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Revisión

Vitamin B₆ status, deficiency and its consequences - an overview

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Abstract

Background: **Vitamin B₆ is thought to be a most versatile coenzyme that participates in more than 100 biochemical reactions. It is involved in amino acid and homocysteine metabolism, glucose and lipid metabolism, neurotransmitter production and DNA/RNA synthesis. Vitamin B₆ can also be a modulator of gene expression. Nowadays, clinically evident vitamin B₆ deficiency is not a common disorder, at least in the general population. Nevertheless, a subclinical, undiagnosed deficiency may be present in some subjects, particularly in the elderly.**

Objective: **This review gives a complete overview over the metabolism and interactions of vitamin B₆. Further, we show which complications and deficiency symptoms can occur due to a lack of vitamin B₆ and possibilities for public health and supplemental interventions.**

Methods: **The database Medline (www.ncvi.nlm.nih.gov) was searched for terms like "vitamin B₆", "pyridoxal", "cancer", "homocysteine", etc. For a complete understanding, we included studies with early findings from the forties as well as recent results from 2006. These studies were summarised and compared in different chapters.**

Results and conclusion: **In fact, it has been proposed that suboptimal vitamin B₆ status is associated with certain diseases that particularly afflict the elderly population: impaired cognitive function, Alzheimer's disease, cardiovascular disease, and different types of cancer. Some of these problems may be related to the elevated homocysteine concentrations associated to vitamin B₆ deficiency, but there is also evidence for other mechanisms independent of homocysteine by which a suboptimal vitamin B₆ status could increase the risk for these chronic diseases.**

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Key words: *Vitamin B₆. Coenzyme. Elderly. Homocysteine. Vitamin B₆ deficiency.*

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ESTADO DE VITAMINA B₆, DEFICIENCIA Y SUS CONSECUENCIAS — UNA REVISIÓN

Resumen

Antecedentes: **se piensa que la vitamina B₆ es la coenzima más versátil que participa en más de 100 reacciones bioquímicas. Está implicada en el metabolismo de los aminoácidos y de la homocisteína, el metabolismo de la glucosa y los lípidos, en la producción de neurotransmisores y en la síntesis de ADN/ARN. Esta vitamina también puede ser un modulador de la expresión génica. Hoy en día, la deficiencia clínicamente evidente de vitamina B₆ no es una afección habitual, al menos en la población general. Sin embargo, puede ocurrir una deficiencia subclínica no diagnosticada en algunos individuos, especialmente en los ancianos.**

Objetivo: **esta revisión aporta una visión de conjunto completa sobre el metabolismo y las interacciones de la vitamina B₆. Además, mostramos qué complicaciones y síntomas por deficiencia pueden ocurrir por la falta de vitamina B₆ y las posibilidades de intervenciones de salud pública y de suplementos.**

Métodos: **se buscó en la base de datos Medline (www.ncvi.nlm.nih.gov) con los términos "vitamin B₆", "pyridoxal", "cancer", "homocysteine", etc. Para una mayor comprensión, incluimos estudios con hallazgos iniciales de los años cuarenta, así como estudios recientes del año 2006. Se resumieron estos estudios y se compararon por capítulos diferentes.**

Resultados y conclusión: **de hecho, se ha propuesto que el estado sub-óptimo de vitamina B₆ se asocia con ciertas enfermedades que afligen en especial a la población anciana: función cognitiva alterada, enfermedad de Alzheimer, cardiopatía y distintos tipos de cáncer. Algunos de estos problemas podrían relacionarse con concentraciones elevadas de homocisteína asociadas con una deficiencia de vitamina B₆, pero también existe la evidencia de otros mecanismos independientes de la homocisteína por los que un estado sub-óptimo de vitamina B₆ podría aumentar el riesgo de padecer estas enfermedades crónicas.**

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Palabras clave: *Vitamina B₆. Coenzima. Anciano. Homocisteína. Deficiencia de vitamina B₆.*

Introduction

Vitamin B_6 sufficiency is required for optimal health. This is due to the participation in many different biochemical reactions. Vitamin B_6 and its derivatives are needed, especially in there coenzyme functions, for the main metabolic pathways in the human body. For that reason, it is clear that a vitamin B_6 deficiency, even in mild forms, has effects on the human metabolism. Several diseases and impairments of health are connected to the wide variety of B_6 functions in suboptimal status. This can also be worsened through ageing.

This article gets deep into metabolic functions of vitamin B_6 and gives an overview about associated diseases to vitamin B_6 deficiency with new insights from recently published studies, including prevention and treatment potentials.

Vitamin B_6 – Chemistry, metabolism and bioavailability

Vitamin B_6 comprises a group of three related 3-hydroxy-2-methyl-pyrimidine derivatives. The derivative pyridoxine (PN) is an alcohol, pyridoxal (PL) is an aldehyde and pyridoxamine (PM) contains an amino group. Their respective 5´-phosphate esters: pyridoxine 5´-phosphate (PNP), pyridoxal 5´-phosphate (PLP)

and pyridoxamin-5´-phosphate (PMP) are the biological active coenzyme forms (fig. 1). They are water-soluble and can be inter-converted in normal human metabolism[1]. PLP is the major form that is used by pyridoxine-dependent enzymes. These enzymes catalyse more than 100 essential biochemical reactions in human metabolism.

B_6 vitamers are similarly absorbed in the upper jejunum and little in ileum, but before absorption, phosphate esters must be hydrolysed by alkaline phosphatase or other intestinal phosphatases. The non-phosphorylated form enters the mucosal cells by two different processes according to luminal concentration. At low concentrations, vitamers enter the cell by an active process regulated by requirements. At high concentrations, transport is by non-saturable passive diffusion mechanisms[1]. Once in the cell, vitamers are phosphorylated by ATP dependent pyridoxine kinase in a process referred to as *metabolic trapping*. The phosphorylated form can not traverse cell membranes and must therefore be dephosphorylated again before traversing the basolateral membrane side of the mucosal cell. Once in the circulation, most of B_6 vitamers are transported to the liver where they are again phosphorylated to PNP, PLP or PMP, and then released to plasma[1,2]. Vitamin B_6 in plasma is mainly PLP (60%), PN (15%) and PL (14%). These vitamers circulate bound to albumin[1]. In peripheral tissues, in order to cross cell membranes, the phosphorylated
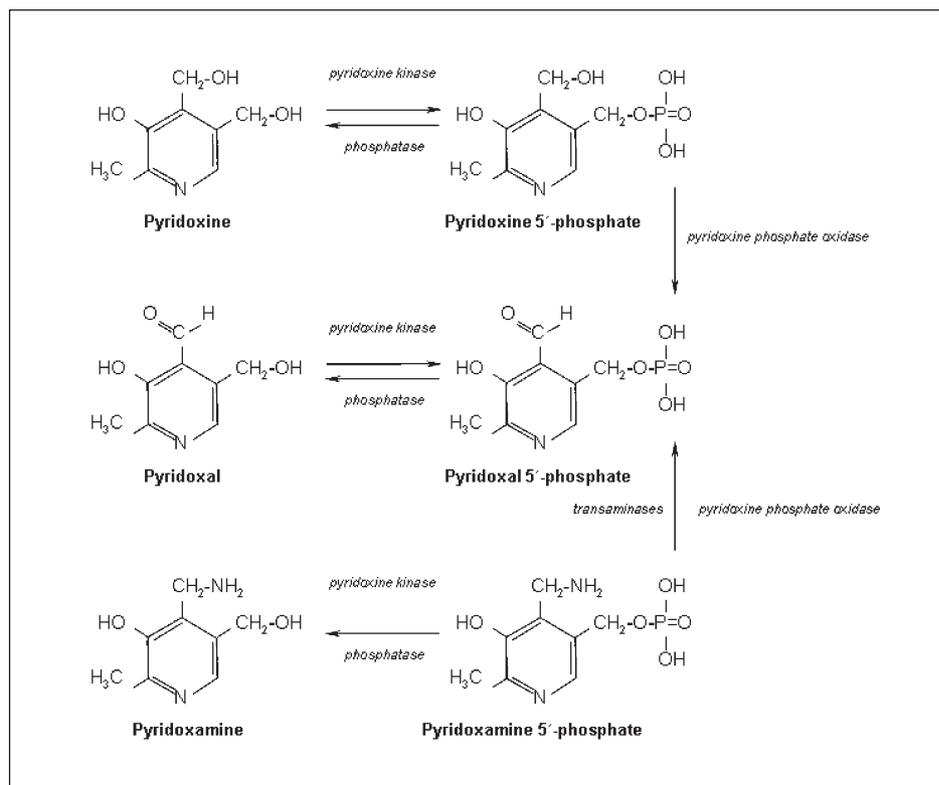

