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Mitochondrial function and toxicity: Role of the B vitamin family on mitochondrial energy metabolism

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Abstract

The B vitamins are water-soluble vitamins required as coenzymes for enzymes essential for cell function. This review focuses on their essential role in maintaining mitochondrial function and on how mitochondria are compromised by a deficiency of any B vitamin. Thiamin (B1) is essential for the oxidative decarboxylation of the multienzyme branched-chain ketoacid dehydrogenase complexes of the citric acid cycle. Riboflavin (B2) is required for the flavoenzymes of the respiratory chain, while NADH is synthesized from niacin (B3) and is required to supply protons for oxidative phosphorylation. Pantothenic acid (B5) is required for coenzyme A formation and is also essential for α-ketoglutarate and pyruvate dehydrogenase complexes as well as fatty acid oxidation. Biotin (B7) is the coenzyme of decarboxylases required for gluconeogenesis and fatty acid oxidation. Pyridoxal (B6), folate and cobalamin (B12) properties are reviewed elsewhere in this issue. The experimental animal and clinical evidence that vitamin B therapy alleviates B deficiency symptoms and prevents mitochondrial toxicity is also reviewed. The effectiveness of B vitamins as antioxidants preventing oxidative stress toxicity is also reviewed.

Keywords: B vitamins; Mitochondria; Oxidative stress; Vitamin B therapy

1. Introduction

The aim of this review is to better understand the relationship between clinical outcome and underlying mitochondrial disturbances due to B vitamin deficiency. The B vitamins are water-soluble vitamins required as cofactors for enzymes essential in cell function and energy production. For each vitamin, we review the biochemical evidence of absorption, metabolism and the role of the active form of B vitamins on cellular function, focusing on reactions relevant to mitochondrial activity and energy metabolism. In this review the term mitochondrial damage refers to disorders of mitochondrial integrity and to reactions leading to or involved in energy production. The term mitochondrial toxins refers to molecules, exogenous or endogenous, that are known to affect mitochondria or those reactions. Thiamin (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5) and biotin (B7) are reported here, while pyridoxal (B6), folate and cobalamin (B12) are reported elsewhere in this issue [1]. We report on current and possible biomarkers for detection of each of the deficiencies and on evidence of the therapeutic potential of vitamin supplementation. The structures of the vitamins are shown in Table 1. Table 2

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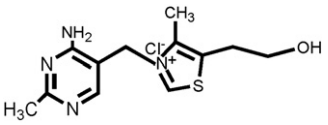
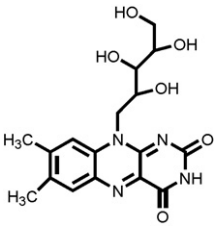
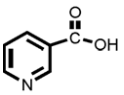
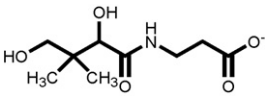
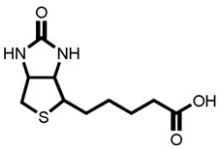
Table 1
Chemical structures of B vitamins

Type	Name	Chemical structure
B1	Thiamin	
B2	Riboflavin	
B3	Niacin	
B5	Pantothenic acid	
B7	Biotin	

summarizes the clinical conditions generally associated with specific B vitamin deficiencies, as well as suggested associations. Table 3 lists a number of inborn errors of metabolism that are linked to B vitamin deficiency, either through inhibition of vitamin metabolism or a vitamin-dependent pathway. The data collected in these tables were mostly obtained from references [2,3].

2. Thiamin (Vitamin B1)

Thiamin is active in the form of thiamin pyrophosphate (TPP). As a cofactor, TPP is essential to the activity of cytosolic transketolase and pyruvate dehydrogenase, as well as mitochondrial dehydrogenases α-ketoglutarate dehydrogenase and branched-chain ketoacid dehydrogenase. Vitamin B1 was among the first vitamins discovered and was identified as the dietary factor responsible for beriberi and missing in polished rice. Since then several additional conditions resulting from thiamin deficiency have been observed in normal populations and in populations affected by genetic mutations (Tables 2 and 3). Most recently, we suggested a link between thiamin deficiency and colon carcinogenesis [4].

2.1. Thiamin delivery from the diet to the mitochondria

Thiamin is found in raw foods, such as cereals, green vegetables, nuts, egg yolk and pork meat. Little is found in refined foods, such as sugar, fat or alcohol, as well as foods heated for a long time or at high temperature. Processed cereal products (e.g. flour, bread, cereals) have been fortified with thiamin since the early 1940s. Thiamin uptake is enhanced by thiamin deficiency and is decreased by thyroid hormone, diabetes, ethanol and age. Chronic alcohol consumption is the most common cause of acute thiamin deficiency in affluent societies [2,3].

Thiamin absorption from the diet takes place primarily in the proximal small intestine. Thiamin is positively charged and at low concentrations the vitamin's movement across cellular membranes requires saturable high affinity, low capacity transporters, i.e. THTR1 and THTR2. The entry of thiamin across the brush border occurs via THTR1, a transporter that saturates in the micromolar range and is inhibited by thiamin analogues. Uptake by THTR2 system is saturable in the nanomolar range [5]. Human intestinal cells (Caco2 cells in culture) have both thiamin uptake systems in a functional state [5], suggesting that this system is capable of absorbing thiamin at physiological concentrations, estimated at 0.1–2.0 μM. At higher concentrations, however, thiamin uptake appears to be through simple passive diffusion [6]. The colon can also absorb thiamin via THTR1 and a thiamin/H^+ exchange, which appears to be under the regulation of an intracellular Ca^{2+}/calmodulin-mediated pathway [7] and could be a route for absorption of bacterially synthesized thiamin. The exit of thiamin from the enterocyte to the blood stream by the basolateral membrane is Na^+ dependent, directly coupled to ATP hydrolysis by Na^+/K^+ ATPase, and is inhibited by thiamin analogues [6]. The total thiamin in the body is only about 30 mg [8], with 40% in the muscle and stores also in the brain, heart, liver and kidney.

