

P5P (B6) Focused Genetics, Epigenetic Glance to Health

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Abstract: B6 is a versatile vitamin with many functions and is involved in more than 100 enzymatic reactions. B6 is a term that refers to six compounds that function similarly in the body. The first three are pyridoxamine (PN, an alcohol), pyridoxal (PL, an aldehyde), pyridoxamine (PM) containing an amino group. The corresponding 5'-phosphate esters form the remaining three: pyridoxal 5'-phosphate (PLP or P5P), pyridoxine 5'-phosphate (PNP), pyridoxamine 5'-phosphate (PMP). Vitamin B6 must be taken from outside foods. In a healthy body, the liver converts B6 taken from the outside into its active form, P5P. If this transformation is not done in sufficient amounts due to genetic, epigenetic or social reasons, it can cause physiological, psychological and social consequences. In such cases supplementation with P5P may be necessary. Vitamin B6 is found in the structure of proteins and basic compounds in our body, transmitters, erythrocytes and prostaglandins in the nervous system. Vitamin B6 is also important in maintaining hormonal balance and regulating immune system functions. P5P, the body-activated form of vitamin B6, is involved in many reactions, is a part of amino acid synthesis, and others are related to single carbon metabolism, lipid metabolism, glyconeogenesis, heme and neurotransmitter biosynthesis. P5P, aside from functioning as a co-factor, is defined as radical cleaner of reactive oxygen types, a metal chelator and a chaperone in the enzyme folding process. Since it is involved in so many enzymatic reactions, sufficient levels are important to promote and maintain a healthy body. Vitamin B6 is depleted by stress, which can affect your social position, mood, sleep and pain level. In this study, the literature was reviewed by focusing on B6 (P5P) and the effects of P5P on chronic inflammatory diseases and other diseases. It was concluded that the use of P5P would be beneficial for the prevention and treatment of these diseases.

Key words: Vitamin B6, coenzyme, pyridoxal 5 phosphate, PLP, P5P, chronic, recurrent, inflammation, vitamin B6 deficiency.

1. Introduction

In this study, firstly, general definitions about the subject and vitamin B6 forms, metabolism and related enzymes are discussed. Then, respectively, vitamin B6 (P5P) status and diseases, functions of P5P, vitamin B6 (P5P) immune function and inflammation, B6 (PLP) vitamin and inflammation indicators, B6 catabolism and PAr index, immune dysfunction, P5P in autoimmune diseases, epilepsy, vitamin B6 and homocysteine, cognitive decline and Alzheimer's disease, depression, cancer, kidney stone, pregnancy nausea and vomiting, premenstrual syndrome (PMS), renal function disorders and P5P, people with alcohol dependence, metabolic diseases and chaperone effect, PLP-hereditary diseases responsive to pyridoxine, X-linked sideroblastic anemia, primary hyperoxaluria type I, aromatic amino acid decarboxylase (AADC)

deficiency were investigated.

Finally an evaluation was made.

2. General Definitions

Enzymes are protein catalysts that regulate the rate at which physiological processes occur. They are encoded by some special genes stimulated with hormones. There are two types of enzymes; enzymes that require a co-enzyme, such as the oxidoreductase (oxidizing-reducing) enzyme, belong to the first type. Twenty-two percent (22%) of known enzymes require a co-enzyme to function. The second type is enzymes that do not require co-enzymes in performing their functions such as digestive enzymes [1].

Vitamins are organic compounds which are sufficient in small amounts for cellular metabolic reactions and cause some problems in their deficiencies. Since they are either not made at all by the human body or not made in sufficient amounts, they must be supplied with food from outside. Some vitamins are

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active in foods and others are available as provitamins. Provitamins are converted to active form in the body; vitamins play an important role in cellular metabolic reactions [2].

Vitamins and minerals are also nutritional cofactors and co-enzymes. Pyridoxine (B6) is a substance that is easily soluble in water and alcohol and sensitive to light and ultraviolet radiation [3].

3. Vitamin B6 Forms, Metabolism and Related Enzymes

Vitamin B6 is a generic name that contains three different pyridine derivatives which represent pyridoxal (PL), pyridoxamine (PM) and pyridoxine (PN), modified in the 4-position, bearing an aldehyde, aminomethyl and hydroxymethyl group, respectively. All three forms exist as phosphorylated derivatives at the 5-position. These are pyridoxal 5'-phosphate (P5P), (PLP), pyridoxamine 5'-phosphate (PMP) and pyridoxine 5'-phosphate (PNP) [4].

PLP is not synthesized in the human body as *de novo*; it is taken from various foods including meat, dairy products, beans, nuts, potatoes and some fruits and vegetables

While animal products mostly contain P5P and PMP, PN(P) is the most common form of B6 in vegetable products. Food-borne PLP, PMP and PNP are dephosphorylated by tissue-specific intestinal phosphatase prior to absorption. The portal circulation transmits PL, PM and PN to the liver where they are re-phosphorylated by pyridoxal kinase (PDXK), and PMP and PNP are converted to P5P by reactions catalyzed by pyridoxine (pyridoxamine) oxidase (PNPO). In the liver, PLP production is regulated so that the content remains relatively constant, even after a very high PN intake. The liver is the production site of PLP for release into plasma. PLP is delivered from the liver attached to albumin. PLP is firmly bound to albumin in plasma and increases intake of vitamin B6 over a wide range. Plasma PLP must be dephosphorylated to PL, the transport form, before it is

absorbed by the tissue or cells or before it crosses the blood-brain barrier. The dephosphorylation is catalyzed by non-tissue-specific alkaline phosphatase (ALP), an ectoenzyme located on the outer membrane of cells, including tissue-specific phosphatases and erythrocytes expressed in placenta and germ cells [4].

As seen Fig. 1, vitamin B6 forms, metabolism and enzymes involved. Pyridoxal 5'-phosphate (PLP) is the metabolically active co-enzyme form of vitamin B6, the transfer form passing through the pyridoxal (PL) biological membranes, the types of vitamin B6 present in the pyridoxine (PN) supplements and the 4-pyridoxic acid (PA) vitamin B6 catabolite. PL, PN and pyridoxamine (PM), are phosphorylated by pyridoxal (pyridoxine, vitamin B6) kinase (PDXK; EC 2.7.1.3511) [5] to PLP, pyridoxine 5'-phosphate (PNP) and pyridoxamine 5'-phosphate (PMP) in this specific order. PNP and PMP are oxidized to PLP in reactions catalyzed by pyridoxamine 5'-phosphate oxidase. (PNPO; EC 1.4.3.5) [6]. PLP in the liver is bound to albumin and transferred to the circulation for delivery to tissues. Prior to cellular uptake, extracellular PLP is dephosphorylated to PL by ectoenzyme, non-tissue-specific alkaline phosphate (ALP; EC 3.1.3.1) [7]. The dephosphorylation of PLP into PL is catalyzed with intracellular enzyme, i.e. pyridoxal (pyridoxine, vitamin B6) phosphatase (pyridoxal phosphate phosphatase, PDXP; EC 3.1.3.74) [8]. The irreversible oxidation of PL to PA is catalyzed by aldehyde oxidase (s) (AOX) [9]. Abbreviation: AT, aminotransferase taken from Ref. [4].