Fig. 1.—Metabolic interconversion of pyridoxine, pyridoxal, pyridoxamine and their respective phosphate esters (modified after Bässler, 2002).

forms need to be dephosphorylated by alkaline phosphorylase. Transport into tissue cells is by gradient diffusion. This gradient is maintained thanks to the immediate rephosphorylation occurring inside the cells. Intracellular accumulation of PLP seems to be limited by intracellular protein binding capacity. In circulation, PLP may enter red blood cells where PLP bounds to haemoglobin. In red cell mass, PLP is at higher concentration (4 to 5 times) than in plasma. PLP increases the oxygen affinity of haemoglobin[3]. Total body stores of B_6 have been estimated to be as little as 167 mg[4]. Up to 80% of that amount is found in muscle tissue[2]. PLP in metabolism occurs mainly in the liver where it is oxidized to 4-pyridoxic-acid (4-PA) which is released and excreted. Almost 50% of urinary vitamin B_6 is 4-PA. In total, some 2 mg vitamin B_6 can be excreted every day. In cases of B_6 deficiency, excretion appears to be lower. PN is also excreted in faeces but to a limited extent and can therefore be neglected[4].

Gregory has reviewed the bioavailability of vitamin B_6[5]. In a diet typically containing about 15% PN glucoside, which is about 50% as bioavailable as the other vitamers, vitamin B_6 is about 75% bioavailable. The bioavailability of non-glucosides of the vitamin is greater than 75%. In the absence of food B_6 compounds are absorbed similarly, even at high doses. A loading dose of 50 mg PL or PLP is found up to 70% in the urine within 24 hours. This demonstrates that the phosphate esters are effectively hydrolysed and absorbed in the gut[6,7]; whereas only 40% PN can be accounted for in the urine under the same conditions, it does raise the plasma PLP concentration and is retained more effectively than is PL[7]. Dietary PM and PL are about 10% less effective than PN in raising the plasma PLP concentration, and slightly more of these vitamins are excreted in the urine as 4-PA[8]. Most controlled B_6 studies have used PN as the added source, but requirements calculated from these studies would underestimate the B_6 requirement by 5% or less for individuals deriving most of their B_6 as PLP and PMP from animal source.

Functions and vitamin B$_6$-dependent enzymes

Since vitamin B_6 is a coenzyme in many different metabolic reactions, we can consider that vitamin B_6 has a wide variety of functions in the human body. PLP, as coenzyme, is required for reactions in different metabolic pathways. To carry out its catalytic action, the carbonyl group present in PLP forms a Schiff's base with the α-amine of lysine of the apoenzyme and then the initial catalytic step proceeds by the formation of a Schiff's base between the α-amino group of an amino-acid and the carbonyl group of PLP[2]. The electron sink properties of PN enable the holo-enzyme for its catalytic reaction[9]. In reactions associated with amino acid metabolism, PLP binding enables the amino acid for further reactions. Some of the reactions PLP is involved in are: transamination of amino acids to keto acids (which can be then used for gluconeogenesis), formation of α-aminolevuline acid (a precursor of heme group), as coenzyme in serin-palmityl-transferase (implicated in sphingomyelin synthesis), decarboxylation of L-amino acids (to yield amines, which function as neurotransmitters, hormones or biogenic amines). In the nervous system, PN-dependent enzymes fall into two categories: transaminases and L-amino acid decarboxylases. The crucial PLP-dependent steps of the synthesis of several neurotransmitters are: the enzymatic decarboxylation of 3,4-dihydroxyphenylalanine (DOPA) to dopamine, the conversion of tryptophan to both nitric acid and serotonine, and the conversion of glutamic acid to α-aminobutyric-acid (GABA)[10]. Another important metabolic pathway is the metabolism of one-carbon units. One-carbon (methyl) units, usually obtained from serine, are transferred to tetrahydrofolate (THF) yielding 5,10-methylene-tetrahydrofolate (5,10-methylene-THF). This reaction is catalysed by serine-hydroxymethyltransferase (SHMT), a PLP dependent enzyme (fig. 2). 5,10-methylene-THF is subsequently used as a methyl donor for the synthesis of thymidylate and purines (for nucleic acid synthesis) or methionine (for protein synthesis or biological methylations). A considerable proportion of methionine is activated by ATP to form S-adenosylmethionine (SAM), which serves primarily as a universal methyl donor in a variety of reactions. In the brain, for example, SAM-dependent methylations are extensive and the products of these reactions include several neurotransmitters such as catecholamines and indoleamines, phospholipids and myelin[11].

The interaction with the homocysteine (Hcy) metabolism through methionine transformation into cysteine (by cystathionine β-synthase and cystathionine-? lyase) (fig. 2) is of growing interest since high levels of Hcy have been related to cardiovascular disease (CVD), cognitive impairment or cancer. Hcy is a sulphur-containing amino acid which is not a constituent of proteins and, in fact, an intermediate compound in methionine metabolism. There are two pathways for Hcy disposal in the human body and both are related to methionine status and vitamin B_6.

In cases of methionine excess, or when cysteine is required, Hcy enters the transsulfuration pathway which consists of condensation of Hcy with serine to form cystathionine (catalysed by cystathionine-β-synthase). Cystathionine is then degraded to cysteine and α-ketobutyrate by cystathionine ? lyase. Both reactions require PLP as cofactor[12]. In cases of methionine deficiency, Hcy enters the remethylation pathway which is a folate and B_{12} dependent process[13].

