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4. B-vitamins role in cellular metabolism and clinical nutrition

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Abstract. Vitamins are important micro-molecules needed in minute but adequate quantities to maintain the body's normal physiologic and metabolic homeostasis. The B-vitamins thus play very important role in cellular metabolism and homeostasis. In all, there are eight (8) individual members of the vitamin B family often referred to as vitamin B-complex. Assaying of Vitamin status as part of a medical regiment for assessing the nutritional status of patients with suspected nutritional disorders may be relevant in modern medical practice, where evidence-based data is mandatory in the management of such patients. The B-vitamins represent a major class of vitamins that are water-soluble. Meat products are the major sources of the B vitamins, however, other good sources also include; whole grains, potatoes, bananas, lentils, chili peppers, beans, nutritional yeast, and molasses. Various studies have established the health and physiological relevance of these vitamins. The B-vitamin complex is important in the maintenance of a healthy skin and muscle tone. They also enhance the immune and nervous function and promote healthy cell division and haemopoetic activity. Vitamin B_2 for example, aids in the release of

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energy from food and promotes growth, good vision, and healthy skin. Adequate nutritional intake of B-vitamins has been shown to reduce the risk of cancers and prevent anemia. Vitamin B_6 for example, has been shown as an important co-factor in hemoglobin formation, red blood cell metabolism, and synthesis of neurotransmitters. Again, studies have confirmed that, Vitamin B_{12} maintains healthy nerve and blood cells and is needed in the synthesis DNA.

Introduction

B vitamins are a group of water-soluble chemical substances that play very important roles in cellular metabolism and homeostasis even in minute quantities. In all, there are eight individual members of the vitamin B family often referred to as vitamin B complex (listed in Table1). Most of the B vitamins must be replenished regularly, since any excess is excreted in the urine. Various studies have established the health and physiological relevance of these vitamins. Indeed, the vitamin B complex is important in the maintenance of a healthy skin and muscle tone. They also enhance the immune and nervous function and promote healthy cell division and haemopoetic activity. It is of interest also to note that adequate nutritional intake of vitamin B has the propensity to reduce the risk of cancers and prevent anemia (Schernhammer *et. al;* 2007). Deficiency of vitamin B has variously been shown to cause disease state. For example, folate deficiency has been shown to induce DNA breaks and may alter cellular capacity for mutation and epigenetic methylation (Kim *et. al;* 1997). However, few studies have examined the influence of one-carbon nutrients on pancreatic cancer risk, although recent studies suggest a potential protective effect for one-carbon nutrients from food sources, but not from supplements (Schernhammer *et. al;* 2007). Despite the enormous health benefits of vitamin B, by the same token, excess amount of the vitamin can result in serious health effects, although most B vitamins are eliminated regularly in the urine. Few studies have indicated a probable high-dose anaphylactic effect caused by thiamin injections into the vein or muscle. However, the doses were observed to be greater than the quantity humans can physically absorb from oral intake (Proebstle *et. al;* 1995). In a study, a type of acne eruption induced by vitamin B_{12} was observed (Sherertz, 1991). The eruption is monomorphic and of a particular type and consists of *voluminous folliculitis lesions* which develop acutely after the first injections of vitamin B_{12} and disappear rapidly when treatment is discontinued. It has also be observed that high-doses of nicotinamide and its derivative acid, nicotinic acid is associated with nausea, vomiting, and signs and symptoms of liver toxicity. Again, it has been observed that intake of more than 1000 mg/day of

vitamin B_6 (pyridoxine) is associated with peripheral sensory neuropathy. Other effects also include probable dermatological lesions. Newborn associated-Vitamin B_6 dependency disorders have also been reported (NAS, 1998). It should be emphasized here that, these observed side effects rarely occurs, except in situations where certain individuals have abused vitamin intake by taking excessive amounts of co-formulated vitamin tablets. Despite all these probable side effects arising from vitamin abuse, the B vitamins continue to be recognized as very important nutritional adjunct of health and vitality.

