

# Something more to say about calcium homeostasis: the role of vitamin K2 in vascular calcification and osteoporosis

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**Abstract. – BACKGROUND:** Vascular calcification and osteoporosis share similar etiopathogenetic mechanisms. Vitamin K2 deficiency could be responsible of the so called “calcium paradox”, that is the lack of calcium in the bone and its storage in the vessel wall. These events may have clinically relevant consequences, such as cardiovascular accidents, and bone fractures.

**AIM:** To review the biological function of vitamin K2 metabolism, the main factors related to its deficiency and the consequent clinical significance.

**DISCUSSION:** Vitamin K2 is essential for the function of several proteins, involved in the maintenance of the normal structure of arterial wall, osteoarticular system, teeth, and for the regulation of cell growth. It has been demonstrated to have a pivotal role in the inhibition of vascular foci of calcification, and in the regulation of calcium deposition in the bone. Vitamin K2 deficiency is often subclinic in a large part of healthy population. This deficiency is related to the interaction of various factors, such as the reduced dietary intake, the alteration of intestinal absorption or production, with a possible role of intestinal microbiota and the increased consumption at the vessel wall.

**CONCLUSIONS:** Vitamin K2 deficiency has recently been recognized as a protagonist in the development of vascular calcification and osteoporosis. Data reported so far are promising and, dietary supplementation seems a useful tool to contrast these diseases. However, large studies or solid clinical correlations regarding vitamin K2 deficiency and its pathologic consequences are needed to confirm these preliminary experiences.

*Key Words:*

Vascular calcification, Calcific atherosclerosis (CA), Cardiovascular disease (CVD), Coronary heart disease

(CHD), Osteoporosis (OP), Calcium paradox, Vitamin K1, Vitamin K2, Vitamin K dependent proteins (VKDP), Carboxylation.

## Introduction

Cardiovascular diseases (CVD) and osteoporosis (OP) are major health problems in Western Countries, due to their increasing incidence related to population ageing, and the consequent impact on the cost of public health<sup>1</sup>. It is also easy to argue how these two diseases may negatively affect the condition of chronic patients and, more generally, the physiologic process of ageing. Therefore, there is a great interest in the prevention of both CVD and OP<sup>2</sup>.

Calcific atherosclerosis (CA) is a notable determinant of CVD complications, and shares with OP similar risk factors, such as smoking, sedentary lifestyle and dyslipidemia<sup>3,4</sup>. Another feature common to CA and OP is the long-lasting latency period, often extended over decades during the lifetime, that could be interrupted at every moment by either an acute (e.g. myocardial infarction, sudden death, or bone fractures without the evidence of significant trauma) or chronic event (e.g. *claudicatio intermittens*, or pre-senile and senile round back). The evidences regarding other risk factors are discordant; the effect of gender is doubtful, at least until the fifth/sixth decade of life, and obesity, seems protective on OP but deleterious for CA<sup>5,6</sup>.

