Are vitamin supplements necessary for all? A role for vitamin status assessment

Nutristasis covers the development and application of analytical tools to support the assessment of vitamin status. Here, Agata Sobczyńska-Malefora and Dominic J Harrington look at the laboratory's role in vitamin status assessment.

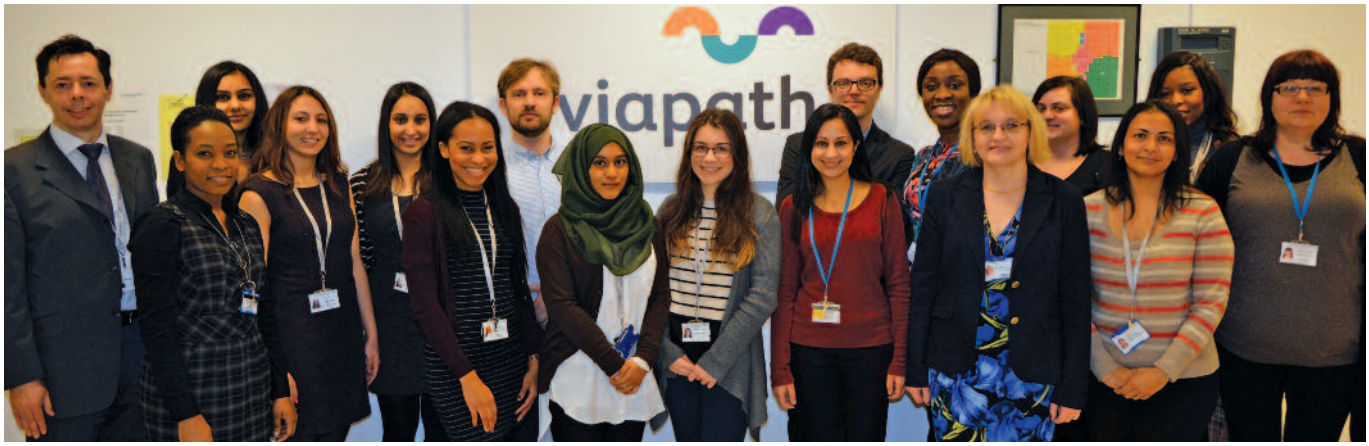
The word 'vitamine' was first used in 1911 by the Polish scientist Dr Kazimierz Funk who, while working in London, referred to an amine-containing compound which was found to be vital for life – thiamine (now also known as vitamin B_1). It was subsequently found that vitamins do not share any commonality in their structure, nor do they all contain amines, hence the word 'vitamin' was coined. Today, although the role that 13 recognised vitamins now play in numerous developmental and metabolic processes has been well defined, many other functions need to be better understood. Similarly, the clinical utility of vitamin supplementation in the treatment and prevention of disease has been well established for some disorders (ie vitamin C to prevent scurvy, vitamin D for rickets and vitamin K prophylaxis to prevent bleeding) while controversies exist regarding others (ie folic acid fortification leading to the presence of unmetabolised folic acid in blood, or vitamin E supplementation in smokers).[1]

Although a balanced diet provides a sufficient daily intake of all vitamins needed for most people, sustaining an adequate status can be challenging for some (ie the elderly because of impaired absorption or during pregnancy because of increased requirement). In many cases optimal treatment regimes have yet to be established. Self-administered supplementation and adverse effects due to an excessive intake of vitamins from pharmaceutical preparations (ie methylcobalamin or adenosylcobalamin for vitamin B_{12} deficiency) is also a concern. Research in the past few decades related to the importance of vitamins in health and disease, coupled with media attention, has led to the impression by some people that taking vitamins in supplement form is necessary. Although high exposure to most vitamins is generally thought to cause no harm, and vitamin toxicity is normally associated with a very high intake (ie vitamin B_6 leading to sensory neuropathy, vitamin A causing a rise in intracranial pressure or bone and joint pain), the adverse effects of long-term supplementation have not been studied for most vitamins.