Sources of B vitamins

Meat products are the major sources of the B vitamins. For example turkey meat, liver and tuna are rich sources of the B vitamins (Stipanuk, 2006). Other good plant sources of these vitamins include; whole grains, potatoes, bananas, lentils, chili peppers, beans, nutritional yeast, and molasses. Many foods are excellent sources of folate; fruits and vegetables, whole grains, beans, breakfast cereals, and fortified grains and grain products. Good sources of vitamin B_6 include fortified cereals, beans, poultry, fish, and some vegetables and fruits, especially dark leafy greens, papayas, oranges, and cantaloupe (Stipanuk, 2006). It should be emphasized here that, although the yeast used to make beer is a source of vitamin B (Winklera *et. al*; 1995), their bioavailability is relatively poor since various studies have confirmed that drinking ethanol inhibits absorption of thiamine (B_1) (Hoyumpa, 1980), riboflavin (B_2), (Pinto et. al; 1987), niacin (B_3), (Spirak and Jackson, 1987), biotin (B_7), (Said et. al; 1990), and folic acid (B_9), (Halsled, 1990). Of all the members of Vitamin B complex, vitamin B_{12} has been shown not to be available from plant sources thus, making B_{12} deficiency a concern for vegetarians. Apart from dietary sources, B vitamins have been added in co-formulated iron tablets and other trace minerals like zinc, cobalt etc. as food supplements.

Biochemical indicators and relevance of B vitamin status determination

In a study to determine the effect of mass folic acid fortification on concentrations of serum and red blood cell (RBC) folate, blood specimens were collected from a nationally representative sample size of nearly, 7300 subject with a mean age ≥3 years to assess their B vitamin status. It was observed that mass/population fortification with the B vitamins were

effective and beneficial (Christine *et. al;* 2005). The study recommended regular and continuous monitoring of B vitamin concentration in the population.

The concentration of circulating total homocysteine is a sensitive marker of inadequate folate and vitamin B_{12} status. A cross-sectional analysis carried out in the Framingham study to describe the distribution of plasma homocysteine concentration in relationship to the vitamin intake among the elderly, revealed that plasma homocysteine concentration positively correlates with plasma folate, vitamin B_{12}, pyridoxal-5'-phosphate (PLP). It was further observed that after controlling for age, sex, and levels of other vitamins, homocysteine exhibited a strong inverse association with plasma folate (Selhub et. al; 1993). The study concluded that, the observed results portrayed a strong association between homocysteine concentration and folate, vitamin B_{12}, and vitamin B_6 status, as well as age and it is likely that a substantial majority of the cases of high homocysteine in studied older population could be attributed to vitamin status.

Despite the fact that studies have confirmed serum homocysteine as a sensitive functional indicator of intracellular folate, vitamin B_{12}, and vitamin B_6 status, chronic alcoholism is known to interfere with one-carbon metabolism, for which the above vitamins serve as coenzymes (Cravo *et. al;* 1996). In a study to ascertain this observation, these vitamins were assessed in a case controlled setting, using 32 chronic alcoholics and 31 healthy volunteers. Both the vitamins and homocysteine levels were measured in the blood of these subjects. It was observed that, in the chronic alcoholics, serum pyridoxal 5'-phosphate and red blood cell folate concentrations were significantly lower than in the control subjects. The mean serum homocysteine was however, twice as high in chronic alcoholics than in nondrinkers. The study suggested that by interfering with folate or vitamin B_6 metabolism, chronic alcohol intake may impair the disposal of homocysteine through the trans-methylation or trans-sulfuration pathways (Cravo *et. al;* 1996).

Metabolically, it has been shown that the enzyme; *methylenetetrahydro-folate reductase* supplies the folate needed for the metabolism of homocysteine, thus, a reduction in the enzyme activity, as occurs in the homozygous state, is associated with an increase in plasma total homocysteine (tHcy) (McNulty *et. al;* 2002). In a bid to investigate whether a reduced activity of the enzyme is associated with the inappropriate loss of its riboflavin cofactor, a study was carried out on tHcy and relevant B-vitamin status by *methylenetetrahydrofolate reductase* genotype in a cross-sectional study on 286 healthy subjects aged between nineteen and sixty-three years (19–63years). The effect of riboflavin status was examined by dividing the sample into tertiles of erythrocyte glutathionine reductase

activation coefficient, a functional index of riboflavin that status. The study observed that lower red blood cell folate and higher tHcy concentrations were found in the homozygote (*TT*) group than in the heterozygous (*CT*) or wild-type (*CC*) groups (McNulty *et. al;* 2002). The study concluded that perhaps, the high tHcy concentration typically associated with homozygosity occurs only with poor riboflavin status. The study outcome may have important implications for vitamin fortification programes aimed at the prevention of diseases for which this genotype is associated with increased risk.