4. P5P Functions

The active form of vitamin B6, pyridoxal 5'-phosphate (PLP), acts as an auxiliary factor (cofactor) in more than 150 enzymatic reactions. PLP, an effective vitamin B6, acts as a co-factor for more than 150 enzymes, which account for about 4% of all enzyme activities. These enzymes catalyze a wide variety of reactions including amino acids and amines, including transamination, aldol cleavages,

α -decarboxylations, racemizations, β - and γ -elimination, and replacement reactions. Many reactions are part of amino acid synthesis and degradation, while others are related to single carbon metabolism, lipid metabolism, glyconeogenesis, heme

and neurotransmitter biosynthesis. Vitamin B6, aside from functioning as a co-factor, is defined as a metal chelator, a radical cleaner of reactive oxygen species, and a chaperone in the enzyme folding process [4].

Vitamin B6 functions are shown in Fig. 2 [10].

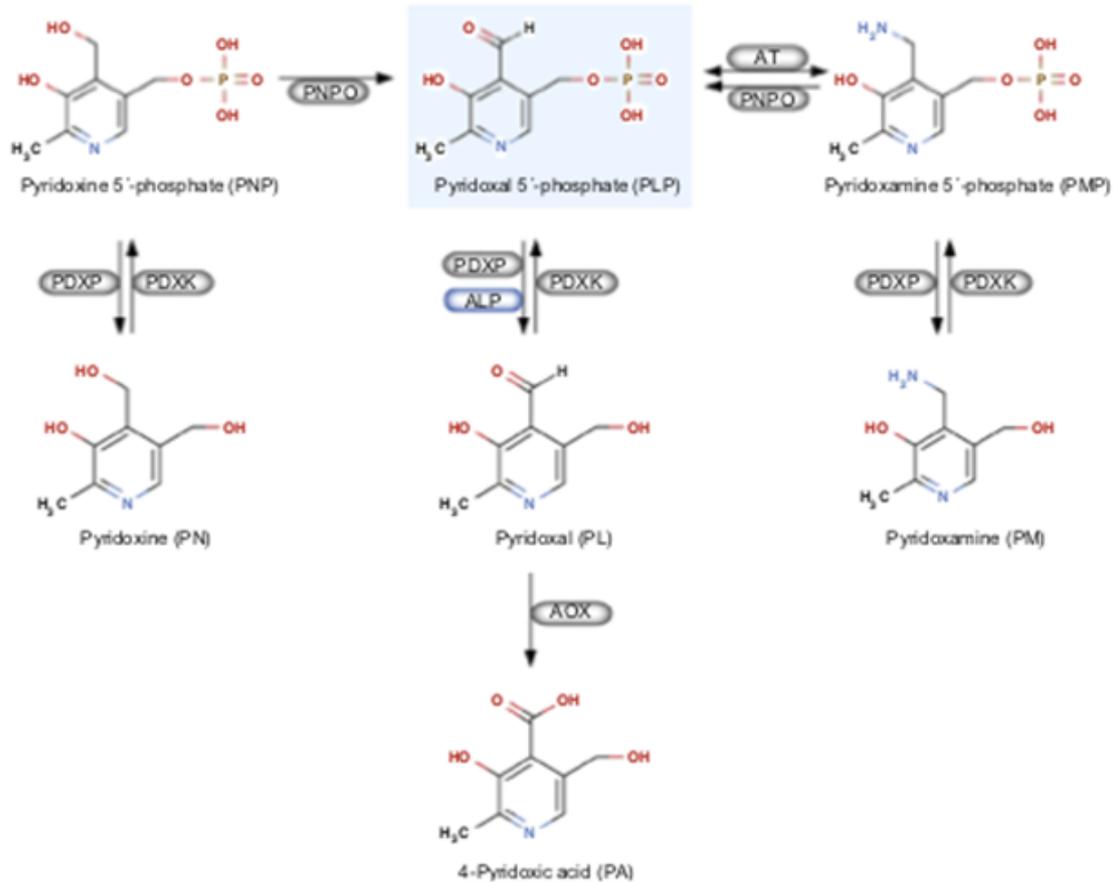


Fig. 1 Forms of vitamin B6, metabolism and related enzymes.

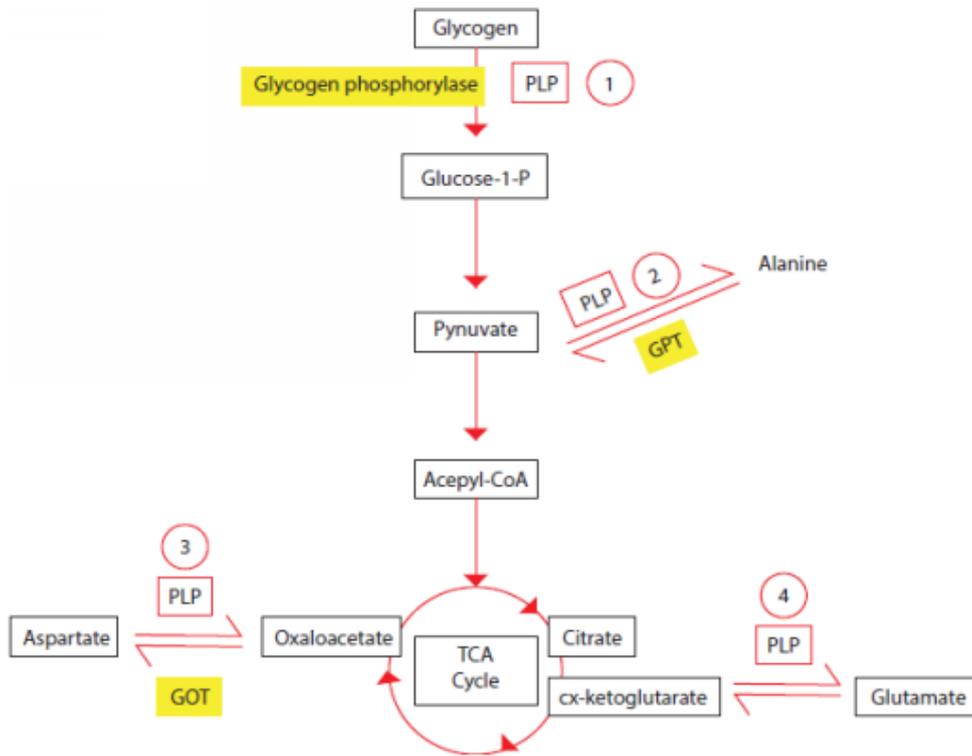


Fig. 2 Vitamin B6 functions.

Source: İnci Özden, p. 576 [10].

(1) PLP covalently links to glycogen phosphorylase and stabilizes the enzyme. Therefore, it plays an important role in glycogenolysis, (2) and is involved in the transamination reaction. During this reaction (3) and (4), the carbon skeletons of amino acids are released and metabolized in the TCA cycle. Therefore, vitamin B6 is required for catabolism of amino acids.

