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Vitamin K Deficiency and Vascular Calcification. Is There Any Evidence about Its Impact on Coronary Artery Disease?

Theodoros Michailidis, Asterios Karakanas, Nikolaos Schizas and Petros Keryttopoulos

Abstract

Nowadays cardiovascular disease remain globally the leading cause of mortality. Coronary artery disease is the predominant clinical entity related to fatal cardiovascular events, while its development is mostly associated with progressive atherosclerosis of the vessels combined with gradual vascular calcification. It is well described and understood that vascular calcification is strongly associated with the occurrence of CVD and increased mortality rates. Therefore, it is essential to understand the metabolic pathways leading to its formation in order to develop effective therapies. A group of vitamin-k dependent proteins seems to play a significant role on the prevention of the arterial wall. Several past studies have shown that in cases of vitamin-k deficiency the process of vessel calcification is accelerated. Vitamin-k depletion and high levels of uncarboxylated and dephosphorylated forms of the aforementioned proteins are considered as important factors that contribute significantly to this rapid progression. Promising studies are giving the stimulus for further research in the field of vitamin-k supplementation and the suspension of vascular calcification.

Keywords: vitamin K, deficiency, vitamin-K dependent proteins, vascular calcification, coronary artery disease

1. Introduction

Cardiovascular diseases are the leading cause of mortality worldwide. Their constant increasing rate is attributed to many factors as the adoption of poor dietary habits and sedentary lifestyle accompanied in some cases with hereditary background. The so-called Western way of living has led also to the rise of other diseases such as dyslipidemia, hypertension, obesity, diabetes mellitus and chronic kidney disease, which are the main risk factors of CVD. Those conditions are mainly responsible for the rapid progression of vessel's atherosclerosis and plaque formation. Intimal calcification of large vessels is the main effect of those disorders responsible for plaque's progression. However, atherosclerosis is not the only factor. Vascular calcification is another major and independent risk factor strongly related with the development of vessel plaque leading to CVD [1]. It is a chronic

and multi-factorial procedure related mainly with disorders such as chronic kidney disease and diabetes mellitus [2]. The metabolic pathways which are responsible for the progression of vascular calcification is not well-established and consequently treatment remains a challenge. Media arterial layer is the target area of calcification and can involve all vessel sizes regardless of the concurrence of atheromatic plaque [3]. The negative effect of this procedure can gradually lead to the development of valvular heart disease and coronary artery disease (CAD).

Matrix degradation and modification is the main reason of medial arterial calcification. Matrix Gla protein (MGP), Gla rich protein (GRP) and growth arrest specific gene-6 (Gas-6) are a group of vitamin-k dependent proteins that is considered to effectively inhibit the progression of vascular calcification. For example, in cases of vitamin-k deficiency, glutamic acid residues do not convert to the amino acid γ -carboxyglutamic acid residuals (Gla) which is a vital part for the normal function of MGP. Consequently, inactive forms of MGP (dp-ucMGP) accumulate in calcified tissues such as the blood vessels. Past studies have proposed ucMGP as a potential marker of vitamin-K deficiency, vascular calcification and cardiovascular disease [4]. Other trials have showed that patients under treatment with vitamin-k antagonists (VKAs) had higher scores of vascular calcification compared to a group of controls. Animal trial connected the administration of VKAs with an accelerated progression of medial calcification. On the other hand, when high doses of vitamin-K were administered, calcification lesions started slowly to improve [5]. Perspectives about the benefit of vitamin-K administration in specific population remain controversial, and therefore further research is required in this domain.