As shown in Fig. 1, cells take up plasma thiamin via THTR1. Plasma thiamin monophosphate (TMP) is taken up by cells via THTR1 and reduced folate carrier (RFC1). RFC1 is an anion exchanger that

Table 2
Clinical disorders associated with vitamin deficiencies

B cofactor/substrate	Established condition of deficiency	Suggested condition of deficiency
Thiamin (B1)	Beriberi (peripheral neuropathy, cardiomyopathy with cardiac hypertrophy and dilatation) Polyneuritis Wernicke–Korsakoff encephalopathy	Alzheimer's disease Cataract Colon cancer Atherosclerotic vascular diseases Diabetes and its complications Fetal alcohol syndrome
Riboflavin (B2)	Seborrheic dermatitis Anemia Neuropathy Deficiency in upper GI crypt fission	Esophageal cancer Parkinson's disease
Niacin (B3)	Pellagra (dermatitis, dementia and diarrhea) Mental depression Dementia, insomnia, delusions	Esophageal cancer Other cancers Leukemia
Pantothenic acid (B5)	Dermatitis Hypoglycemia, convulsions Encephalopathy with liver failure	
Biotin (B7)	Cutaneous (skin rashes, alopecia, conjunctivitis) Neurological (depression, seizures, paresthesias) Diabetes	Congenital abnormalities Insulin resistance

This table is a list of clinical consequences of deficiency of thiamin, riboflavin, niacin, pantothenic acid and biotin. The links between the clinical symptoms and vitamin deficiency were based on clinical measurements in an affected population, regression of symptoms upon vitamin supplementation, or animal models. Conditions that have been established are listed on the left, and others that have been suggested are found on the right.

transports reduced folate and TMP but not thiamin itself [9]. Intracellular thiamin is rapidly phosphorylated to form thiamin diphosphate (TPP). The reaction is catalyzed by thiamin diphosphokinase located in the cytosol. Vitamin B1 is present in the body as thiamin or its phosphorylated forms, TMP or TPP.

TPP is rapidly taken up by mitochondria. The transfer occurs via a TPP/thiamin antiporter and may be the same high affinity transporter (THTR1, THTR2) found in the

Table 3
Genetic disorders associated with vitamin deficiencies

Disease	Vitamin	Genetic mutation	Vitamin link
Breast cancer	B1	THTR2	Absorption
Cancer	B1	TKTL1	Cofactor
Diabetes	B1	TKTL1	Cofactor
Glutaric aciduria	B2	ETFA	Cofactor
Hartnup disorder	B3	SLC6A19	Tryptophan absorption
Leigh syndrome	B1	PDH	Cofactor
Maple syrup urine	B1	BCKDH	Cofactor
Multiple carboxylase deficiency	B7	Biotinidase, HCS	Metabolism, metabolism
Neurodegenerative diseases	B1, B5	TKTL1, PANK2	Cofactor metabolism
Propionic aciduria	B7	PCC	Cofactor
Rogers syndrome (TRMA)	B1	THTR1	Absorption
Sudden infant death syndrome (SIDS)	B1, B7	TK, PC	Cofactor, cofactor
Wernicke predisposition	B1	TK	Cofactor

This table is a list of genetic disorders (often present as autosomal recessive mutations) affecting absorption, metabolism or cofactor activity of B vitamins. *Note*: THTR1/THTR2, thiamin transporter; TKTL1, transketolase-like; ETFA, electron transport flavoprotein; SLC6A19, solute carrier (neutral amino acid transporter); PDH, pyruvate dehydrogenase; BCKDH, branched-chain ketoacid dehydrogenase; HCS, holocarboxylase synthetase; PANK2, pantothenate kinase; PCC, propionyl-CoA carboxylase; TRMA, thiamin responsive megaloblastic anemia; TK, transketolase; PC, pyruvate carboxylase.

