REVIEW ARTICLE

Water soluble vitamins: mechanism and metabolism. A narrative review

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ABSTRACT

Vitamins are essential nutrients that are classified into two groups, fat soluble vitamins (FSVs) and water-soluble vitamins (WSV). WSVs include thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxal phosphate (B6), biotin (B7), folate (or folic acid) (B9), cobalamin (B12), and ascorbic acid (C). Although deficiency of WSVs is uncommon, it can still be seen among individuals with different disorders such as short bowel syndrome, chronic alcoholism, and malnourished or post bariatric surgery patients. Despite the use of advanced technologies such as chromatography and mass spectrometry to measure vitamins, there is a need for more consistent standardisation and reference intervals. Covariates such as requirement for sample collection, fasting and avoiding supplementation, require more work to assess their effects in the interpretation of vitamin results. The aim of this review is to highlight pre-analytical factors such as fasting and cessation of supplementation before sampling. There are no comprehensive guidelines found in the literature describing how long vitamin supplementation should be ceased before sampling, and further studies are warranted.

Keywords: water soluble vitamins, reference range, B vitamins, vitamin C, vitamin supplement, fasting and non-fasting vitamins.

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OVERVIEW

Vitamins play a crucial role in many biochemical mechanisms in the human body. Water soluble vitamins (WSVs) are found in a variety of foods such as fruits, vegetables and meat and play vital roles in body growth, skin, nerve, red blood cells (RBCs) and heart function (1). Although deficiency of WSVs is uncommon in New Zealand, it can be seen among malnourished people, such as: chronic alcohol abuse; strict veganism; and malabsorption syndromes. Unlike fat soluble vitamins (FSVs) where excess amounts of vitamins can be stored in the human body (2), WSVs are readily excreted in the urine and most do not have large stores. The structures of the biologically active forms of WSVs are shown in Figure 1.

WSVs are measured by immunoassay, high performance liquid chromatography (HPLC) and liquid chromatography tandem mass spectrometry (LC-MS/MS). HPLC and LC-MS/MS assays have been reported for vitamins B1, B2, and B6 (3, 4). Vitamins B9 and B12 are routinely measured on auto-analysers, while vitamins B3, B5 and B7 are not routinely measured in most clinical laboratories. This may be due to both analytical challenges and lack of clinical demand. Little information is available about the bio-accessibility and bioavailability of many WSV's. Currently, there are no New Zealand specific reference intervals for WSVs, and overseas reference intervals may not be appropriate.

In this review we provide an overview of WSVs and challenges for measurement and interpretation of results.

Vitamin B1 (Thiamine)

Introduction

Thiamine was described more than 4000 years ago in Egypt (5) and was the first member of the B complex to be discovered.

Chemistry and measurement

The main active form of thiamine is thiamine pyrophosphate (TPP) (6) (Figure 1). Thiamine is often measured using erythrocyte thiamine transketolase activity (ETKA) (6). However, due to the limited sensitivity and specificity of ETKA, HPLC and LC-MS assays are considered more reliable assays (3). TPP is usually measured in whole blood because thiamine is more concentrated in RBCs.

Indications for measurement

Thiamine testing can be considered in patients with suspected beriberi, particularly in the context of poor diet. Measurement of thiamine can help confirm deficiency in patients with poor intake. Patients with suspected thiamine deficiency should be treated with thiamine to prevent the development of Wernicke encephalopathy and Korsakoff psychosis. Thiamine testing can also be considered in patients with behavioural changes, ocular disorders, delirium, and encephalopathy (7), and in patients who undergo insulin therapy, due to thiamine deficiency lowering insulin synthesis and secretion (8).

Limitations of measurement

Systemic inflammation, variations in testing methods between laboratories, and the presence of hypoalbuminemia may interfere with the measurement of thiamine (9). Thiamine can degrade if samples are not light protected. Non-fasting and thiamine supplementation may falsely increase thiamine results.

Sources

High levels of thiamine are found in foods such as yeast, legumes, pork and brown rice. However, high temperature and modified pH can destroy thiamine (6).

Metabolism and action

Thiamine is mainly absorbed in the small intestine (6). Thiamine is dephosphorylated to facilitate entering the blood through an adenosine triphosphate (ATP) dependent pump (*i.e.*, active transport), then phosphorylated intracellularly to the active form. Thiamine is most commonly passed via active transport into to most cells and by passive diffusion to RBCs (6).

Thiamine has a 10–20-day half-life in the body. Therefore, it is necessary to maintain thiamine levels with continuous intake. Thiamine is mostly excreted in the urine and partially in bile (6). TPP is a crucial cofactor for enzymes involved in carbohydrate and branched-chain amino acid metabolism. In the Krebs cycle, TPP works as a catalyst to convert pyruvate to acetyl CoA.(6).