2. Vitamin-K dependent proteins

The correlation between low vitamin-K levels and rapid progression of vascular mineralization is supported by researchers in the past [6]. The pathophysiological pathways that justify such claims involve a group of vitamin-K dependent protein and calciprotein particles. Those proteins are the matrix Gla protein (MGP), gamma-carboxylated Gla-rich protein (GRP) which are both considered as strong inhibitors of vascular calcification and the growth specific arrest gene-6. Matrix Gla protein (MGP) is a vitamin-k2 dependent protein which is secreted by many cells, including the vascular smooth muscle cells (VSMC) and has high affinity to calcium crystals. Its effect on atherosclerotic plaque may probably derive from the blocking of calcium accumulation. It's present in bones and cartilage, in kidneys but also in blood vessels, endothelium and coronary artery. Matrix Gla protein prevents from the calcification development in soft tissues such as the blood vessels. Additionally, its preventive effect is exerted by inhibiting the bone morphogenetic protein-2 and 4 (BMP-2, BMP-4). This effect is of utmost importance because it prevents the VSMC from the transdifferentiation into osteoblasts like-cells which lead into progressive calcification of atherosclerotic plaques. In order to exert its effect, MGP has to be synthesized locally in the VSMCs. Vitamin-k2 is used as co-factor to its formation through the post-translational γ -carboxylation and phosphorylation of inactive MGP [7]. Carboxylated MGP (cMGP) is the active protein form responsible for the prevention of the calcification of the arterial wall [8]. MGP's expression from the VSMCs and the endothelium may give us the opportunity to measure its circulating levels. As it was mentioned before, cMGP has high affinity to calcium crystals and binds strongly to them preventing from their accumulation into the arterial wall. Considering that cMGP could evolve into a very promising biomarker for the prognosis and the progression of calcification

as well as a prognostic factor to severe cardiovascular outcomes [9]. On the other hand, it should be mentioned that inactive forms of MGP have been associated with accelerated rate of vascular calcification but also with increased mortality [10]. Whereas the active form of MGP is both carboxylated and phosphorylated, the inactive forms have not undergone one of those two metabolic steps or both of them (uncarboxylated, dephosphorylated MGP, dp-ucMGP). cMGP is the only factor that under specific conditions may promote the reversal of vascular calcification [11]. It is worth mentioning that calcified arteries have high concentrations of MGP and the severity of such lesions was related to MGP serum levels [12]. Gla rich proteins is another vitamin K dependent protein which is present in both bone and cartilage and also has high affinity to calcium. High concentrations of γ -carboxylated GRP have been observed in individuals with increased vascular calcification. However, suggested data derive mainly from animal studies and its functionality in humans is not well established. Calciprotein particles are particles that prevent from vascular calcification by blocking the formation of the calcium/phosphate crystals. These particles also contain high levels of MGP and GRP, so in cases of vitamin-k deficiency, their effect may be impaired. Vascular smooth muscle cells excrete also the growth arrest specific gene 6 protein (Gas-6) which is one of the stimulating factors of their growth. It exerts its preventive effect through several pathways. Initially it stimulates the bcl-2 anti-apoptotic protein which is responsible for the inhibition of caspase-3. Caspase-3 is a crucial protein for the pro-apoptotic cell procedure, so through that way Gas-6 protects VSMCs from transdifferentiation into cells with osteoblast-like effects. Apoptotic cells could be used as substrate for the development of constant inflammation and excessive calcification. Thus, Gas-6 through this way Gas-6 prevents the arterial from this process. However, in order to exert

