Vitamin K2 and arterial calcification

NEW ROLE FOR VITAMIN K

Atherosclerosis is a gradual process that, after many years, often results in severe cardiovascular disease like heart infarct, heart failure or stroke. In most Western countries, atherosclerosis is the main cause of illness or death. In 2003, atherosclerosis in the Netherlands was responsible for approximately 50,000 deaths. Despite significant advances in medical science, heart and brain infarcts induced by atherosclerosis are still one of the major causes of death.

Calcification of the arteries – which deteriorates arterial function – is an independent risk factor for the development of atherosclerosis. Recent studies have shown that calcification (mineralization by calcium deposits) of the vascular walls occurs in an early stage of atherosclerosis and adversely affects its severity and progression[1]. This contradicts the supposition that the vascular wall does not calcify until the final stage of the disease. In view of this it is logical to try to prevent or revert calcification in order to prevent or slow down the development of cardiovascular diseases. Presently however, there are no medicines for the prevention or reduction of calcification. The medicines developed to control cardiovascular diseases are mainly aimed at reducing blood pressure and cholesterol, at vascular dilatation and blood dilution. Recent research suggests that vitamin K2 could possibly play the role of an effective inhibitor of vascular calcification. However, additional documentation from human intervention studies are needed.

Along with new insights into vascular calcification as an independent risk factor, it was discovered that vitamin K – in particular vitamin K2 – plays a prominent role in the prevention of the calcification of arterial vascular walls and heart valves. By now, it has also been discovered that the performance of this newly found function requires a daily vitamin K level in excess of what is needed for the coagulation of blood. However the recommended daily intake of vitamin K is based on the needs for blood clotting. A recent experiment on rats showed that a high vitamin K dosage can partially remove the calcification already existing in the arteries, and also restore vascular performance. These new findings may mean that vitamin K can play a promising role in the prevention of cardiovascular diseases.

VITAMIN K: NEW ‘BLOOD-CURDLING’ DISCOVERIES

It is now apparent that vitamin K is not only involved in blood coagulation, but is vital for many more important biological functions. Recent scientific research shows that inadequate intake of vitamin K may contribute significantly to the development of arteriosclerosis (hardening of arteries) and osteoporosis (bone decalcification).

FUNCTIONS OF VITAMIN K

Vitamin K is essential for the conversion of certain proteins into their active forms. These proteins are found throughout the body and have many functions. In addition to coagulation of the blood, the best characterized vitamin K dependent proteins are involved in calcification (mineralization) of bones during bone formation and in inhibiting of calcification of soft tissues (cartilage and organ tissues).
Other less well known vitamin K dependent proteins play roles in various cell-regulatory processes related to, for example, cellular growth and differentiation and apoptosis\(^2\).

Although the role of vitamin K on coagulation factors has been known for over 60 years, other functions of this vitamin became generally known only recently. In the last ten years, its role in the prevention of osteoporosis in the elderly has been the subject of much scientific interest. Today the interest in the role of vitamin K in cardiovascular disease is growing. Over a decade ago, the first link was made between the vitamin K status and the risk of atherosclerosis\(^3\).

**MECHANISM OF VITAMIN K**

Vitamin K dependent proteins contain glutamic acid groups (Glu) that must become carboxylated into gamma-carboxyglutamic acid groups (Gla) in order to activate the proteins. This conversion is performed by an enzyme (carboxylase) which needs vitamin K as a co-factor. The Gla groups enable these proteins to specifically bind calcium ions. Binding calcium will contribute to building and strengthening of bones, to prevention of calcification in arteries or activation of specific coagulation factors in the liver. Lack of vitamin K causes ineffective enzymatic carboxylation, or ‘under-carboxylation’, which makes vitamin K dependent proteins less effective or completely ineffective. In the presence of inadequate amounts of vitamin K, coagulation usually continues with normal activity because the liver rapidly absorbs what it needs. This may leave too little vitamin K for the general circulation, creating deficiencies in bone and arterial cells. This means that normal coagulation may conceal vitamin K deficiency for other functions.

The body’s vitamin K status can be estimated (in blood serum) by measuring the degree of carboxylation of the vitamin K dependent bone protein osteocalcin. At an intake of vitamin K sufficient for completely carboxylate blood clotting factors in the liver, 20-30% of osteocalcin can be found in the undercarboxylated (inactive) form\(^4\). Low serum levels of carboxylated osteocalcin signals a poor vitamin K status.

In patients suffering from osteoporosis, under-carboxylated osteocalcin is almost always found in the blood. Active osteocalcin is necessary in order to preserve the bone strength and mass (density of bone minerals). The presence of under-carboxylated osteocalcin indicates that it is highly probable that other vitamin K dependent proteins outside the liver are also less active, such as matrix-Gla-protein (MGP), which counteracts the calcification of soft tissues. Documented findings of the simultaneous occurrence of both bone decalcification and vascular calcification in the same person is often referred to as the “calcification paradox”\(^5\).