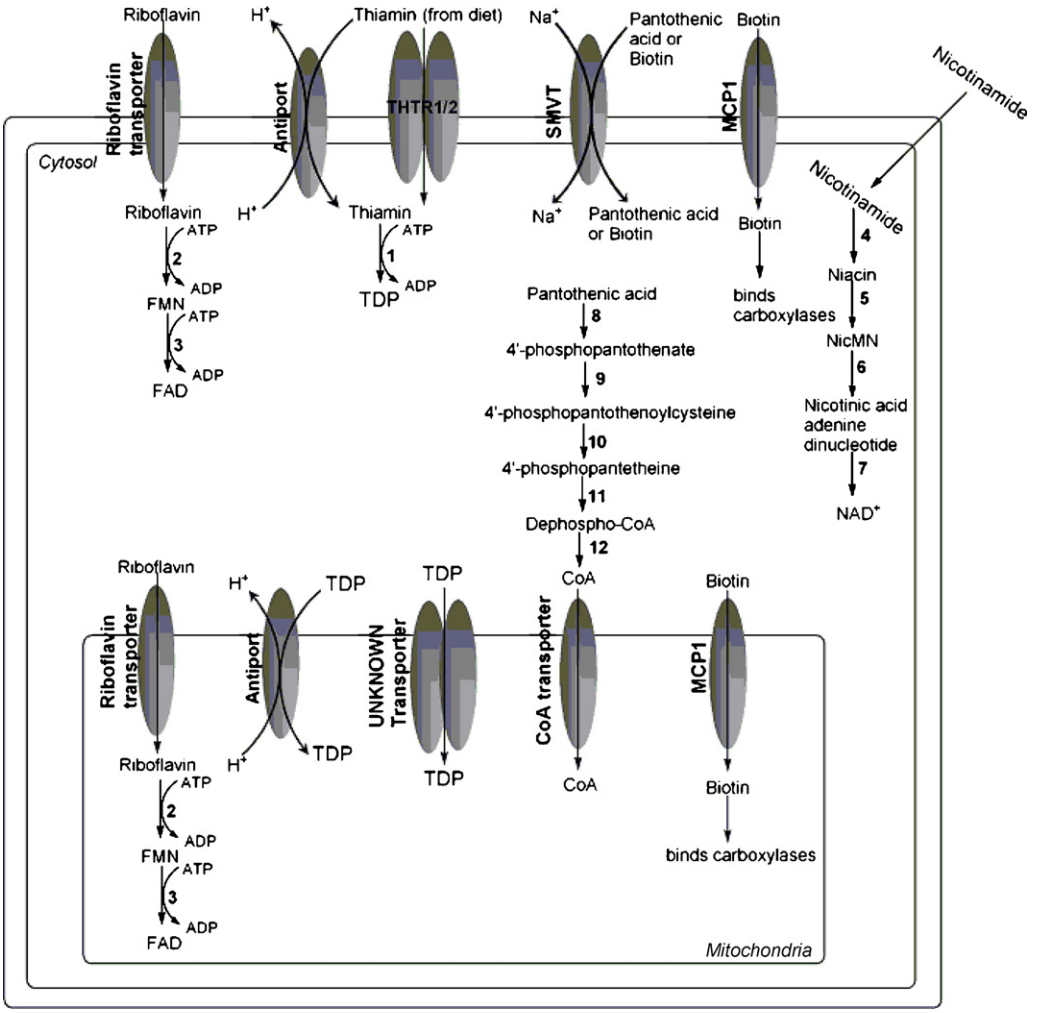


Fig. 1. Vitamin absorption and biosynthesis of active factors. Thiamin enters the cell via THTR1/2 cell membrane receptor or the antiport. (1) Thiamin diphosphokinase. Thiamin diphosphate (TDP) enters the mitochondria via the antiport or an unknown transporter. Riboflavin is taken up by hepatocytes by a riboflavin transporter that is energy dependent and is regulated by Ca^{2+}/calmodulin; (2) flavokinase; (3) FAD synthetase. The riboflavin taken up forms flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) via riboflavin kinase and FAD synthase, respectively. Niacin is taken up by cells as nicotinamide by simple diffusion and is converted to NAD^+ by a series of reactions; (4) deaminase; (5) nicotinic phosphoribosyl transferase; (6) NMN ATPase; (7) NAD synthetase. NAD^+ is trapped in the cells as it cannot permeate the cell membranes or the mitochondrial membrane. The NADH generated in the cytoplasm by glycolysis is oxidized by the respiratory chain by the glutamate/aspartate shuttle, and dihydroxyacetone phosphate (DHAP) shuttle, which are used predominately in the liver and muscle, respectively. Pantothenic acid and biotin are taken up by the sodium-dependent multivitamin transporter (SMVT). (8) Pantothenate Kinase (PANK) ATP dependent; (9) PP cysteine synthetase (PPCS) ATP dependent; (10) PP cysteine decarboxylase (PPCDC); (11) PP adenyltransferase (PPAT) ATP dependent; (12) Dephospho-CoA kinase ATP dependent. CoA transporter is energy-dependent and is sensitive to electrochemical and pH gradients. Biotin can also be transported into the cell via the monocarboxylate transporter (MCP1), which is present in both the mitochondrial and plasma membrane. Biotin binds to carboxylases. When carboxylases turnover by proteolysis the biotin-ε-aminolysine is released and biotin is regenerated by hydrolysis catalyzed by biotinidase.

plasma membrane [10,11]. In the matrix, where TPP is mostly bound to enzymes, the intracellular TPP concentration is estimated to be 30 μM with only 2 μM unbound [12] and an estimated 30% of cellular TPP located in the mitochondria. Mitochondrial TPP can also undergo hydrolysis by thiamin pyrophosphatase to form TMP. TMP can efflux the mitochondria and then be hydrolyzed by a cytosolic TMP phosphohydrolase to form thiamin.

2.2. Essential role of thiamin in mitochondrial and cellular functions

TPP is involved in dehydrogenase reactions as a cofactor in three mitochondrial enzyme complexes: pyruvate, α-ketoglutarate and branched-chain ketoacid dehydrogenases (PDH, KGDH and BCKDH, respectively). These catalyze the oxidative decarboxylation of