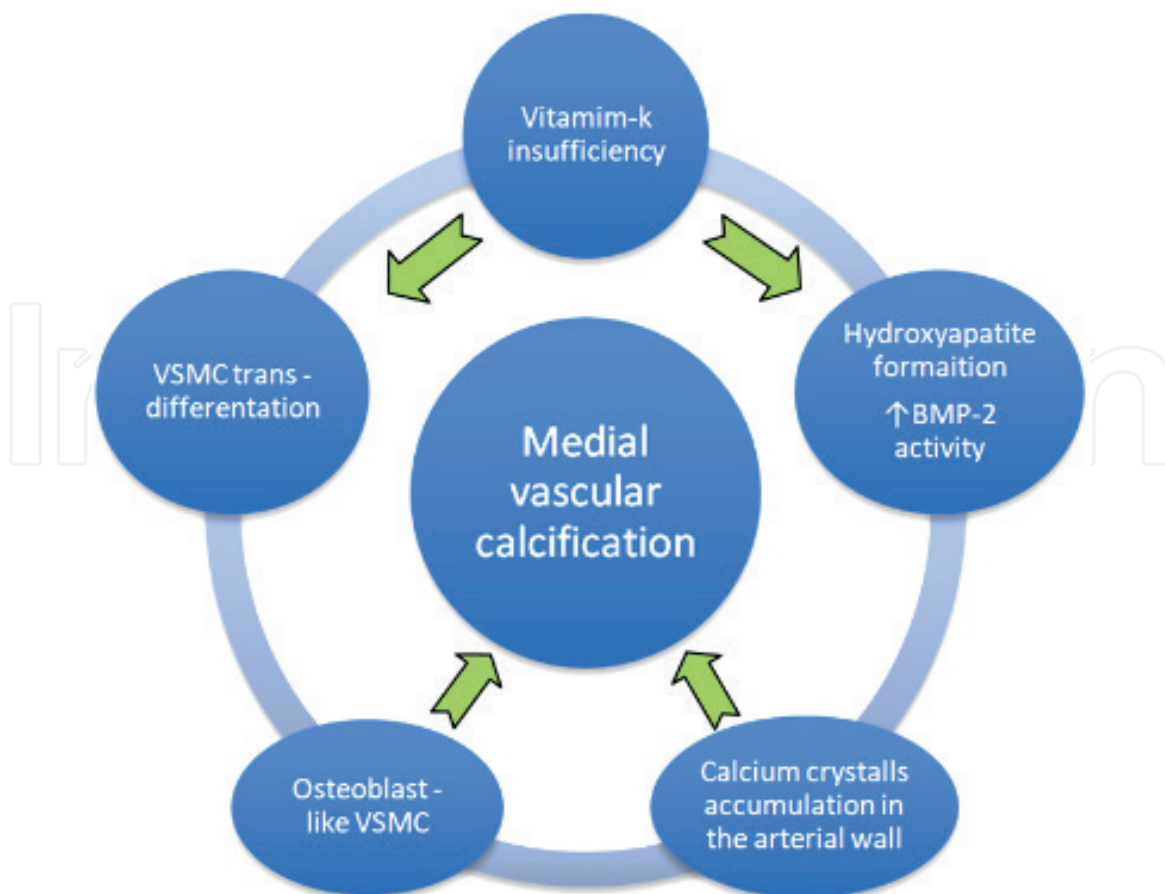


Figure 1. Summary of the effect of vitamin K deficiency on vascular calcification. Abbreviations: BMP-2, bone morphogenetic protein-2; VSMCs, vascular smooth muscle cell.

its effects Gas-6 needs γ -carboxylation a process in which vitamin-k is a necessary co-factor [13]. Taking all that into consideration, Gas-6 is vitamin-K dependent and very important factor, against the VSMCs apoptosis and the progression of calcified plaque. In cases of vitamin-k deficiency the lack of Gla proteins affect negatively the growth of VSMC [14] (**Figure 1**).

3. Postmenopausal women, vitamin-k and vascular calcification

In past studies it was also observed that post-menopausal women with osteoporosis had increased rates of arterial calcification [15]. Based on that, researchers have investigated the potential correlation of vitamin-k status, bone formation rate and vascular calcification of post-menopausal women. Initially, lower bone formation rate in parallel correlation with low levels of serum vitamin-k. The same also applies for the increased frequency of vascular calcification observed in post-menopausal women [16]. Concerning, the lower bone mass of post-menopausal women, it seems that vitamin-k levels and osteocalcin induced remodeling bone process are closely related. Though not with the same metabolic way to vascular calcification but in both situations vitamin-k is necessary [17]. It is important to mention that few trials, evaluated the potential benefit of vitamin k2 supplementation in post-menopausal women with aortic calcification and osteoporotic lesions. According to the authors, calcified lesions were at least sustained or in some cases decreased. However, these beneficial effects were observed in individuals that received vitamin-k2 supplementation but not vitamin-k1 [18]. Finally, results from the study of Gast et al. in this group of patients, have showed that vitmamin-k2 had the greatest preventive effect in CAD [19]. There are data that suggest that osteoporotic women are on higher risk of cardiovascular disease compared to match-aged non-osteoporotic individuals. These evidence imply that this difference occurs due to increased vascular calcification of both large vessels and coronary artery in those patients. Vitamin-k2 may have an essential role both to bone formation via its effect on osteocalcin as well as to the reduction of vascular mineralization. Further and larger studies are needed in order to assess the potential benefit of vitamin-k2 supplementation in both bone mass loss, atherosclerotic progression and prevention of cardiovascular disease in this specific population [20].