It should also be noted that some vitamins impede the action of others. The excessive intake of one vitamin may lead to an increased utilisation or masking deficiency of the other vitamin, resulting in the depletion of this vitamin or exacerbating clinical symptoms. Examples include the interaction of folate (vitamin B_9) with vitamin B_{12}, or the negative impact of high-dose vitamin E supplementation on vitamin K status.[2]

Nutristasis Unit

The Nutristasis Unit was established in 2002 to develop and apply analytical tools to support the assessment of vitamin status. The unit currently performs 300,000 tests annually, using various analytical techniques to estimate the abundance of a particular



The Nutistasis Unit team, with Dr Dominic Harrington (far left) and Dr Agata Sobczyńska-Malefora (fifth from the right)

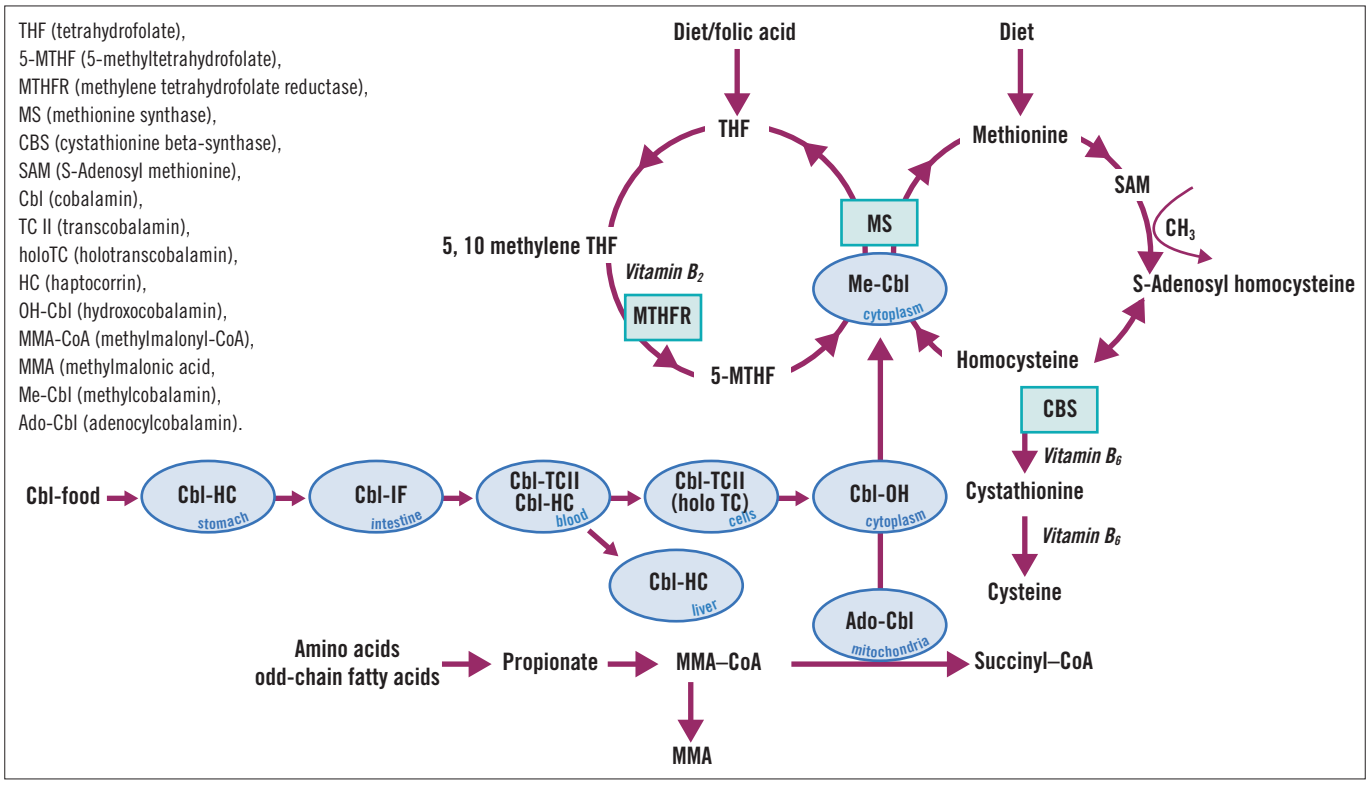


Fig 1. Homocysteine, folate and vitamin B_{12} metabolism.

vitamin, as well as functional assays that establish utilisation within cells. Functional assays include total plasma homocysteine to evaluate folate and B_{12} status, methylmalonic acid (MMA) for vitamin B_{12} and PIVKA-II for the evaluation of vitamin K. Values that fall within the laboratory reference ranges are suggestive of adequate vitamin status respective to the metabolic process where the vitamin is being utilised.

