



Vitamin K in Cystic Fibrosis

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The importance of vitamin K to coagulation is well known but there is increasing evidence that vitamin K has a diverse role in the body including the maintenance of bone health, preventing inappropriate calcification in soft tissues, and a protective role in inflammation. There is evidence that most, if not all, of these non-coagulation functions require higher dietary intakes than those required to maintain coagulation. Disturbances in the intestinal absorption of vitamin K in people with CF (PWCF) are common and several studies suggest that the majority of PWCF have suboptimal vitamin K stores. At present there is no consensus on the need for routine vitamin K supplementation, or when implemented on the vitamin K preparation that should be used, its dosage and the frequency of administration. Modern laboratory tests for specific vitamin K status offer a way to resolve these dilemmas.

Role of vitamin K in the body

It was called the anti-haemorrhagic vitamin by Henrik Dam who in 1929 discovered that certain foods contained an essential factor that prevented a dietary-induced deficiency syndrome in chicks. Dam, a Danish scientist, named the new vitamin "K" after the first letter of the Germanic spelling for coagulation. The processes that lead to the cessation of external or internal bleeding are exceedingly complex, and it took several decades to discover all the components. As each component or factor of the coagulation pathway was identified, it was represented by a Roman numeral. It turned out that four of these factors, all synthesized in the liver, needed vitamin K for biological activity. These factors are proteins that were named prothrombin (factor II) and factors VII, IX and X. Without an adequate supply of vitamin K to the liver the levels of functional factors II, VII, IX and X decline and blood clots more slowly. Eventually the entire coagulation machinery shuts down resulting in potentially catastrophic bleeding. In 1974 it was shown that vitamin K was required as a cofactor (helper molecule) for the conversion of certain amino acid (glutamic acid) residues in the protein target to a new amino acid identified as γ -carboxyglutamate (Gla) residues. Put simply this means that vitamin K transfers carbon dioxide to the protein in a process called carboxylation.

Discovery of new vitamin K-dependent functions

The elucidation of the unique molecular role of vitamin K quickly led to the discovery of several new vitamin K-dependent proteins. These proteins, also known as Gla-proteins, are now known to have a widespread tissue distribution. They include a



major protein in bone called osteocalcin and another called matrix Gla protein (MGP). It has been more difficult to find out how these new proteins work. The function of osteocalcin has proved the most intractable to unravel, but latest evidence suggests that it promotes the quality of the bone mineralization process. In elderly people, several studies have suggested that a high level of circulating undercarboxylated osteocalcin is an independent risk predictor of bone fractures. Other studies have shown links between either low vitamin K intakes or low blood levels and bone disease. Intervention trials, again in elderly people, suggest an overall beneficial effect of vitamin K in preventing bone loss, although the effect is modest. Randomized, controlled trials with fractures as an endpoint have been limited to large doses of menaquinone-4 (MK-4) in Japan. Nearly all showed a reduced fracture incidence although a recently published statistical analysis of all these trials concluded that the high success rate claimed by some should be treated cautiously. The disparity between effects in preventing bone loss and fracture rates may suggest that MK-4 improves bone quality without affecting bone loss.

More is known about MGP function and this is the first protein to have been shown to be able to prevent the process of calcification of arteries and cartilage in living animals. A lack of MGP in humans has already been shown to have important pathological consequences and recent research suggests that undercarboxylated forms of MGP are associated with sites of calcification in the vasculature. The implication that MGP is a central regulator of tissue calcification is important to patients with diabetes in whom vascular calcification is a well recognized problem. This may have relevance to PWCF in whom CF-related diabetes is increasing.

An intriguing finding is that two Gla-proteins (proteins C and S) that are important to maintaining the balance between bleeding and clotting also have other functions unrelated to coagulation. Protein C in its activated form has a direct anti-inflammatory function and indeed when administered to patients with severe sepsis, reduces their mortality rates. A recent epidemiological study showed that a high vitamin K status was associated with the lower production of inflammatory markers. Protein S (and a related Gla-protein called Gas6) are widely distributed in tissues and are thought to have more general roles in protecting the integrity of cellular function, particularly in the brain. Again maintaining the structural integrity of these Gla-proteins may be very relevant to PWCF, particularly in view of the pro-inflammatory processes characteristic of CF.

The discovery of these new Gla-proteins has led to a renaissance in vitamin K research and it is important to emphasize that, for all those Gla-proteins so far discovered, the presence of the Gla region is essential for their biological function. This implies that in states of vitamin K deficiency, the functions of these proteins will be compromised.

