REVIEW ARTICLE

Fat-soluble vitamins: mechanisms and metabolism. A narrative review

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ABSTRACT

Vitamins A, D, E and K are fat-soluble vitamins that play a crucial role in the metabolism and homeostasis in the human body. Differences in methods used to measure vitamins can lead to variations between laboratories, making it difficult to establish universally agreed reference intervals. Using robust and reliable harmonised methods helps clinicians and Healthcare Scientists to improve the diagnosis and monitoring of vitamin deficiencies and toxicity. Immunoassay and high-performance liquid chromatography (HPLC) methods have been traditionally used to measure vitamins. However, liquid chromatography tandem mass spectrometry (LC-MS/MS) is increasingly utilised for its improved selectivity and accuracy when using isotopic internal standards. In this review, we will summarise analytical and clinical aspects of fat-soluble vitamins.

Keywords: Fat-soluble vitamins, HPLC, LC-MS/MS, Retinol, Tocopherol, Cholecalciferol, Phylloquinone.

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OVERVIEW

Vitamins are essential nutrients present in trace amounts that cannot be synthesised in the human body, except for Vitamin D which is synthesised by the skin in the presence of ultraviolet light. Other vitamins need to be ingested in food or taken as supplements. Vitamins are classified as either water soluble (Vitamin B group or C) or fat-soluble (Vitamins A, D, E and K). An insufficiency of vitamins can lead to a range of serious diseases. Toxicity is also possible at high intakes of fat-soluble vitamins which cannot be easily metabolised and excreted in the urine.

Requests to measure vitamins (especially, A, E and D) have been increasing significantly since 2000, in particular for vitamin D. For example, in Australia vitamin D measurement requests have risen from 23000 in 2000 to more than 2 million in 2010 (1). The increased number of requests may be related to an increasing number of underlying potential risk factors, such as significant liver issues, poor supplementation adherence and poorer diet (junk foods) in comparison with two decades ago. Measurement of some fat-soluble vitamins can be performed using immunoassay or high-performance liquid chromatography (HPLC). Immunoassay methods are faster, cheaper and easier to troubleshoot, while reliability, precision, and accuracy are greater in HPLC methods (2).

Liquid chromatography - tandem mass spectrometry (LC-MS/MS) analysis is a more robust technique that has three different modes of separation: 1. chromatographic separation, 2. separation based on mass, and 3. mass transitions based on specific fragmentation patterns of the analytes (1,3). LC-MS/MS techniques utilising isotope dilution mass spectrometry are increasingly referred to as the “gold standard” and can often be used to quantify vitamins with higher accuracy, precision, sensitivity, and specificity compared to other methods.

Fat-soluble vitamins are all absorbed in the small intestine by different mechanisms (3). Symptoms of deficiency include night blindness (vitamin A), increased oxidative cell stress (vitamin E), osteomalacia (vitamin D), and haemorrhage (vitamin K). In this review we tried to briefly review the importance of FSVs in human body.

Vitamin A

Introduction

Vitamin A deficiency was first recognised by the ancient Egyptians, and later as a fat-soluble compound (retinol) which was extracted from liver and named vitamin A (4).

Chemistry and measurement

Vitamin A (retinol) consists of four isoprenoid units joined in a head-to-tail fashion. There are only two main forms; provitamin A (carotenoids) and preformed vitamin A (retinol). Retinol measurement in the serum or plasma is the best test to assess vitamin A status.

The amount of retinol is tightly regulated in the body unless the liver has significantly depleted the store of vitamin A. Therefore, serum retinol not only indicates vitamin A sufficiency, but it is also as an indicator of vitamin A stores. In addition, serum β-carotene may be useful to help diagnose vitamin A deficiency (5). Serum carotene can be used as a surrogate marker of malabsorption and nutrition status, as the carotene level is usually very low among people with vitamin A deficiency (6).