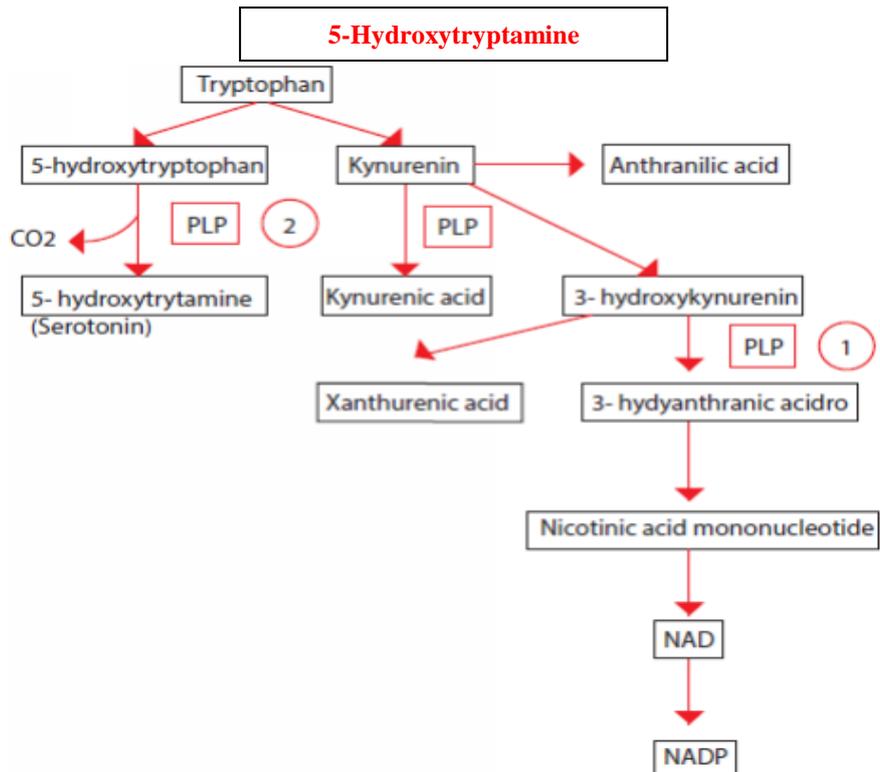


Fig. 3 Participation of P5P in tryptophan catabolism as well as serotonin (neurotransmitter) production.

Source: İnci Özden, p. 577 [10].

- (1) The incorporation of P5P into the production of serotonin, a transmitter as well as tryptophan catabolism.
- (2) P5P is required for serotonin synthesis in the decarboxylation step.

As seen Fig. 3, in the synthesis of dopamine and norepinephrine, P5P is required in the decarboxylation step. P5P is also needed for histamine synthesis by histidine decarboxylation.

5. Vitamin B6 (P5P) Status and Diseases

Vitamin B6 deficiency associated with isolated diet is rare in underdeveloped countries; however, low vitamin B6 circulation has been found in patients who use oral contraceptives (contraceptives) or some drugs, smokers and patients with alcoholic, celiac disease or diabetes [4].

5.1 Vitamin B6, Immune Function and Inflammation

Vitamin B6 (P5P) deficiency affects cellular immunity and to a lesser extent humoral immunity in both animal and human studies. Experimental studies in rodents with B6 deficiency showed a significant reduction in lymphocyte proliferation, T-cell mediated cytotoxicity, delayed-type hypersensitivity, allograft

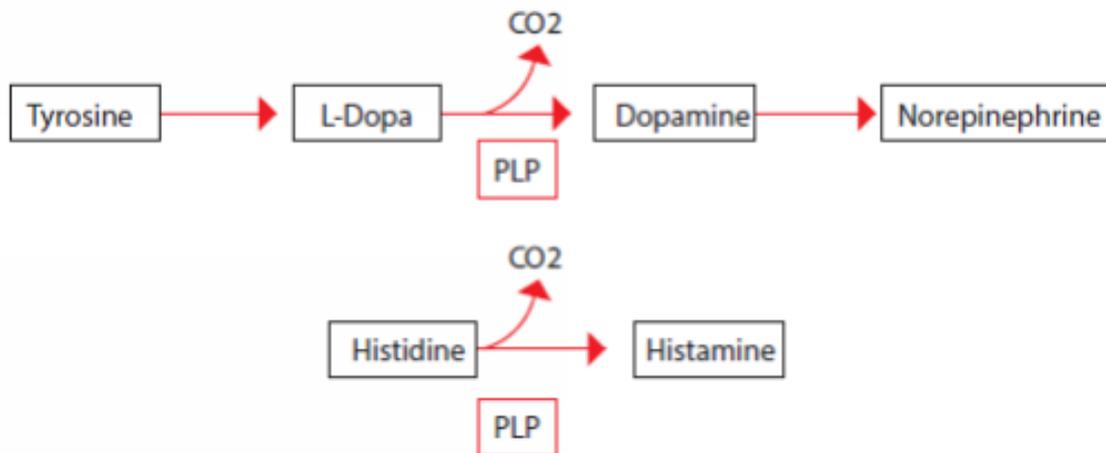


Fig. 4 Neurotransmitter synthesis.

Source: İnci Özden, p. 577 [10].

In Fig. 4, P5P is needed for the synthesis of the sphingolipids required for myelin formation. P5P is needed for ALA synthesis. Therefore, it is also necessary for heme synthesis.

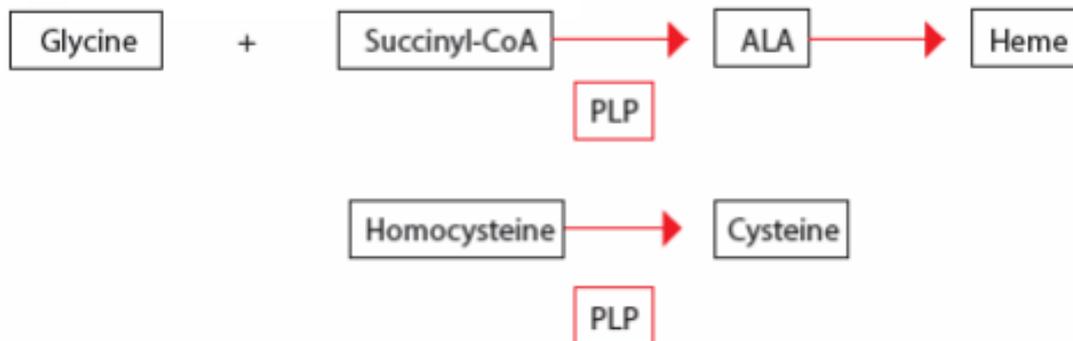


Fig. 5 Heme and cysteine synthesis.

Source: İnci Özden, p. 578 [10].

As seen Fig. 5, the conversion of homocysteine to cysteine is important because hyperhomocysteinemia is a risk factor for cardiovascular disease.

rejection and altered cytokine profile. Immune response is improved by pyridoxine supplementation in older individuals, patients with renal failure and critical patients. Immune responses in the elderly and young women have been investigated in well-controlled metabolic environments as a function of variable vitamin B6 status. In the elderly, vitamin B6 deficiency decreased the number of blood lymphocytes and mitogen response as well as interleukin (IL) 2 production, particularly in T-helper cells. Immune indices were normalized based on vitamin B6 repetition [4].

Several enzymatic reactions in the pathway of tryptophan-quinurenine depend on vitamin B6 coenzyme, pyridoxal 5'-phosphate (PLP). The main intermediates in the pathway of tryptophan-quinurenine play a role in regulating immune responses. It has been found that tryptophan induces apoptosis or blocks the proliferation of certain immune cells such as lymphocytes (particularly T-helper 1). They can also inhibit the production of pro-inflammatory cytokines. There is evidence that adequate vitamin B6 intake is important for adequate immune system function, especially in elderly individuals. However, triggering tryptophan degradation and underlying chronic inflammation of many diseases (such as cardiovascular diseases and cancers) may increase the loss of PLP and requirements [11].