Deficiency

Thiamine deficiency may cause a reduction in carbohydrate metabolism, which can affect amino acid homeostasis leading to decreased formation of acetylcholine needed for neural function (10). Thiamine deficiency is mostly reported in people who consume polished rice, or milled white cereals, due to the refining process (6). Thiamine deficiency leads to beriberi, a neurological disorder which is related to the synthesis of glutamate and γ-aminobutyric acid as well as myelin sheath maintenance (6). Thiamine deficiency occurs in both children and adults. Infantile beriberi usually happens in babies who were born to a mother with thiamine deficiency with features such as cardiomegaly, tachycardia, and pulmonary hypertension (6). In older babies thiamine deficiency can be present as meningitis with no abnormalities on cerebrospinal fluid analysis (6). The main reason for thiamine deficiency in babies and new-borns is inadequate parenteral nutrition (6). In adults there are two types of beriberi. Wet beriberi which is characterised by signs of cardiac disorders such as cardiomegaly and heart failure. Dry beriberi is recognised by development of a symmetrical peripheral neuropathy classified through sensory and motor impairments (6).

Another neuropathic complication of thiamine deficiency is Wernicke-Korsakoff syndrome (WE). WE is often recognised by short-term impaired memory, confabulation (honest lying), nystagmus, ophthalmoplegia, ataxia, and confusion.

Toxicity
There are no reports of thiamine toxicity, due to the rapid clearance of excess thiamine from the kidneys (6).
Therapeutic application
The role of thiamine deficiency in diabetic nephropathy was proposed a long time ago, but is still controversial (11).

Vitamin B2 (Riboflavin)
Introduction
Vitamin B2 is a member of the flavin family with a critical role in a myriad of biochemical reactions.
Chemistry and measurement
Riboflavin is mostly present in the form of flavin-adenine dinucleotide (FAD) (6) (Figure 1). Flavins in food are derivatives of FAD, flavin mononucleotide (FMN) or free flavins. After ingestion and absorption of dietary riboflavin, FAD and FMN are hydrolysed into free riboflavin through gastric acid, proteolytic enzymes and bile salts (6). Free riboflavin binds to albumin and immunoglobulins (6) and is absorbed in the proximal small intestine. When Free riboflavin reaches cells of the liver, heart and kidney it is metabolised into FMN and FAD (6). Riboflavin is first phosphorylated to form FMN. FMN can be either further phosphorylated into FAD or become part of coenzyme flavin complex. Both reactions are ATP dependent.

Plasma and urine riboflavin measurement reflect recent dietary intake. Urinary riboflavin is primarily used as a test to determine dietary intake in a population for riboflavin deficiency (6). FMN has been introduced as a biomarker to predict outcomes after kidney transplantation (12).

Vitamin B2 is usually measured as the active form, FAD. FAD can be measured in serum or whole blood by HPLC-fluorescence directly without the need for derivatisation (13).
Indications for measurement
Riboflavin deficiency can be seen in people who consume few dairy products (*i.e.*, lactose intolerant people) (14). In addition, malabsorption syndromes, HIV, malignancy, and some inborn genetic defects (*e.g.*, acyl-coenzyme A (CoA) dehydrogenases) have been linked to riboflavin deficiency (15, 16). Medications such as tricyclic phenothiazine, barbiturates and chlorpromazine also affect measurement.

Non-fasting or taking a dietary supplement can falsely increase the blood level of vitamin B2.
Sources
Riboflavin is present in different foods such as milk, eggs, meats and green vegetables (17).
Metabolism and action
FAD is often combined with proteins to form flavoproteins. Flavoprotein is the most common form of riboflavin storage in the human body (6). Riboflavin is an essential cofactor in energy producing respiratory pathways such as Krebs cycle, beta-oxidation of fatty acids, as a catalyst in mitochondrial oxidative and reductive reactions and electron transporters (6).

The absorption of riboflavin is regulated by bile salt in two ways. Firstly, bile salts induce solubility and permeability of riboflavin in the small intestine. Secondly, bile salts assist gastric emptying and increase the dephosphorylation time of FMN and FAD to riboflavin in the small intestine; then, riboflavin is stored in the liver as FAD (16).
Deficiency
A deficiency of vitamin B2 results in a disorder called ariboflavinosis. Symptoms include sore throat, hyperaemia of pharyngeal mucous membranes, oedema of mucous membranes, cheilitis, stomatitis, glossitis, normocytic-normochromic anaemia, and seborrheic dermatitis (6). Whether these symptoms are due to riboflavin deficiency is sometimes unclear, and the condition can be confused with other WSV deficiencies (6).