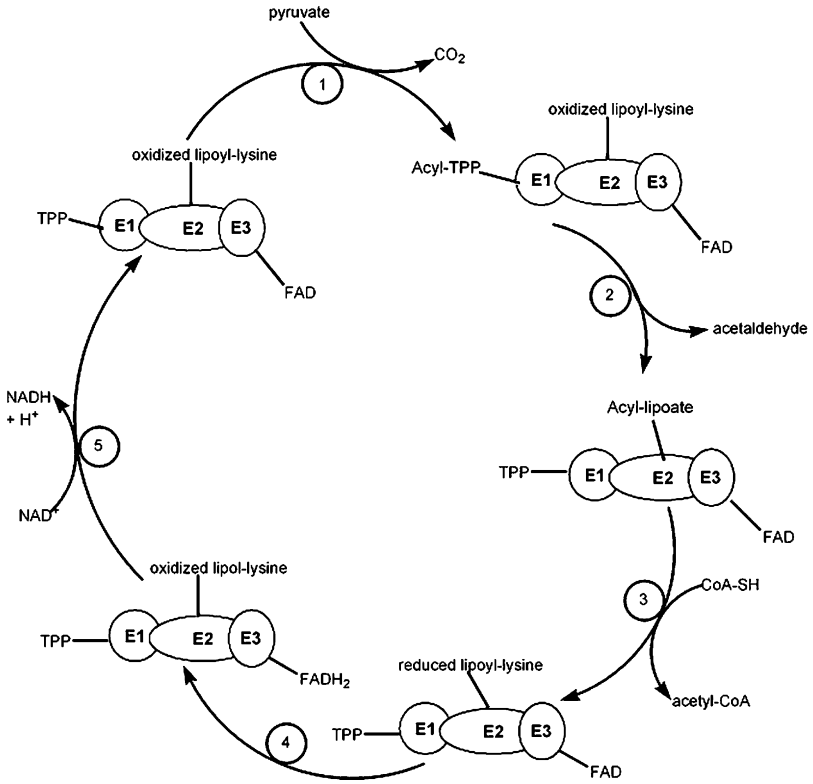


Fig. 2. Dehydrogenase enzyme complex. The oxidative decarboxylation of pyruvate (in this example) is catalyzed by a multi enzyme complex containing: (E1) pyruvate dehydrogenase, (E2) dihydrolipoyl transacetylase and (E3) dihydrolipoyl dehydrogenase. The cycle follows five steps: (1) decarboxylation of pyruvate is catalyzed by E1 (cofactor: TPP), (2) acetaldehyde reacts with the oxidized lipoyllysine, (3) acetaldehyde is transformed into acetyl-CoA by E2 (cofactor: lipoic acid), (4) FAD (cofactor) oxidize lipoyllisine catalyzed by E3, (5) NAD^+ (cofactor) oxidize reduce $FADH_2$ back to FAD. It is important to note the role not only of TPP (E1), but also CoA (E2), FAD and NAD^+ (E3) for both catalytic activity and regeneration of the enzyme.

α-ketoacids to release CO_2. The ketoacids include pyruvate, the isocitrate metabolite, α-ketoglutarate and the branched-chain amino acid metabolites, ketoisovalerate, ketoisocaproate and ketomethylvalerate. The three enzyme complexes have similar structures and mechanisms. Fig. 2 shows the details of the five-step process of the dehydrogenase complex activity. In the process, NAD^+ is converted to NADH, which can be reoxidized to NAD^+ by the mitochondrial electron transport chain to generate three ATP molecules for each NADH formed. Pyruvate, the endproduct of glycolysis, forms acetyl-CoA, the entry point of the tricarboxylic acid (TCA) cycle. The third step of the TCA cycle involves α-ketoglutarate metabolism to succinyl-CoA. As part of amino acid catabolism, α-ketoisocaproate, α-keto-β-methylvalerate and α-ketoisovalerate are transformed by BCKDH into isovaleryl-CoA, α-methylbutyryl-CoA and isobutyryl-CoA, respectively. Isovaleryl-CoA and isobutyryl-CoA can then form succinyl-CoA, which is part of the TCA cycle. The three enzyme complexes are thus central to mitochondrial energy production.

Thiamin pyrophosphate is also a cofactor of transketolase (TK), a reversible cytosolic enzyme that catalyzes the first and last step of the pentose phosphate pathway which plays a major role in cellular function in the production of NADPH for maintaining cellular redox, glutathione (GSH) levels and protein sulphydryl groups, as well as fatty acid synthesis. The pentose pathway also supplies ribose for nucleic acid synthesis. In this enzymatic system, TPP serves as a cofactor that helps transfer a glycolaldehyde from a ketose donor to an aldose acceptor. The thiazole ring of TPP does this by forming a complex with the xylulose phosphate substrate releasing glyceraldehyde-3-phosphate and forming a thiamin/glycolaldehyde complex. The glycolaldehyde is then transferred to ribose phosphate to form sedoheptulose phosphate.

2.3. Thiamin deficiency

Vitamin B1 was among the first vitamins discovered and was identified as the dietary factor responsible for

beriberi and missing in polished rice. Since then several additional conditions resulting from thiamin deficiency have been observed in normal populations and in populations affected by genetic mutations (Tables 2 and 3). From animal studies, we also suggested a link between thiamin deficiency and colon carcinogenesis [4]. Thiamin status is commonly estimated through measure of plasma thiamin, or erythrocyte transketolase activity. Although TK activity does not always correlate with reported thiamin intake, it is a good marker of biochemical thiamin status. Inhibition of this and other thiamin-dependent enzymes is a clear sign of a thiamin deficient status. Other assays of thiamin status include measurement of urinary thiamin excretion, as well as plasma pyruvic or lactic acid concentrations after exercise or glucose load.

A measure of thiamin deficiency is afforded by evidence of oxidative stress in thiamin-responsive neuropathology. The antioxidative stress properties of thiamin become apparent during thiamin deficiency when biomarkers indicate oxidative stress occurs in vivo at or before pathological changes occur. Early animal studies, in which diets very low in thiamin were prepared by autoclaving, caused central nervous system damage, but no apparent peripheral neuropathy. It was only when the marginal thiamin deficiency was extended over longer periods of time that animals with the degenerated peripheral nerves and enlarged hearts were observed (reviewed by Carpenter [13]). Marginal thiamin deficiency combined with various sources of oxidative stress or glucose loading can produce the neurodegenerative changes similar to those seen in Alzheimer's disease, Parkinson's disease, Huntington's disease and the Wernicke–Korsakoff syndrome [14].

Thiamin deficiency can also be assessed by decreased activities of thiamin-dependent enzymes in the brain. In the rat, thiamin deficiency reduces brain KGDH more markedly than PDH activity [15]. The reduced activity was observed after 12 days on a deficient diet with a daily administration of the thiamin analogue and antagonist, pyrithiamine, when the animals had lost their righting reflex [16]. By 14 days brain TK activity and protein had declined by 40% and was accompanied by neuronal cell loss and pathology similar to that of Wernicke–Korsakoff syndrome. The location of the sites of TK inhibition, however, did not correlate with the sites of brain damage suggesting that cytotoxicity is more likely related to KGDH inhibition. In thiamin-responsive areas of the brain, thiamin deficiency is associated with induction of oxidative stress enzymes, such as heme oxygenase-1 or superoxide dismutase, nitration products, such as peroxynitrite, nitrotyrosine and lipid peroxidation products, such as 4-hydroxynonenal which eventually resulted in neuronal cell death [17].