Diagnosis of vitamin A deficiency is usually based on clinical findings (5). Light protected samples with a low number of freeze-thawings should be obtained in the fasting state in order to avoid the possibility of pre-analytical interference (e.g. lipaemic samples) and non-fasting samples may also reflect the recent intake (7). Vitamin A (retinol) and E (α-tochopherol) are often measured together. They are both non-polar molecules containing native chromophores allowing measurement by HPLC with ultraviolet (UV) detection (8). Pre-concentration of samples is not necessary. Samples can be prepared using a protein precipitation in a solvent containing internal standards, *i.e.,* retinyl acetate and α-tochopheryl acetate. Separation can be achieved using a reversed phase column with a mobile phase containing methanol and water. The wavelength spectrums of vitamin A and E are different. The UV detector can be set at 325nm and 295nm detect vitamins A and E respectively. The measurement of vitamin A is a reliable indicator for epidemiological studies to estimate vitamin A deficiency in a population (5,9).

Indications for measurement

Patients at high risk of Vitamin A deficiency should have routine monitoring of vitamin A status, including patients with malabsorption, cystic fibrosis (CF), short gut syndrome, or steatorrhea. Measurement may also be performed to confirm toxicity.

Limitations of measurement

Serum retinol may not be a reliable indicator of total vitamin A stores in some situations, such as samples that are exposed to light or heat. In systemic inflammation there may be a decreased serum concentration due to reduction in many

carrier proteins, such as retinol binding protein (10) and in severely malnourished people (11). Vitamin A can be artificially higher in the serum of people who are under vitamin A supplementation.

Sources
High levels of preformed vitamin A are found in liver, kidney, egg yolk and butter, while provitamin A (carotinoids) are mostly found in yellow and red vegetables and fruits. About 70 to 90% of vitamin A is absorbed in the presence of intestinal fluid and bile salts in the gut (12).

Metabolism and action
Provitamin A must be cleaved to retinal by 15,15'-mono-oxygenase then converted to retinol by retinal reductase before absorption, while preformed vitamin A is hydrolysed to retinol and free fatty acids in the lumen of the small intestine with help of bile salts and pancreatic enzymes (13).The metabolism of vitamin A depends on the form of vitamin A ingested. Metabolism is highly regulated with less toxicity from plant sources (provitamin A). Absorption and storage of animal sources (preformed vitamin A) are more efficient, and higher consumption may cause toxicity.

In the small intestine retinols are re-esterified into retinyl esters, merged with chylomicrons, and secreted into the lymphatic system and plasma (11). Chylomicrons are broken down into several retinyl ester remnants, such as apolipoproteins B and E. These apolipoproteins are taken up by the liver cells through a receptor-mediated endocytosis on the surface of the liver and lead to the release of retinyl esters (11). Later these are metabolised to be combined with fat-soluble vitamins. In order for vitamin A to approach its target organs, retinol-RBP complex pathway is crucial. About 50 to 85% of the total retinol is stored in the liver (14).

The best known role of vitamin A is related to sight where low levels of vitamin A can lead to night blindness and dysfunctional regulation of carbohydrates, lipid and protein metabolism (13). Vitamin A has also been shown to have an inhibitory effect on the growth of tumour cells in vitro, controlling cell division, differentiation, and apoptosis (2).

Deficiency
Globally, vitamin A deficiency is still a big burden. More than 50% of pre-school aged children and pregnant women are at the risk of deficiency (2). Vitamin A deficiency is uncommon in developed countries, but may be seen among people who have undergone bariatric surgery, e.g. biliopancreatic diversion or duodenal switch procedures (14). Deficiency of vitamin A may lead to dry skin, inflammation of the respiratory, gastrointestinal system, and urogenital tracts, disruptions in white blood cell development, and reduction in the number of natural killer cells. These changes predispose to infection and communities with Vitamin A deficiency have a higher prevalence of infections such as respiratory tract infections (5,15).

Vitamin A deficiency is observed among people with fat malabsorption disorders, such as coeliac disease, cholestatic liver disease (such as primary biliary cholangitis), small bowel Crohn's disease, short bowel syndrome, very restricted diet from mental health issues or autism (16). Therefore, anyone with fat malabsorption who experiences vision changes, especially in low light or night, should be checked for vitamin A deficiency.