As a coenzyme for glycogen phosphorylase, PLP is also involved in glucose metabolism. In this case, the 5-phosphate group of PLP acts as a proton donor or acceptor. In addition to these functions, PLP
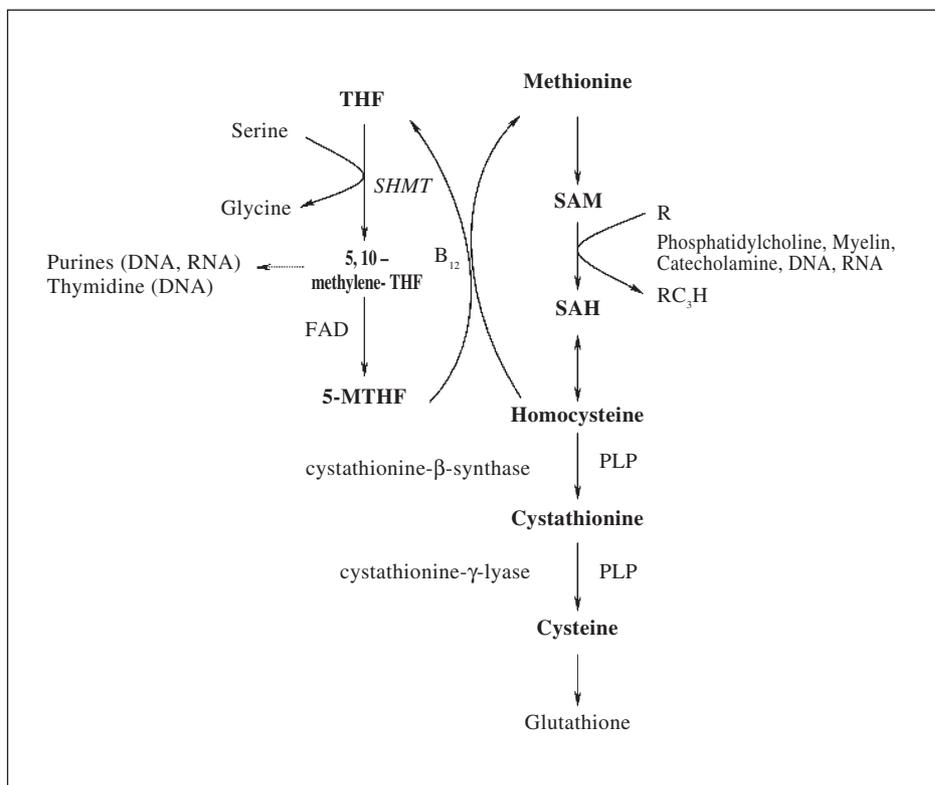
Fig. 2.—One-carbon unit generation and Hcy metabolism. Vitamin B_6 is involved as coenzyme PLP. PLP: Pyridoxal 5'-phosphate; SAM: S-adenosylmethionine; SAH: S-adenosylhomocysteine; SHMT: Serine-hydroxymethyltransferase; THF: tetrahydrofolate; 5-MTHF: 5-methyl tetrahydrofolate; R: methl group acceptor.

interacts with different proteins and plays many different roles including regulation of steroid hormone receptors, modulation of the affinity of haemoglobin into oxygen or inhibition of some transcription factors[1].

Measurement of vitamin B_6

Over the last decades several indicators of B_6 status have been developed. Indicators of vitamin B_6 status include direct measures, plasma or erythrocyte vitamin concentration, or urinary 4-PA; and functional measures, stimulation or activation of erythrocyte aspartate aminotransferase (α-EAST)

and alanine aminotransferase (α-EALT) by PLP, or determination of tryptophan metabolites, such as urinary xanthurenic acid (XA). The increase in methionine metabolites (e.g. Hcy, cystathionine) after a methionine load is also used as an indicator of B_6 status[14]. All of these and their respective reference values are summarised in table I and briefly summed up below.

1. Direct measures in blood and urine.
   (A) Plasma PLP, other vitamers and total vitamin B_6

The PLP concentration is a direct indicator for the activity of vitamin B_6 in the organism. Plasma

Table I
Parameter for assessment of vitamin B_6 status and reference values (modified by Leklem, 1994)

Parameter		Reference value
Direct	Plasma PLP	> 30 nmol/L 20 nmol/L
	Total vitamin B_6	> 40 nmol/L
	Urinary 4-PA excretion	> 3 µmol/day
	Urinary total vitamin B_6	> 0.5 µmol/day
Indirect	α-EAST	< 1.8 (< 80%)
	α-EALT	< 1.25 (< 25%)
	XA excretion (2g L-Try)	< 65 µmol/day
	Cystathionine excretion (3 g L-Methionine)	< 350 µmol/day

PLP is used as the primary index of whole-body PL levels. Protein bound PLP in the plasma is in equilibrium with free PLP. Binding of PLP to protein protects it from hydrolyses by alkaline phosphatase. The plasma PLP concentration reflects liver PLP[15], and changes fairly slowly in response to changes in vitamin intake, taking about 10 days to reach a new steady state[4]. PLP generally correlates with other indicators of B_6 status[16]. Leklem has suggested a plasma PLP concentration of 30 nmol/L as the lower end of normal status[17], whereas other investigators have proposed a cut-off of 20 nmol/L[4]. Generally, values lower than 20 nmol/L indicate B_6 deficiency. Table II shows different physiological and other parameters which influence PLP plasma concentration[14,18]. Usually, men show higher levels than women, because of their higher muscle tissue mass. During gestation lower values of this vitamin have been observed, because of heme dilution due to changes in plasma volume. A release of muscle glycogen phosphorylase in prolonged fasting may mask an underlying deficiency, because most PLP in the body is usually bound to this enzyme. Plasma total vitamin B_6 and plasma PL concentration are additional direct measures that have utility. Since PL is the form that enters the cell, its measurement may be more relevant than that of PLP.

A number of methods have been developed for the assay of B_6 vitamers in plasma for clinical and nutritional purposes. These include microbiological or enzymatic methods. Currently, the quantitative determination of PLP in plasma is commonly performed with procedures that utilise tyrosine apodecarboxylase. Another extensively used method is a chromatographic determination. High-performance liquid chromatography (HPLC) procedures with appropriate detection systems are regarded as the most convenient method for the evaluation of vitamin B_6 nutritional status since it is possible to quantify all vitamers and 4-PA in one assay.

(B) 24-hour urinary excretion of 4-PA and total vitamin B_6

4-PA is the major inactive metabolite of PN metabolism. Urinary 4-PA has been used extensively to evaluate B_6 requirements. Approximately 50% of the B_6 intake is excreted as 4-PA, but this proportion can vary somewhat. 4-PA excretion responds almost immediately to changes in dietary intake and therefore reflects recent dietary B_6 intake rather than tissue saturation status[4]. Thus, 4-PA excretion should not be used for status assessment. Nevertheless, low urinary excretion implies a low intake. Leklem has suggested a value greater than 3 µmol/day as indicative of adequate status[17]. Urine levels of 4-PA are lower in females than in males and will be reduced in persons with riboflavin deficiency. Further, different pharmaceuticals (isoniacid, penicillin, and cyclosporine) increase urinary excretion and interfere with the results. Neither age nor alcohol intake affects the measured level.

2. Indirect functional measures
   Activation coefficient (AC) of α-EAST and α-EALT

The stimulation of these enzymes by external (added) PLP is a frequently used functional measure of vitamin B_6 status. These indicators are considered long-term measures because of the length of the erythrocyte's lifespan (120 days). Because there is no protein synthesis in mature erythrocytes, the holo-enzyme: apo-enzyme ratio will reflect the availability of PLP at the time the erythrocytes were released into the circulation. In vitamin B_6 deficiency, a greater than normal proportion of aminotransferases is present in form of the catalytically inactive apo-enzyme. This can be converted to the catalytically active holo-enzyme by incubation *in vitro* with PLP. Since the seventies, some authors suggest that α-EALT is a more sensitive index of vitamin B_6 nutritional status than is α-EAST[19-21]. In order to overcome some of the diffe-

Table II
Parameter by which PLP concentration is influenced (modified after Leklem, 1994)

	Parameter	Plasma-PLP
Nutrition	↑ vitamin B_6	↓
	↑ protein	↓
	↑ glucose	↓, acute
	↓ bioavailability	↓
Physiology	↑ physical activity, aerobic	↑, acute
	↑ age	↓
	prgnancy	↓
	↑ activity of alkaline phosphatase	↓
	Smoking	↓
	Hypophosphatasia	↑

rences in methods of measurement and in erythrocyte transaminases activities between normal healthy individuals, the results are generally expressed as an AC (ratio of activity with added coenzyme/activity without added coenzyme). Although complete saturation of aminotransferases with PLP (AC = 1.0) does occur, some degree of unsaturation (AC > 1.0) is considered to be normal. Assuming that erythrocytes must compete with other tissues for the vitamin, and hence that erythrocyte aminotransferase saturation with PLP reflects the status of other tissues, such incomplete saturation may be a factor in normal metabolic regulation[22]. Indicator of deficiency is an AC of EAST of greater than 1.6 and of EALT of greater than 1.25[17]. As mentioned above, EAST-AC reduction lags behind the onset of the PN deficiency. Thus, a low aminotransferase AC value confirms a subacute to chronic deficiency state. Chronic alcoholism causes these indices to be falsely low, and these indices decrease with age.