Secondary metabolites and by-products provide a still further assessment of thiamin deficiency. Lactate concentrations are increased in thiamin-responsive areas of the brain. In a rat model of thiamin deficiency, concentrations of dopamine and intermediate metabolites of dopamine catabolism are increased in the thalamus whereas noradrenaline is decreased in the thalamus and hypothalamus [18]. Metabolic enzymes involved in this process, such as mitochondrial monoamine oxidase (MAO) or cytosolic catechol-*o*-methyltransferase (COMT), however were not affected [18]. Thiamin deficiency has long been known to increase the concentrations of pyruvic acid and lactic acid in the blood [19]. Methylglyoxal concentrations are increased in the urine and cerebrospinal fluid in thiamin deficiency [20], which has been associated with inhibition of the methylglyoxal detoxification enzyme, glyoxalase. Glyoxalase is not a thiamin-dependent enzyme, but thiamin deficiency decreased levels of reduced glutathione, the cofactor for glyoxalase. We recently reported that thiamin deficiency in the rat was followed by increased plasma levels of both glyoxal and methylglyoxal, as well as both their advanced glycation endproducts (AGE) adducts [21]. Isolated rat hepatocytes became susceptible to glyoxal and cellular GSH levels were decreased under thiamin deficient conditions [22].

2.4. Prevention of oxidative stress and mitochondrial toxicity by thiamin

Thiamin can act directly as an antioxidant. At 100 μM it prevents microsomal lipid peroxidation as well as oleic acid oxidation. In the process, thiamin is oxidized to form thiochrome (by tricyclic thiamin formation) and thiamin disulfide (by opening of the thiazole ring). This antioxidant effect probably involves the successive transfer of protons from the pyrimidine NH_2 group and thiazole ring [23]. Thiamin is also oxidized by H_2O_2/peroxidase or ascorbate/Cu or lipid peroxidation [24].

Three potentially important consequences of thiamin deficiency on enzymatic antioxidant activity have been described. Neurodegenerative diseases, such as Alzheimer's, Parkinson's, prion diseases and ALS are associated with increased brain protein/Cu complexes and mitochondrial dysfunctions [25]. Interestingly, copper added to neuroblastoma cells markedly increased mitochondrial reactive oxygen species (ROS) formation and inhibited PDH, KGDH and respiratory complex I [26]. Supplementation with the mitochondrial dehydrogenase cofactors, lipoic acid or thiamin, prevented

copper cytotoxicity and dehydrogenase inhibition [27]. Thiamin pyrophosphate also prevented the inhibition of KGDH-mediated mitochondrial respiration by H_2O_2 [28]. Thiamin also prevented hepatocyte cytotoxicity and formation of reactive oxygen species induced by the mitochondrial respiratory inhibitors rotenone or cyanide [29].

Thiamin can also prevent renal stone formation. The molecular basis of this phenomena has not been fully clarified, however, it is known that mitochondrial TPP dependent enzymes on the oxalic acid pathway include hydroxypyruvate decarboxylase (α-ketoglutarate: glyoxalate carboligase), an activity of the decarboxylase moiety of KGDH complex [30] and that thiamin deficiency inhibits hepatic carboligase activity. This may explain why thiamin deficiency increased glyoxalate and oxalic acid levels. Glyoxalate accumulation in the mitochondria could then react with oxaloacetate to form oxalomalate, which causes mitochondrial toxicity and ATP depletion [31]. Oxalomalate is a competitive inhibitor of mitochondrial $NADP^+$-dependent isocitrate dehydrogenase that supplies NADPH, which helps maintain GSH in the reduced form. It is also a competitive inhibitor of aconitase, a rate-limiting enzyme of the TCA cycle [32]. Thiamin supplementation could thus prevent this cascade of events by reducing glyoxalate production.

Thiamin may also be important in diabetes risk. It is known that a high glucose concentration increases apoptosis in mammalian cells cultured in vitro, and that apoptosis is inhibited by increased thiamin [33]. Thiamin therapy can also counter the development of streptozotocin-induced diabetes in rats [34] as well as complications, such as dyslipidemia, atherosclerosis or nephropathy in rodent models. Thiamin pyrophosphate was found to be better than the antidiabetic agent aminoguanidine at preventing the non-enzymatic oxidative glycation of proteins by glucose. The mechanism is unknown but is likely to involve its amine group forming Schiff bases with the carbonyls of open-chain sugars, dicarbonyl fragments, Amadori products or with post-Amadori intermediates, thus preventing AGE formation [35]. Of particular interest is that TPP, and other thiazolium derivatives, such as ALT711 (alagebrium chloride), can selectively cleave AGE derived protein crosslinks that involve an alpha-diketone moiety [35].

Thiamin therapy appears to be effective in alleviating a wide range of chronic conditions. Treatment, however, is often inadequate or delayed. It can be effective in the early stages, but delayed treatment often results in permanent damage, as observed in Korsakoff syndrome. It is important that physicians be aware of which patients could be susceptible to thiamin deficiency and that they recognize the symptoms as early as possible.

3. Riboflavin (Vitamin B2)

Riboflavin is a precursor to flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). As prosthetic groups they are essential for the activity of flavoenzymes including oxidases, reductase and dehydrogenases.

3.1. Riboflavin delivery from the diet to mitochondrial enzymes

Most plant and animal tissue contain at least small amounts of riboflavin. Major sources of riboflavin are eggs, lean meats, milk, broccoli and enriched bread and cereal products. Riboflavin is easily destroyed upon exposure to light.

Riboflavin bound to proteins, and FMN and FAD in flavoproteins are released by stomach acid and gastric/intestinal proteases. The FMN and FAD released are hydrolyzed by alkaline phosphatase and FMN/FAD pyrophosphatases on the brush border of the ileum enterocyte to free riboflavin that is transported into the enterocyte by an energy-dependent and sodium independent riboflavin transporter. Most of the riboflavin effluxes across the enterocyte basolateral membrane into the portal blood and to the liver using the riboflavin transporter. The liver is the body's main storage site, storing riboflavin mostly as FAD. The spleen, kidney cardiac muscles are also stores which protect these tissues from riboflavin deficiency. The circulating plasma contains 50% riboflavin, 40% FAD, 10% FMN, with a total concentration of 0.03 μM riboflavin [2].