Available assays for the determination of B vitamin status

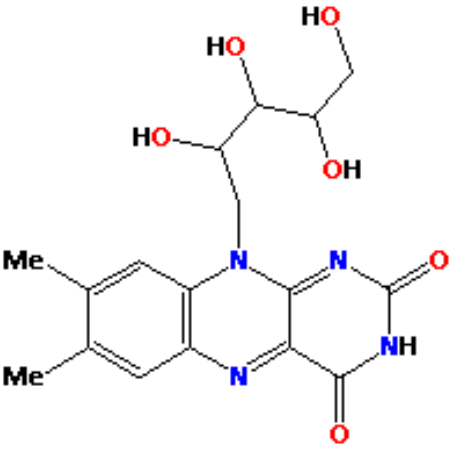
The use of an integrated High Performance Liquid Chromatography (HPLC) system by various investigators has enabled the analysis of most B vitamins (Aslam *et. al;* 2008) status in patient of malnutrition or other nutritional disorders e.g. persons suffering from intestinal disorders that prevent the normal physiological absorption of vitamins from food nutrients. Again, in such conditions like pregnancy or even in lactating mothers, the need may arise for the assessment of the status of these vitamins to aid in their proper antennal or post-natal management. The experimental conditions employed in the HPLC analysis for these water-soluble vitamins include; a dual mobile phase comprising; A: MeOH, B:0.023M H_3PO_4, at a pH of = 3.54 and a flow rate of 1.1 or 0.5 mL/minute and a column temperature of 25^0C. It has been shown that under these conditions, the LC-2010 is suitable for the analysis of both fat and water-soluble vitamin samples. The LC-2010 is a fully integrated HPLC system with quaternary pump, high-speed auto-sampler, column oven, and detector in one unit. In a study to assess the B vitamins content in germinated chickpea (*Cicer arietinum* L.), a comparative HPLC analysis of water-soluble vitamins (B-group) was carried out both *in vitro* and *ex vitro.* The investigators observed the germinated seedlings contained significant content of these vitamins (Aslam *et. al;* 2008). By employing a combination of Uv-Vis scan and a Fluorescence scan, the concentration of B vitamins in various energy drinks have also been analysed (Brivett and Nirode, 2005).

Overview of the various B vitamins and their nutrition relevance

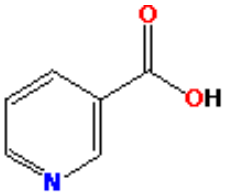
Vitamin B_1 (*thiamine*)

Deficiency of vitamin B_1 causes *beriberi*, a polyneuritis disease. Thiamine has a pyrimidine structure linked by a methylene group to a

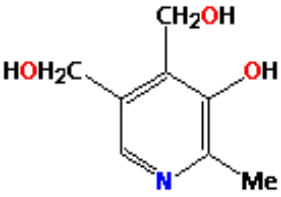
thiazole (Figure 1). It is pH-labile and easily destroyed in alkaline media but resistant to high temperatures. Due to its high water-solubility, thiamine can easily also leach out from food during washing or cooking. Whole grain, wheat and yeast are some of the rich sources of thiamine. Dietary deficiency consumes a lot of polished rice or persons with anorexia, vomiting or even



Vitamin B_2, $C_{17}H_{20}N_4O_6$



Vitamin B_3, $C_6H_5NO_2$



Vitamin B_6, $C_8H_{11}NO_3$

Figure 1. Structures of some B-Vitamins.

diarhoea may show signs and symptoms of thiamine deficiency. It has been shown that, exogenous thiamine is absorbed in the intestine by a carrier-mediated process that is saturated at an oral intake of approximately 10mg (Flint and Prinsley, 1981). As a cofactor, thiamine catalyzes the formation of ketols in its thiamine triphosphate form (TPP). Chemical indices of thiamine deficiency is indicated by a reduction in urinary thiamine, a reduction in activity of the enzyme; erythrocyte tranketolase and a concomitant stimulation of this enzyme by TPP. However a prolonged deficiency of the vitamin may lead to a decreased synthesis of the transketolase (Brass, 1981).