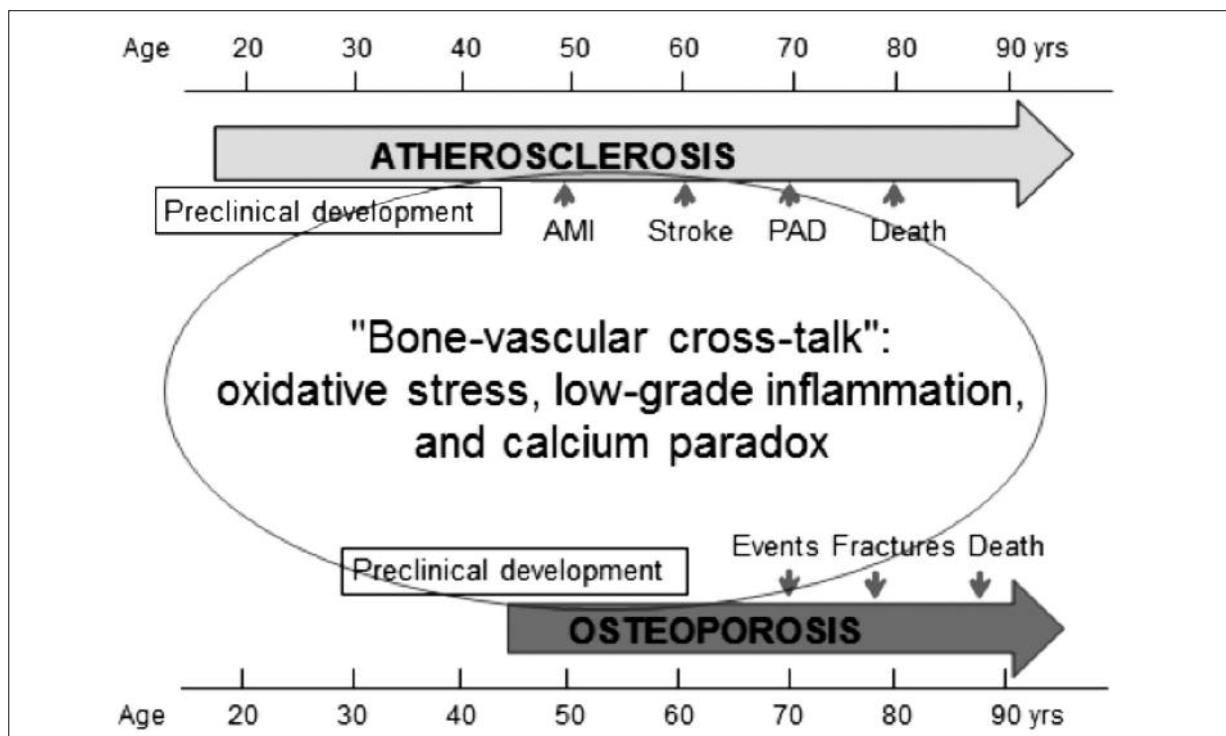
Beyond the large prevalence of these conditions among old people, at the origin of the strict association between CA and OP there are the

same physiopathologic and pathogenic mechanisms, such as subclinic inflammation, oxidative stress, and, in particular, calcium dysmetabolism (Figure 1). Recent evidences have cast a new light on CA and OP association, and have suggested the possibility to program common preemptive and therapeutic strategies<sup>7</sup>. Several studies emphasizing the presence of a “bone-vascular crosstalk” have been published, and the expression of its altered equilibrium is the association between CVD-related mortality and osteoporotic bone fractures. The innovative concept of “calcium paradox”, which means the deposition of calcium in the vessel wall with the simultaneous reduction of calcium deposition in the bone, has been provided to explain this phenomenon. However, at present, an unequivocal demonstration of the causal relationship between these two events has not been provided yet<sup>8</sup>.

**Vascular Calcifications: What do they Mean?**

The renewed interest in the study of the physiopathogenetic mechanisms of calcium deposition in arterial walls comes from the great number of recent studies highlighting the prognostic impact of coronary arteries calcifications (CAC). CAC are

detected by computed tomography (CT), providing a score based on the quantification of calcium in tunica intima and media of coronary arteries<sup>9</sup>. The CAC score has been demonstrated to be related with the incidence of new cardiovascular events and CVD mortality<sup>10</sup>. Moreover, CAC score is a useful instrument to provide a classification of those patients with a less defined prognosis; indeed, using the CAC score, individuals previously included in the intermediate class of cardiovascular risk could be moved either in the high or in the low class, with consequent important changes in the diagnostic and therapeutic programs<sup>11</sup>. The need to find new indicators of cardiovascular risk, such as the CAC, derives from the frequent observation that about 50% of patients who already had a first episode of myocardial infarction do not actually have an increased cardiovascular risk, and that many subjects belonging to the high categories of risk will never have a cardiovascular accident during their life. One of the possible matters of the epidemiologic research on CAC is the unfeasibility of population studies, nevertheless the difficulty to evaluate the different modality of intervention, since CT imaging follow-up is not advisable, due to the high risk related to radiation exposure<sup>12</sup>.



**Figure 1.** Common physiopathologic aspects of atherosclerosis and osteoporosis. AMI acute myocardial infarction; PAD peripheral artery disease.