Whenever possible and applicable, we combine functional assays with assays which assess the abundance of vitamins in blood/plasma, referred to as 'static' assays to reflect storage and transport. However, the interpretation of the combined results is not always straightforward, since discrepancies do occur as a result of test limitations in certain clinical and analytical conditions. Our scientists assist clinicians with the interpretation of such results. We also provide regular teaching sessions to doctors, scientists in training and students from medical schools and universities. The Nutristasis Team also actively leads or participates in research projects and studies, aiming to better understand the role of vitamins and improve our services for better patient care.

Currently, the Nutristasis Unit routinely assesses vitamin status of the following water-soluble vitamins: B_1, B_2, B_6, B_9, B_{12} and C, and fat-soluble vitamins: A, E, K and D. It also provides analysis of vitamin K antagonists such as warfarin (oral anticoagulant) and superwarfarins (rodenticides). Although services are primarily provided for clinical specimens, the unit can also perform analyses for veterinary surgeries and the food industry.

Folate, vitamin B_{12} and homocysteine

Vitamin B_9 (folate) and vitamin B_{12} (cobalamin) are essential for metabolism, DNA synthesis and regulation of gene expression. Deficiencies of folate and/or vitamin B_{12}, resulting in megaloblastic anaemia and neurological impairment, are one of the most common vitamin deficiencies found in patients and the general population. In our recent study of 17,875 patients from primary care, we found serum folate below our deficiency cut-off of 7 nmol/L in 14% and serum B_{12} <138 pmol/L in 4.5% of patients.[3] Deficiency of folate and B_{12} is also the most frequent reason for elevated plasma homocysteine (hyperhomocysteinaemia [HHcy]), a risk factor for cardiovascular disease and dementia. As an example, the prevalence of HHcy in our cohort of patients with a history of thromboembolic disease was 27%. Hyperhomocysteinemia due to folate and/or vitamin B_{12} insufficiency could be explained in about 56% of patients of these patients.[4,5]

The diagnosis of folate and B_{12} deficiency remains problematic and there is no 'gold standard' test. There is no consensus on cut-off points used by laboratories, given the variety of methodologies and diagnostic approaches used. Our approach to the diagnosis of vitamin B_{12} deficiency for hospital patients includes the use of the holotranscobalamin (holoTC) assay as a first-line marker, followed by confirmatory testing with MMA if indicated. HoloTC measures only the biologically active fraction of vitamin B_{12}, as opposed to the widely used serum B_{12} test which quantifies both 'inactive' holohaptocorrin (Cbl-HC) and 'active' B_{12} (Cbl-TC II) (Fig 1). If the holoTC result falls within 25–70 pmol/L, we perform MMA analysis.[3]

Methylmalonic acid is a very sensitive test for identifying patients with suboptimal/deficient vitamin B_{12} status. To assist our users with the diagnosis of folate deficiency, in addition to commonly used serum and red cell folate tests, we provide analysis of 5-methyltetrahydrofolate (plasma, whole blood and CSF)[6] and homocysteine. 5-methyltetrahydrofolate (5-MTHF) is the main form of folate and an essential methyl donor for methionine synthase and vitamin B_{12}-mediated conversion of homocysteine to methionine (Fig 1). Low 5-MTHF most commonly suggests folate deficiency, but can also indicate vitamin B_2 deficiency or methylene-tetrahydrofolate reductase (MTHFR) polymorphism (TT genotype). Patients with this polymorphism often have raised homocysteine, and hence are at greater cardiovascular risk.[7,8] Our Molecular Unit performs analysis of this polymorphism and other common polymorphisms as well as full sequencing of *MTHFR* and *CBS* genes (Fig 1).