Toxicity
High accumulation of vitamin A may cause hypervitaminosis A or vitamin A toxicity. There are three forms of vitamin A toxicity, acute, chronic and teratogenic (17). Acute toxicity occurs in adults who ingest a single but high dose of vitamin A. Most vitamin A toxicity occurs with chronic ingestion of large amounts of synthetic (or "preformed") vitamin A (17). Symptoms include nausea, vomiting, headache, dizziness and blurred vision, and can eventuate in death (13). Vitamin A is very teratogenic in the first trimester of pregnancy and is associated with spontaneous abortions and fetal malformations, such as microcephaly and cardiac abnormalities (17).

People with high intake of provitamin A may develop yellow tinged skin (carotenaemia) without developing vitamin A toxicity (18). In infants and toddlers, carotenaemia is common when large amounts of pureed vegetables (particularly carrots and green leafy vegetables) are eaten. This can be mistaken for jaundice. Dietary reduction of intake will result in the discolouration reversal (19). Although carotenaemia may have no severe complications, it may cause nephrosis, diabetes mellitus, anorexia nervosa, liver disease, and hypothyroidism due to reduced conversion of beta carotene into retinol, and can lead to death if severe (2).

In children with CF, excessive vitamin A supplementation increases risk of vitamin A toxicity which may lead to CF-associated liver and bone disorders (20). Chronic toxicity of vitamin A may cause adverse effect on bone metabolism in females and males (2,20). Retinyl ester concentrations >10 percent of the total vitamin A pool are indicative of hypervitaminosis A. Chronic vitamin A toxicity is often associated with elevated serum levels of alanine aminotransferase, aspartate aminotransferase and/or calcium (21).

Therapeutic application
Currently, vitamin A supplementation is not recommended for new-borns, infants aged one to five months, and to mothers during postpartum period living in endemic areas (13). Vitamin A supplementation is not indicated in the absence of deficiency, except for children at high risk of vitamin A deficiency with conditions such as measles, diarrhoea, respiratory disease, or severe malnutrition, and not receiving supplements within the past one to four months while living in among populations at risk for vitamin A deficiency and for xerophthalmia (22).

Recommendations for future research
Currently, there is no accurate data about the prevalence of vitamin A deficiency in New Zealand. Harmonisation of retinol measurement between laboratories will enable better comparisons of results.

Vitamin E
Introduction
Vitamin E or "anti-sterility factor" was introduced several decades ago and later was called tocopherol from Greek root, where "toc" and "phero" coined "baby" and "to bring forth" respectively (4).

Chemistry and measurement
Vitamin E is an antioxidant. The two naturally occurring groups of vitamin E are the tocopherols and tocotrienols, each of which has four isomers, α, β, γ and δ according to the position and number of methyl groups on the chromanol ring system. Vitamin E has eight isomers, four which are actively maintained in the human body (2).Vitamin E comes mainly from diets that are rich in γ-tocopherol. α-tocopherol is the most bioavailable and active metabolite (23). α-Tocopherol is the best marker of vitamin E status in the blood. The level of α-tocopherol in the blood may be related to several factors, such as gender, age, and cholesterol level. The ratio of α-tocopherol to total cholesterol has been recommended as a biomarker of vitamin E status (2). The measurement of vitamin E is often performed with Vitamin A, as previously discussed in this review.

Indications for measurement
Patients at high risk of vitamin E deficiency should have routine monitoring of vitamin E, including patients with malabsorption, CF, short gut syndrome, or steatorrhea, liver and pancreatic disorders, or some genetic disorders. Measurement may also be performed to confirm clinical deficiency or toxicity.

Limitations of measurement
Lower vitamin E levels in patients with hypoproteinaemic states should be interpreted with caution because fat-soluble vitamins are transported by proteins. In addition, people who are hyperlipidaemic are recommended to be tested for "effective serum vitamin E" because serum vitamin E does not reflect tissue vitamin E levels (2).

