The Forgotten Vitamin

Almost 80 years after its initial discovery, vitamin K is now emerging from relative obscurity as a result of recent long-term studies, demonstrating a range of health benefits. Could it offer a solution to the recent controversy over calcium supplements?

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In 1943, Danish scientist Henrik Dam and American scientist Edward A Doisy jointly received the Nobel Prize for the discovery of vitamin K and the determination of its chemical nature, respectively.

The work leading to the discovery of vitamin K began in 1929 at the Biochemical Institute of the University of Copenhagen, Denmark, and was conducted by Dam, who experimented on chickens receiving a diet very low in fat. The researcher noticed that, after some time, the chicks developed haemorrhages in different parts of their bodies and also that their blood samples coagulated slower than those of the chicks fed a regular diet. A similar observation was made a few years later, in 1931 and 1933, by American investigators Roderick, Holst and Halbrook.

At first, Dam hypothesised that the haemorrhagic changes in chickens were due to scurvy, but he found that neither vitamin C nor any other known vitamin could prevent or check the haemorrhagic tendencies in the laboratory animals.

Blood Coagulation

In 1934, in cooperation with fellow scientist Schoenheyder, Dam found that an addition of hempseeds to the food prevented the occurrence of bleeding in experimental chickens. This led him to believe that hempseeds contained a still-unknown substance that had a protective effect against the haemorrhaging tendency. This substance, which was apparently necessary for blood coagulation, was described by Dam as the coagulation ("koagulation" in Nordic spelling) vitamin, or vitamin K. Later, Dam discovered that vitamin K occurs not only in the vegetable kingdom – for example, in green vegetables, cabbage seeds, tomatoes, soya beans and lucerne – but also in certain animal organs, especially in the liver.

Then, in 1938, American scientist Almaquist and his co-workers showed that vitamin K is also produced by the gastrointestinal bacteria. Therefore, vitamin K can be provided to the body either via food, or by our own healthy

gastrointestinal flora. Almaquist's discovery also set in motion the differentiation between what are now known as vitamin K1 (phylloquinone) and vitamin K2 (menaquinones).

While Dam and his colleagues took the lead in discovering vitamin K and denoting it with the letter "K", it was Doisy who determined its chemical nature. Doisy was already a world-renowned biochemist when he started his work on vitamin K chemistry. In 1939, together with his colleagues, Doisy succeeded in isolating two different forms of vitamin K, namely K1 from lucerne seeds and K2 from fish meal, both in pure crystalline forms. In the same year, he determined the chemical structure of vitamin K as a derivative of naphthoquinone, and also created a synthesised version, identical to the natural one. Doisy's work greatly facilitated the medical use of vitamin K in maintaining blood homeostasis.

The role of vitamin K in human health

The life-saving clotting of the blood (for example, in a cut-wound) is the outcome of sequential physiological processes. The wound is ultimately closed with lattice formed of a protein fibrin. The fibrin is formed via the activity of thrombin, another protein. Thrombin has a large array of functions. Its primary role is the conversion of fibrinogen to fibrin – the 'plug' or lattice that stops bleeding. Thrombin is formed from prothrombin, a substance synthesised in the liver and activated by vitamin K.

Although vitamin K activates several other proteins regulating blood coagulation, its most critical role is probably reserved for the formation of biologically active prothrombin. Deficiency of vitamin K leads to a lack of prothrombin, and thus to a lack of thrombin. Subsequently, the fibrinogen cannot be converted into the fibrin necessary for the coagulation of the blood.

Despite controlling the critical homeostatic mechanism, vitamin K is one of the safest vitamins for general populations. Deficiency of vitamin K for blood coagulation occurs only in a disease state. While practically 100 per cent of a general, healthy population is sufficient in vitamin K for supporting blood coagulation factors, up to 98 per cent of the same general population is insufficient in vitamin K, especially vitamin K2, for functions supporting healthy bones, the cardiovascular system and the metabolic system.

Since vitamin K's important role at the molecular level is as a calcium chaperone, its supplementation – in combination with calcium and vitamin D – is particularly important in view of the recent controversy of the overburden of calcium supplementation on the cardiovascular system.

Transforming Modern Nutrition

While vitamin K was recognised through systematic research some 80 years ago and introduced to healthcare 70 years ago, it has in fact been consumed in various cultures for hundreds of years. Probably the most recognised food rich in vitamin K, as we know it now, is the traditional dish known in Asia as natto, prepared from fermented soya beans.