5.2 Vitamin B6 and Indicators of Inflammation

Plasma PLP has an inverse relationship with inflammatory markers in clinical and population-based studies. Population-based cohort studies have shown that plasma PLP is inversely related to many inflammatory markers, including CRP, IL-6 receptor, α -1 antitrypsin, serum amyloid A, white blood cell count (WBC), kynurenine/tryptophan ratio (KTR), and neopterin.

In the population based NHANES study, vitamin B6 intake with nutrition and supplements is inversely

related to CRP. It was found that high plasma uptake but also sufficient plasma PLP (> 20 nmol/L), independently of uptake, protects against inflammation. Vitamin B6 deficiency (plasma PLP < 20 nmol/L) was not common in individuals with a B6 vitamin intake of 2-3 mg/d (mg^3 mg/mL) with low CRP (mg^3 mg/mL); however, it was more frequent (50%) in patients with high CRP (> 10 mg/L). In particular, in a small study on healthy individuals, controlled dietary vitamin B6 restriction did not affect CRP levels, and levels of inflammatory markers such as CRP, neopterin or soluble CD40 ligand, in patients with stable angina pectoris, did not change either in high doses (40 mg/d) only in PN supplementation or in combination of folic acid and vitamin B12. A strong inverse relationship between CRP and plasma PLP was observed in cardiovascular patients even after supplementation. Therefore, intervention studies show that the reverse relationship between CRP and plasma PLP reflects the altered vitamin B6 distribution during inflammation rather than high B6, which protects against inflammatory reactions. Plasma PLP, while CRP was inversely related to WBC, KTR and neopterin, PA showed positive correlation with neopterin and KTR in patients with stable angina pectoris. These relationships were mainly supported after being supplemented with high dose pyridoxine for 28 days. After supplementation, all B6 vitamins increased 9-60 times, but there was a sharp decrease in PL and PA in subjects with CRP > 7 mg/L. These data show that acute-phase reaction (as a reflection of high CRP) leads to increased vitamin B6 uptake into tissues, whereas cellular Th1 immune activation (neopterin and KTR) increases uptake and promotes simultaneous catabolism to PA.

5.3 B6 Catabolism and PA Index

4-Pyridoxic acid (PA) is a vitamin B6 catabolite consisting of PL in the liver. PA in plasma increases after vitamin B6 intake, is not protein bound, has high renal clearance and is excreted in urine. Unlike PLP,

PA is not associated with the acute-phase inflammatory state in the general population, but is positively associated with cellular immune activation markers and is significantly increased in critical patients.

PLP, PL and PA, the main circulating B6 vitamers, are measured concurrently with contemporary methods based on mass spectrometry and show strong intercorrelation reflecting strict metabolic control. PAr has some unique features. Compared to isolated vitamin B6 in plasma, PAr is less affected by renal function, smoking, and vitamin B6 intake.

The PAr response can be divided into changes in PA, PL and PLP based on CRP and markers of cellular immunity (KTR and neopterin). PA was positively associated with KTR and neopterin but not with CRP; whereas PLP and PL were more strongly associated with CRP than with KTR and neopterin. These observations show increased PLP and PL uptake during the acute phase (reflected by CRP), while increased PL degradation predominates in PA by cellular immune activation.

Cellular intake of vitamin B6 involves dephosphorylation across the cell membrane to PLP and is retained in cells after re-phosphorylation to PLP catalyzed by PL kinase. PLP and PL are interchangeable, and most tissues have PLP-specific phosphatases. The oxidation of PL to PA is irreversible and is thought to be catalyzed by liver aldehyde oxidase 1 (AOX 1) expressed by signaling pathways associated with oxidative stress. PL is also oxidized to PA by aldehyde dehydrogenase (ALDH), shown in many tissues. Thus, the expression (expression) of both AOX 1 and ALDH increases during oxidative or aldehyde stress. This may explain the strong association of PAr with KTR, a marker of cellular immune activation; this reflects the activation of an enzyme with redox control and peroxidase activity, i.e. indoleamine 2,3-dioxygenase (IDO).

The PAr index has recently been shown to be a precursor of cancer in the general population. The relationship was the strongest for lung cancer.

Inflammation has been shown to play a role in lung carcinogenesis, also predicted by the markers of IDO activation and cellular immunity. The association with PAr is the strongest in patients with no previous coronary event [4].

5.4 P5P and Hormone Function

Steroid hormones such as estrogen and testosterone show their effects in the body by binding to steroid hormone receptors in the nucleus of target cells. Nuclear receptors bind themselves to specific regulatory sequences in DNA and alter the transcription of target genes. Experimental studies have demonstrated a mechanism by which PLP can affect the activity of steroid receptors and reduce its effects on gene expression. It has been found that PLP can interact with RIP140/NRIP1, a nuclear receptor inhibitor known for its role in reproductive biology. However, additional research is needed to confirm that this interaction can increase RIP140/NRIP1 repressor activity on steroid receptor mediated gene expression. If the activity of steroid receptors estrogen, progesterone, testosterone or other steroid hormones can be inhibited by PLP, it is possible that one of the statuses of vitamin B6 will affect the risk of developing steroid hormones such as breast hormones and prostate cancers [12].

5.5 Insulin Resistance, Aging and P5P

Tryptophan (TRP)-quinurenine (KYN) and KYN-nicotinamide adenine dinucleotide (NAD) metabolic pathways are seen to be one of the insulin resistance (ID) mechanisms. The first and rate-limiting step of the TRP-KYN pathway is regulated by enzymes induced by pro-inflammatory factors and/or stress hormones. The essential enzymes of the KYN-NAD pathway necessitate pyridoxal-5-phosphate (P5P), an active form of vitamin B6 as cofactor. P5P deficiency directs KYN-NAD metabolism from NAD production to excessive xanthurenic acid (XA) formation. Human and experimental studies have shown that XA and

some other KYN metabolites may impair the production, release and biological activity of insulin. Vitamin B6 deficiency may contribute to the prevention and treatment of ID, for example in cases associated with type 2. Our data show that inflammation or stress-related regulation of TRP-KYN metabolism, which results in overproduction of KYN, is one of the predisposing factors to IR. The deficiency of P5P, a common factor of the essential enzyme of the KYN-NAD pathway, leads from excessive NAD formation of KYN to the production of XA (and other) diabetogenic derivatives of KYN. Improved insulin resistance has been identified as one of the factors that increase aging [13].

5.6 Autoimmune Diseases

People with rheumatoid arthritis generally have low P5P concentrations, and P5P concentrations tend to decrease as the severity of the disease increases. These low levels of vitamin P5P result from the disease-induced infection and also increase the inflammation associated with the disease. Although vitamin P5P supplements can normalize vitamin B6 concentrations in rheumatoid arthritis patients, they do not suppress the production of inflammatory cytokines or reduce inflammatory marker levels. As assessed by a functional group of vitamin B6 biomarkers, RA patients had low plasma PLP with normal erythrocyte PLP not explained by low B6 uptake, congenital defects in vitamin B6 metabolism, or vitamin B6 deficiency.

Patients with celiac disease, Crohn's disease, ulcerative colitis, inflammatory bowel disease, and other autoimmune diseases tend to have low plasma PLP concentrations. The mechanisms for this effect are unknown. However, celiac disease is associated with low pyridoxine absorption and low PLP concentrations in inflammatory bowel disease may be due to inflammatory response.