3. Metabolites (A)
Tryptophan load test (fig. 3)

One of the earliest markers used to determine B_6 deficiency was the urinary excretion of XA, which is normally a minor tryptophan catabolite. The major pathway of tryptophan catabolism proceeds via the PLP-dependent kynureninase reaction[23]. The XA pathway also involves PLP-dependent enzymes, but kynurenine aminotransferase seems to be less sensitive to PLP deficiency. Under conditions of B_6 deficiency, this minor pathway is used to a greater extent, leading to the increased excretion of abnormal tryptophan metabolites such as XA, and as the deficiency worsens, 3-Hydroxykynurenine (3-HK) and kynurenine. The tryptophan load test has been widely used as an index

of vitamin B_6 nutritional status. Various challenge doses have been used in different studies such as 2 or 3 grams, as well as 4 grams[14,17,24-26]. However, as for many of the tests of B_6 status, it is not clear what level of excretion represents adverse B_6 status under the conditions of the tryptophan challenge dose. A 24-hour urinary excretion of less than 65 µmol xanthurenate after a 2 g tryptophan oral dose has been suggested[17]. The use of an oral loading dose of L-tryptophan to detect abnormalities in tryptophan and vitamin B_6 status is an underutilised laboratory procedure. If used only to measure B_6 status, it may be less indicative than other indices, such as the methionine load test (urinary cystathionine), plasma PLP, urinary vitamin B_6, or 4-PA. It may, however, be more indicative than erythrocyte aminotransferases. Marginal B_6 or magnesium deficiencies cause significant alterations. This test is influenced by protein intake, exercise, lean body mass, and pregnancy. Hormonal factors and infections enhance tryptophan-to-niacin conversion. Thus, this test is most useful for monitoring an individual's response to PN supplementation rather than for diagnosing a deficiency.

(B) Methionine load test (fig. 4)

A methionine loading test has also been studied as a possible indicator of vitamin B_6 status. Three enzymes of the methionine metabolism, cystathionine β-synthase, cystathionine-? lyase and cysteine sulphinic acid decarboxylases, are PLP-dependent. In vitamin B_6 deficiency, a test dose of methionine (3 g in an adult) results in abnormal accumulation and excretion of Hcy and the mixed disulphide of Hcy and cysteine, as well as cystathionine and cysteine sulphinic acid, and reduced excretion of taurine[27-30]. More recently a standardi-
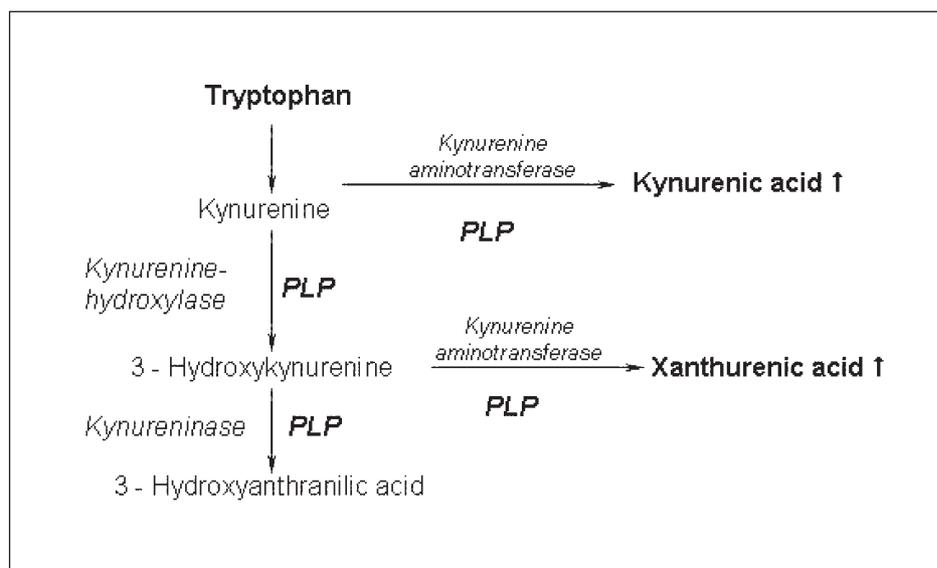

Fig. 3.—Tryptophan load test. PLP: pyridoxal 5'-phosphate.

$$S\text{-adenosylmethionine} \longrightarrow S\text{-adenosylhomocyst(e)ine}$$

Methionine $\xleftarrow{\ B_{12} + Folate\ }$ Homocysteine ↑

cystathinonine β-synthase ⇌ **PLP**

Cystathionine

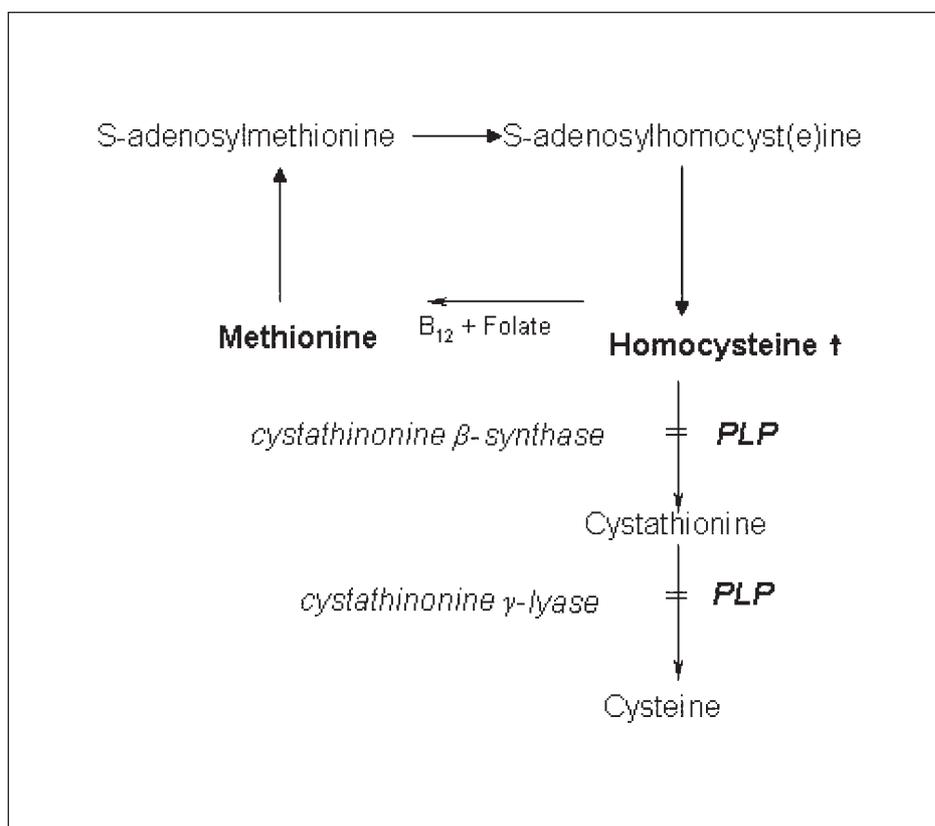cystathinonine γ-lyase ⇌ **PLP**

Cysteine

Fig. 4.—Methionine load test. PLP: pyridoxal 5'-phosphate.

sed methionine load test has been successfully used[31]. The general procedure involves the administration of 100 mg of L-methionine per kilogram of body weight. The increase in Hcy concentrations are evaluated in terms of the vitamin B_6 status. For several reasons, the methionine load test has been used less often as an index of vitamin B_6 nutritional status than has the tryptophan load test.