Vitamin B_2 (*riboflavin*)

This occurs as a yellowish pigment flavin which is attached to a carbohydrate moiety, D-ribitol. It principally exists in two active forms with two active cofactors namely; riboflavin 5'-phosphate (flavin mononucleotide; FMN) and flavin dinucleotide (FAD). Of these forms, the FAD is the most water-soluble, however, aqueous solution of flavins are resistance to heat and oxidizing agents. Among, the rich sources of vitamin B_2 are; liver, egg yoke, meat, milk and green vegetables. The various forms of this vitamin is inter-convertible by enzymatic reactions. Flavin is excreted in its free riboflavin form in the urine and only a small amount of it is actually metabolized in the body. This vitamin plays an important role in cellular respiration by acting as prosthetic groups on enzymes such as pyruvate dehydrogenase, xanthine oxidase, NADH dehydrogenase and glutathione reductase among others. Studies have also indicated that, riboflavin also plays an important role in iron and folate metabolism (Komindr and Michaels, 1980). Among the clinical symptoms of riboflavin deficiency (Aribоflavinosis) are; neurological alterations, glossitis, stomatitis and certain dermatological and muscle disorders. Again it has been shown that, severe deficiencies of this vitamin can occur as a result of disease state including prolonged febrile illness, malignancy, hyperthyroidism, diabetes mellitus and cardiac disorders (Bates, 1987).

Vitamin B_3 (*niacin or niacinamide*)

Vitamin B_3 (niacin) is another water-soluble vitamin that works in the glycogen stage of the energy cycle and also in the fatty acid oxidation for energy process which aids tissue respiration. However, excess niacin above 30 mg can cause vascular dilation accompanied with flushing, burning, and itching. This is commonly called a "niacin flush". This is uncomfortable, but not toxic; and is sometimes the body's adaptation strategy for breaking

through a migraine headache (ref). Deficiency, along with a deficiency of the amino acid tryptophan has been shown to cause causes pellagra (Spivak and Jackson, 1977). Symptoms of niacin deficiency may include aggression, dermatitis, insomnia, weakness, mental confusion, and diarrhoea. In advanced situations, pellagra may lead to dementia and even death.

Vitamin B_5 (*pantothenic acid*)

Pathothenic acid is a growth factor that occurs in both animal and plant sources. It is a very important nutrient for energy metabolism. It is also a precursor for the synthesis of brain neurotransmitters and natural body steroid hormones. Dietary sources of this vitamin include; such animal products as liver, meat, milk etc. Foods such as eggs, legumes, mushrooms, salmon and whole cereals are also rich in vitamin B_5. Deficiency of pantothenic acid in rats causes highly notable failure of cartilage growth and lesions such as occurs in osteoarthritis (White-O'Connor and Sobal, 1986). The free form of this vitamin is found in the urine and serum, whereas coenzyme A is the major erythrocytic form. Low urinary excretion and reduced serum levels of this vitamin have been reported in patients with chronic malnutrition, acute alcoholism and also in patients with acute rheumatism (Dastur et. al; 1976).

Vitamin B_6 (*pyridoxine, pyridoxal, or pyridoxamine*)

Vitamin B_6 (Pyridoxine) functions in the biosynthesis of structural body proteins and amino acid metabolism. It also plays a very important role in nerve transmission, red blood cells, and prostaglandins synthesis. This vitamin is involved in cell division and therefore plays a vital role in pregnancy and in the proper function of the immune system, mucous membrane, skin, red blood cells, and brain chemistry (Coursin, 1969). Major dietary sources of vitamin B_6 are; meat, poultry products, fish, dairy products, potatoes, grains and vegetables. It is interesting to note that pyridoxine does not bind to plasma but binds mainly to albumin and that, erythrocytes rapidly take up this vitamin and convert it to pyridoxal phosphate and pyridoxal the latter of which is released into the plasma. Again, the metabolism of vitamin B_6 has been shown to occur predominantly in the liver with a concomitant excretion of its metabolite; 4-pyridoxic acid. Vitamin B_6 supplements have been utilized in the treatment of several diseases including, celiac disease, acute alcoholism, ulcerative colitis, renal calculi etc. (Diepersloot et. al; 1990). Autism has also been linked to a decrease in certain brain neurotransmitters that require vitamin B_6 for normal brain chemistry.

Vitamin B_7 (*biotin*)

Vitamin B_7 (Biotin) is a component of the B-complex vitamin which act as precursors for enzymes that enhance metabolic processes in the human body. There are several sources of biotin, although no one food is especially rich in this vitamin. It has been shown that dietary intake of biotin is low in the neonatal period despite the high concentration levels in newborns (Roth, 1981). Physiologically, biotin is absorbed in the proximal area of the small intestine and circulates in the blood bound to plasma proteins. Biotin acts a coenzyme in carboxylation reactions involving such enzymes as acetyl CoA carboxylase, pyruvate carboxylase, propionyl CoA carboxylase etc. Deficiency of biotin have been found to be associated with dermatitis, mental and neurological disorders accompanied by nausea, anorexia etc. Again, it has been shown that dietary deficiency of biotin is associated with reduced urinary and plasma levels as well as increased urinary organic acids, indicating functional deficiency of β-methylcrotonyl CoA carboxylase and propionyl CoA carboxylase (Roth et. al; 1981). Indeed, without biotin, the body cannot effectively utilize fats or glucose for energy, and the metabolism would be severely impaired.

