

The key question of vitamin K nutrition in humans may be how much is enough. As with other vitamins, the answer could depend on which biological effect is observed. Vitamin K was first discovered as an antihemorrhagic factor, and it is now known to be required for the synthesis of several proteins involved in blood coagulation (prothrombin; Factors VII, IX, and X; proteins S and C). One measure of vitamin K nutritional status is therefore the time it takes blood to clot. By this measure vitamin K nutritional deficiency in the U.S. population is largely limited to neonatal infants.

Other measures of vitamin K nutritional status have arisen from an understanding of the molecular mechanism of vitamin K action. Vitamin K is now known to be a cofactor for the posttranslational modification of specific glutamic acid residues to form the  $\text{Ca}^{2+}$  binding amino acid,  $\gamma$ -carboxyglutamic acid (Gla). Since Gla is not metabolized further in humans, urinary Gla levels provide a measure of the total turnover of all Gla-containing proteins in an individual. If dietary levels of vitamin K are low, the extent of the vitamin K-dependent formation of some or all Gla residues in these proteins should be reduced and less Gla should appear in urine.

In the study by Ferland et al. (1), vitamin K deficiency was induced in young and old healthy human subjects by restricting vitamin  $\text{K}_1$  intake to 10  $\mu\text{g}/\text{d}$ , a level which is below the 80  $\mu\text{g}/\text{d}$  recommended daily allowance for the vitamin. Plasma levels of vitamin K fell over sixfold within 3 d of dietary restriction in both the young and old subjects. Although this level of vitamin  $\text{K}_1$  depletion had no effect on blood coagulation time or on the activity of Factor VII or protein C, it did reduce urinary Gla levels by a consistent 10% in the younger group. How can urinary Gla decline if the synthesis of the vitamin K-dependent coagulation factors is normal? It is clear that other Gla proteins must contribute to urinary Gla levels, and that the normal synthesis of these proteins must be reduced at levels of vitamin K which are adequate for normal blood coagulation. The authors estimate that these other Gla proteins contribute 36% of urinary Gla, and consequently a 10% drop in urinary Gla translates to a 28% decrease in synthesis of these other Gla proteins. Although a number of novel Gla-containing proteins may remain to be discovered, only two other Gla-containing proteins have been isolated to date, osteocalcin (bone Gla protein) and matrix Gla protein (2). Osteocalcin is synthesized by osteoblasts in bone and is one of the most abundant noncollagenous proteins in the extracellular matrix of bone. Osteocalcin is also found in plasma, where its levels have been shown to correlate with bone turnover. MGP is synthesized by many cells and tissues, with the highest levels in heart, lung, kidney, and cartilage.

Since the function of osteocalcin and matrix Gla protein are presently unknown, it is not possible to access directly vitamin K nutritional status by measurement of the physiological activity of these proteins as it is for the coagulation factors. Osteocalcin, however, does have a Gla-dependent property that can be used to access vitamin K nutritional status: the ability to bind strongly to the hydroxyapatite mineral phase of bone. The measurement of the fraction of serum osteocalcin that cannot bind to hydroxyapatite provides a simple measure of the amount of undercarboxylated osteocalcin (ucOC) secreted into serum by bone cells. This assay was first applied to the analysis of human nutrition in a study of pre- and postmenopausal women by Knapen et al. (3). Postmenopausal women were found to have elevated levels of serum ucOC while premenopausal women did not, and administration of high oral doses of vitamin  $\text{K}_1$  (1 mg/d for 2 wk) restored normal carboxylation.

In this issue of *The Journal*, Szulc et al. (4) also report that serum ucOC is elevated in elderly women. They make the striking observation that the serum level of ucOC correlates with the subsequent risk of hip fracture during follow-up. The relative risk of hip fracture was in fact six times higher in women with abnormally high values of serum ucOC. Other biochemical indices of bone metabolism had no predictive value for risk of hip fracture. Although the mechanisms that link vitamin K deficiency and risk of hip fracture in elderly women are unclear, it is interesting that a recent study has found that the administration of high doses of vitamin K to postmenopausal women for 24 and 48 wk does significantly increase bone mass compared to women treated with placebo (5).

The studies in this issue (1, 4) are significant advances in the understanding of vitamin K nutrition in humans, and indicate that impaired synthesis of some vitamin K-dependent proteins may be far more prevalent in the human population than coagulation assays alone indicate.

Paul A. Price  
Department of Biology  
University of California, San Diego

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