As shown in Fig. 1, riboflavin is taken up by the hepatocyte riboflavin transporter that is energy dependent and is regulated by an intracellular Ca^{2+}/calmodulin. On entry the riboflavin is phosphorylated by ATP and flavokinase to FMN and then converted to FAD by ATP and FAD synthetase. The mitochondria likely take up riboflavin by a riboflavin transporter and then form FMN and FAD as they contain riboflavin kinase and FAD synthetase. The FAD may then be incorporated into newly imported apoflavoproteins. Alternatively, FAD may be converted to riboflavin in the outer mitochondrial membrane. An FAD carrier, Flxlp, may efflux the FAD from the mitochondria into the cytosol and from the plasma membrane into the plasma [36].

3.2. Essential role of riboflavin in mitochondrial energy production and cellular function

FAD and FMN act as electron carriers. FAD or FMN are essential prosthetic groups of flavoenzymes which are reversibly reduced by NAD(P)H or succinate. Flavin oxidases use oxygen as an electron acceptor and transfer two electrons to form H_2O_2 or four electrons to form water. An example of flavin oxidase is glycolate oxidase, which use FMN to form glyoxylate from glycolate. Flavin reductases catalyze the reduction of substrates. $NAD(P)H + H^+$ reduces FAD to $FADH_2$ which then reduces cytochrome or oxidized glutathione (GSSG). A typical example of a flavin reductase is glutathione reductase, using NADPH as reducing substrate. Flavin dehydrogenases catalyze the removal of hydrogen from a substrate and the transfer of the hydrogen to an acceptor in an oxidation–reduction reaction.

Mitochondria contain five acyl-CoA dehydrogenases that require FAD. They are isovaleryl CoA dehydrogenase (IVDH), branched-chain acyl CoA dehydrogenase (BCADH), 2-methyl-branched-chain acyl CoA dehydrogenase (MBCADH), isobutyryl CoA dehydrogenase (IBDH) and short-chain acyl CoA dehydrogenase (SCADH). These dehydrogenases catalyze the first step in each cycle of β-oxidation. For example, acyl CoA dehydrogenases catalyze α-β-dehydrogenation of acyl CoA thiol esters to the corresponding trans-2,3-enoyl CoA products with concomitant reduction of enzyme-bound FAD. The reoxidation of the flavin involves transfer to the electron transferring flavoprotein. Protoporphyrinogen oxidase requires FAD and catalyzes the oxidation of protoporphyrinogen to protoporphyrin needed for heme/cytochrome synthesis.