The other condition that can lead to riboflavin deficiency is multiple acyl-CoA dehydrogenation deficiency (MADD), a genetic disorder that is usually manageable with riboflavin supplementation (18).
Toxicity
There are no adverse effects reported after high doses of riboflavin. This may be due to limited absorption in the intestine (6).
Therapeutic application
Riboflavin can be used to prevent migraines. Riboflavin has shown some promise in preventing Parkinson's disease, though more research is required to confirm these findings (19).

Vitamin B3 (Niacin)
Introduction
Vitamin B3 (nicotinic acid or niacin) (Fig. 1) is essential in the synthesis and metabolism of carbohydrates, fatty acids, and proteins. A deficiency of this vitamin results in a disease called pellagra. This condition can progress to both physical and mental deterioration if left untreated.
Chemistry and measurement
Niacin is rapidly taken up by a passive process in liver, kidney and RBCs. During metabolism of niacin dietary nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) are converted to nicotinamide and nicotinic acid for intestinal absorption by hydrolysis and microbial mediation and released through passive and facilitated diffusion. Then, they are returned to NAD and NADP form to be used in cellular functions (20). Many enzymatic redox reactions need NAD and NADP as a cofactor.

High levels of niacin metabolites, such as N-methyl nicotinamide are the indicators of sufficient concentrations of intracellular niacin (21). Niacin can be measured by urinary N-methyl nicotinamide or by measuring the erythrocyte NAD: NADP (ratio). Nevertheless, this test is not offered in many laboratories (6). Niacin can be measured in serum by HPLC using derivatisation and fluorometric detection (22), or using LC -MS methods (23).
Indications for measurement
High consumption of corn or corn meal can lead to niacin deficiency due to inadequate tryptophan, a precursor of niacin synthesis, in maize. For instance, niacin deficiency has been seen in resource-rich countries where people consume a lot of corn (24). Niacin measurement should also be considered for chronic alcoholics, people undergoing bariatric surgery, anorexia or malabsorption disorders (6).
Limitations of measurement
Non-fasting and niacin supplement can provide falsely elevate niacin results. Some medications for tuberculosis (e.g., isoniazid and pyrazinamide) can falsely reduce plasma levels (24).
Sources
High levels of niacin are found in plant and animal foods such as yeast, meats (especially liver), legumes and grains (6).
Metabolism and action
NAD and NADP are necessary for enzymes involved in redox reactions. For instance, NAD dependent enzymes work in oxidation of fatty acids and producing of NADH via glycolysis and Krebs cycle (6).
Deficiency
Deficiency of niacin is called pellagra or "raw skin" which is characterised by a photosensitive pigmented dermatitis, diarrhoea, and dementia, and eventually death (if not treated) (6).
Toxicity
Although toxicity of niacin is not normally problematic, high doses of niacin may lead to nausea, vomiting, pruritus, hives and liver dysfunction with elevation of serum aminotransferases (6).
Therapeutic application
Niacin is well-known as an antihyperlipidemic agent by reducing very low density lipoprotein (VLDL) as a result lower low density lipoprotein (LDL), thus increasing the level of high density lipoprotein (HDL) (6).

Vitamin B5 (Pantothenic acid)

Introduction

Pantothenic acid was discovered less than a century ago (25) and plays a crucial role to the metabolism of CoA and acyl-carrier-protein (26).

Chemistry and measurement

Pantothenic acid got its name from a Greek word meaning 'everywhere' because it is found in nearly all foodstuffs (25). Due to the wide availability of pantothenic acid, deficiencies are uncommon and there is low interest in measuring it clinically.

Different methods that have been used to quantify pantothenic acid include: microbiological assays; radioimmunoassay; and HPLC (27). In addition, measurement of pantothenic acid in different sample matrices can produce different results (6). The ideal sample for pantothenic acid measurement is urine because urinary pantothenic acid is well-correlated to dietary intake (6). Measurement of pantothenic acid with LC-MS/MS is considered the "gold standard" (28).

Indications for measurement

It is generally unnecessary to measure pantothenic acid in a clinical setting (29). However, measurement might be considered in malnourished people (30).

Limitations of measurement

Literature regarding the interferences of pantothenic acid not readily available.

Sources

Pantothenic acid is found in many dietary sources and is also partially produced via gut bacteria (6). Pantothenic acid levels are high in egg yolk, chicken, animal organs such as liver and kidney, and vegetables such as broccoli and potatoes (6).

Metabolism and action

Pantothenic acid is a coenzyme involved in mitochondrial carbohydrate and lipid biosynthesis, the Krebs cycle, and in the biosynthesis of haemoglobin and cytochromes (26). CoA is hydrolysed in the small intestine by gut microbiota to form pantothenic acid. It is absorbed in the jejunum and secreted into the blood circulation through a sodium-dependent transport system. In the cell, pantothenic acid undergoes phosphorylation and re-conversion to CoA. Any excess of pantothenic acid is hydrolysed and excreted as cysteamine and pantothenate through the renal system (6).