**TWO TYPES OF VASCULAR CALCIFICATION**

There are two types of vascular calcification (arteriosclerosis), namely atherosclerosis and Mönckenberg’s arteriosclerosis. In both types, the vascular wall hardens and loses elasticity. In atherosclerosis, calcification occurs in the inner layer of the vascular wall (tunica intima) and the formation of atherosclerotic plaques is typical. As the plaques increase in size, arteries become narrower and can even become entirely blocked by blood clots (thrombi). Thrombi are formed when some plaque is torn or burst open. Atherosclerosis can cause heart or brain infarct. The risk factors include high blood pressure, increased cholesterol, smoking and insufficient physical exercise.

In Mönckenberg’s arteriosclerosis, the calcification occurs in the middle layer of the vessel wall (tunica media) and there is no plaque formation and obstruction of the vessels, as is the case in atherosclerosis. The stiffness of the blood vessels disables the blood circulation. Mönckenberg’s sclerosis is generally found in patients with diabetes mellitus, kidney diseases and in ageing. In diabetes, Mönckenberg’s sclerosis is a marked risk factor for death from cardiovascular disease and the occurrence of stroke\(^6\).

**NEW INSIGHTS INTO THE CALCIFICATION PROCESS**

Calcification of the vascular wall is a dynamic and complex process that has a highly regulated course and shares features of mineralization of bone\(^5,7\). As mentioned before vascular calcification occurs in an early stage and affects the severity and progression of atherosclerosis\(^8\). These new insights implicate that it could be of great benefit to inhibit the calcification process. It has become apparent that various vitamin K dependent proteins play a role in the calcification process\(^8,9\). Of these, the role of matrix-Gla-protein (MGP) is currently the clearest. Furthermore, a very recently published experiment in rats has shown that calcification of the arteries is not an irreversible phenomenon as presumed\(^11\).
Matrix-Gla-protein (MGP) inhibits the calcification of soft tissue and is one of the strongest inhibitors of calcification of vascular walls and heart valves. This was first brought to light in 1997 in an experiment with mice. Matrix-Gla-protein (MGP) is mainly synthesized in blood vessels and cartilage, and furthermore in other soft tissues such as the heart, lungs and kidneys.

Matrix-Gla-protein inhibits calcification in both types of vascular calcification (i.e., atherosclerosis and Mönckenberg’s arteriosclerosis). In healthy arteries, MGP is synthesized by endothelial cells (cells that line the lumen of vessels) and by smooth muscle cells in the tunica media (middle layer of vascular walls). The matrix protein is deposited in the extracellular matrix (connective tissue) of the vascular wall, especially in the elastic fibres. In pathological circumstances, local calcification of the extracellular matrix occurs. As a countermeasure, the generation of MGP around the place of calcification is considerably increased.

In order to adequately counteract the calcification it is important that there is enough vitamin K present to activate and sustain the activation of the increased MGP. Activated matrix-Gla-protein prevents the formation of calcium crystals and deactivated a factor that stimulates calcium deposition (BMP2, bone morphogenetic protein-2).

**UNDER-CARBOXYLATION OF MGP IN VASCULAR CALCIFICATION**

Under-carboxylated (and therefore inactive) MGP appears to be a risk factor for the formation of vascular calcification. In healthy arteries, the MGP occurs in the carboxylated form and no under-carboxylated MGP is found, while in the arteries of patients suffering from atherosclerosis or Mönckenberg’s arteriosclerosis, under-carboxylated MGP is found in calcified areas. In calcified areas of the media, the MGP may even be entirely absent. Also in rats receiving the vitamin K antagonist warfarin, non-carboxylated MGP is found in vascular calcium deposits.

**EPIDEMIOLOGICAL STUDIES**

In 1995, Vermeer’s research group at the University of Maastricht were the first to describe the connection (in post-menopausal women) between vitamin K intake and the risk of atherosclerosis. Data from the Nurses’ Health Study (1984 to 2000) and from the Health Professionals’ Follow-up Study (1986-2000) have brought to the fore a high vitamin K intake (K1) as indicator of a low risk of cardiovascular disease. The Nurses’ Health Study covered 72,874 nurses aged between 38 and 65 years. The Health Professionals’ Follow-up Study concerned 40,087 male health-care workers.

The Rotterdam Study (1990-2000) has shown a protective effect against cardiovascular disease and death from heart attack given a higher consumption of foodstuffs rich in vitamin K2. This study was done among 4,807 subjects aged 55 years or above. Such a relation was not found for vitamin K1. Furthermore, the epidemiological study has shown that in many cases the dietary intake of vitamin K was inadequate for the protective function of vitamin K from atherosclerosis.