Sources
Vitamin E is high in different foods such as almonds, vegetable oils, and cereals, while α-tocopherol and γ- tocopherol levels are particularly high in olive, sunflower, soybean and corn oils.

Metabolism and action
Like any other fat-soluble vitamins, activities of α-tocopherol depend on physiological mechanisms of fat digestion and absorption. In the human body, the small intestine is where vitamin E is absorbed with help of biliary and pancreatic secretions to raise vitamin E solubility (24). The metabolism of vitamin E occurs in the liver, but only α-tocopherol is re-secreted into the blood circulatory system. This process is facilitated by α-tocopherol transfer protein *(α-TTP),* which maintains the concentration of α-tocopherol in the blood. Thus, any defects in *α-TTP* leads to vitamin E deficiency (2).

α-Tocopherol is a free radical scavenger, protecting polyunsaturated fatty acids from peroxidation. Vitamin E also has non-antioxidant activities (2). Activities such as inhibitory effect on platelet aggregations have been thought to play a protective role against atherosclerosis and cardiovascular diseases, and vascular and immunological disorders (2). Vitamin E has been investigated for prevention of cancer due to its anti-oxidation, anti-inflammatory and NO_2 detoxification roles (2). However, data from randomised controlled trials and Mendelian Randomisation have been largely disappointing (25).

Deficiency
Vitamin E deficiency is usually clinically diagnosed in adults and children when neuropathic and myopathic disorders are seen, such as, ataxia, hyporeflexia, loss of proprioceptive and vibratory sensation, a skeletal myopathy, and haemolysis due to the shorten lifespan of erythrocytes (26), as well as other haemoglobinopathies (27). The other early indicators of vitamin E deficiency include elevated creatine kinase and pyruvate kinase in plasma (13).

Vitamin E deficiency is highly associated with genetic and malabsorption disorders such as cystic fibrosis, chronic hepatitis and gastrointestinal problems (2). Vitamin E metabolism requires functioning pancreatic enzymes, bile salt and ileal mucosa (2). Vitamin E deficiency is not very common in humans except in people with underlying medical conditions such as malnourishment and fat malabsorption disorders (28). These disorders include pancreatic exocrine insufficiency due to insufficient lipase to enable optimal lipid absorption, such as cystic fibrosis, pancreatectomy, chronic pancreatitis and cholestatic liver disease (due to insufficient bile in the small intestine for fat solubilisation), biliary atresia, primary sclerosing cholangitis, primary biliary cholangitis, and familial intrahepatic cholestasis. The level of vitamin E may be high in people with cholestasis due to hyperlipidaemia. Although cholestasis is a potent risk factor for deficiency of fat-soluble vitamins, vitamin E deficiency is very uncommon in patients unless they suffer from severe and prolonged cholestasis (29). In this case, any patients with bilirubin >2mg/dL (34.2 μmol/L) are highly recommended to be routinely monitored for fat-soluble vitamins (28).

Small intestinal resection or short bowel syndrome leads to malabsorption due to insufficient intestinal absorptive area. In particular, ileal resection leads to bile acid malabsorption and eventually further fat malabsorption. Rare causes of vitamin E deficiency are congenital intestinal lymphangiectasia, amyloidosis or lymphoma, and Crohn's disease. Rare genetic disorders and mutations may also occur in *α-TTP* (e.g. ataxia) and abetalipoproteinemia leading to vitamin E deficiency (26).

Toxicity
No significant toxicity level has been reported for vitamin E.

Therapeutic application
Vitamin E supplementation is mainly indicated for deficiency. In addition, vitamin E supplementation is beneficial in non-alcoholic fatty liver disease due to the improvements in aminotransferase (30), and may delay progression of macular degeneration caused by aging (31). By contrast, vitamin E supplementation does not prevent macular degeneration (32). Also, vitamin E supplementation has not been proven to prevent CVD and cancer (25).