Pathophysiological implications related to vitamin B_6 deficiency

Since vitamin B_6 is present in almost all foods, B_6 deficiency due to insufficient dietary supply is rare. Additionally, isolated vitamin B_6 deficiency is uncommon; it usually occurs in combination with deficiencies of other B-complex vitamins. Often PN deficiency is caused by absorption disorders, genetic factors, interactions with drugs or elevated requirements as shown in table III. Because of its wide variety of functions in the body, clinical vitamin B_6 deficiency results in a broad spectrum of impaired features. On the other hand, borderline vitamin B_6 deficiency may represent a subtle, undiagnosed and frequent cause of disease in some population groups. In fact, a reduced B_6 status close to borderline concentrations or even mild deficiency could persist in an individual for months or years without the appearance of any of the diagnostic features or symptoms suggesting clinical deficiency. In this situation, some PLP related functions may be adequately compensated but others may not[32]. This subclinical deficiency can be attributed to inadequate intake and/or to the effect of ageing on physiological and metabolic processes, which may act in conjunction with many other factors. The most common pathophysiological implications related to vitamin B_6 deficiency are the following:

1. Hypochromic, microcytic, iron-refractory anemia

B_6-deficiency anemia is one form of sideroblastic anemia, characterised by ineffective erythropoiesis with hypochromic, microcytic anemia, splenomegaly, elevated tissue and serum iron, and large numbers of ringed sideroblasts in the bone marrow[33]. Microcytic anemia reflects decreased haemoglobin synthesis. Vitamin B_6 deficiency or a genetic defect of the enzyme aminolevulinate synthase can therefore lead to an iron refractory, microcytic anemia. In animals, it is well established that a deficiency of vitamin B_6 results in a severe microcytic, hypochromic anemia accompanied by strongly elevated serum iron levels[34]. In humans, vitamin B_6 deficiency anemia is very rare. Therefore, literature is very scarce. In the seventies, Ofori-Nkan-

Table III
Conditions that increase risk for PN deficiency

Advanced age
Medical conditions
   Severe malnutrition
   Hospitalization
   Celiac disease
   Hepatitis and extrahepatitic biliary obstruction
   Hepatocellular carcinoma
   Chronic renal failure
   Kidney transplant
   Hyperoxaluria types I and II
   High serum alkaline phosphatase level such as in cirrhosis and tissue injury
   Catabolic state
Medical procedures
   Hemodialysis
   Peritoneal dialysis
   Phototherapy for yperbilirubinaemia
Social-behavioral conditions
   Excessive alcohol ingestion (except for pyridoxine-supplemented beer)
   Tobacco smoling
   Severe malnutrition
Other risk factors
   Poisoning, such as Gyromitra mushroom poisoning
   Perinatal factors, such as pyridoxine-deficient mother
   Inherited conditions, such as pyridoxine dependent neonatal seizures
Other patient history
   Sideroblastic anemia
   Pregnancy
   Physical exercise

Drug-interaction	Mechanism of interaction
Isoniazid (hydrazines)	Reacts with PL and PLP
Cycloserine	Reacts with PLP, forms oxime
L-3,4-Dihydroxyphenlalanine	Reacts with PLP, forms
derivative	tetrahydroquinoline derivative
Penicillamine	Reacts with PLP, forms thiazolidine
Ethinylestradiol, mestranol	Increased enzyme levels and retention of PLP in tissue
Ethanol	Increased catabolism of PLP
Theophylline, caffeine	Inhibition of pyridoxal kinase

sah *et al.* reported that the prevalence is higher in men than in women[35]. Further, Fishman and colleagues suggest that a treatment with B_6 may be effective in correcting the haematological abnormalities of sideroblastic anemia[36].

2. Immune function

Animal studies in the forties demonstrated that a deficiency of vitamin B_6 results in large effects on lymphoid tissues. Thymic atrophy occurs and lymphocyte depletion in lymph nodes and spleen has been found in monkeys, dogs, rats, and chickens[37,38]. More recently, studies performed in humans confirm that vitamin affects immunocompetence[39]. Low vitamin B_6 intake and status have been associated with impaired immune function, especially in the elderly[40,41]. Decreased lymphocyte and interleukin (IL)-2 production has been observed in B_6-deficient individuals. Restoration of adequate vitamin B_6 status resulted in normalisation of lymphocyte proliferation and IL-2 production. These results suggest that adequate vitamin B_6 intake is important for optimal immune system function, at least in older individuals[40,41]. Further, experimental deficiency in elderly humans has been shown to reduce total blood lymphocyte numbers and decrease the proliferative response of lymphocytes to mitogenic substances. It has been suggested that the mechanism for the effect of vitamin B_6 on immune function relates to the importance of PLP in the synthesis of one carbon compounds and hence DNA and RNA synthe-

sis. Low synthesis rates of the compound will thus impair cell replication[39]. Further, U937 monocytes which were cultured with PN, PL or PM demonstrated that these vitamers can prevent the oxygen radical generation and lipid peroxidation caused by hydrogen peroxide[42]. Others suggest a connection between inflammation and vitamin B_6 status[43,44]. They found significantly lower PLP concentrations in patients with rheumatoid arthritis than in healthy subjects. In contrast, no difference was found between patients and controls in erythrocyte PLP or erythrocyte α-EAST or 4-PA levels. Therefore, they suggest that the lower vitamin B_6 concentration in patients with rheumatoid arthritis is tissue-specific. Additionally, the lower circulating PLP levels observed in rheumatoid arthritis could reflect a decrease in hepatic PLP pools, because plasma PLP is a good indicator of liver B_6 status during inflammation. Further, the study indicates that inflammation directly affects vitamin B_6 metabolism differently in different tissues[44]. The following study of the research group analysed the effect of B_6 supplements (50 mg PN hydrochloride/day) on B_6 and inflammation status. All markers of vitamin B_6 status improved significantly in the B_6 supplementation group. Although, the authors found no effect of vitamin B_6 supplementation on inflammatory cytokines, plasma CRP, ESR, or rheumatoid factor levels. They suggest that it is likely that inflammation causes vitamin B_6 deficiency and/or impaired vitamin B_6 status contributes to more severe inflammation in patients with rheumatoid arthritis. But as improving vitamin B_6 status did not alleviate inflammation, it is unlikely that vitamin B_6 inadequacy directly causes or worsens the inflammatory condition[43].

3. Cancer

Some studies on diet and cancer have disclosed a significant inverse correlation between serum PLP (and vitamin B_6 intake) and different types of cancer. There are several potential mechanisms by which vitamin B_6 may influence carcinogenesis. First, B_6-deficiency causes a decrease in the enzyme activity of SHMT. This results in a lack of methylene groups for 5,10-methylene-THF production[45]. Consequently, methylation of deoxyuridylate to deoxythymidylate may be impaired resulting in misincorporation of uracil instead of thymidine into DNA. As a consequence, a greater potential of chromosome strand breaks[46] and/or an impaired DNA excision repair may exist[47]. Evidence of this has been reported[48]. In addition, disruption of the above mentioned reactions may lead to imbalances in the methyl groups required for methylation processes, resulting in DNA hypomethylation. Altered DNA methylation has been observed in different types of tumors[49,50]. The B_6 connection to the immune system could be a mechanism by which low vitamin B_6 status or intake also contributes to

development of cancer. The two different PLP-dependent enzymes which are implicated in the transsulfuration pathway also generate cysteine, an important component of glutathione. Glutathione S-transferases and glutathione peroxidases are detoxifying agents of several carcinogenic compounds[51,52]. PLP is also involved in steroid hormone action; consequently, PLP can be implicated in some types of steroid related cancer. Some experimental studies suggest that borderline-deficient B_6 status increases sensitivity to steroid hormones, and this may have implications for breast, prostate, and uterine carcinogenesis[53]. Furthermore, one study found a steroid independent inhibition of *in vitro* breast cancer cell growth induced by PL and this was present in oestrogen-dependent and oestrogen-independent mammary carcinoma cell lines[54]. Many studies have shown a relation to special types of cancer. Thus, a more detailed overview may be helpful.