Deficiency

Deficiency of pantothenic acid is characterised by paresthesia and dysesthesias or 'burning feet syndrome (6). Although pantothenic acid deficiency is a rare disorder in humans it can still be seen where chronic starvation occurs (6).

Toxicity

No toxicity has been reported as pantothenic acid is readily excreted by the kidneys.

Therapeutic application

Pantothenic acid is used in skin care, in particular for dry skin and acne (31).

Vitamin B6 (Pyridoxine)

Introduction

Vitamin B6 consists of pyridoxine, pyridoxamine, pyridoxal, and the phosphorylated derivatives of each of these compounds. Vitamin B6 plays a vital role in the metabolism of fats, carbohydrates and protein.

Chemistry and measurement

Vitamin B6 has seven forms: pyridoxine (PN), pyridoxal (PL), pyridoxamine (PM), pyridoxine 5′-phosphate (PNP), pyridoxal 5′-phosphate (PLP), pyridoxamine 5′-phosphate, and pyridoxine -5-β-D-glucoside (PNG). However, the biologically active form PLP is the form most commonly measured. (Figure 1.)

Methods available for measuring vitamin B6 include erythrocyte transaminase activity, urinary 4-pyridoxic acid and xanthurenic acid detection (6). However, HPLC and LC-MS are the most reliable methods to measure vitamin B6 as PLP. PLP can be measured by HPLC with fluorescence detection using either serum or whole blood (32). Derivatisation is required to increase assay sensitivity (33, 34).

Indications for measurement

Early markers of vitamin B6 deficiency include decreased plasma PLP and urinary 4-pyridoxic acid. Other signs of deficiency include reduced circulating lymphocytes and normocytic, microcytic, or sideroblastic anaemia (10). Plasma PLP is the best single indicator to reflect storage of vitamin B6 in tissues (10).

Limitations of measurement

Pre-analytical factors such as: not protecting samples from light; non-fasting samples; and multivitamin supplements may affect the result. As this vitamin is photosensitive, a non-light protected specimen may report a falsely-lowered measurement. Non-fasting samples falsely increased the vitamin B6 level. Vitamin B6 can be reduced in alcoholics, and those with coeliac disease.

Sources

Pyridoxine and pyridoxamine are high in plant foods while pyridoxal is most predominantly in animal-based foods. However, cooking, food processing and canning may reduce the availability of vitamin B6 by up to 50% (6).

Metabolism and action

PLP is the active form found in circulation. It acts as a coenzyme in more than 100 known enzymatic reactions (35). It is also involved in the synthesis of NAD from tryptophan and homocysteine metabolism. Pyridoxine is converted to PLP in the liver and catabolised to 4-pyridoxic acid and excreted in the urine.

Vitamin B6 is a crucial cofactor in transamination of amino acids, gluconeogenesis, decarboxylation of amino acids (*e.g.* tryptophan to niacin), haem synthesis, sphingolipid biosynthesis, neurotransmitter synthesis, immune function and transsulfuration of homocysteine to cystathionine and to cysteine; Vitamin B6 insufficiency can result in elevation of plasma homocysteine concentration and this can indirectly lead to atherosclerosis and vascular thromboembolism (6).

Deficiency

Although vitamin B6 deficiency is rare, marginal deficiency is relatively common. Vitamin B6 deficiency may lead to a variety of clinical abnormalities such as heart disease, lung cancer, diabetes mellitus, gastrointestinal and ocular disorders, and depression (17). Vitamin B6 deficiency usually occurs with other B-complex deficiencies. For instance, hypovitaminosis B6 may occur with riboflavin deficiency because riboflavin is needed to make PLP. Non-specific manifestations include dermatitis, glossitis, microcytic anaemia, irritability, confusion, depression, and possibly peripheral neuropathy and seizures (5, 6). Lack of vitamin B6 is reported in diseases such as asthma, diabetes, alcoholism, heart disease, pregnancy, breast cancer, Hodgkin lymphoma, and sickle-cell anaemia, cardiovascular diseases, colon and lung cancers, diabetes mellitus complications, digestive system disorders (5, 6). Moreover, medication such as isoniazid, penicillamine, hydralazine, and levodopa are associated with deficiency of vitamin B6 (5, 6). Infants are the most susceptible to vitamin B6 insufficiency, which can lead to epileptiform convulsions, dermatitis with cheilosis and glossitis (5).

Toxicity

Vitamin B6 toxicity is common and may present with peripheral neuropathy, dermatoses, photosensitivity, dizziness, and nausea.

Therapeutic application

Vitamin B6 is used for several complications such as reducing the severity of nausea in the first trimester, and to reduce the risk of dental decay in pregnant women (36, 37). Vitamin B6 is also prescribed for pre-menstrual syndrome (due to its same effect as serotonin and dopamine), carpal tunnel syndrome (due to its effect on the rising pain threshold through analgesic property) and other neurologic disorders (10).