High vitamin B_{12} and folate concentrations are also prevalent. In our hospital and primary care cohort,[3] we found 20% of patients with holoTC results >128 pmol/L (upper limit of the linear range of the method) and 9% of total B_{12} >652 pmol/L (upper limit of our reference range), respectively. Elevated folate concentrations (>45.3 nmol/L) were found in 3.8% of hospital and 10.3% of primary care patients, respectively. We also found that of those with high B_{12} status, 6% of

hospital and 10% of primary care patients had serum folate within our deficiency range (<7 nmol/L). Conversely, within patients with very high folate status, there were 4% of hospital patients with holoTC <25 pmol/L and 2.3% of primary care patients with total B_{12} <138 pmol/L (our cut-offs for deficiency).

It is possible that over-supplementation of one vitamin exacerbated the deficiency of the other. Some of the evidence which supports this hypothesis comes from the analysis of data from the National Health and Nutrition Examination Survey (NHANES), which found associations of high folate status with impaired activity of the two vitamin B_{12} enzymes methionine synthase and MMA-coenzyme mutase (Fig 1), as well as anaemia and cognitive impairment in elderly subjects.[9] Recently, it has been also suggested that high folic acid consumption reduces MTHFR protein and activity, creating pseudo-MTHFR deficiency and leading to hepatocyte degeneration.[10]

The potential adverse effects of high maternal folic acid have also been linked with higher adiposity, and low vitamin B_{12} and high folate have predicted high insulin resistance in children.[11,12] There is little known about the adverse effects of excessive intake of vitamin B_{12} in general. Some hypotheses include the formation of antibodies to transcobalamin, leading to a high plasma concentration of the vitamin.

Vitamin B_6

Vitamin B_6 is involved in over 100 enzymatic reactions, including the metabolism of amino acids, carbohydrates, neurotransmitters and lipids. Low vitamin B_6 status can lead to HHcy (Fig 1) and has been associated with severe malnutrition and venous thromboembolism, while very high doses of pyridoxine (B_6) supplementation lead to toxicity presenting as sensory neuropathy. Pyridoxine is used in the treatment of many conditions (ie cystinuria, homocystinuria, seizures or peripheral neuropathy associated with isoniazid and hydralazine therapy). The prevalence of vitamin B_6 deficiency is low due to its availability in various foods.

We only found pyridoxal 5'-phosphate (PLP), the active coenzyme form of vitamin B_6, below the lower cut-off of the reference range, suggesting deficiency, in 2% of patients referred for B_6 assessment.[13] However, 38% of our patient cohort had PLP values above the upper limit of the reference range, including 7% of patients with concentrations >540 nmol/L (five times the upper limit). Of these 19 patients, sensory neuropathy was present in three cases. Vitamin B_6 toxicity should have been considered as a contributing factor to this neuropathy.

Vitamin K

The major naturally occurring K vitamins are the plant form phylloquinone (vitamin K_1) and multiple forms of menaquinones (MK-n or vitamins K_2) predominantly of bacterial origin. In Western diets, K_1 and MK-n account for 90% and 10% of the vitamin K intake, respectively. Vitamin K is required for the conversion of peptide-bound glutamate to γ-carboxyglutamate (Gla), essential for the synthesis of seven vitamin K-dependent proteins that have a crucial role in blood coagulation (factors II, VII, IX and X; proteins C, S, and Z). Other vitamin K-dependent proteins (Gla proteins), with a widespread tissue distribution, have now been shown to be essential for functions such as the regulation of bone turnover and calcification, inhibition of vascular calcification, and roles in vascular repair processes, cell cycle regulation, cell-cell adhesion and signal transduction.

Green leafy vegetables are a rich source of vitamin K_1, while dietary MKs are present in animal tissues. Like other fat-soluble vitamins, vitamin K is absorbed from the duodenum, where it is dependent on bile and pancreatic secretions for solubilisation. Any condition causing the malabsorption of fat leads to a secondary deficiency of fat-soluble vitamins.