3.1. Colorectal cancer

Komatsu and coworkers demonstrated that vitamin B_6 intake decreased both the incidence and the number of colon tumors in an animal model; it decreased colon cell proliferation and expressions of c-myc and c-fos proteins in a dose-dependent manner[55]. More recently, they also showed that the preventive effect of vitamin B_6 against colon tumorigenesis in mice is mediated by a reduction of oxidative stress and nitric oxide production[56]. In humans, Wei *et al.* found out that in women plasma concentrations of PLP is inversely associated with risk for colorectal cancer (RR = 0.54 for those in the highest *vs* lowest quartiles, 95% CI = 0.31 to 0.92)[57,58]. Other researchers found an inverse association of vitamin B_6 intake and colon cancer[59,60]. Nevertheless, epidemiologic evidence is still scarce.

3.2. Pancreatic cancer

In a large nested case-control study (included in the ATBC Cancer Prevention Study cohort) a statistically significant inverse dose-response relationship was found between plasma PLP levels and pancreatic cancer risk: the risk of subjects in the highest PLP tertile was half the risk of the subjects in the lowest tertile (OR = 0.48). Interestingly, as much as fifty percent of the study population presented lower than adequate PLP plasma levels (< 30 nmol/L)[61]. This is the first study that observed an inverse association between plasma PLP concentrations and pancreatic cancer risk. Deficiencies in PLP have been shown to impair pancreatic exocrine function in experimental animals. Plasma and pancreatic amylase, trypsin and chymotrypsin activities were found to be significantly decreased in B_6 deficient rats[62]. This situation can theoretically lead to incomplete digestion of food, greater duodenal cholecystokinin release, and stimulation of

pancreatic enzyme production, hypertrophy, and hyperplasia of exocrine tissue, thereby increasing the susceptibility of the pancreas to carcinogens. In fact, chronic hypercholecystokininemia has been shown to enhance pancreatic carcinogenesis in experimental animals[63,64]. Finally, animals receiving inhibitors of cellular methylation reactions develop acute hemorrhagic pancreatitis as a consequence of autolytic destruction of the pancreas[65-67] and it is well known that chronic pancreatitis increases the risk of pancreatic cancer.

3.3. Gastric, oral and pharyngeal cancer

Several case-control studies have found that high B_6 intake was associated with a decreased risk of gastric adenocarcinomas[68-70] and oral or pharyngeal cancer[71].

3.4. Lung cancer

A nested case-control study within the ATBC Cancer Prevention Study found a significantly lower risk of lung cancer among men who had higher plasma B_6 levels. Men presenting high B_6 plasma concentration had about half the risk of lung cancer compared to men with the lowest vitamin B_6 concentrations (OR = 0.51). This is the first report from a prospectively conducted study to suggest a role for vitamin B_6 in lung cancer[72].

3.5. Prostate and breast cancer

At present, there are no conclusive data relating vitamin B_6 levels and prostate or breast cancer[73,74]. One prospective, nested case-control study showed associations between the average intake of vitamin B_6 (highest *vs* lowest quintile) and the risk of breast cancer after adjusting for folate intake[75]. Thus, the authors suggest that folate and vitamin B_6 may have the potential to be chemopreventive against breast cancer.

4. Cognitive function

Early findings supporting the implication of vitamin B_6 status in neurocognitive functions are derived from:

a) animal experiments on B_6 deficiency[76-78];
b) reported cases of neurological abnormalities associated to clinical vitamin deficiency (peripheral neuropathy, seizures)[79];
c) familial studies on homozygous defects of genes encoding Hcy metabolism enzymes (such as cystathionine-β-synthase defect) which result in mental retardation, psychiatric disturbances and seizures (homocystinuria)[80,81].

4.1. Findings in the elderly

There are several studies providing evidence for the importance of vitamin B_6 and brain function in the elderly[82-84]. Significant correlations between vitamin B_6 status and memory were found in the Boston Normative Aging Study. Higher concentrations of serum vitamin B_6 were associated with better performance in two memory tests in men (aged 54-81 years) and this association was independent of plasma Hcy levels[82]. Others found a significantly higher Wechsler memory score in the top 90% of vitamin B_6 intake[83]. Furthermore, Deijen and coworkers reported that supplementation with vitamin B_6 could significantly improve memory in elderly men[84]. Additionally, one researcher showed a statistically significant improvement in most of the cognitive function tests in a group of elderly people, supplemented with multivitamins[85]. Subjects with lower blood levels of one or more nutrients showed lower responses on all cognitive-function tests, but there was no significant correlation between single nutrients and cognitive function test scores. This evidence favours the hypothesis that poor vitamin B_6 status could be, at least in part, responsible for the cognitive decline observed in some elderly persons. Further, several studies and meta-analyses have found inverse associations between objective measures of cognitive function and plasma or serum Hcy concentrations[82,86-94], but others not[95,96]. The increased risk for cerebrovascular disease could be due to a toxic effect of Hcy on vascular tissue[97]. Regarding dementia, the interaction between B_6 and Hcy is not clearly established. A recent study[98] showed a prevalence of low B_6 levels in 5.3% (B_6 < 30 nmol/L) of a Canadian long-term care population. 46 of 75 residents had a diagnosed dementia and 41.3% showed elevated Hcy levels (Hcy > 13.3 µmol/L), but no association with B_6. Nevertheless, low vitamin B_6 status has been associated with Alzheimer's disease (AD) and vascular dementia. Miller and others have recently reported that low PLP levels were strongly associated with AD, but not with cerebrovascular disease[99]. On the contrary, Faßbender and coworkers reported that compared with patients without cerebrovascular disease, patients with subcortical vessel encephalopathy showed significantly decreased plasma concentrations of vitamin B_6[100]. In both studies, there was an association between Hcy and AD and microangiopathic vascular dementia, respectively, while there was no correlation between Hcy levels and vitamin B_6. Others found that individuals with hyperhomocysteinemia had a higher risk of developing AD compared with those who had normal Hcy levels (OR = 4.6)[101]. Another study found a strong relationship between periventricular and subcortical white matter lesions and low vitamin B_6 levels in patients with AD[102].

4.2. Convulsive seizures

Since the fifties and sixties, PN deficiency is known to cause convulsive seizures in humans and experimen-

tal animals. Hence, a number of investigators reported disorders in central nervous system (CNS) activity in adults and infants[103-107]. The neurological disorders in the infants manifested itself within 6 weeks up to 4 months by convulsive seizures, hyperirritability, and abnormal acuteness of the sense of hearing. Two reviews from the eighties summarised research on causative factors responsible for convulsions during vitamin B_6 deficiency[108,109]. They propose two hypotheses explaining the onset of convulsive seizures. First, it has been shown that a particular nutritional deficiency leads to the accumulation of a potentially hazardous tryptophan metabolite, 3-HK in the CNS. Kynurenine transaminases and kynureninase catalyse are the main routes of 3-HK catabolism and require PLP. Reductions in their activities should lead to a build-up of 3-HK in the body and brain. Experimentally produced B_6 deficiency by Guilarte and Wagner caused a 200-fold increase in brain regional 3-HK levels. This increase led the authors speculate that such levels of 3-HK could be responsible for the convulsions that typically occur in infant B_6 deficiency[110]. Another possible cause could be changes in the concentration of the neurotransmitter GABA, a major inhibitory transmitter in the CNS. GABA concentrations are reported to be low in the brains of vitamin B_6 deficient infant animals, and such animals are often seen to convulse[111-113]. Furthermore, pharmacologic studies indicate that drugs diminishing GABA transmission in the CNS promote convulsions[114].