4. Vitamin-k-antagonists and vascular calcification

Vitamin-k antagonists are oral anticoagualants that reduce the levels of vitamin-k by interfering in vitamin's recycle. They essentially inhibit vitamin-k reductase complex-1 and consequently reducing active vitamin-k reserves. Thus, they prevent from the formation of blood clots. Nevertheless, vitamin-k depletion in serum has some detrimental effect on many sequences of steps in metabolic pathways like those mentioned before. Taking that in mind, previous studies have demonstrated the negative effect of VKAs on the progress of vascular calcification [5]. Win et al. examined the potential benefit of apixaban administration in patients with atrial fibrillation in terms of the atherosclerotic plaque progression. Study results showed that apixaban administration was associated with slower plaque progression as well as lower calcium scores compared to warfarin treated group. Taking into consideration the usual co-existence of atrial fibrillation and coronary artery disease, apixaban could be a preferable choice in patients with atrial fibrilation not only for the

prevention of thromboembolic events but also to slow down coronary plaque progression and prevent from fatal cardiovascular events [21]. Accordingly, outcomes from a trial that compared the use rivaroxaban against warfarin have also indicated the beneficial effect of NOAGs into the slower plaque progression arterial calcification [22]. Therefore, in cases of individuals with established CAD or high level of vascular atherosclerosis in need for anticoagulation, the physician should choose wisely the appropriate treatment. These data suggest the preferable choice of NOACs instead of VKAs though further studies are needed for more robust results. However, what about patients that are in need for VKAs coagulation, or the use of NOACs is contraindicated? Such dilemmas are common in clinical practice and therefore the presence of clearly defined guidelines is essential.

5. Chronic kidney disease, vitamin-k status and vascular calcification

Chronic kidney disease is strongly associated with the presence of increased cardiovascular risk. The most frequent cause of death in this population is coronary artery disease as well as stroke events. Hypertension, dyslipidemia, diabetes mellitus, which are common comorbidities in patients with renal failure, are important factors that can lead to excessive atherosclerosis and the development of CVD. Moreover, vascular calcification is also excessive in patients with chronic kidney disease (CKD) and so it constitutes an independent risk factor for the development of CVD [23]. Age, time on hemodialysis, persistent hyperphosphatemia and hypercalcemia are some of the causes of accelerated vascular calcification. High calcium and phosphate levels are associated with the deposition of hydroxyapatite into the arterial wall [24]. Whereas the presence of intimal calcification, which is related mainly to large vessels, is almost the same between individuals with or without end-stage renal disease, this is not the case for median calcification of the arterial wall. For example, aorta calcification is worse in patients with renal disease compared to those without. Aorta calcification increases arterial stiffness, which consequently aggravates the present hypertension and finally contributes to the development or the deterioration of a pre-existing left ventricular hypertrophy and left ventricular insufficiency. Patients with end-stage renal disease have increased vascular calcification compared to non-CKD individuals, especially those that are under hemodialysis [25]. Coronary artery calcification is common among older patients with CKD. However past studies have also showed that younger individuals with end-stage renal disease have also a high percentage of vascular calcification as well as coronary artery calcification [26]. Using high resolution computed tomography; researchers have proved that coronary calcification was higher in young adults that were on dialysis compared to those that were not. At this moment, the main treatment approach against the vessel mineralization of CKD patients is the strict regulation of calcium and phosphate balance. Additionally, clinician focus their attention to better treatment management of concomitant disorders that affect atherosclerotic plaque, such as diabetes, dyslipidemia and smoking cessation. Even though there is no strong evidence that support the administration of agents that inhibit calcification, it is easy to understand that those interventions might play a crucial role to the prevention of CVD in CKD-individuals. In a microenvironment of constant inflammation, vascular smooth cells are gradually transformed in osteoblasts like-cells promoting then the development of medial artery calcification. This process is mediated by multiple proteins and is facilitated by the presence of systemic or local inflammation. The latter is of outmost importance for the reason that macrophages excrete among others, matrix-metalloproteinase which