Recommendations for future research
Due to the role of vitamin E in boosting the immune system and its anti-inflammatory activity, it is recommended to evaluate if DNA synthesis, immune responses, anti-inflammatory, cell signalling and proliferating, and infectious diseases like COVID-19 (33) are influenced by vitamin E levels.

Vitamin D
Introduction
Vitamin D (cholecalciferol) deficiency was first observed in the mid-1800s as rickets in children and osteomalacia in adults (4). Several foods, such as fatty fish livers, contain vitamin D, along with fortified milk. Unlike other vitamins, vitamin D is produced endogenously by dermal synthesis from cholesterol (13). Nevertheless, vitamin D, obtained either from food or sunlight, is biologically inactive and needs enzymatic conversion to the active form, 1,25-dihydroxy vitamin D (34).

Chemistry and measurement
The most common form of vitamin D in the blood is 1, 25-hydroxy vitamin D (25(OH)D). Vitamin D has two main forms, ergocalciferol (also called vitamin D2) and cholecalciferol (also known as vitamin D3). The best biomarker to evaluate vitamin D status in the blood is 25(OH)D, for a few reasons such as its role to reflect dietary and endogenous vitamin D, very tightly regulated inactive metabolite, relatively higher concentrations in the blood compared to other metabolites. It also has a relatively long half-life of about three weeks (35).

Vitamin D metabolism follows a regulated pathway. First, the inactive form of circulating vitamin D, 25(OH)D, is formed in the liver then 1,25-dihydroxyvitamin D (5), the active form, is formed in the kidney (35). 25(OH)D is typically measured in serum by immunoassay in high volume laboratories (8). Immunoassays react well with 25(OH)D3 but have variable cross-reactivity to 25(OH)D2 and the C3 epimer which is inactive. All major vitamin D contain epimerisation at C3 position. C3 epimer formation is associated with overestimation of vitamin D status measurement in routine laboratory tests (36). However, it is increasingly measured by LC-MS/MS (8). LC-MS/MS is particularly useful for measuring vitamin D in dried blood spots. LC-MS/MS assays can be established to reliably quantify both 25(OH)D3 and 25(OH)D2. Chromatographic separation is recommended to distinguish the C3 epimers, particularly for neonatal samples which contain high concentrations of the inactive C3 epimer indistinguishable from 25(OH)D3 and 25(OH)D2 by mass and fragmentation characteristics (3). Vitamins A, E, and D can be measured by LC-MS/MS in a single assay (2).

Due to the different factors, such as geographical locations, genetics and season, a lower limit of a reference interval is controversial (e.g. vitamin D) (37). However, parathyroid hormone appears to rise when the concentration of 25(OH)D drops below 50 nmol/L, levels above which are generally considered sufficient (37).

Indications and contraindications
25(OH)D should be measured in people at high risk of vitamin D deficiency, such as people in residential care, dark-skinned, chronic disease, obesity, low sunlight exposure (38), and may be useful in investigation of hypocalcaemia or hypercalcaemia. 1,25-(OH)2D measurement may be useful in the investigation of hypercalcaemia, particularly with suppressed parathyroid hormone (PTH) (34).

Laboratory interferences
There are some drugs such as barbiturates, corticosteroids and weight-loss drugs, such as orlistat, which may affect vitamin D measurement by some immunoassays (13).

Source
Vitamin D is synthesised from cholesterol with sunlight exposure (2). Vitamin D2 can be found in plants or supplements. However, a small amount of vitamin D3 can be found in food derived from animals. Endogenous synthesis of vitamin D depends on several factors such as skin colour and thickness, the duration of sun exposure and the season (13).

Metabolism and action
Vitamin D and its metabolites play important roles in the body due to their interrelationship with the calcium homeostasis and bone metabolism. The active form of vitamin D is 1,25-(OH)2D, which promotes enterocyte differentiation and intestinal absorption of calcium. Other functions include stimulation of intestinal phosphate absorption, direct suppression of PTH and metabolism and regulation of osteoblast function (34). Vitamin D is mostly present in food as the inactive forms. Thus, the conversion to the active form requires an enzymatic pathway in the liver and kidney.