5.7 P5P and Nervous System Function

In the brain, the PLP-dependent enzyme aromatic L-amino acid decarboxylase catalyzes the synthesis of two major neurotransmitters: serotonin from amino acid tryptophan and dopamine from L-3,4-dihydroxyphenylalanine (L-Dopa). Other neurotransmitters, including glycine, D-serine, glutamate, histamine, and β -aminobutyric acid (GABA) are also synthesized in reactions catalyzed by PLP-bound enzymes [12].

5.8 Epilepsy

Pyridoxine P5P-responsive epilepsies are severe forms of epilepsy that occur as seizures immediately after birth, sometimes months or years later. Crisis can be effectively treated by lifelong supplementation with pyridoxine or its biologically active form pyridoxal phosphate, but even in this case, patients may become intellectually disabled without currently effective treatment. This may be caused by gene mutations (TNSALP, PIGV, PIGL, PIGO, PNPO, PROSC, ALDH7A1, MOCS2 or ALDH4A1). ALDH7A1, MOCS2 and ALDH4A1 mutations necessitate the accumulation of reactive aldehydes (α -aminoaldehyde semialdehyde, glut-glutamic semialdehyde) that can react non-enzymatically with macromolecules of cells. Such reactions may alter the function of macromolecules and produce "improved glycation end products" (AGEs). AGEs trigger inflammation in the brain. Studies on how aldehydes pass through cell membranes and affect brain function may further enhance the development of treatments for patients with pyridoxine-sensitive epilepsy [14]. Severe neurological disorders, such as convulsions and epileptic encephalopathy, result from a reduced presence of pyridoxal 5'-phosphate in the cell.

Multifactorial neurological pathologies such as autism, schizophrenia, Alzheimer's disease, Parkinson's disease and epilepsy have also been associated with insufficient intracellular levels of PLP [15]. Some rare congenital metabolic disorders including pyridoxine-dependent epilepsy (PDE) and

pyridoxamine 5'-phosphate oxidase (PNPO) deficiency are the causes of early-onset epileptic encephalopathies found to be sensitive to pharmacological doses of B6. In individuals affected by PDE and PNPO deficiency, PLP bioavailability is limited and treatment with pyridoxine and/or PLP is used to alleviate or eliminate epileptic crisis that characterizes these conditions [16].

5.9 Vitamin B6 and Homocysteine

Moderately elevated homocysteine levels in the blood have been associated with cardiovascular disease risk (CVD), including heart failure myocardial infarction and cerebrovascular attack. During protein digestion, methionine-containing amino acids are released. Methionine is an essential amino acid and precursor of S-adenosylmethionine (SAM), the universal methyl donor for most methylation reactions, including methylation of DNA, RNA, protein and phospholipids (Fig. 6). Homocysteine is an intermediate in the metabolism of methionine. Healthy

individuals use two different ways to regenerate methionine from homocysteine in the methionine remethylation cycle (Fig. 7). A first way is via methionine synthase and methyl donor, 5-methyl tetrahydrofolate (a folate derivative) bound to B12 to convert homocysteine back to methionine. The other reaction is catalyzed by betaine homocysteine methyltransferase using betaine as a methyl group source for methionine formation from homocysteine the liver. In addition, two PLP-dependent enzymes are required to convert homocysteine in the homocysteine transsulfuration pathway to amino acid cysteine: cystathionine beta synthase and cystathionine gamma lyase (Fig. 7). Therefore, the amount of homocysteine in the blood can be influenced by the nutritional status of at least three B vitamins, folate, vitamin B12 and vitamin B6. Deficiencies in one or all of the B vitamins can affect both remethylation and transsulfuration processes and cause abnormally elevated homocysteine levels.

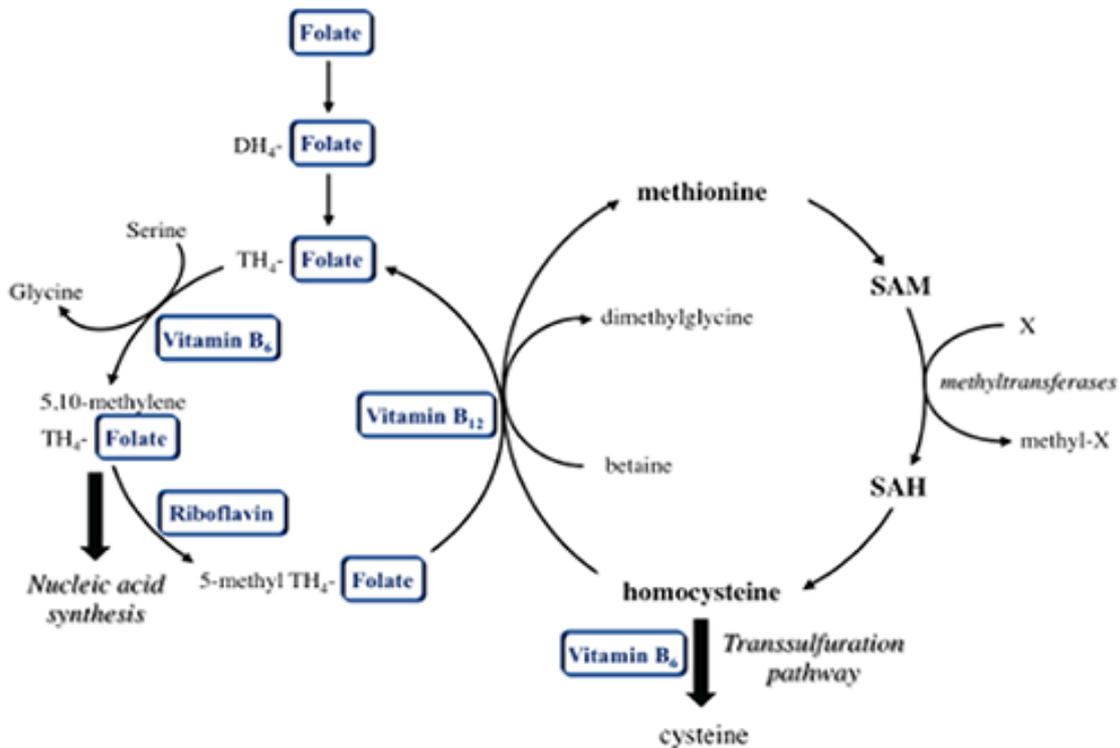


Fig. 6 Single carbon metabolism.

Source: <https://pi.oregonstate.edu/book/export/html/129> [11].