Vitamin B7 (Biotin)

Introduction

Early in the 20th century, growth factors found in yeasts and called "Bios" were identified as *myo*-inositol, pantothenate, and biotin (6). Biotin was introduced as a vitamin when its deficiency

caused a clinical syndrome mediated by carboxylase enzymes (6). Biotin or vitamin B7, also called vitamin H, coenzyme R, factor S, factor W, vitamin B, and protective factor X due to its protective property of biotin to avoid dermatosis and hair loss in animals (38).

Chemistry and measurement

D-Biotin is the active form of biotin (Figure 1.); biocytin (biotin bound with lysine) is the end product of biotin-containing enzyme degradation and is convertible to biotin through the action of biotinidase (6). Biotin is mostly absorbed in the proximal small intestine and to a lesser degree in the caecum. It is either ingested as the biotin form or produced by gut bacteria. Unabsorbed biotin is excreted either in faeces or urine (6). Measurement of biotin is done by immunoassay and LC-MS/MS (39).

Indications for measurement

Biotin may interfere with several immunoassays including thyroid function tests and may give a pattern that mimics Graves' disease (6). In addition, high dose of biotin may interfere with troponin, ferritin, testosterone, B-type natriuretic peptide, digoxin, and progesterone (6). Avoidance of biotin supplement for 2 to 5 days before testing can eliminate the potential interference (39).

Limitations of measurement

Metabolism of biotin is increased in smokers, which may affect biotin measurement because smoking increases the catabolism of the vitamin (40).

Sources

High levels of biotin are found in different plants (e.g., peanuts and soybeans), liver and egg yolk (6). However, egg yolk is the best source of biotin.

Metabolism and action

Biocytin (biotin bound with lysine) is the end product of biotin-containing enzyme degradation and is convertible to biotin through the action of biotinidase (6). It is either ingested as the biotin form or produced by gut bacteria. Unabsorbed biotin is excreted either in the faeces or urine (6). Biotin is necessary for a range of carboxylase enzymes such as acetyl coenzyme A (CoA) carboxylase (ACC), pyruvate carboxylase (PC), propionyl CoA (PCC) and methylcrotonyl CoA carboxylase (MCC) in mammals. The vitamin acts as a CO_2 carrier on the surface of biotin carboxylase, and it is essential in the metabolism of carbohydrates, protein and lipids. PC and PCC are involved in Krebs for the metabolism of lipid (odd-chain fatty acids) and proteins (3-carbon non-carbohydrate precursors for gluconeogenesis) and MCC is needed for the metabolism of leucine (41).

Deficiency

Biotin deficiency may result in dermatitis around the eyes, nose, mouth, and cause conjunctivitis, alopecia, and changes in mental status (42). Most of these clinical manifestations are from malfunctioning of the biotin dependent enzymes mentioned above (6). Deficiency of biotin was firstly detected among patients on parenteral nutrition (6). Biotin deficiency reports are very rare, apart from lack of biotinidase (6). Deficiency of biotin can also result from inherited disorders such as multiple carboxylase deficiency which is usually diagnosed in the first weeks of life, and manifests as lethargy, poor muscle tone and vomiting and biotinidase deficiency. Biotin deficiency occurring later in life leads to loss of biotin in urine and organic aciduria is characterised by ataxia, ketoacidosis, dermatitis, seizures, myoclonus, and nystagmus (43).

Toxicity

No toxicity has been reported for biotin.

Therapeutic application

It has been shown that biotin may have a potential to prevent hair loss and build nail, but these benefits are yet to be proven (44).

Vitamins B9 (Folate) and B12 (Cobalamin)

Introduction

As vitamin B9 and B12 share similarities and are measured simultaneously, both are reviewed together in this section. Vitamin B9 is necessary for the normal function of RBCs and white blood cells and vitamin B12 is required to convert inactive folate to the active form, maintenance of myelin, and methionine synthesis.

Chemistry and measurement

Folate is necessary during early pregnancy for neural tube formation and folic acid supplementation is required to prevent or reduce the risk of neural tube defects.

The diagnosis of vitamin B12 deficiency is complicated due to poor sensitivity and specificity of conventional tests (45). There are many different forms of vitamin B12. Cyanocobalamin (Fig.1) is commonly found in vitamin supplements. However, the cyanide group can be replaced by an adenosyl, methyl, or hydroxyl groups. Holotranscobalamin (or the active protein bound fraction of vitamin B12) was introduced to improve the detection of vitamin B12 (45, 46).