Natto is widely consumed as a breakfast food in Japan, and considered a rich source of dietary menaquinones, particularly menaquinone-7. The tradition of soya-fermented natto has been traced back to Chinese salt-free soy nuggets. A description of the process for making different types of nuggets appears in the *Ch'i-min yao-shu*, the earliest encyclopedia of agriculture, written in 535 AD.

The fermented soya beans were prepared as a common food in Japan sometime between the 1st and 11th centuries. Since the late 18th century, natto preparation has been transformed by the modern science of microbiology. Subsequently, the microbial species responsible for the production of natto were identified as *Bacillus natto* and *Bacillus mesentericus vulgatus* or *Bacillus licheniformis*.

Depending on the specific method of preparation, natto may provide between 775µg and 1,750µg of menaquinone-7 (MK-7) for every 100g (1). Natto is reported to contain over 100 times more MK-7 than cheese – one of the main sources of menaquinones and MK-7 in the Western diet, alongside pork and fish (2).

Clinical Support

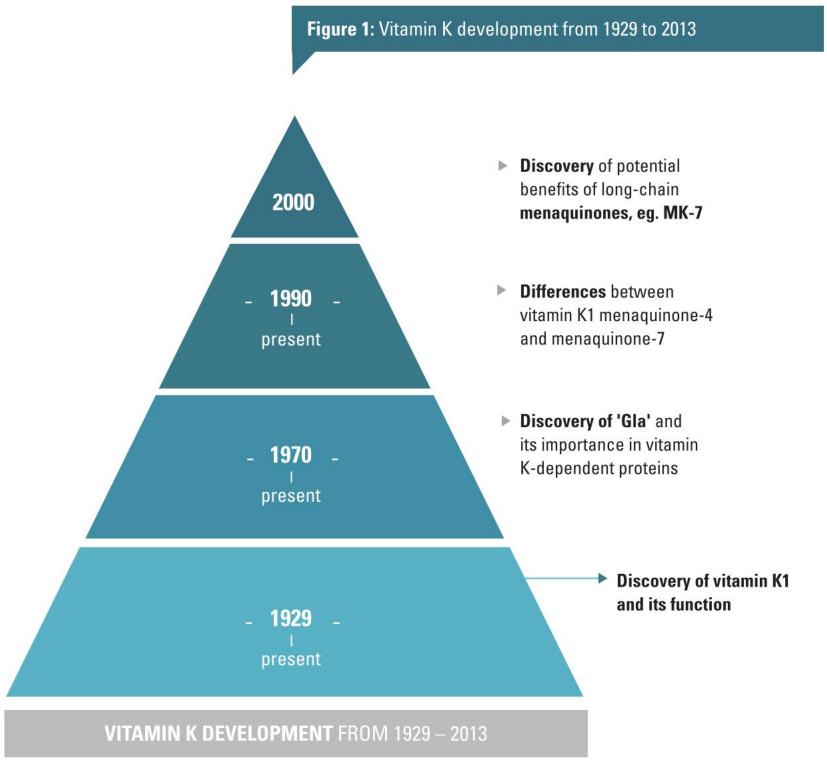
The epidemiological trials show the benefits of eating food rich in vitamin K, especially K2, and the positive health impact on the populations and cultures regularly consuming traditional food sources of vitamin K.

In an epidemiological trial of a Dutch population, the dietary intake of phylloquinone and menaquinone was followed in 4,807 subjects for seven to 10 years (3). The relative risk of mortality due to coronary heart disease was reduced significantly with increased intake of dietary menaquinones from food such as cheese, but not with intake of phylloquinone from vegetables.

For another epidemiological study, the health status of 944 Japanese women (aged between 20 and 79 years) was followed for three years, and natto intake was suggested to be responsible for preventing post-menopausal osteoporosis due to the effects of MK-7 (4).

Despite the strong epidemiological evidence showing that dietary intake of vitamin K2 may improve overall health status, it was not until the three-year 'breakthrough' study of MenaQ7® – a natural supplement of K2 as MK-7 – completed in 2012, that there had been a clinical trial to show that only a long-term (more than one year) supplement of vitamin K, especially MK-7, improves bone mineral density, bone mineral concentration and bone strength (5).

The study was a double-blind randomised clinical trial evaluating the results of a three-year regular intake of natural MK-7 in a 180mcg daily dose by



a group of 244 healthy post-menopausal Dutch women, 55 to 65 years old, randomly assigned to receive either MenaQ7 or identical-looking placebo capsules, daily.