lead to the apoptosis of elastic fibers and thus promote vascular calcification. In addition, vitamin k-dependent matrix Gla protein and Gia-rich protein are both important for vascular calcification. As it was mentioned before, low levels of those proteins have been associated with increased rate of calcification and development of cardiovascular disease. Several studies in the past have proved the increased coronary artery calcification in CKD-individuals by calculating the number of vessel calcifications using high resolution computed tomography. According to the study of Holden et al. in patients with CKD and/or end-stage renal disease vitamin-K deficiency is very common. It is possible that this outcome has some extra effect on the progressive calcification of these patients [27]. At this point, treatment in order to delay the progression of vascular calcification in ESRD and CKD patients targets to the regulation of calcium and phosphate. However, dietary advice in order to prevent hyperkalemia or hyperphosphatemia leads to the avoidance of food rich in vitamin k, inducing the existing deficiency. A recent study with hemodialysis patients have studied the effect of vitamin-k supplementation on the plasma levels of ucMGP, uncarboxylated osteocalcin and PIVKA-II. The results were very promising, because have showed a significant decrease of the inactive form of MGP and also proved the vitamin-k deficiency in this specific population. The outcomes of this study may be the beginning of future randomized controlled trials that will evaluate the effect of vitamin-k supplementation on vascular calcification of CKD patients [28]. Respectively, another study by Oikonomaki et al. in hemodialysis patients have demonstrated the reduction in uc-MGP levels after 1-year of vitamin-k2 supplementation but the progression of vascular calcification remained the same between studied groups [29]. It is possible that only vitamin-k supplementation is not enough in order to overt calcification. This process in such individuals is so multi-factorial that need a comprehensive treatment approach that will slow the progression. Further studies are needed in order to clarify the benefits of vitamin-k supplementation in ESRD or CKD patients.

6. Vitamin-k and coronary calcification

Vascular calcification and especially coronary calcification is a strong predictor of coronary events and this is a process that is regulated actively by vitamin K dependent proteins which are called matrix Gla proteins. There are currently no pharmacological means to improve vascular stiffness and vascular calcification. There is growing evidence that vitamin K, a cheap and safe intervention that can have beneficial effects on cardiovascular health. Vitamin K straightforward administration can reduce the progression of vascular calcification. The biological rationale is that supplementation with vitamin K will carboxylate (activate) Gla proteins, whose role, among others, is to reduce the progression of vascular calcification [30]. Coronary artery calcification which is a significant marker of cardiovascular disease is affected by these dependent vitamin K proteins. As it was mentioned before vitamin-K deficiency promotes coronary artery calcification [6]. In animal studies, MGP removal showed severe progression of vessel calcification and this empowered the theory about vitamin K role in this process [31]. The most of the clinical trials showed that supplementation with Vitamin K2 (menaquinone) had beneficial effects in cardiovascular calcification. But a clinical trial from Shea et al. showed that also supplementation with vitamin K1 (phyloquinone) slowed the progression of coronary artery calcification. Though its effect was enhanced by the parallel administration of vitamin-D and calcium supplements [32]. On the other hand, Beulens et al., with a cross-sectional study among 564 post-menopausal women reached the conclusion that only high intake of menaquinone is associated