Different forms and sources of vitamin K

Vitamin K occurs naturally as two chemically distinct forms called vitamin K₁ (phylloquinone) and vitamin K₂ (menaquinones) respectively [Figure 1]. Vitamin K₁ is synthesised by plants, is the major dietary source, and in most people accounts for 90% of intakes. Vitamin K₂ occurs as a family of different compounds which are only synthesised by bacteria; they have the same nucleus and individual members are named according to their different side chain lengths. The best food sources of

vitamin K₁ are dark-green leafy vegetables followed by certain vegetable oils (e.g. soybean, canola and olive oils). Although K₂ vitamins represent a minority of overall vitamin K intakes, the best common food sources of vitamin K₂ are cheeses and liver. In Japanese culture the fermented soybean food called Natto is an enormously rich source of the K₂ vitamin called menaquinone-7 (MK-7). There is also a synthetic form called vitamin K₃ or menadione, which is used in some countries. Strictly this is a precursor of vitamin K since its activity relies on its conversion in the human body to a vitamin K₂ called menaquinone-4 (MK-4). A fraction of dietary vitamin K₁ is also converted to MK-4. There is presently much interest in the conversion of vitamin K₁ to MK-4 because the latter accumulates in certain tissues (e.g. pancreas, brain) and may have unique functions. Large daily doses of MK-4 are used as an anti-osteoporotic agent in Japan.

Although menadione is still available for clinical use in many countries, we would argue that this should not be used in PWCF (as is common in the UK). The reason is the high reactivity of the position shown in Figure 1 which reacts rapidly with compounds containing sulfur in reduced form thus inactivating them through an oxidative process. Since the transport of the important antioxidant glutathione is thought to be already defective in PWCF, it seems counterintuitive to add to the oxidative stress by giving menadione, a known pro-oxidant. Menadione also combines with haemoglobin. It was the propensity to cause haemolytic anaemia in newborn infants that led to the withdrawal of menadione for vitamin K prophylaxis in the 1960s. Recent studies also show that menadione may promote DNA damage and induce mutations. None of these toxicities is demonstrated by naturally occurring K₁ and K₂ vitamins.

Intestinal absorption and blood transport

The pathway whereby dietary vitamin K is absorbed from the intestine follows the same general principles as for the other fats and fat-soluble vitamins [Figure 2]. The naturally occurring forms of vitamins K₁ and K₂ can only be absorbed in the presence of bile salts that serve to emulsify and solubilise the fat components. This solubilisation is strongly enhanced by the molecular breakdown of food provided by the enzymes secreted into the proximal intestine by the pancreas. The process by which the solubilised vitamin K and fat breakdown products are transported from the intestinal lumen into the intestinal cells is complex but the end result is that vitamin K is packaged into specialised fat transport molecules (lipoproteins) called chylomicrons. These chylomicrons are secreted into the lymphatic system of capillaries which eventually empty into the systemic blood circulation via a large vessel called the thoracic duct. While flowing through the blood capillaries, the chylomicrons are transformed to smaller particles called chylomicron remnants which are taken up by the liver. The vitamin K that reaches the liver is then available for the manufacture of the blood coagulation proteins within this organ. In order to reach other tissues, the vitamin K is probably repackaged into different lipoproteins and re-exported into the bloodstream.

Synthetic preparations of menadione are usually administered in salt form (e.g. menadione sodium bisulfite). The absorption of menadione in the salt form travels by a different route that does not need bile-salts and bypasses the lymphatic pathway. In theory this is advantageous because greater amounts should be absorbed in PWCF

(although this has never been shown). Against this, nobody knows what fraction of the absorbed menadione is converted to the active MK-4 but it is likely to be small.

Risk factors for vitamin K deficiency in cystic fibrosis

As with the other fat-soluble vitamins, the leading risk factors for vitamin K deficiency in people with PWCF stem from pancreatic insufficiency and bile salt deficiency. Factors such as a poor appetite from chronic illness may also lead to reduced dietary intakes. In addition, PWCF are exposed to a specific risk factor that applies to vitamin K alone and this is their frequent need for antibiotics to control chronic infection. Although most evidence suggests that the majority of human vitamin K requirements are supplied by the diet, the contribution of significant amounts of vitamin K₂ (menaquinones) synthesised from the gut flora cannot be discounted and repeated antibiotic use in PWCF would reduce any availability from this source. It is well recognized that antibiotic use is a risk factor for vitamin K deficiency in other patient groups such as intensive care patients and very young infants.