This study is considered by the medical community to be a breakthrough because it shows clinically, for the first time, statistically significant protection with vitamin K against osteoporosis and cardiovascular deterioration with ageing. Specifically, with regards to bone preservation, the study showed significant protection of the vertebrae and the hip (femoral neck) against osteoporosis and fractures due to bone degeneration. This was achieved with only 180mcg daily of MenaQ7, which is considered a 'nutritional dose', meaning it is a dose that can be obtained from a healthy balanced diet.

The Calcium Factor

Importantly, the three-year study showed, for the first time, substantial benefits of nutritional vitamin K2, as compared to the placebo group, in preventing age-related stiffening of arteries manifested by a significant increase of the pulse wave velocity – a sign of ageing and hardening of arterial walls (age-related calcification of arteries).

Enter the paradox of calcium: supplemental calcium is crucial for the maintenance of a strong skeletal system and teeth, but also for nerve transmission, blood coagulation, vascular tone, blood pressure, muscle contraction, enzyme activation and hormone regulation. On the other hand, several recent epidemiological studies – including the results of a 2013 *JAMA*-published study – indicate that supplemental calcium and vitamin D (which increases absorption of calcium) may be detrimental to cardiovascular health, presumably by increasing arterial calcification (6).

In view of the biological role of vitamin K as one of the most important biological compounds regulating calcium in the body, and the cited three-year study showing prevention of age-related hardening (calcification) of arterial walls with vitamin K, the new paradigm for supplemental calcium emerges: the guidelines for such supplements may have to be revised, and calcium and vitamin D supplements may need to be complemented with K2 due to its increasingly recognised role as calcium chaperone.

This emerging trend in vitamin K's (K2 in particular) supplemental use has been further supported by the most recent clinical study and the new clinical methods evaluating deposits of calcium in the arteries. In one just-completed prospective randomised study in patients with chronic renal disease, the cardiovascular effects of oral administration of K2 plus vitamin D – compared to vitamin D alone – were evaluated (7).

In this six-month study, the progression of coronary artery calcification index and common carotid intima media thickness – both markers of calcium deposits in arteries detected with computerised tomography – showed a slower progression of the calcification in the K2/vitamin D group than detected in the vitamin D alone group of patients. Therefore, calcium and vitamin D supplements should not be avoided, but complemented with vitamin K2 supplementation.

Supplemental Improvements

Sensible vitamin K supplementation has become a pressing health matter, since it is increasingly obvious that the current status of its nutritional intake, especially of the K2 sub-types, is insufficient. This is due to a widespread use of chemical preservatives and food refrigeration in food processing, which depletes sources of friendly bacteria that manufacture K2. Up to 98 per cent of the general healthy population may be K2-insufficient, resulting in long-term detrimental impact on bone and cardiovascular health.

To compound the problem, the supplemental offerings of K2 are lacking in quality and stability. Many of the existing products have allergens and contaminants such as soya and anti-foaming agents, as well as impurities carried over from the fermentation process. The quinone moiety in K2's structure is sensitive

to multiple destabilising factors, including visible and ultraviolet light, moisture, oxidising agents, pH changes and several common supplement and food ingredients like omega-3 fatty acids. This makes it difficult to formulate stable compositions with natural K2. The pressing need for a better form has spearheaded the development of a technology to increase the stability, quality and efficacy of the vitamin's raw material through the creation of a pure and natural ingredient in the form of MK-7 crystals.

K2 Breakthrough

The significant technological breakthrough arrived with the development of a multi-step process of purification, condensation and crystallisation of fermentation-derived K2. The MK-7 is prepared by submerged fermentation using *B. licheniformis* (a non-toxicogenic and non-pathogenic strain, selected over other possible fermentation strains due to its stability and safety profile) as the producing strain, and chickpea flour and dextrin as carbon and nitrogen sources. Chickpeas are used in the manufacture instead of soya beans to minimise the potential for allergens, as legumes such as soya are often linked with various forms of allergy.

The MK-7 is extracted in vegetable oil and subject to a process of purification, concentration and crystallisation to obtain the crystals. The resulting product is characterised by:

- No less than 95 per cent content of only trans-MK-7 with less than 0.5 per cent MK-6
- No other detectable menaquinones
- Solvent under the detectable levels
- Improved stability profile in storage and in finished supplement and food preparations

Based on these characteristics, it is implicit that MK-7 crystals will have an improved gastrointestinal absorption rate, a higher rate of activating target proteins – such as osteocalcin and matrix gla protein – and an increased bioavailability of K2-dependent proteins activating target receptors in the body.

Conclusion

In summary, vitamin K has emerged from relative obscurity as a single-function homeostasis vitamin to be re-discovered as a multi-functional vitamin (8), and its potential health benefits for consumers – particularly alongside calcium supplements and vitamin D – are clear.

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