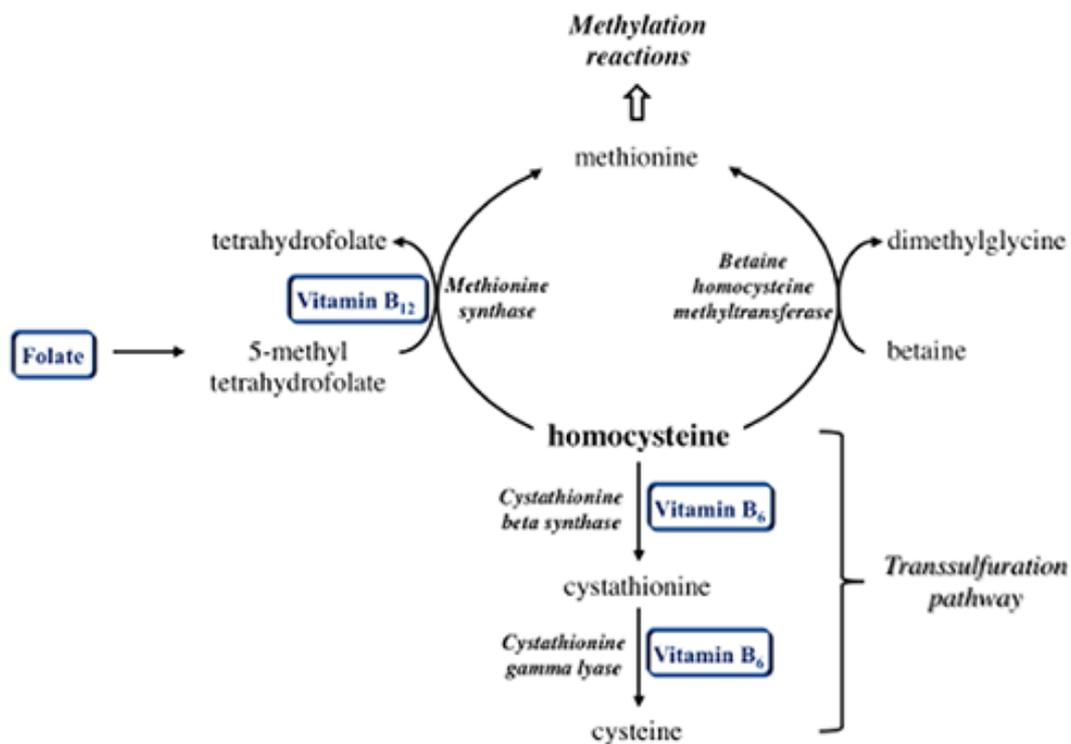


Fig. 7 Homocysteine metabolism.

Source: <https://ipi.oregonstate.edu/book/export/html/129> [11].

Pyridoxine P5P therapy is also used in the treatment of vitamin B6-sensitive homocystinuria resulting from a deficiency of cystathionine synthase, a PLP-dependent enzyme, along with dietary protein restriction [17].

Low vitamin B6 intake is associated with an increased risk of cardiovascular disease and an increased risk of cancer in some, but not all, prospective studies. A prospective study that followed a group of Japanese participants of more than 40,000 middle-aged individuals over 11.5 years reported a 48% lower risk of myocardial infarction in subjects receiving P5P on average 1.6 mg/day [18].

Low plasma PLP has been associated with cardiovascular disease, stroke and venous thrombosis risk in some studies, including three prospective studies. However, systemic inflammation measured by elevated C-reactive protein (CRP) has been hypothesized to predict myocardial infarction by plasma PLP.

In patients with myocardial infarction (heart attack),

plasma PLP showed a temporary reduction of 40% approximately 40 hours after entry; the decrease was accompanied by an equivalent increase in erythrocyte PLP. While plasma PLP returned to normal levels, erythrocyte PLP rose during discharge. Support for critically ill patients with systemic inflammation with high-dose pyridoxine caused no or slight increase in plasma PLP, a moderate (3-fold) increase in erythrocyte PLP, and a radical (15-20-fold) plasma PL increased [4].

Homocystinuria due to cystathionine beta-synthase (CBS) deficiency is the most common cause of mental retardation after phenylketonuria. Developmental delay should be kept in mind in the differential diagnosis of the child with mental retardation. The gold standard in the diagnosis of CBS deficiency is the quantitative assessment of blood amino acids. In cases where this is not possible, the determination of plasma homocysteine gains importance in the diagnosis. It should be kept in mind that qualitative amino acid measurement methods can be omitted especially in

patients who are responsive to pyridoxine. Half of the patients respond to pyridoxine, albeit partially [19].

5.10 Cognitive Disorders and Alzheimer's Disease

Some observational studies have associated with cognitive decline and Alzheimer's disease (AD) in the elderly with folate, vitamin B12 and vitamin B6 deficiency [20]. However, the relationship between B vitamins on cognitive health with aging is complicated with the high incidence of hyperhomocysteinemia and systemic signs of inflammation in the elderly [21]. In a randomized, double-blind, placebo-controlled study of 2,695 stroke survivors with or without cognitive impairment, daily supplementation with 2 mg of folic acid, 0.5 mg of vitamin B12, and 25 mg of vitamin B6 for 3.4 years caused significant results. There was a decrease in average homocysteine levels, as against placebo (28% and 43% respectively in cognitive and intact patients) [22]. However, B-vitamin intervention had no effect on the incidence of newly diagnosed cognitive disorders or on cognitive performance measures compared to placebo [22]. In contrast, another placebo-controlled study showed that a daily vitamin B regimen leading to a significant reduction of homocysteine in high-risk elderly individuals could limit the progressive atrophy of the gray matter brain regions associated with the AD process [23].

Some studies have shown a relationship between vitamin B6 and brain function in the elderly. For example, analysis of the data obtained from the Boston Normative Aging Study found a correlation between higher serum P5P concentrations and better memory test scores in 70 men aged 54-81 years [24].

5.11 Depression

Several cross-sectional studies have reported that depression symptoms co-exist with low B6 vitamin status (plasma PLP level ≤ 20 nmol/L) [25]. In a prospective study of 3,503 free-living people aged 65 and older from the Chicago Health and Aging Project, total vitamin B6 intake was inversely correlated with

the incidence of depressive symptoms over a mean follow-up period [26]. In a randomized, double-blind, placebo-controlled study of 563 subjects who had a stroke in 7.2 years, daily 2 mg folic acid supplementation, 0.5 mg vitamin B12 and 25 mg vitamin B6 reduced the risk of major development in half [27].

5.12 Cancer

Due to chronic inflammation underlying most cancers, vitamin B6 deficiency may develop. Since the methionine cycle is required for mechanisms such as homocysteine catabolism, low P5P vitamin status can affect these pathways and potentially increase the risk for chronic conditions. A systematic review of nine prospective studies found a reverse or positive association between vitamin B6 intake and colorectal cancer (CRC) risk [28]. In the meta-analysis, inconsistent evidence of a link between vitamin B6 intake and breast cancer was also reported [29]. In addition, in a meta-analysis of four interrelated case-control studies, the risk of colorectal cancer was reduced by 49% per 100-pmol/mL increase in blood PLP levels (approximately 2 SD) [28].

5.13 Kidney Stones

A large prospective study examined the relationship between vitamin B6 intake and the occurrence of symptomatic kidney stones in women. Groups of more than 85,000 women with no previous history of kidney stones were followed for 14 years, and those who consume 40 mg or more of vitamin B6 per day may reduce the risk of developing kidney stones than those who consume 3 mg [30]. However, in a group of more than 45,000 men followed for 14 years, no relationship was found between vitamin B6 intake and the formation of kidney stones [31]. Limited experimental data have shown that high-dose P5P supplementation may help reduce calcium oxalate kidney stone formation and reduce urinary oxalate levels, an important determinant of calcium oxalate kidney stone

formation [32].