In the liver, foodborne vitamin D bound to vitamin D-binding protein and with help of chylomicrons and lipoproteins vitamin D3 are synthesised (39). 25-hydroxyvitamin D2 and D3 produced by the liver enter enterohepatic circulation which then in the kidney bind to the vitamin D-binding protein. It is worth noting that only 3 to 5% of the entire circulating binding sites are normally occupied. Therefore, vitamin D-binding protein is not rate-limiting in vitamin D metabolism, unless in diseases such as nephrotic syndrome, where large quantities of vitamin D are lost in the urine (40). Therefore, one of the early indicators of kidney disorders may be measurement of vitamin D in urine.

Deficiency
Vitamin D deficiency is caused by four mechanisms. First, unavailability of vitamin D secondary to inadequate nutritional vitamin D, fat malabsorption diseases and/or least sunlight exposure. Second, disorders in the liver impair hydroxylation of 25(OH)D. Third, disorders in the kidney impair hydroxylation of 25(OH)D. Fourth, genetic disorders leading to end organ insensitivity to vitamin D (41).

Vitamin D deficiency is prevalent throughout the world (42). The prevalence of deficiency is higher with darker skin and increasing latitude (2). People with less exposure to sunlight are more at risk of vitamin D deficiency. Addition of vitamin D supplements to daily food intake of infants, disabled persons, elderly, people from northern latitudes is recommended (13). Low 25(OH)D is associated with numerous conditions, including cardiovascular diseases, respiratory infection, human immunodeficiency virus (HIV), and breast cancer (43). However, randomised controlled trials for supplementation have been largely underwhelming and not shown improved outcomes (44).

Toxicity
High concentrations of vitamin D may cause hypercalcaemia in susceptible individuals, especially those with 24-hydroxylase (CYP24A1) deficiency (45).

Therapeutic application
Vitamin D is used for treatment of deficiency and in groups at high risk of deficiency, as well as in pregnant women to prevent rickets in their offspring. The storage of vitamin D reduces by age, especially in wintertime.

Recommendations for future research
Randomised controlled trials with more ethnic variations and participants are required to evaluate whether there is a clear role for supplementation outside of deficiency.

Vitamin K
Introduction
The vitamin K family has a 2-methyl-1, 4-naphthoquinone nucleus in common while the side chain at the 3 position is what differentiates the vitamers. Vitamin K is made by plants and bacteria. Phylloquinone (vitamin K1) with a phytyl side chain is made by plants, and menaquinones (vitamin K2) are produced by bacteria.

Chemistry and measurement
Vitamin K in food is mainly present as phylloquinone which is absorbed from proximal intestine once solubilised into mixed micelles containing bile salts and pancreatic lipase (13). Vitamin K is mostly metabolised in the liver and released in the urine (~20%) and bile (~40-50%) (13). Vitamin K is a biological cofactor for the carboxyl reaction to transform selective glutamate (Glu) residues to γ-carboxyglutamate (Gla) residues (13). This reaction is catalysed by ag-glutamyl or vitamin K dependent carboxylase, a microsomal enzyme. Coagulation proteins are vitamin K dependent and are made in the liver. They included factors II, VII, IX, and X, which have haemostatic protein C and S to inhibit blood clotting (46).

Vitamin K_1 and K_2, can be measured by HPLC with fluorescence detection or LC-MS/MS (8). However, many assays struggle with insufficient sensitivity near the lower end of the reference interval to reliably diagnose a deficient patient. LC -MS/MS can help to overcome the sensitivity problem, although pre-concentration of the sample, and/or sample clean-up is usually still required. Sample clean-up can be achieved by using either solid phase extraction (SPE) cartridges (47), or two dimensional chromatography. A sensitive LC-MS/MS system with atmospheric pressure chemical ionisation is required for the analysis. Phenyl-hexyl columns are useful for the separation of vitamin K1 as they offer mixed-mode selectivity for non-polar analytes with an aromatic functional group (47).