As regards the physiopathology of vascular calcifications, it has recently been demonstrated that CA is a metabolically active process, rather than a passive, age-related, almost progressive and irreversible, phenomenon. CA resembles bone mineralization, of which shares several cellular and molecular mechanisms. The transformation of vascular smooth muscle cells (VSMC) into osteoblastic-like cell is widely documented in humans and experimental models of atherogenesis, and is the final step of the transformation of VSMC from the contractile phenotype to the secretory phenotype<sup>1,13</sup>. The result is the increase in arterial wall stiffness and a consequent negative influence on hemodynamics, which together with the thromboembolic risk of atherosclerotic plaques and the effects of vascular stenosis or obstruction contributes to increase CVD risk. This is the background of the development of systolic hypertension, hypertrophy/dilation of left cardiac ventricle, risk of myocardial infarction, risk of cerebral hemorrhage, hypertensive nephropathy. Furthermore, there are some evidences that globular subintimal microcalcifications are constantly present within stable atherosclerotic plaques, and exert a de-stabilizing effect dependent on peak circumferential stress<sup>14-19</sup>.

A common pathogenic mechanism between CA and OP has recently been identified, but it has been only partially clarified; it is represented by subclinical deficiency of vitamin K2 (VK2), which could have an important role in the development of “calcium paradox”<sup>20</sup>.

**Biological Function of Vitamin K1 and K2 and Physiopathology of Cerebral Status**

In the last decade, two wide epidemiologic studies conducted in Central European population evidenced that an adequate dietary intake of VK2 isoforms menaquinone 4 and 11 (MK 4 and 11) is of great importance for cardiovascular and

bone health. Moreover, it is inversely related to CVD morbidity and mortality and to incidence of bone fractures<sup>21,22</sup>. Furthermore, these studies failed to demonstrate any protective effect of the best-known vitamin K1 (VK1), also named phyloquinone, necessary for the physiologic homeostasis of blood coagulation<sup>23-26</sup>. Other data regarding the detrimental effects of prolonged administration of VK antagonists have been reported. Anticoagulant treatment with dicumarols is associated with an increased number of vascular and cartilaginous calcifications, and non-traumatic bone fractures. These evidences reinforce the evidence of the central role of VK2 in the prevention of ectopic calcifications and bone demineralization<sup>27-33</sup>.

VK1 and VK2 are activators of specific hepatic and extra-hepatic proteins with important biological functions, called “Gla-proteins”, which are VK dependent (VKDP). Considering the physiopathologic effects and the distribution of VKDP, extra hepatic sites are the main targets of VK2 absorbed from the gut, while hepatic ones of VK1<sup>34</sup>. The “Gla-proteins” owe their name and differences to the richness of glutamic acid residues, that are carboxylated in a VK-dependent process necessary for the acquisition of the biological function. Thus, un-carboxylated forms of VKDP are biologically inactive, and their presence in the blood is a sign of relative or absolute VK deficiency<sup>35-37</sup>. The 17 VKDP identified so far, their sites of synthesis and biological functions are reported in Table I. VK1 acts mainly in the liver, activating the coagulation factors II, V, IX and X, and the antithrombotic proteins C, S and Z. Indeed, spontaneous or pharmacologically induced VK1 deficiency has heavy consequences on blood coagulation process, and leads to serious polydistrectual hemorrhagic complications. The administration of activated coagulation factors, in the short term, and of VK1, in the mid-

**Table I.** Classification of the 17 Gla-proteins based on their function.

Function	Protein
Hemostasis (pro-coagulant activity)	Prothrombin, factor VII, IX and X
Hemostasis (anti-coagulant activity)	Protein C, S and Z
Inhibition of arterial calcification	Matrix-gla-protein (MGP)
Bone metabolism	Osteocalcin
Cell growth regulation	Growth-arrest sequence 6 protein (Gas6)
Unknown functions	Gla-rich-protein (GRP)
Periostin	
Periostin like factor	
Trans-membrane Gla-protein	

dle-long term, are necessary to overcome VK1 deficiency. Among the 17 VKDP identified so far, 3 have been recently identified and are VK2 dependent. These VK2DP have a well-characterized biological function, essential for the maintenance of the normal structure of arterial wall, osteoarticular system, teeth, and for the regulation of cell growth. In particular, the Matrix-gla-protein (MGP) is the gatekeeper of vascular “ossification”. MGP inhibits the precipitation of calcium, in the form of hydroxiapatite crystals, at the site of elastic lamellae, an event that blocks the removal of the initial foci of calcification. Thus, VK2 has a pivotal role in the prevention of CA, as it is the only one, indispensable activator of MGP. The second VK2DP with a relevant biological function is the Bone-gla-protein (osteocalcin). It acts in the bone, favoring mineralization of trabecula, and in the tooth, favoring synthesis of enamel. Finally, the third “Gla-protein”, named Growth arrest specific-6 gene (Gas-6), is, at present, object of several studies, due to its anti-oncogenic potential. Gas-6 seems able to delay tumor growth<sup>38</sup>, and participates to anticalcificant function of MGP, inhibiting apoptosis of the SMC of the vessel wall; indeed, SMC process of apoptosis provides the major number of initial structured foci of calcification. Other VKDP are object of study, but their clear role in the regulation of cellular processes has not been found yet.