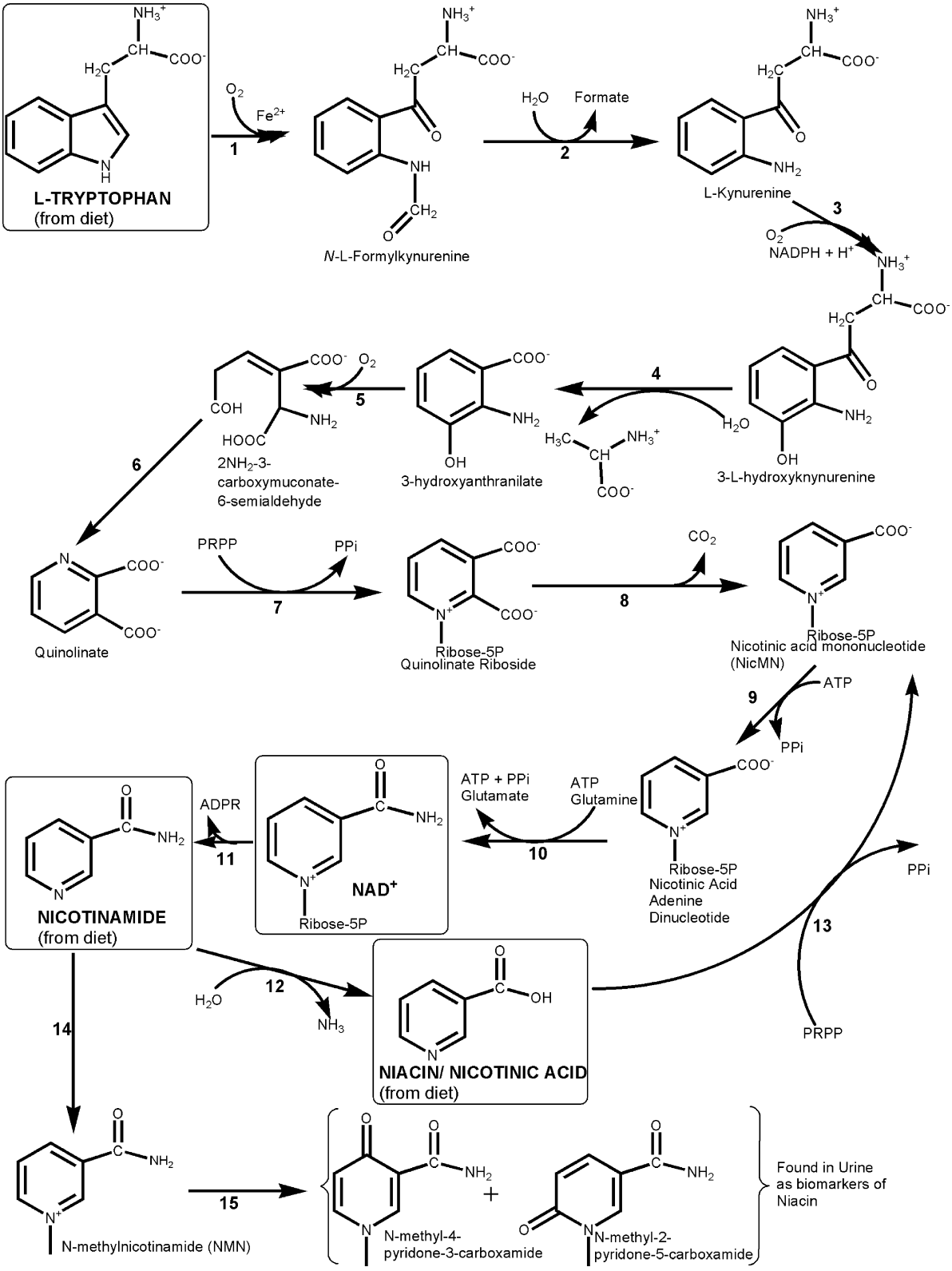
3.3. Riboflavin deficiency

Riboflavin was discovered shortly after thiamin. In experimental animals, it was found that a diet fraction that promoted growth consisted of a heat-labile fraction (thiamin) and another heat stable fraction that had a yellow color, riboflavin [2]. A typical symptom of ariboflavinosis is inflammation of the lip and tongue. Other conditions associated with riboflavin deficiency are listed in Tables 2 and 3. Riboflavin status is commonly assessed by measuring erythrocyte glutathione reductase activity, or erythrocyte or urinary flavin levels.

FAD-dependent dehydrogenases are affected by riboflavin deficiency. Rats deprived of riboflavin firstly undergo a drastic decrease in acyl-CoA dehydrogenase activities, particularly short chain acyl-CoA and isovaleryl-CoA dehydrogenases. Addition of FAD restored only 10–25% of the dehydrogenase activity, attributed to a large loss of apoenzyme in the FAD deficient mitochondria [37]. As a result of dehydrogenase inhibition, the mitochondrial oxidation of fatty acids and branched chain amino acids were severely inhibited [38]. In rats, riboflavin deficiency inhibited succinate dehydrogenase and β-oxidation of fatty acids with the consequence of increased $18{:}2n-6$ and decreased $20{:}4n-6$ fatty acids levels [39]. The unmetabolized long-chain fatty acids are ω-oxidized to medium chain dicarboxylic acids, which can accumulate, with an increased excretion in peroxisome β-oxidation of fatty acids resulting from a decreased mitochondrial response [37].

Vitamin B2 is a cofactor for mitochondrial protoporphyrinogen oxidase. In brain tissue, ATP production pathways are selectively preserved with B2 deficiency. There is, however, a decrease of the less critical FMN/FAD dependent pathways, leading to decreased ferritin synthesis and hemin catabolism, increased cytosolic free iron levels and H_2O_2 accumulation, and decreased glutathione levels [40]. Iron metabolism is also affected by riboflavin deficiency. Riboflavin deficiency decreased heme formation in red blood cells resulting in normochromic, normocytic anemia. In other cells, the deficiency is expressed as a loss of mitochondrial complex IV, induction of oxidative stress and increased concentrations of free iron [41]. In riboflavin responsive anemia, ferritin iron mobilization is decreased as is iron absorption. There is thus increased intestinal iron loss.

Riboflavin deficiency has still other enzymatic effects. Glutathione reductase (NADPH: glutathione oxidoreductase) requires FAD as a prosthetic group. Riboflavin deficiency results in decreased FAD levels. A consequence of this is that glutathione reductase activity and levels of reduced glutathione are lower [42]. Riboflavin deficiency also decreases the activities of glutathione peroxidase (H_2O_2: glutathione oxidoreductase), catalase, aldose reductase (alditol: NADP oxidoreductase) and sorbitol dehydrogenase [42] without affecting superoxide dismutase activity [43]. Similarly, the mitochondrial oxidation of succinate, glycerophosphate, β-hydroxybutyrate, ketoglutarate, glutamate, pyruvate and malate through their FAD-dependent dehydrogenases was decreased by 50% in a rat model of riboflavin deficiency [44]. Folate metabolism is also affected by riboflavin deficiency. Decreased FAD levels inhibited MTHFR activity which decreased dTTP


L-TRYPTOPHAN
(from diet)
O2
Fe2+
1
N-L-Formylkynurenine
H2O
Formate
2
L-Kynurenine
3
O2
NADPH + H+
3-L-hydroxyknynurenine
4
H2O
3-hydroxyanthranilate
5
O2
2NH2-3-carboxymuconate-6-semialdehyde
6
Quinolinate
PRPP
PPi
7
Ribose-5P
Quinolinate Riboside
8
CO2
Ribose-5P
Nicotinic acid mononucleotide (NicMN)
9
ATP
PPi
Ribose-5P
Nicotinic Acid Adenine Dinucleotide
ATP + PPi
Glutamate
ATP
Glutamine
10
NAD+
Ribose-5P
ADPR
11
NICOTINAMIDE
(from diet)
H2O
12
NH3
NIACIN/ NICOTINIC ACID
(from diet)
13
PPi
PRPP
14
15
N-methylnicotinamide (NMN)
N-methyl-4-pyridone-3-carboxamide
N-methyl-2-pyridone-5-carboxamide
Found in Urine as biomarkers of Niacin

synthesis, CpG island methylation, increased uracil incorporation and the risk for chromosome breaks [1]. Another consequence of FAD deficiency is increased circulating homocysteine levels. Other observed effects are on peripheral nerve demyelination, thyroxine metabolism, increased DNA strand breakage and increased induction of DNA repair enzymes [39]. FAD can also have an impact on choline metabolism through the coenzyme role to sarcosine dehydrogenase. Thus, all methyl-carrier systems can be affected by riboflavin deficiency. Although FAD is also involved in methylcobalamin synthesis, there is no clear evidence that riboflavin deficiency leads to cobalamin deficiency. Finally, FAD is a cofactor of the erythrocyte pyridoxamine phosphate oxidase, the enzyme that catalyzes the phosphorylation of pyridoxamine or pyridoxine phosphate to the active pyridoxal phosphate. Riboflavin deficiency has been found to induce pyridoxal deficiency [39].