Indications for measurement

One of the biggest concerns about folate and vitamin B12 deficiency is that most people with deficiency are asymptomatic. As a result, the detection of the insufficiency is usually diagnosed by laboratory analysis or gradual development of symptoms. In some situations, such as pregnancy and symptomatic anaemia (or neurologic or neuropsychiatric findings) the treatment with folate and vitamin B12 should be the first priority. For symptomatic patients the first line of treatment is parenteral administration, and after symptoms have resolved, oral therapy. However, in those who do not have the ability to absorb oral therapy, parenteral administration should be considered (6).

Due to the link between vitamin B12 deficiency and anaemia, screening tests such as a complete blood count (CBC) with a peripheral smear (morphology studies) along with measurement of serum B12 and folate can be performed. Folic acid deficiency may also lead to macrocytic anaemia and can be confused with vitamin B12 deficiency. Therefore, testing for serum vitamin B12 and folate at the same time can help to differentiate between them. However, if the case is still unclear the measurement of methyl malonic acid (MMA), homocysteine, and active B12 is useful. Total B12 has a large borderline interval, and testing for MMA, homocysteine, and active B12 can help in these cases. For instance, active vitamin B12 usually makes up 10% to 30% of the entire B12 in the body, thus someone whose B12's range is normal might be actually B12 deficient (49). Homocysteine is elevated in either folate or B12 deficiency, whereas MMA is elevated in B12 deficiency but not folate deficiency.

Limitations of measurement

There is no standardised or harmonised assay available for the measurement of folate, B12 and active B12. Vitamin B12 deficiency is seen among people with small intestine disorders, neonates from B12 deficient mothers, people who are exposed to nitrous oxide (N_2O) and aging (47).

Sources

Vitamins B9 and B12 are derived from plants (e.g., green vegetables) and animal products (e.g., red meat), respectively. Foods such as breads and cereals in NZ are fortified with a range of vitamins including B9 and B12.

Metabolism and action

Folate is needed for metabolism of purines and pyrimidines, which are precursors for DNA synthesis. Vitamin B12 has other roles such as being a cofactor for methionine synthesis. In the absence of vitamin B12, homocysteine cannot be converted to methionine, thus homocysteine accumulates. As a result, DNA synthesis is decreased, and this results in megaloblastic anaemia. Malfunctioning DNA synthesis results in polymorphonuclear leukocytes. Altogether, vitamin B12 deficiency leads to the formation of hyper-segmented neutrophils (a classical indicator of megaloblastic anaemia).

The other role of vitamin B12 is converting methylmalonyl-CoA to succinyl-CoA via methylmalonyl-CoA mutase. Patients with vitamin B12 deficiency have accumulated MMA because MMA cannot be converted to succinyl-CoA. It has been shown that an increased level of MMA and homocysteine is associated with neurologic deficits and myelin damage. The latter affects various parts of spinal cord and results in ataxia, peripheral neuropathy, and dementia (47).

Folate is absorbed through the intestinal tract and a decreased level of folate indicates deficient dietary absorption. Folate can be also found in the tissues and RBCs (48).

Deficiency

Folate deficiency is uncommon. A decreased level of folate in RBCs means either there is tissue folate deficiency or the patient has primary vitamin B12 deficiency resulting in an inability of cells to uptake folate. The former needs folate therapy while the latter requires vitamin B12 therapy (47). However, it can be seen in some conditions such as inflammatory bowel disease (IBD) severe malnourishment, alcoholism, diets low in green leafy vegetables, chronic haemolytic anaemia, and disorders with high cellular turnover.

Vitamin B12 deficiency usually occurs by three mechanisms: 1.) autoimmune; 2.) malabsorption, and 3.) dietary insufficiency. Vitamin B12 deficiency is usually indicated by clinical manifestations such as gastrointestinal, neurologic findings plus fatigue and pallor manifestations along with laboratory findings such as macrocytic anaemia, haemolysis, and jaundice. There are other nonspecific findings such as peripheral neuropathy, glossitis, diarrhoea, headaches, and neuropsychiatric disturbances. A history of coeliac or Crohn's disease, bowel resection, surgical history of gastrectomy and strict vegan diet increase suspicion for vitamin B12 deficiency.

Toxicity

There is an association between high folic acid consumption and increased risk of cancer (e.g. cutaneous melanoma, a type of skin cancer) and developmental delay in new-borns (47).Toxicity for vitamin B12 has not been reported.

Therapeutic application

Restricted vegans and vegetarians often need to take vitamin B12 supplements (49). In addition, vitamin B9 is prescribed to women who are planning a pregnancy to prevent neural tube defects (50). Folic acid deficiency is not very common in NZ as many foods are now fortified with folic acid (51).

Vitamin C (Ascorbic Acid)

Introduction

Vitamin C (or ascorbic acid) is an antioxidant, and a crucial cofactor for collagen, carnitine, catecholamine and iron metabolism. The human body is unable to produce vitamin C, therefore we need to obtain vitamin C through the regular dietary intake of fruit and vegetables.