VK2 seems to have a wide range of action in human, even only in relation to the physiopathologic roles that have been demonstrated so far (prevention of calcium precipitation in the matrix of vessel wall, cartilage and soft tissue, the involvement in neoformed bone trabecula ossification and the potential regulation of cell growth). This is the reason why VK2 is defined by the most expert authors in the field as “a vitamin that became omnipotent”<sup>39</sup>.

VK2 deficiency could be one of the key factors involved in the “calcium paradox”, providing an explanation for the recent evidence of increased CVD risk and mortality related to the exogenous administration of high calcium dose, eventually associated with vitamin D<sup>40-43</sup>. Such a deficiency could be the connection between CA and OP, opening new intriguing therapeutic scenarios for both diseases.

#### ***Vitamin K2 Deficiency and Therapeutic Potential of Dietary Supplementation***

As reported by the studies published so far, in the Western Population the percentage of MGP

carboxylation and activity seem to be about 60-70% only. This frequency is compatible with a real VK2 deficiency, maybe secondary to modifications of dietary habits occurred in the last 50 years, to population ageing, since the absorption of liposoluble vitamins is reduced in old people, and to other factors related to intestinal metabolism. According to the recent theory of “trriage”, VK2 deficiency may be secondary to a kind of preferential “choice”, that favors the VK1 in conditions of reduced dietary intake and/or intestinal absorption<sup>44</sup>. Indeed, the role of VK1 in maintaining homeostasis of blood coagulation is mandatory for survival, differently from the role of VK2 whose deficiency has almost long-term effects on individual health.

Cheese and fermented soya beans called “natto” are food rich of VK2, which is also present in lower measure in eggs, meat, and fish. VK2 intake can be evaluated by specific food frequency questionnaires, or measuring plasmatic levels, a method affected by blood lipid alteration. However, the functional evaluation of VK2 activity seems the most useful to assess VK2 intake in the clinical practice; it can be tested by quantification of un-carboxylated VK2DP.

Based on this physiopathologic background, dietary supplementation with VK2 in subjects with a proven deficiency, according to the principles of translational medicine, may contribute, in the long term, to the reduction of cardiovascular events and osteoporotic bone fractures, improving osteoarticular dynamic in old patients too. At present, published data regarding VK2 deficiency are restricted to special populations of patients, such as those who received a previous renal transplant and those undergoing dialysis<sup>8,45-46</sup>, but the efficacy of VK2 administration on MGP and osteocalcin carboxylation has been demonstrated. Two trials, one in postmenopausal women and the other in patients with CAC, are still ongoing<sup>47,48</sup>.

Bacteria are the producers of VK2 present in food; indeed, high levels of VK2 can be found especially in fermented cheeses or in the natto, which contains high levels of MK-7 due to the presence of *Bacillus Subtilis Natto*<sup>49</sup>. Based on these findings, a role of gut microbiota in maintaining the physiologic balance of VK in humans could be hypothesized.

#### ***The Role of Gut Microbiota in Vitamin K Homeostasis***

As like as food bacteria, intestinal bacteria are the main non-dietary source of menaquinones.

Our gut microbiota is made of 10 to 100 trillion of microbes, distributed along the gastrointestinal tract with a relative major abundance in the distal gut<sup>50</sup>. Among 70 divisions of bacteria, there are only two predominant ones: the *Bacteroides* and the *Firmicutes*<sup>51</sup>. However, only *Bacteroides* are able to synthesize menaquinones<sup>52-54</sup>, which are used as substrates for electron transport and oxidative phosphorylation.