5.14 Pregnancy Nausea and Vomiting

Nausea and vomiting during pregnancy (NVP) are often referred to as morning nausea; it can affect 85% of women in early pregnancy, and usually lasts 12 to 16 weeks [33]. Vitamin B6 has been used to treat nausea during pregnancy since the 1940s. Vitamin B6 is considered safe during pregnancy [34]. A systematic review of randomized controlled trials of NVP symptoms in early pregnancy showed that vitamin B6 supplementation was somewhat effective [35]. It should be noted that NVP is usually resolved without any treatment and it is difficult to conduct well-controlled trials. Pyridoxine and drug doxylamine supplementation significantly improved NVP symptoms compared to placebo. In the United States and Canada, vitamin B6 (pyridoxine hydrochloride, 10 mg) and doxylamine succinate (10 mg) have been recommended as primary care [33].

5.15 Premenstrual Syndrome (PMS)

Premenstrual syndrome (PMS) refers to a group of symptoms that begin after ovulation, including but not limited to fatigue, restlessness, moodiness, depression, fluid retention, and breast tenderness, which begin with menstruation. In a systematic review and meta-analysis of nine randomized, placebo-controlled trials, it was determined that supplementary vitamin B6 up to 100 mg/day may be important to treat PMS, including mood symptoms [36].

5.16 Impaired Kidney Functions and P5P

People with renal dysfunction, including those with end-stage renal disease and chronic renal failure, generally have low vitamin B6 concentrations. Plasma PLP concentrations are low in patients undergoing renal dialysis or intermittent peritoneal dialysis, as well as in people who have undergone renal transplantation, perhaps due to the metabolic clearance of PLP. Patients with kidney disease often have clinical symptoms

similar to those with vitamin B6 deficiency [24].

5.17 Alcohol Addiction

Plasma PLP concentrations tend to be very low in people with alcohol dependence. Alcohol produces acetaldehyde, which reduces PLP formation and competes with PLP in protein binding. People with alcohol dependence can benefit from P5P supplementation [24].

6. Metabolic Diseases Chaperone Effect

While the main role of PLP in acquiring the biological activity of many proteins has long been known, the finding that some enzymes require coenzymes for refolding in an artificial environment indicates an additional role of PLP as a chaperone in the folding process. Mutations in genes encoding PLP-enzymes cause several rare hereditary diseases. Some of these diseases are AADC deficiency, cystathionuria, homocystinuria, gyrate atrophy, primary hyperoxaluria type 1, xanthurenic aciduria, and X-linked sideroblastic anemia. Affected patients, though in different degrees, may obtain benefit from the application of pyridoxine which is a PLP precursor. The effect of coenzyme is not limited to mutations affecting the enzyme-coenzyme interaction but also includes those that cause fold defects; which reinforces the notion that PLP can play the role of a chaperone and that the misfolded variants increase the folding efficiency [37].

6.1 The Role of PLP in Folding Enzymes

More than 30% of all proteins require binding of cofactors to perform their biological activities. Since these molecules fold in a cellular environment with co-origin cofactors, it is important to understand whether the cofactor binds to the corresponding proteins before, after, or during folding, to learn the role of the cofactor in the folding process. Emphasis on the interaction between cofactor interactions and protein folding is also important for an increasing

number of diseases found to be due to protein misfolding.

The role of PLP binding during non-folding of PLP-enzymes, the relationship between PLP transport and protein folding, may have important implications for the role of protein folding in B6-related hereditary diseases.

6.2 Hereditary Diseases Responsive to Pyridoxine due to PLP-Enzyme Deficiencies

Some innate metabolic problems are known to be due to mutation of a gene encoding a PLP-linked enzyme including metabolic, neurological and blood disorders. Although individually rare, they form a class with an incidence of about 1:10,000. In most cases, genetic disorders underlying the disease are inaccurate mutations that are not always easy to predict the effect on the protein level. Recent biochemical, bioinformatic and cellular studies have highlighted that many pathogenic mutations lead to the synthesis of an abnormal protein; it has significant or nearly normal catalytic activity but is characterized by low stability and/or reduced expression level. In these cases, disorders affecting PLP-enzymes can be considered as misdiagnosed diseases, implying that treatments aimed at increasing the folding efficiency or intracellular stability of the mutated protein are possible, and PLP—treated with pharmacological doses of pyridoxine known to be converted to PLP in artificial

medium lists innate problems due to lack of enzymes. Symptoms, the enzyme involved and the catalyzed reaction are also indicated. The percentage of patients who respond may vary from 5% to 90% depending on the enzyme involved and the mutated residue, and the mechanism of action of the coenzyme is not clearly understood. In principle, the coenzyme is expected to have a prosthetic role and is only effective in increasing the catalytic activity of the variants with reduced coenzyme binding affinity (sensitivity) due to residual mutations in or near the active site that alter the PLP microenvironment. This is valid in the ornithine aminotransferase gene, which causes gyrate atrophy in the case of B6-sensitive mutations in the quinureninase gene which leads to xanthurenic aciduria, and the γ -cystathionase gene that leads to cystathioninuria, and the cystathionine β -synthase gene that causes homocystinuria. However, various evidences show that exogenous PLP may have a chaperone effect by increasing the folding efficiency of mutated proteins and/or increasing their stability against intracellular degradation. This explains why pyridoxine administration is effective in rescuing variants that carry mutations of residues away from the active site (i.e. mutations that cause a protein conformational change but do not affect coenzyme binding affinity).

The following (Table 1) are examples of hereditary disorders involving B6-dependent enzymes, in which PLP plays a role of chaperone.

Table 1 Hereditary diseases with chaperone effect of B6.

Disease	Findings	Related enzyme
Aromatic L-amino acid decarboxylase deficiency	Growth retardation, abnormal movements, oculogyric crisis vegetative symptoms	DOPA decarboxylase (EC 4.1.1.28)
Cystathioninuria	Abnormal cystathionine excretion, fibrotic liver	Cystathionine γ -lyase (EC 4.4.1.1)
Homocystinuria	Increased homocysteine, endothelial damage, risk of other arterial vein diseases	Cystathionine β -synthase (EC 4.2.1.22)
Deficiency of ornithine aminotransferase (gyrate atrophy)	Poor vision at night or dim light, loss of peripheral vision	Ornithine aminotransferase (EC 2.6.1.13)
Primer hyperoxaluria	Kidney stone	Alanine: glyoxylate aminotransferase (EC 2.6.1.44)
Xanthurenic aciduria	Vomiting, jaundice, high xanthurenic acid excretion	L-quinurenine hydrolase (EC 3.7.1.3)
X-linked sideroblastic anemia	Abnormal iron deposition in microcytic red blood cells, hypochromic and red blood cells	δ -aminolevulinate synthase (EC 2.3.1.37)

6.2.1 X-linked Sideroblastic Anemia

Sideroblastic anemia is a group of diseases characterized by anemia, hypochromic and microcytic erythrocytes, elevated serum iron and cyclic sideroblasts. The most common form of sideroblastic anemia is caused by a lack of erythroid-specific δ -aminolevulinic synthase (ALAS2). ALAS2 is a PLP-enzyme that catalyzes the first step in heme biosynthesis, i.e. the decarboxylation of glycine and succinyl-coenzyme A, which yields 5-aminolevulinic acid, coenzyme A and CO₂.

Biochemical studies on purified recombinant proteins show that exogenous P5P also plays a chaperone role by supporting the inherent structure of folding defective variants of ALAS2. In fact, the T388S mutation has been shown to not alter PLP binding affinity but to cause a structural change on ALAS2 and reduce the affinity for the substrates of the enzyme. The presence of exogenous coenzyme probably promotes the correct folding of the protein and thus forming larger amounts of the catalytically active enzyme, thus explaining the specific activity of the enzyme in the treated patients. Moreover, Cotter et al. [37] found that the F165L mutation did not significantly reduce the catalytic activity of ALAS2, but disrupted the overall structure of the protein, and that PLP could have a stabilizing effect, explaining pyridoxine activity.