Indications and contraindications
Although vitamin K deficiency is rare, it can manifest with bruisability, mucosal bleeding, splinter haemorrhages, melena, haematuria, and other coagulopathies. Vitamin K deficiency can occur in people with fat malabsorption due to bile or pancreatic dysfunctional secretion, for example CF, primary biliary cholangitis, primary sclerosing cholangitis, familial intrahepatic cholestasis (and other inherited disorders associated with cholestasis), active coeliac disease, inflammatory bowel disease, or short bowel syndrome and liver failure (48). The indications of vitamin K deficiency are found in patients with bleeding symptoms by measuring prothrombin time and international normalized ratio (INR) (elevated in vitamin K deficiency) in predisposed patients, measuring levels of PIVKA-II (protein induced in vitamin K absence, also known as des-gamma-carboxy prothrombin) is more sensitive than prothrombin time (49).

Laboratory interferences
Analytically, some drugs can interfere with the result of vitamin K, such as warfarin, antacids, blood thinners, antibiotics, aspirin (50).

Sources
Animal liver and fermented foods, such as cheeses, are typically high in long-chain menaquinones. In addition, a high level of vitamin K is found in green vegetables, gut bacteria, meat, cheese, and eggs (13).

Metabolism and action
Vitamin K has roles in bone turnover and coagulation (48). Although scarce information exists on the effectiveness of different hepatic forms of vitamin K in the matter of coagulation, further work is required on vitamin K dependent coagulation pathways.

Deficiency

Vitamin K deficiency is rare but is seen in neonates and people with hepatobiliary or pancreatic disease and thus it is a significant public health problem around the world (51). The deficiency of vitamin K in infants was traditionally called haemorrhagic disease of the new-born. Nowadays, the term used is vitamin K deficiency bleeding (VKDB). VKDB onset time is unpredictable and vitamin K injection is highly recommended in new-borns (52). The deficiency of vitamin K is classified as early, within 24 hrs of life (53), classic VKDB develops between the second and seventh day of life (largely preventative by vitamin K administration) (54). Late VKDB occurs between three weeks and eight months of age (highly linked with central nervous system) (55). Acquired vitamin K deficiency occurs as a side effect in some medications such as antibiotics, high dose of vitamin E, and prolonged fasting or starvation (56). Interestingly, vitamin K deficiency has been linked with higher mortality in Covid-19 patients (57).

Toxicity

Toxicity of vitamin K is rare. Although the synthetic form of vitamin K (menadione) can cause haemolytic anaemia, hyperbilirubinemia, jaundice, and kernicterus in infants (55), natural orally taken forms of vitamin K from food do not show any harmful effects (55).

Therapeutic application

Patients at risk of deficiency and with coagulation defects suspected to be from vitamin K deficiency, such as with liver disease, may be given a trial of vitamin K supplementation to correct a coagulopathy. New-borns are at risk of vitamin K deficiency due to the inability of their immature livers to utilise vitamin K efficiently and low concentration of vitamin K in breast milk, this maybe further exacerbated by maternal ingestion of coumarin-like anticoagulants (e.g. warfarin), antibiotics and anticonvulsants (55).

Recommendations for future research

To find out the prevalence, causes, and prevention of VKDB in the different age and ethnicity groups. Evaluate if there is any possibility to fortified food with phylloquinone and menaquinones. Determine the significance of menaquinones to humans.

CONCLUSIONS

The measurement of some vitamins, such as 25(OH) D, has substantially improved over time. Yet the measurement of some other vitamins, such as A and E, are a challenge for many laboratories due to result variability (2). The measurement of fat -soluble vitamins can be improved by using LC-MS/MS and adoption of harmonised methods across laboratories in order to avoid unnecessary variation in the results (1,3,58). To harmonise an analytical method it is necessary including five significant factors in practice: 1, reference materials; 2, reference method; 3, reference intervals; 4, reference labs and 5, external quality controls (59). Nevertheless, there is always some degree of analytical variation in any assay. Having a harmonised method with higher reproducibility and repeatability is a priority and can help clinicians and healthcare scientists to harmonise patient care pathways.

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