*Bacteroides* are mainly able to synthesize the very long chain forms (with higher number of prenyl units) of menaquinones, the MK-10 and MK-11, while the MK-7, MK-8, MK-9 and MK-12 represent the minor part<sup>52-55</sup>. Moreover, *Bacteroides* produce VK2 isoprenologues, in which one or more of the prenyl units are saturated<sup>53,54,56,57</sup>. About 200-300 nmol of vitamin K are stored in an adult human liver, with a large inter-individual variation<sup>58,59</sup>. Phylloquinone represents a minor part, while menaquinones are predominant, especially the MK-7 to MK-13, with a 90% to 10% menaquinone to phylloquinone ratio<sup>58-60</sup>. The MK-10, MK-11, MK-12 and their isoprenologues are the main fractions of liver menaquinones; since they are mainly produced by *Bacteroides*, while are only in a little part introduced with diet<sup>53,55-57</sup>, gut microbiota is the main responsible for VK2 storage in the liver.

The debate whether liver storage may supply for VK2 necessary for Gla-proteins carboxylation in physiologic conditions or when dietary intake is insufficient, is still open. Studies in rat models are difficult to be conducted, since coprophagy represents a major bias in evaluating the dietary vitamin K intake. Moreover, in such animal models, the only reliable data regard the effects of acute VK deficiency<sup>61</sup>, which is an improbable event in humans. Studies conducted inducing VK deficiency in humans, reported that phylloquinone stores in the liver show a faster reduction than long-chain menaquinones ones, due to the different lipid content between the two compounds<sup>59,62</sup>. However, when the VK intake is nearly to or equal to zero, after an initial condition of buffering due to liver stores, deficiency becomes evident, leading to Gla-proteins decarboxylation and alteration in coagulation parameters<sup>63,64</sup>. Even if these considerations suggest that gut microbiota alone is not able to produce adequate amounts of menaquinones to maintain their physiologic functions in absence of an external source, its role in conditions of reduced (and not completely absent) intake has not yet been assessed. Moreover, the previous mentioned studies

examined only the restriction of dietary phylloquinone (VK1), and in one of them<sup>59</sup> individuals under study received also antibiotic treatment, which can alter gut microbiota composition and function. Nevertheless, the specific contribution of gut microbiota in maintaining VK2 stores in conditions of severe and mild deficiency needs to be further investigated, and may be not be related to phylloquinone metabolism. Indeed, although some authors postulated that MK-4 can be produced from intestinal conversion of dietary VK1<sup>53,55,65-68</sup>, such an hypothesis has been successively denied<sup>69-71</sup>.

## Conclusions

Calcific atherosclerosis plays a pivotal role in the development of cardiovascular disease, which is a main cause of morbidity and mortality in developed Countries. Quantification of risk and the knowledge of the mechanism at the origin of the development of arterial calcifications are important to prevent acute and chronic complications and to increase patients' survival. Vitamin K2 has been demonstrated to be involved in the inhibition of vascular foci of calcification, especially through the carboxylation of proteins that regulate calcium deposition in atherosclerotic plaques. At present, the evaluation of VK2 activity is the most reliable tool in the assessment of VK2 metabolism and its deficiency. Data regarding VK2 supplementation are available in special populations of patients, such as dialyzed ones, but large randomized clinical trials are lacking. Nevertheless, there are no internationally recognized guidelines and also indications for the every-day medical practice are scarce. In all patients diagnosed with calcific atherosclerosis, the assessment of the overall cardiovascular risk is mandatory, and an accurate evaluation of VK2 dietary intake is crucial for the investigation of carential status. Probably, particular attention should be paid to patients suffering from intestinal dysmicrobism. However, gut microbiota intervention in VK2 metabolism is still debated and should be object of specific studies including microbial metagenomic characterization and functional correlations.

In conclusion, we agree with the opinion that vitamin K is "a vitamin that became omnipotent", and, in particular, a vitamin that became promising in the prevention of cardiovascular accidents and complications; an adequate dietary supple-

mentation and a possible role of the correction of bacterial dysbiosis could be determinant for its physiologic effects on vascular calcifications. In this regard, in our center a multidisciplinary study group is investigating VK2 status in selected patients. The analysis includes the determination of MGP carboxylation level in blood samples and a food frequency questionnaire compilation, in order to correlate the presence of the eventual VK2 deficiency to intimal-medial thickness and stiffness of carotid arteries, presence of CA and signs of intestinal microbiota alterations.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

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