Poor absorption of vitamin K quickly leads to the depletion of its tissue stores, which is indicated by a decrease in circulating levels of the vitamin long before pathological changes develop. Cases of vitamin K deficiency are often missed, or detected late, due to the use of inappropriate laboratory markers of vitamin K status, commonly the international normalised ratio (INR). The INR, which is

based on the prothrombin time, is designed to detect bleeding tendencies and is a very insensitive and non-specific marker of vitamin K status.

Vitamin K status is evaluated by the measurement of K_1 in serum. This is supported by the analysis of PIVKA-II (under-carboxylated prothrombin, an abnormal species of factor II that is only detectable in the circulation of patients with suboptimal vitamin K status or those taking vitamin K antagonists (ie warfarin; Fig 2). By running these two assays in tandem we are able to monitor the two most important determinants of vitamin K status, availability and utilisation. A vitamin K_1 concentration within the laboratory reference range (suggestive of adequate vitamin K status) does not preclude an elevated PIVKA-II concentration, which is suggestive of hepatic deficiency.

In the UK, vitamin K supplementation is commonly used for the prophylaxis of vitamin K deficiency bleeding (VKDB) of the newborn and to reverse anticoagulation by vitamin K antagonists (ie warfarin). The role of vitamin K supplements and their potential benefits for bone and vascular health and treatment of certain cancers is still being evaluated.[14] Toxicity related to vitamin K ingestion has been mainly associated with menadione (vitamin K_3) formulations leading to haemolytic anaemia. It is important to note that vitamin K_3 is only indicated for the prophylaxis and treatment of vitamin K deficiency in malabsorption syndromes.[15]

Conclusions

Deficiencies of some vitamins are prevalent in patients and general populations, and it is important that these are diagnosed and treated early. However, the prevalence of elevated vitamin concentrations is much more common, often resulting from excessive intake. The adverse effects of over-supplementation for many vitamins have not yet been very well studied. These need to be taken into consideration when treatment is reviewed/prescribed or self-administration takes place. Assessment of vitamin status coupled with investigations of causes of vitamin status should precede any future treatment.

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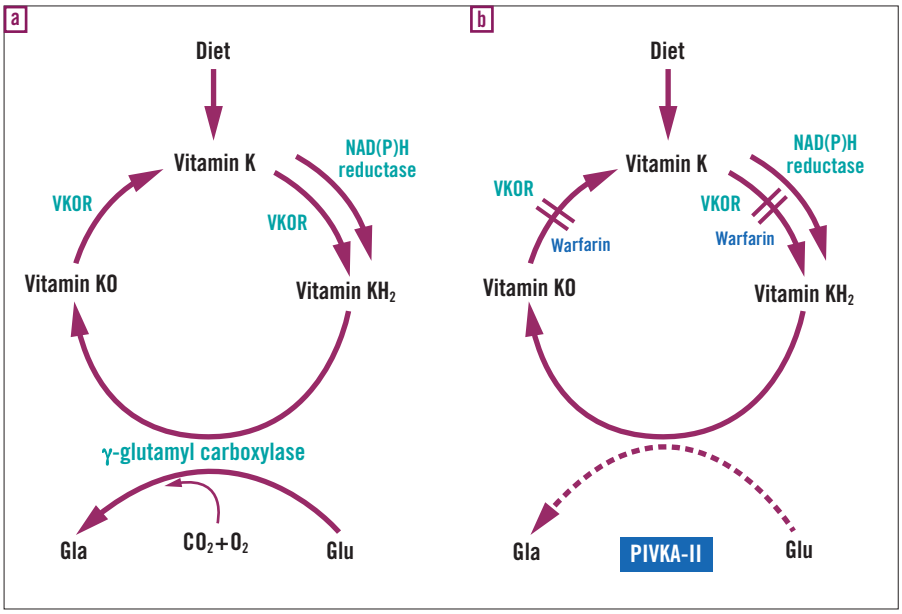

Fig 2. a) The vitamin K cycle and associated enzymes. b) Warfarin action on the vitamin K cycle. Warfarin exerts its anticoagulant effect by inhibiting the vitamin K epoxide reductase (VKOR) enzyme complex that recycles vitamin K 2,3-epoxide (KO) to vitamin K quinol (KH2).

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