007;61(3):366-370.
46. van Summeren MJ, van Coeverden SC, Schurgers LJ, et al. Vitamin K status is associated with childhood bone mineral content. *Br J Nutr.* Feb 18 2008:1-7.
47. Price PA, Nishimoto SK. Radioimmunoassay for the vitamin K-dependent protein of bone and its discovery in plasma. *Proc Natl Acad Sci U S A.* Apr 1980;77(4):2234-2238.
48. Koyama N, Ohara K, Yokota H, et al. A one step sandwich enzyme immunoassay for gamma-carboxylated osteocalcin using monoclonal antibodies. *J Immunol Methods.* May 17 1991;139(1):17-23.
49. Takahashi Y, Endo H, Tange T, et al. Des-gamma carboxy prothrombin (PIVKA-II)- and alpha-fetoprotein (AFP)-producing gastric cancer. *J Gastroenterol.* Apr 2005;40(4):432-433.
50. Cornelissen M, Steegers-Theunissen R, Kollee L, et al. Increased incidence of neonatal vitamin K deficiency resulting from maternal anticonvulsant therapy. *Am J Obstet Gynecol.* Mar 1993;168(3 Pt 1):923-928.
51. Motohara K, Takagi S, Endo F, Kiyota Y, Matsuda I. Oral supplementation of vitamin K for pregnant women and effects on levels of plasma vitamin K and PIVKA-II in the neonate. *J Pediatr Gastroenterol Nutr.* Jul 1990;11(1):32-36.
52. Sokoll LJ, Sadowski JA. Comparison of biochemical indexes for assessing vitamin K nutritional status in a healthy adult population. *Am J Clin Nutr.* Apr 1996;63(4):566-573.
53. Nishiguchi T, Matsuyama K, Kobayashi T, Kanayama N. Des-gamma-carboxyprothrombin (PIVKA-II) levels in maternal serum throughout gestation. *Semin Thromb Hemost.* Jun 2005;31(3):351-355.
54. Miskad UA, Yano Y, Nakaji M, et al. Histological study of PIVKA-II expression in hepatocellular carcinoma and adenomatous hyperplasia. *Pathol Int.* Dec 2001;51(12):916-922.
55. Oshiro Y, Takada Y, Enomoto T, Fukao K, Ishikawa S, Iijima T. A resected case of metachronous liver metastasis from lung cancer producing alpha-fetoprotein (AFP) and protein induced by vitamin K absence or antagonist II (PIVKA-II). *Hepatogastroenterology.* Jul-Aug 2004;51(58):1144-1147.
56. Ronden JE, Groenen-van Dooren MM, Hornstra G, Vermeer C. Modulation of arterial thrombosis tendency in rats by vitamin K and its side chains. *Atherosclerosis.* Jul 11 1997;132(1):61-67.
57. Munroe PB, Olgunturk RO, Fryns JP, et al. Mutations in the gene encoding the human matrix Gla protein cause Keutel syndrome. *Nat Genet.* Jan 1999;21(1):142-144.
58. Luo G, Ducey P, McKee MD, et al. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. *Nature.* Mar 6 1997;386(6620):78-81.
59. Levedakou EN, Torsten G. Strohmeyer, Peter J. Effert, Edison T. Liu *Human Cancer*
60. Expression of the matrix Gla protein in urogenital malignancies *International Journal of Cancer.* 18 Jul 2006 2006;52(4):534-537.