4.3. EEG alterations

Abnormal EEG-tracing changes were reported in young adult men during experimentally induced vitamin B_6 deficiency in the sixties[106,107]. The young men were maintained for 21 days on purified diets that provide daily intakes of ≤ 0.06 mg vitamin B_6 and either 30 or 100 g protein. The type of EEG abnormalities observed in the young men generally consisted of a slowing of activity with either increased or decreased wave amplitude, and minimal to marked build-up with hyperventilation, particularly in the frontal and parietal leads[106]. More recently studies show EEG abnormalities can develop in women with short-term vitamin B_6-depletion (< 2 weeks)[115]. Abnormal patterns could be reversed administering 0.5 mg vitamin B_6/day. EEG changes are probably caused by altered neurotransmitter metabolism in the brain and are thought to occur only in cases of frank B_6 deficiency. However, before ruling out the possibility of EEG abnormalities occurring at marginal levels of vitamin B_6 intake, research employing quantitative analysis of the EEG spectral data should be conducted[115]. To our knowledge such research is still lacking.

4.4. Neuropathies

In the sixties, peripheral neuropathies have been reported in PN deficiency[34]. Demyelination of peripheral nerves has been observed, which could be caused by a disturbance of sphingomyelin synthesis owing to a lack of PLP as cofactor for serine-palmityl-transferase. Recently, peripheral neuropathy caused by vitamin B_6 deficiency has been reported in patients on chronic peritoneal dialysis. Symptoms included paresthesia, burning and painful dysthestias, and thermal sensations[116]. Interestingly, Gorson and Ropper found in diabetic patients with distal sensory polyneuropathies as most common laboratory abnormalities low levels of vitamin B_6 or B_1. They concluded that the frequency of low vitamin levels suggests that vitamin B_1 and B_6 levels should be assessed in future studies of diabetic patients with distal sensory polyneuropathies[117].

5. CVD and Hcy

Suboptimal vitamin B_6 nutriture was first associated with vascular disease following development of atherosclerosis in monkeys fed a B_6 deficient diet[118]. Later, some researchers[119] reported that estimated dietary intake of vitamin B_6 and folate, and plasma concentrations of PLP and folate, were lower in patients who had myocardial infarction than in controls. They also reported that the frequency of myocardial infarction was negatively correlated with both folate and vitamin B_6 nutriture. Others confirmed the association of Hcy and vitamin B_6[31,119-126]. Results of epidemiologic studies suggest that moderately elevated plasma or serum Hcy levels are prevalent in the general population and are associated with increased risk for CVD, independent of classical cardiovascular risk factors[94,127-129]. It is still unknown if this is due to the effect of vitamin B_6 on platelet function[130-132], connective tissue[133], blood pressure[134], thrombogenesis[135] or, indirectly by causing hyperhomocysteinemia[136]. Further, low circulating vitamin B_6 levels have been associated with elevation of the inflammation marker C-reactive protein independently of plasma Hcy[137].

How to handle vitamin B_6 deficiency?

Although overt clinical deficiency of vitamin B_6 occurs only rarely, nutrition surveys indicate that vitamin B_6 intakes may be marginal or inadequate in segments of the population. Consequently, the presence of a subclinical deficiency may be fairly widespread. Especially in the elderly there is a high prevalence of deficient or borderline vitamin B_6 status. Herrmann and Knapp found vitamin B_6 deficiency in 23% of 65 – 75 year old and 40% in > 85 year old persons, respectively[138]. According to the SENECA study, 23.3% of the European elderly are B_6 deficient (PLP < 20 nmol/L)[139]. A borderline deficient status, which might persist in an individual for a long time without developing clinical manifestations, could have pathological

consequences. It may contribute to the development of certain diseases, as already stated above.

Treatment of B_6 deficiency

1) Treatment of clinical deficiency

Treatment of vitamin B_6 deficiency implies mega doses of this vitamin via oral. Usually around 50 mg/day are administered depending on the cause of the deficiency; higher doses are given if vitamin B_6 deficiency is related to medication use. Some drugs (table III) such as isoniacid, penicillamine, hydralazine, L-Dopa and cycloserine interfere with PLP reacting with the carbonyl groups[140,141]. The requirements of vitamin B_6 increase in eclampsia and preeclamsia[142,143] and haemodialysis. B_6-dependent syndromes, which require pharmacological doses of the vitamin, are seldom. These include cystathionine-β-synthase deficiency, PN dependent anemias (especially sideroblastic anemia), and homocystinuria. Treatment consists of very high pharmacological doses of PN[144]. Supplementation of PN hydrochloride in various medical conditions and their doses are shown in table IV.

2) Preventive treatment of borderline, subclinical deficiency

There are some studies showing that especially in the elderly there is a high prevalence of deficient or borderline vitamin B_6 status[138,139]. Since a subclinical vitamin B_6 deficiency has been associated to several chronic diseases especially in the elderly, a supplementation in this group seems to be indicated. While vitamin B_6 supplementation is an effective treatment for lowering excessively high Hcy levels in patients with PN-responsive homocystinuria due to cystathionine β-synthase deficiency[145,146], its role in the prevention or treatment of mild hyperhomocysteinemia remains unclear. Results of 11 studies[147-158] that investigated the effect of vitamin B_6 on fasting plasma Hcy concentrations are inconclusive. Seven of the aforementioned studies[147-149,151-155] found no change in fasting Hcy, and one found a significant increase[150]. Some studies found a significant reduction in fasting Hcy after vitamin B_6 supplementation[156-158]. The first of these studies, carried out in young women who had clinical and biochemical vitamin B_6 deficiency, found a significant lowering of fasting Hcy of 19.7% after 15 days of treatment with 20 mg PN hydrochloride/day[156]. The trial, however, was not blinded or placebo-controlled and had no washout period. The second study, a recent placebo-controlled trial, showed a significant lowering of fasting Hcy of 17% ($p < 0.011$) in response to extremely large doses (120 mg/day) of vitamin B_6 in healthy subjects[157]. McKinley et al. found that in healthy elderly persons who are folate and riboflavin replete, low-dose vitamin B_6 supplementation (1.6 mg/day) effectively lowers fasting Hcy levels by 7.5% ($p < 0.008$)[158]. Since there is more than one vitamin involved in Hcy metabolism, most intervention trials use a combination supplement containing folic acid, vitamin B_{12}, and vitamin B_6[159-161] and therefore it is somewhat difficult to conclude the effect of a single vitamin.