with reduced coronary calcification, as it was measured via computed cardiac tomography [31]. Also Vossen et al. wanted to study a sample of patients with coronary artery disease and follow them up via Agatston calcium score about the progression of coronary calcification as long as the individuals were under vitamin-k supplementation [33]. The Rotterdam Study, a prospective population-based study, showed in a sample of 7983 men and women aged 55 y and over in a follow up to 10 years, that dietary intake of menaquinone had a protective effect against coronary heart disease [18]. Also a meta analysis of 3 US Cohorts, among 3891 patients, who measured fasting circulating phyloquinone levels, showed that low phyloquinone levels was associated with increased all – cause mortality but not of CVD [32]. Many clinical trials as referred above showed a possible correlation between vitamin K and increased artery calcification mainly in high risk patients. A systematic review from Hartley et al. tried to show if there is primary prevention from CVD in healthy individuals who received supplementation with vitamin K. They only found a small clinical trial with only 60 patients that fulfilled their criteria and there was no significant impact in primary prevention of CVD and other CVD factors such as blood pressure and plasma lipids level (Hartley). However, this was a small clinical trial with short duration. Further studies are necessary about the benefit of vitamin-k supplementation in the primary prevention of CVD.

7. Vitamin-K and valvular calcification

Valvular calcification is a common degenerative disease characterized by progressive valvular calcification. Nowadays the incidence of valvular disease is higher probably due to increased life expectancy. The most commonly calcified valves is aortic valve followed by mitral valve. Based on the role of vitamin K as protective agent for cardiovascular health and especially as a protector against vascular calcification, some trials evaluated the potential effect of vitamin-k as a protective factor against the development of valvular calcification. Bradenburg et al. with a small prospective, open label clinical trial with 99 patients, selected individuals with asymptomatic or mild symptoms with aortic calcified valve and separated them in two study arms with vitamin-k1 supplementation and placebo. Then, they calculated the amount of calcification of the valve via cardiac computed tomography, at the beginning of the trial and after 12 months. Also they measured the dephosphorylated undecarboxylated MGP as a circulating marker for vitamin-k deficiency. Over the 12 month period, aortic valve calcification volume score progressed 10% in the arm with vitamin K supplementation compared with 22% in the placebo group. Also plasma dp-ucMGP were significantly reduced by 45% in the vitamin K group. On the other hand, this trial had many limitations such as the small sample, the short duration of follow up, the open-label design and the broad spectrum of severity of valvular disease at baseline [34]. Another clinical trial that is planned and want to study the effect of vitamin K in aortic calcification is from Peeters et al. concerning especially the calcification progress of bicuspid aortic valve. Bicuspid aortic valve, a common congenital abnormality, occurring in 13,7 per 1000 people in general population is associated with early development of calcific aortic valve stenosis. In this double-blind study they will supply vitamin K2 and follow up 44 people in a period over 18 months. The follow up of the sample will be every 6 months with PET/cardiac MRI, cardiac CT and echocardiography. This trial will provide us with more information for calcium activity on aortic valve and the potential effect of vitamin K. It will also open the way for large scale randomized clinical trials in order to develop potential treatment option against the progression of calcific aortic valve stenosis [35].

8. Vitamin-k supplementation and vascular calcification

Vitamin-K deficiency is associated with low-levels of MGP and Gas-6 and the accumulation of GRP, ucMGP and dpMGP forms in soft tissues and vessels. As it was described before, vitamin-K is used as co-factor for the post-translational γ -carboxylation of MGP and Gas-6 in order to maintain preventive effect on vessels and VSMCs respectively. In addition, vitamin-k deficiency is associated with higher circulating levels of uncarboxylated or dephosphorylated forms of MGP. A three arm randomized controlled trial assessed the levels of dephospho-carboxylated MGP levels after 12 weeks of menaquinone-7 supplementation. The dp-ucMGP levels were reduced significantly. It is important to mention that according to the authors the outcome was dose-dependent and increased in time [4]. In a systematic review meta-analysis of trials with different study-design by Lees et al., dp-ucMGP levels were significantly reduced with the administration of vitamin-k. However, that effect did not lead to the improvement of vascular calcification with the exception of a small number of studies. Concerning the use of dp-ucMGP as biomarker of CVD the results are controversial and so that is not suggested. It is important to mention that the greater efficacy was achieved with vitamin-k2 supplementation rather than k1 [30]. These data suggest that dp-ucMGP could be an important marker of vitamin-k status. It is worth mentioning that the co-administration of vitamin-k and vitamin-D have showed some promising results concerning the prevention of fatal CVD. However, larger randomized controlled trials are needed in order to delineate if vitamin-k supplementation alone or in combination with vitamin-D could benefit patients with progressive vascular calcification [36] (Table 1).