Vitamin B_9 *(folic acid*)

Food which serve as rich sources of folic acid includes; green and leafy vegetables, fruits, meat and yeast. Cooking methods which involves excessive boiling and the use of large amounts of water tend to destroy folate. Folate is a cofactor for enzymatic reactions involving a single carbon transfer. After cellular uptake, folic acid is converted to tetrahydrofolate while transferring a carbon to homocysteine to yield methionine. Recent analysis in multiple studies, suggests that folic acid supplements can reduce the risk of stroke in people who have not already suffered a stroke, but they do not reduce the risk of second stroke in people who have already had one (Lee et. al., 2010). Observational studies have shown that people who get higher than average amounts of folate from their diets or folic acid supplements for 15 years or more have lower risks of colon cancer (Giovannucci et. al., 1998) and breast cancer (Zhang et al., 1999). This has biochemical implications especially for those take in alcohol, since alcohol interferes with the proper metabolism of folate and inactivates circulating folate as already stated. An interesting observation is that, higher intake of folate appears to mask the increased risk of breast cancer seen among women who have more than one alcoholic drink a day (Zhang et al., 1999). More recent studies have had similar findings, including one from Sweden that

found that sufficient folate intake can protect against breast cancer even in women who have only one drink a day or less (Ericson et. al., 2007). Previous studies have shown that phenytoin therapy accelerates folate excretion and interferes with its absorption and metabolism (Infante-Rivard et. al., 1986).

Vitamin B_{12} (*cobalamins*/cyanocobalamin)

Vitamin B_{12} consist of a corrin ring linked to a cobalt atom in the center similar to a porphyrin ring. Rich sources of Vitamin B_{12} include mostly animal products like meat, milk and eggs. The average daily diet contains approximately 3-30μg of this vitamin but paradoxically, only 1-5 μg is absorbed. Vitamin B_{12} is actually a coenzyme that is necessary to metabolize fats and carbohydrates and also assists in the proper digestion and absorption of foods, and promotes normal growth (Scott, 1997). Unlike the other members of the B vitamin family, VitaminB_{12} is not excreted quickly in the urine, but rather accumulates and is stored in the liver, kidney and other body tissues. As a result, a Vitamin B_{12} deficiency may not manifest itself until after 5 or 6 years of a diet deficient in this vitamin. Physiologically, Vitamin B_{12} functions as a methyl donor and works with folic acid in the synthesis of DNA and red blood cells (Schernhammer et. al; 2007). Besides, it is vital in maintaining the health of the insulation sheath (myelin sheath) that surrounds nerve cells. Clinically, the major symptom of vitamin B_{12} deficiency is pernicious anaemia, a condition characterized by large, immature red blood cells (Berlin et. al., 1986). Again, a deficiency may also manifests itself in neurological dysfunction that is almost indistinguishable from senile dementia and Alzheimer's disease. Low levels of vitamin B_{12} have also been associated with asthma, depression, AIDS, multiple sclerosis, tinnitus, diabetic neuropathy and low sperm counts (Schneede and Ueland, 2005, Carmel, 2008). Several surveys have shown that long-term and strict vegetarians are vitamin B_{12} deficient (Stabler and Allen, 2004). Vitamin B_{12} has been shown to be deficient in the elderly perhaps due to a decline with age in the production of the intrinsic factor in the small intestine needed to absorb the vitamin.

Conclusion

The B-vitamins represent a major class of vitamins that are required as part of the body's nutritional requirement to enhance normal metabolic and physiological homeostasis. Almost all the classes of vitamin B family are water-soluble. B vitamins continue to be recognized as very important

nutritional adjunct of health and vitality. Despite the enormous health benefits of vitamin B, excess amount of the vitamin can result in serious health effects, although most B vitamins are eliminated regularly in the urine. The clinical relevance of assaying the body's vitamin status as part of the medical regiment for assessing the nutritional status of patients with suspected nutritional disorders may thus be relevant in modern medical practice, where evidence-based data is mandatory in the management of such patients.

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