Chemistry and measurement

Vitamin C is an alpha-ketolactone (Figure. 1). Although the measurement of vitamin C in leukocytes was previously shown to be a better indicator of vitamin C in body stores (52), this method is not widely utilised now.

Vitamin C (ascorbic acid) can be measured in plasma using HPLC with either UV or electrochemical detection (53). HPLC is often performed using a reversed phase column with an ion-pairing agent in the mobile phase(54).

Indications for measurement

Vitamin C should be measured in patients with disorders such as alcoholism, anorexia, cancer, asthma, glaucoma, and collagen disorders (55). It is worth noting that people with autism may also require to be monitored for vitamin C due to the possibility of an inadequate diet (56).

Limitations of measurement

Samples for vitamin C need to be stored in light protected tubes. The levels of vitamin C increase significantly among people who take a vitamin C supplement before sample collection. Measurement of vitamin C is difficult. For instance, pre-analytical sample treatment is important due to the limited stability of vitamin C, which is readily oxidised to dehydroascorbic acid (DHA). Metal ions present in blood increase the oxidation of vitamin C; the choice of chelating reagent is important. It is recommended that samples are collected in lithium heparin tubes and kept cold until analysis. DHA can be reduced back to ascorbic acid using strong reducing agents such as tris (2-carboxyethyl) phosphine (TCEP). This is often added to convert all DHA to ascorbic acid, so that a total vitamin C is measured. If the samples have been stored at room temperature for extended periods, then DHA can oxidise to diketogulonic acid, which cannot be converted back to ascorbic acid (57), but frozen (*i.e.* -80℃) samples seem to be more stable (58).

Sources

Vitamin C is found in citrus fruits, berries, tomatoes, potatoes, and green leafy vegetables (6). It is an essential nutrient for humans, and other living creatures.

Metabolism and action

Vitamin C, a biological electron donor or reducing agent, plays a crucial role in the maintenance of enzymes involved in iron and copper metabolism, and stabilisation of vitamin E and folic acid (6). Other functions of vitamin C include fatty acid transport (6), a crucial cofactor in wound healing (6), neurotransmitter synthesis for instance, synthesis of norepinephrine (6), prostaglandin metabolism, an anti-inflammatory (6) and a potent vasodilator (i.e. nitric oxide synthesis). It is absorbed in the distal small intestine via an energy-dependent active transport pathway (6) and regulated by kidney excretion. Vitamin C is necessary for the integrity of the skin, mucous membranes, blood vessels, and bone via its nature in collagen synthesis (59).

Deficiency

Deficiency of vitamin C is called scurvy. It is a historical disease, which is manifested in haemorrhage, hyperkeratosis, and haematological abnormalities bruising, gingivitis, arthralgia, and impaired wound healing (6). Vitamin C deficiency is mainly diagnosed by clinical symptoms based on a history of insufficient vitamin C intake. Early onset of the condition usually occurs by follicular hyperkeratosis and perifollicular haemorrhage, with petechiae and coiled hairs, ecchymoses, gingivitis, anaemia and impaired wound healing (6).

Although vitamin C deficiency has generalised systemic symptoms such as weakness, malaise, depression etc., (6), it can have life-threatening symptoms such as dyspnoea, hypotension and sudden death (6). It occurs mainly among people with severe malnourishment, chronic drug and alcohol abuse, children with autism, iron overload such as sickle cell anaemia or thalassaemia, and bone marrow transplantation because ferric deposits increase the catabolism of vitamin C (6).

Toxicity

Large doses of vitamin C can be toxic and associated with diarrhoea, and abdominal bloating, and oxalate kidney stones in males. Higher doses of vitamin C interfere with guaiac results (give a false negative result) but not immunological faecal haemoglobin tests (6, 60)

Therapeutic application

Vitamin C has several therapeutically and prophylactic roles such as cardiovascular diseases and cancer prevention (61), but does not prevent common colds except in people doing extreme physical exertion (62-64).

CONCLUSIONS

Requests for vitamin testing are increasing. However, there is no standardised or harmonised procedure for pre-analytical sample treatment, measurement, and interpretation of vitamin results. There are different references used for recommended ranges, reference range and interval range that can be very overwhelming and confusing, not only for health professionals but also for patients. Establishing a universally adopted recommended interval is not a simple task. In order to establish a normal range, a large number of healthy subjects with tight inclusion and exclusion criteria (e.g., not fasting and not taking supplementation) would need to be analysed for WSVs in the New Zealand population. Most samples measured in clinical laboratories are not taken from healthy people, and often, little consideration is given as to whether they are taking a supplement and what effect that has on the interpretation of the results, especially when testing for a vitamin deficiency or how long it will take to excrete from the body. An effective vitamin working group with input from Pathologists is useful in order to widely agree and adopt a standardised approach to vitamin testing.