**IMPROVEMENT OF THE ARTERY FUNCTION**

Braam et al. (University of Maastricht) carried out a randomized, placebo controlled study into the effect of a 3-year supplementation with vitamins D and K1 in post-menopausal women. The women (181) were divided into three groups:

- a placebo group
- a minerals + vitamin D group (MD-group)
- a minerals + vitamin D + vitamin K1 group (MDK-group).

The data of 108 of the women were used in the analysis. After three years, the elasticity of the carotid artery in the placebo and in the MD-group decreased, while this was not the case with the MDK-group. The values measured in the MD-group hardly differed from those in the placebo group.

The difference between the vitamin K group (MDK) and the placebo group was considerable. This study opted for the combination of vitamin K with vitamin D since it is known that matrix-Gla-protein is a strong inhibitor of vascular calcification and depends on vitamin K (for activation) and vitamin D (for gene expression).
In a very recent (2006) experiment on rats, Schurgers et al. (University of Maastricht) made the unexpected discovery that a (very) high dosage of vitamin K can partially remedy vascular calcification and restore blood vessel elasticity. In this experiment a model for Mönckeberg’s sclerosis was used. Calcification was generated in rats during six weeks by administering the vitamin K antagonist warfarin, in a dose that prevents the carboxylation of matrix-Gla-protein. In the next six weeks the rats were divided into four groups. One group received a high dose of vitamin K1 and an other group a high dose of vitamin K2.

The researchers surprisingly observed a partial disappearance (about 40% reduction) of the calcificated areas in the rats that had received the high dosage of vitamin K (both vitamin K1 and vitamin K2). This brought the elasticity of the arteries back to normal (same as in the control group). This startling result might have important therapeutic consequences. Clinical studies in patients are required in order to establish whether extra intake of vitamin K can effectively contribute to the control of vascular calcification.

Earlier experiments performed by Vermeer’s research group at the University of Maastricht showed that vitamin K2 is more effective than vitamin K1 in preventing osteoporosis and arteriosclerosis\(^\text{[22, 25-27]}\). This is especially true for the vitamin K2 type menaquinone-7 (MK-7). Vitamin K2 as a group is made up of a series of menaquinones (MK-4 to MK-10). Vitamin K1 remains mainly in the liver (blood coagulation factors). Vitamin K2 is more distributed to the other tissues and organs, including blood vessels and bones. Tissues outside the liver preferably take up and use vitamin K2. The much longer half-life of MK-7 (3 days) compared to vitamin K1 and MK-4 (several hours) makes it possible to build up a 7-8 times higher concentration in the tissues than can be obtained with vitamin K1 or MK-4. In addition MK-7 is the most efficient co-factor in the carboxylation reaction.

**RECONSIDERATION OF THE DAILY ALLOWANCE OF VITAMIN K**

The current (American) recommended daily allowance (RDA) of vitamin K is based on its function in coagulation and has not been adapted to the new insights gained in the last decade. The American RDA for adults is 90-120 micrograms vitamin K per day. In the Netherlands, an official RDA for vitamin K for adults has not yet been established.

However, according to the Rotterdam study as well as several other papers looking at bone and arterial health, it is now clear that we should consider which form of vitamin K and which daily doses are optimal for bone and cardiovascular health. Presently Schurgers et al. suggest that 370 micrograms of vitamin K1 and 45 micrograms of vitamin K2 would be a sufficient daily dose for the protection of blood vessels and bones from calcification or decalcification\(^\text{[28]}\).

Taking these new estimates into account, a considerable section of the Dutch population could have a chronic moderate deficiency in vitamin K (especially vitamin K2), which might contribute to the occurrence of bone fractures and cardiovascular disease\(^\text{[5, 16, 29]}\).

Vitamin K1 is found in vegetable foodstuffs — especially green leafy vegetables (spinach, cabbage species) — and vegetable oils. Vitamin K2 is of bacterial origin. Rich sources of vitamin K2 are fermented dairy products (soft curd cheese, cheese) and fermented soy products. A very rich source of vitamin K2 (especially of MK-7) is ‘natto,’ a traditional Japanese soy product. Approximately 100 grams of cheese or 5 grams of natto will provide about 45 micrograms of vitamin K2.

**INTERACTION WITH ANTICOAGULANTS**

Vitamin K food supplements should not be taken without physician’s advice, when oral anticoagulants are used. From tests in healthy young adults it is estimated that vitamin K supplements can be safely used in combination with oral anticoagulants (coumarin derivatives such as warfarin) as long as the supplemented dosage does not exceed 100 micrograms of vitamin K1 and 50 micrograms of vitamin K2\(^\text{[27]}\). More studies are underway to document the safety in combination with anticoagulants.