Assessment of vitamin K status

With newer knowledge of the multiplicity of vitamin K-dependent functions in different organs and tissues, the concept has arisen that vitamin K sufficiency should be considered in relation to the site at which these Gla -proteins are synthesised. In other words, vitamin K deficiency states may be organ or tissue-specific. In part, this concept is based on studies in healthy people that show that higher amounts of vitamin K are required to carboxylate osteocalcin in the bone than those amounts needed to carboxylate the coagulation proteins synthesised in the liver.

Available methods

Historically, the most common laboratory test for vitamin K deficiency with respect to its coagulation function is the prothrombin time (PT) which in healthy people is usually in the range of 11-13 seconds (the precise range is laboratory dependent). However it is not commonly appreciated that the PT is an insensitive and non-specific test for diagnosing vitamin K deficiency. A significant laboratory lengthening of the PT by 1-2 seconds does not occur until the blood levels of one or more of the vitamin K-dependent proteins has fallen below ~50% of normal. By this time the liver stores of vitamin K will be severely depleted. Furthermore the PT only reflects the vitamin K stores in the liver available to manufacture the coagulation factors and does not give any information on the degree of undercarboxylation of Gla proteins in other tissues. Even if the PT were raised by 1 second, this would certainly mean that osteocalcin in bone and MGP in the vasculature would be present in a highly undercarboxylated state. For this reason scientists have sought to develop tests that give an early warning of impending vitamin K deficiency.

Some specialised laboratories such as our own have developed and validated methods for the measurement of circulating levels of vitamin K₁. Low levels of serum vitamin K offer an early indicator of declining body stores that may affect the carboxylating ability of vitamin K. This functional capability can then be addressed by measuring the carboxylation status of individual Gla-proteins. At present, tests are available for undercarboxylated coagulation factor II (known as PIVKA-II) and for undercarboxylated osteocalcin, that reflect the vitamin K status of the liver and bone respectively. We generally recommend that the combination of serum vitamin K and



a sensitive test for PIVKA-II offer sufficient information to assess vitamin K status. For several reasons the measurement of undercarboxylated osteocalcin is more difficult to interpret (especially in children) but can offer valuable information for assessing the efficacy of supplementation.

Evidence for vitamin K deficiency in CF

The development of an acute bleeding event due to severe vitamin K deficiency is fortunately rare in PWCF but sporadic cases are still reported in the literature. As with the population at large, the group most at risk of vitamin K deficiency bleeding (VKDB) are young infants below the age of 6 months, particularly if they are breast-fed (breast milk has low concentrations of vitamin K compared to milk formulas) and did not receive any vitamin K prophylaxis at birth (common practice for all infants in most countries worldwide). In this age group VKDB may be the first presenting symptom of CF and the bleeding site is commonly intracranial with potentially devastating consequences.

Although bleeding is still rare there are now many studies that suggest that subclinical vitamin K deficiency is common in PWCF. A recent study in the UK using the assays that we have developed showed that 70% of children with CF had a suboptimal vitamin K status based on a low serum vitamin K, raised PIVKA-II or both these abnormalities. This was consistent with other studies in the USA and Canada based on PIVKA-II alone. These studies imply that the vitamin K status of bone would be more affected. This was borne out by a small Dutch study that showed that the carboxylation status of osteocalcin in children with CF was much worse than in healthy children.

Should PWCF receive regular vitamin K supplementation?

Although the practice of routinely supplementing patients with CF with the fat-soluble vitamins A, D and E is well established and near universal in developed countries this is not the case with vitamin K. In a 2004 review, the director of a large regional CF unit in the UK argued that it is illogical to routinely supplement vitamins A, D and E and to ignore vitamin K. However, a 2007 survey in the UK still showed that whereas >90% of pancreatic insufficient patients received extra vitamins A, D and E, only 18% of centres routinely supplemented with vitamin K, and in those centres <10% of their CF patients actually received vitamin K. Why is there this resistance? Historically the reason probably lies with an understandable perception that vitamin K is only important for coagulation and that the PT test is adequate for monitoring vitamin K status. The evidence presented in this article suggests otherwise. Natural forms of vitamin K are extremely safe but further work is necessary to determine an effective dosage regime that at least corrects the existing abnormalities.