PLP can be stabilized consistently with the positive response of pyridoxine to patients carrying the mutation A172T variant [37].

6.2.2 Primary Hyperoxaluria Type I

Human liver peroxisomal alanine: glyoxylate aminotransferase (AGT) catalyzes the conversion of alanine and glyoxylate to pyruvate and glycine. The reaction is largely shifted towards glyoxylate-glycine processing, indicating that AGT has an important role in hepatic glyoxylate detoxification. Inherited mutations on the AGXT gene encoding AGT are the

cause of primary Hyperoxaluria Type I (PH1); this is a rare disorder characterized by the overproduction of oxalate and the accumulation of insoluble calcium oxalate crystals leading to urolithiasis and nephrocalcinosis in the kidneys and urinary tract (approximately 1 incidence in 120,000 newborns). As a result, renal insufficiency leads to a systemic oxalosis with calcium oxalate accumulation in the whole body.

Administration of P5P is one of the currently available therapeutic therapies, the increased concentration of PLP demonstrating a chaperone role by altering balance to more stable haloform than apo, possibly not only a prosthetic role but also by improving productive folding and/or dimerization of variants [37].

6.2.3 AADC Deficiency

Dopa decarboxylase (DDC), called the aromatic amino acid decarboxylase (AADC), catalyzes the conversion of L-Dopa and L-5 hydroxytryptophan (5-HTP) to dopamine and serotonin, respectively. AADC deficiency is a rare hereditary neurometabolic disease characterized by loss of function of DDC, leading to an excessive deficiency of dopamine and serotonin together [37].

To date, variants causing 29 AADC deficiencies have been detected from the genetic screening of the AADC gene [38]. It was strongly demonstrated that AADC deficiency may be associated with B6-sensitive forms [37].

In this study, we focused on B6 hereditary diseases (X-linked sideroblastic anemia, primary hyperoxaluria type I, AADC deficiency) in which the role of chaperone in PLP is demonstrated by both biochemical and cell biology analyzes. The studies examined demonstrate how the elucidation of the molecular degradation of pathogenic variants is simple/direct in predicting and/or explaining the response to vitamin administration and thereby enabling more rational remedial approaches. The validity of these observations can also be estimated for the management

of other inherited metabolic disorders including vitamin-dependent enzymes.

7. Nutrition and P5P

Plasma PLP is the most common measure of vitamin B6 status. PLP concentrations more than 30 nmol/L are conventional indicators of adequate vitamin B6 status in adults.

Vitamin B6 is found in a wide variety of foods. The richest sources of vitamin B6 include fish, beef liver and other organ meats, potatoes and other starchy vegetables and fruits (excluding citrus).

Comparative changes of nutritional values of foods by years are given in Table 2. There has been a significant decrease in the nutritional value of foods over the years. For example, the amount of B6 in banana is seen to be 95% decreased. B6 is a vitamin that must be taken from outside via foods. It should be taken from outside in sufficient quantity with sufficient quality.

8. Conclusion

The active form of vitamin B6, pyridoxal 5'-phosphate (PLP), has a wide range of effects because it acts as an auxiliary factor (co-factor) in more than

150 enzymatic reactions. It is required in single carbon metabolism, lipid metabolism, glyconeogenesis, heme and neurotransmitter biosynthesis, and it is also defined as chaperone in the process of enzymes folding, radical cleaner of reactive oxygen species and metal chelators. In this study, literature review related to P5P (B6) was made.

Mutations in genes encoding PLP-enzymes lead to a few rare known hereditary diseases. Affected patients may benefit from pyridoxine (P5P) practice, although they are in different degrees. Misunderstanding mutations related to P5P are the most common type of genetic disorder, but in some cases have been identified as folding mutations. The rationale behind this approach is that vitamins can bind to the protein to have a chaperone-like effect, thereby increasing folding efficiency or stability to cellular degradation. When the molecular disorder is not known, vitamin supplements are also used. This may explain, at least in part, why the level of response to vitamin supplementation is variable.

In the last 15-20 years, as a result of clinical and basic research, coenzymes or their precursors or derivatives have been used as curative drugs for a number of congenital problems resulting from the lack

Table 2 Content change of some foods.

Minerals and vitamins per 100 grams	Contents reviewed	1985	1996	2002	1985-1996	1985-2002
Broccoli	Calcium	103	33	28	-68%	-73%
	Folic acid	47	23	18	-52%	-62%
	Magnesium	24	18	11	-25%	-55%
Beans	Calcium	56	34	22	-38%	-51%
	Folic acid	39	34	30	-12%	-23%
	Magnesium	26	22	18	-15%	-31%
	Vitamin B6	140	55	32	-61%	-77%
Potato	Calcium	14	4	3	-70%	-78%
	Magnesium	27	18	14	-33%	-48%
Carrot	Calcium	37	31	28	-17%	-24%
	Magnesium	21	9	6	-57%	-75%
Spinach	Magnesium	62	19	15	-68%	-76%
	Vitamin C	51	21	18	-58%	-65%
Apple	Vitamin C	5	1	2	-80%	-60%
Banana	Calcium	8	7	7	-12%	-12%
	Folic acid	23	3	5	-84%	-79%
	Magnesium	31	27	24	-13%	-23%
	Vitamin B6	330	22	18	-92%	-95%
	Potassium	420	327	-	-24%	-
Strawberry	Calcium	21	18	12	-14%	-43%

Vitamin C	60	13	8	-67%	-87%
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Source: 1985 Pharmakonzern Geigy (Schweiz), 1996/2002 Lebensmittellabor Karlsruhe/Sanatorium Oberthal [39].

of enzymes that require cofactors for their activities.

They are used under doctor control and at the recommended doses in obesity, insomnia and depression, immunocompromised children and adults with frequent infections, anemia, mouth and tongue sores, dementia, senile memory impairment, asthma, autism cardiovascular diseases (arteriosclerosis), carpal tunnel syndrome, glucose intolerance, diabetic neuropathy, pregnancy diabetes, kidney stones, pregnancy-related nausea, vomiting, bone resorption, high blood cholesterol and lipid levels, people using birth control pills.

In addition, it is used as a food supplement in children and adults in cases where it is not thought to be balanced and adequate nutrition for any reason. Daily B6 intake puts a question mark in the minds due to the state of ownership of the digestive system in health required for absorption and synthesis and the nutritional value of nutrients approaching to zero according to years in Table 2. Therefore, societies should determine their P5P levels by carrying out their own wide-ranging studies and prophylactic guidelines should be prepared according to the situation. It is concluded that this trend is important for the next generation of public health. Because diseases caused by P5P deficiency are monitored as chronic, aggressive, and costly diseases.

This publication was needed because it was observed that chronic diseases which usually occur in families are the results of different pathways of the same enzyme deficiencies. When this situation is observed, prophylactic P5P food supplementation is recommended to family members.

P5P is the activated form of B6. It is thought to be useful in breaking the chronic cycle when used in disease states.

In addition, it is considered necessary to determine the amount of P5P in food in terms of public health and to carry out the studies envisaged in this regard.

Further and detailed studies on vitamin P5P (B6) are still needed.

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