There is further work to do to for better agreement on best practices to be achieved in New Zealand (and Australian) laboratories. Deficiency of some WSVs is a serious clinical issue that can lead to severe morbidity and mortality. Therefore, reliable, accurate and standardised measurement is needed to assist in better diagnosis of vitamin deficiencies.

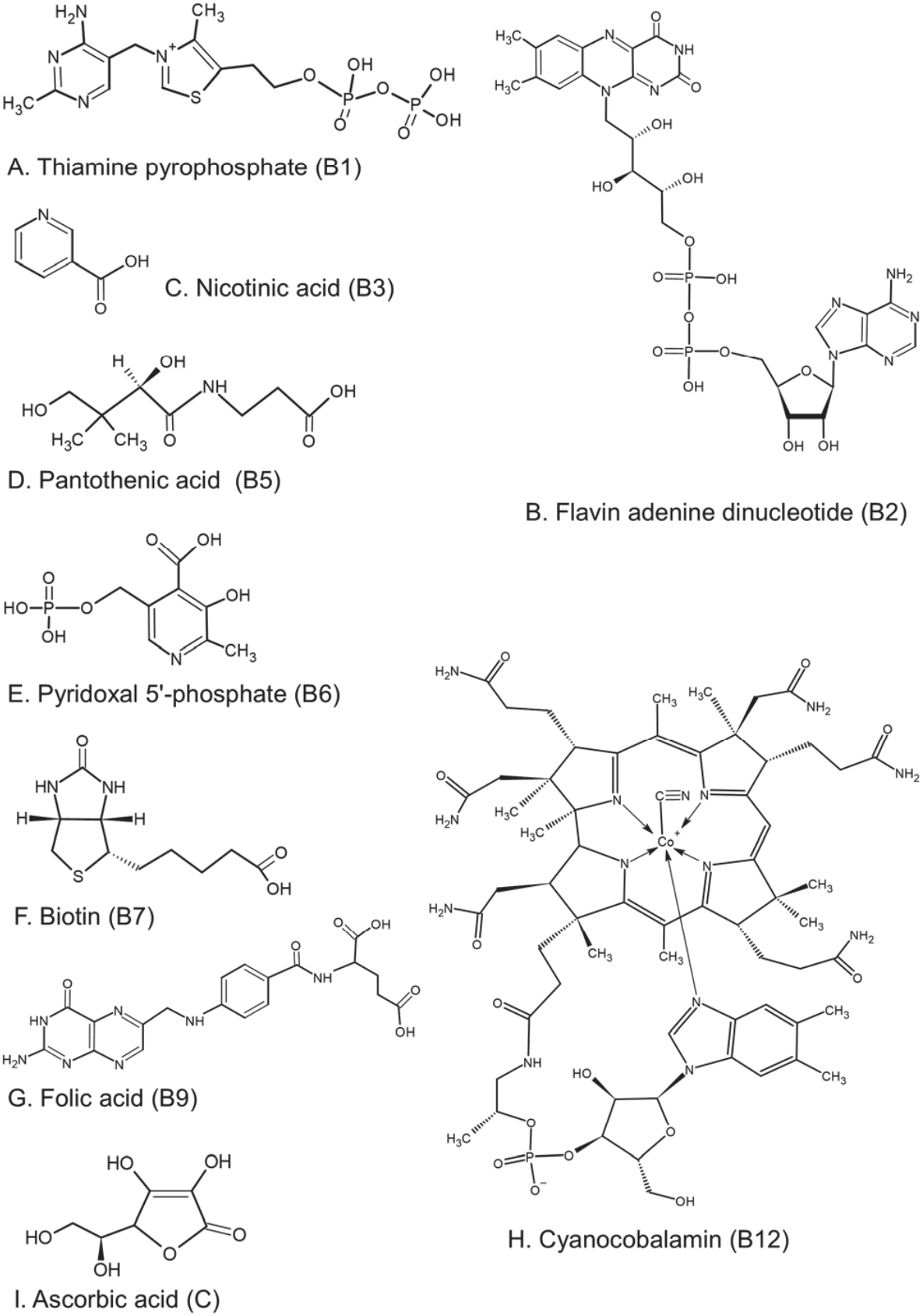

Figure 1. Structures of biologically active forms of WSVs: A.) thiamine pyrophosphate (vitamin B1); B) Flavin adenine dinucleotide (B2); C) nicotinic acid (B3); D) pantothenic acid (B5); E) pyridoxal-L-phosphate (B6); F) biotin (B7);G) folic acid (B9); H) cyanocobalamin (B12); I) ascorbic acid (vitamin C).

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