	Vitamin-K sufficiency	Vitamin-K deficiency (ex. Inadequate intake, VKAs administration)
MGP (vitamin-K dependent carboxylation)	<p>↑ active cMGP prevents:</p> <ol style="list-style-type: none"> 1. The hydroxyapatite formation 2. The accumulation of calcium crystals 3. Transdifferentiation of VSMCs into osteoblast like-cells 4. Decreased BMP-2 activity 	Significantly lower levels of MGP-2 and accumulation of its inactive forms (dp-uc MGP) with devastating impact on VC
Gas-6 protein (γ -carboxylation with vitamin-K as co-factor)	<ol style="list-style-type: none"> 1. Activation of anti-apoptotic protein Bcl-2 2. Inhibition of pro-apoptotic protein Caspase-3.* 	↓Gas-6 levels, inhibition of VSMCs growth and increased apoptosis
VSMCs	Inhibition of apoptosis and transdifferentiation into osteoblast like-cells	Increased apoptosis and transdifferentiation into osteoblast like-cells
Vascular Calcification	Suspended and possibly reversed medial VC**	Increased medial VC

VSMCs status and progression of vascular calcification depending on vitamin-k serum levels. Abbreviations: MGP, matrix Gla protein; Gas-6 protein, growth arrest specific gene 6 protein; VSMCs, vascular smooth muscle cell; VC, vascular calcification.

**Apoptotic cells are used as substrate for accumulation of calcium crystals.*

***Observed in small trials with co-administration with vitamin D. Further studies are needed.*

Table 1.
Summary of changes in vitamin-k dependent proteins.

9. Conclusion

Vitamin-k is a fat-soluble vitamin mostly known for its significant role in the coagulation sequence of steps. However, vitamin-k is also important to other also important metabolic pathways. Vascular calcification has proved to be a multi-factorial procedure that leads to the transdifferentiation of VSMCs into osteoblast phenotype like-cells and a vicious circle of arterial wall calcification. Vitamin-K dependent proteins MGP, GRP and Gas-6 play an important role in the regulation of this constant progressive process. In cases of vitamin-k deficiency, the preventive effect of those Gla proteins on the arterial wall declines and consequently the process of vascular calcification is enhanced. The calculation of the uncarboxylated and dephosphorylated forms of those proteins are considered a marker of vitamin-k deficiency. However, existing data do not suggest the use of dp-ucMGP as predicting factors of cardiovascular disease. Vitamin-k supplementation have been associated with a significant reduction in the dp-uc MGP circulating levels, although this reduction was not related with a reduction in the process of vascular calcification. Due to the multi-factorial process for the formation of calcified plaques, it cannot be suggested at this point that vitamin-k supplementation alone will reverse those lesions. Emerging evidence support the co-administration of vitamin-D and vitamin-K has a greater effect against the progression of vascular calcification. Concerning the effect of vitamin-k deficiency and cardiovascular health, evidence remains controversial. It is a given that more studies are needed in this area in order to draw safe and robust conclusions. Vascular calcification and atherosclerotic plaques are strongly related with arterial stiffness, hypertension and impaired cardiac function. It is of outmost importance to find solution of this degenerative procedure in order to develop additional preventive and therapeutic strategies against the development of cardiovascular diseases.

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