Prevention of B_6 deficiency

Dietary reference intakes (DRI)

According to data from nationally representative US surveys, the median daily intake of B_6 by men is approximately 2 mg/day and the median intake by women is approximately 1.5 mg/day. German data show

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Table IV
Supplementation of PN hydrochloride in various medical conditions (Frye, 2002)

Medical conditions	Doses
Cirrhosis	50 mg/day
Hemodialysis	5-50 mg/day
Peritoneal dialysis	2.5-5 mg/day
Chronic renal failure	2.5-5 mg/day
Sideroblastic anemia	50-600 mg/day
PN-dependent seizures	100 mg/day
Homocystinuria	100-500 mg/day
Homocysteinemia	100-500 mg/day
Gyromitra poisoning	25 mg/kg
Prophylactic administration should be provided when using:	
isoniacid	30-450 mg/day
penicillamine	100 mg/day
Estrogen-induced reduction in tryptophan metabolism may require supplementation	20-25 mg/day

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the same pattern[162]. This indicates that the general population is not at risk for developing clinical vitamin B_6 deficiency.

Further, the new DRIs set by different international organisations, are now taking into account new aspects to estimate requirements. They do not only focus on the prevention of clinical deficiency, but try to take into account health effects that certain nutrients might have. Even though there is evidence that vitamin B_6 could help to prevent CVD, certain types of cancer, and cognitive impairment, present data are insufficient to set EARs based on these new aspects. DRIs and DACH reference values for vitamin B_6 are summarised in table V. The recommended dietary allowance (RDA) for adults is 1.3 mg food vitamin B_6/day, which lies in the same range as the DACH-settings for men and women, 1.5 and 1.2 mg, respectively[163]. Most studies of B_6 requirements have focused on adults and have been depletion-repletion studies. Clinical symptoms of vitamin B_6 deficiency have only been observed during depletion with very low levels of B_6 and have never been seen at intakes of 0.5 mg/day or more, suggesting that an intake of 1 mg/day is sufficient for most adults[2]. The current RDA (1998) for older adults and elderly people (age > 51) are set higher than for younger adults, based on the results of several repletion-depletion studies. Ribaya-Mercado and coworkers employed a vitamin B_6 depletion-repletion protocol in healthy elderly and showed that higher than RDA (1989: men: 2.0 mg/day, women: 1.6 mg/day) intakes were necessary to normalise several parameters of vitamin B_6 status including enzyme activity, tryptophan metabolism, and plasma vitamers. In a related series of experiments, the amount of vitamin B_6 essential to restore several immunologic indices (lymphocyte number and percentages, mitogenic responses, and IL-2 production) to baseline values was also greater than the current DRI (1998)[40]. Higher levels of fasting insulin and plasma glucose were also observed during the periods of low vitamin B_6 status[164]. Despite evidence that the requirement for vitamin B_6 may be slightly higher in older adults, several surveys have found that over half of individuals over age 60 consume less than the current DRI (1.7 mg/day for men and 1.5 mg/day for women). Several studies have found elderly populations to have plasma PLP concentrations below the cut-off threshold for deficiency[165]. A high prevalence of marginal vitamin B_6 deficiency in the elderly is reflected by their reduced activity of α-EAST and α-EALT, increased excretion of XA, and lowered levels of PLP and plasma total vitamin B_6[166-168]. While 50 to 90% of older adults are reported to have die-

Table V
DRIs and DACH-Reference Values

Age	EAR[a] Men	EAR[a] Women	RDA[b]/AI* Men	RDA[b]/AI* Women	UL[c]	Age	Vitamin B_6 (mg/day) Men	Vitamin B_6 (mg/day) Women
0-6 months	–		0.1 (" 0.014 mg/kg)*		–	0-4 months	0.1	
7-12 months	–		0.3 (" 0.033 mg/kg)*		–	4-12 months	0.3	
1-3 years	0.4		0.5		30	1-4 years	0.4	
4-8 years	0.5		0.6		40	4-7 years	0.5	
9-13 years	0.8		1.0		60	7-10 years	0.7	
						10-13 years	1.0	
14-18 years	1.1	1.0	1.3	1.2	80	13-15 years	1.4	
						15-19 years	1.6	1.2
19-30 years	1.1		1.3		100	19-25 years	1.5	1.2
31-50 years	1.1		1.3		100	25-51 years	1.5	1.2
51-70 years	1.4	1.3	1.7	1.5	100	51-65 years	1.5	1.2
> 70 years	1.4	1.3	1.7	1.5	100	> 65 years	1.4	1.2
Pregnancy	1.6		1.9		100[1]	Pregnancy	1.9	
Lactation	1.7		2.0		100[1]	Lactation	1.9	

a Estimated average requirement
b Recommended dietary allowances
c Upper level of safe intake; intake of vitamin B_6 as pyridoxine
* Adequate Intake
1 > 19 years; < 19 years: 80 mg/day

tary intakes of vitamin B$_6$ below 1989 RDA levels, several studies indicate that the physiologic requirement may exceed these recommended values[169,170]. Considering this, it is not understandable why the DACH reference values are not set higher for older adults (> 51 years) or even lower for men of the age 71 and older.

Food fortification

The possibility to enrich food with vitamins allows it to increase the health status of the population. The United States and Canada started with the first official guidelines concerning the fortification of food with folic acid. Further, Hungary and Chile followed the guidelines and voluntary fortification is present in Switzerland, Australia and Great Britain. Due to the interrelationship of the vitamins that participate in the Hcy cycle, it has been debated if vitamin B$_6$ (and vitamin B$_{12}$) should be added to folate-enriched foods. From the different countries that enrich their flour with folate, only in Hungary vitamin B$_6$ is added (880 µg/100 g flour) beside vitamin B$_{12}$ with 0.8 µg and folic acid with 160 µg[171].

Toxicity

Values of maximum tolerable intakes are summarised in table VI. As a water-soluble vitamin which is rapidly metabolised and excreted, B$_6$ might be expected to have low toxicity. In fact, no adverse effects have been associated with high intake of vitamin B$_6$ from food sources. Schaumburg and others reported the development of severe sensory neuropathy in 7 patients treated with 2-6 g of PN hydrochloride/day[172]. Further, reports of peripheral sensory neuropathy associated with high-dose PN therapy (1 to 4 g/day) appeared also in the 1980s[173-176]. However, it is noteworthy that none of the reviews of patients with vitamin B$_6$ dependency syndromes, who are treated with 500-1,500 mg/day, mentions the development of peripheral neuropathy[177]. Some patients developed abnormally low plasma concentrations of PLP after high doses of vitamin B$_6$. This rebound avitaminosis presumably reflects induction of pyridoxal oxidase, and hence increased catabolism of the vitamin. Within 4 months, plasma concentrations of the vitamin return to normal without supplementation[178]. The limited data involving lower PN doses reveal that the risk of developing sensory neuropathy decreases rapidly at doses below 1 g/day[179,180]. The safe upper limit for vitamin B$_6$ has been set at 100 mg/day, taking into account a security factor of 5, because the lowest dose at which toxicity (sensory neuropathy) has been observed is 500 mg/day[2].

Table VI
Food sources of PN (vitamin B$_6$)
(modified after Elmadfa et al., 1998)

Food	Content (mg/100 g)
Meat	
Beef liver	0.90
Pork	0.39
Chiken	0.50
Beef	0.50
Fish	
Salmon	0.98
Tuna	0.46
Eggs and Dairy	
Egg	0.12
Whole milk	0.05
Cram cheese (20%)	0.09
Vegetables	
Spinach, raw	0.20
Potatoes, raw	0.21
Pes, green, raw	0.16
Broccoli, raw	0.17
Carrots	0.30
Fruits	
Apple, unpeeled, raw	0.10
Banana, raw	0.37
Grapes, raw	0.13
Walnuts	0.87
Cereals	
Wheat germ	4.00
Wheat flour, whole grain	0.46
Rye bread	0.20
Bread, white	0.04
Rice, white	0.18
Rice, brown	0.67
Miscellaneous	
Spaghetti with tomato sauce	0.12
Pizza, all kinds	0.41
Pancakes	0.02
Yeast	0.81
Human milk	0.01

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