3.4. Prevention of oxidative stress and mitochondrial toxicity by riboflavin

The potential for riboflavin therapy has been reported in rodent studies [45,46]. The authors showed an improvement in mitochondrial respiration (electron transport chain) with supplementation of riboflavin in combination with other energy modulating vitamins. Riboflavin therapy was also shown to improve the chemotherapeutic effect of tamoxifen in a rat model of breast carcinogenesis [45]. A few human case reports have also been published in the recent years [47,48]. The authors showed, in the three patients reported, that riboflavin therapy was effective in cases of multiple acyl-CoA dehydrogenase deficiency. Treatment both recovered the enzyme activity and improved complexes I and II of mitochondrial electron transport chain. Similar effects were reported with riboflavin therapy alone or in association with other vitamins for treatment of complex I deficiency (reviewed in ref. [49]). General improvements of muscular tone and exercise capacity were noted, suggesting riboflavin supplementation as a promising additive to treatment of myopathies.

4. Niacin (Vitamin B3)

Niacin is a precursor to reducing groups nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$). These molecules are involved in more than 500 enzymatic reactions. For the focus of this review, it is important to note that NAD/NADP are involved in reactions pertaining to mitochondrial respiration, glycolysis or even lipid β-oxidation.

4.1. Niacin delivery from the diet to mitochondrial enzymes

Foods rich in niacin include mixed dishes rich in meat, fish or poultry, whole or enriched grain cereal and bread products, and most fresh foods. Cereal products are fortified with niacin (nicotinic acid); multivitamin tablets contain nicotinamide. Both nicotinamine adenine dinucleotide phosphate ($NADP^+$) and tryptophan in the diet are major sources of nicotinamide or niacin with a conversion ratio of 60:1 [2].

Niacin exists mostly as NAD^+ and $NADP^+$ in the diet. Both are hydrolyzed by intestinally secreted NAD^+ pyrophosphatase to NMN and ATP. NMN is hydrolyzed to nicotinamide riboside and phosphorylated, releasing ribose-1-phosphate and nicotinamide.

As shown in Fig. 1, nicotinamide is rapidly taken up by enterocytes by simple diffusion. In the enterocyte it is immediately metabolized via NMN to NAD^+, catalyzed by phosphoribosyltransferase and adenosyltransferase, respectively. NAD^+ is trapped in cells as it cannot permeate membranes. Excess NAD^+ can, however, follow the reverse process. NAD^+ is hydrolyzed, catalyzed by glycohydrolase, to form nicotinamide that diffuses out of the enterocyte into the portal blood. It is transported to the liver where it is taken up by diffusion and converted in the hepatocytes via NMN to NAD^+ and $NADP^+$. Excess $NAD(P)^+$ is hydrolyzed to nicotinamide, releasing ADP-ribose.

Tryptophan is an essential amino acid and forms NMN and hence NAD^+ via quinolinic acid, as detailed in Fig. 3. The pathway is dependent on the activity of quinolinate decarboxylase, the rate-limiting enzyme of tryptophan catabolism to NAD^+. Excess hepatocyte $NAD(P)^+$

Fig. 3. Nicotinic acid metabolism from tryptophan. The reactions are catalyzed by: (1) tryptophan oxygenase, (2) kynurenine formylase, (3) kynurenine hydroxylase, (4) kinureninase, (5) oxidase, (6) decarboxylase, (7) quinolinic phosphoribosyl transferase, (8) nicotinate nucleotide pyrophosphorylase, (9) NMN ATPase, (10) NAD synthetase, (11) NADase, (12) deaminase, (13) nicotinic phosphoribosyl transferase, (14) *N*-L methyltransferase and (15) aldehyde oxidase. It is important to note the role of B vitamins (formate, PLP) in the pathway. *Abbreviations*: PPi, pyrophosphate anion; ADPR, adenosine diphosphate ribose; PRPP, phosphoribosyl pyrophosphate.

is hydrolyzed to niacin which it is released from the hepatocyte. There is little storage of nicotinamide and excess nicotinamide will efflux the hepatocyte into the plasma or will be methylated (catalyzed by nicotinamide N1-methyltransferase) to form *N*-methylnicotinamide, effluxed into the plasma and excreted in the urine. The latter is also oxidized by liver aldehyde oxidase to form N1-methyl-2-pyridone-5-carboxamide and N1-methyl-4-pyridone-5-carboxamide, which are also excreted in the urine. The urinary or serum levels of these *N*-methylated nicotinamides are used to clinically assess niacin deficiency that occurs in alcoholic pellagra, malnourishment, AIDS and other diseases [50].

4.2. Essential role of NAD^+ in mitochondrial energy production and cellular function

As mentioned earlier, more than 500 enzymes need niacin coenzymes. All of our energy production including mitochondrial oxidative phosphorylation/citric acid cycle and cytosolic glycolysis is dependent on these enzymes. On entering the cells, nicotinamide is mostly immediately metabolized to NAD^+, which functions as an electron carrier to form ATP by mitochondrial respiration or glycolysis, and $NADP^+$, which functions as a hydrogen donor in the reductive biosynthesis of fatty acids, steroids or as a coenzyme in pentose synthesis in the pentose phosphate pathway. NAD^+ and $NADP^+$ are involved in oxido-reduction reactions (tissue respiration), or reduction of poly(ADP)ribose synthase. NAD^+ is a coenzyme of dehydrogenase enzyme complexes (as in Fig. 2) as well as other enzymes of glycolysis (glycerophosphate, glycerophosphate, isocitrate, glyceraldehydes, glucose-6-phosphate and lactate dehydrogenases). Niacin is central to our energy metabolism. The NADH reduced during the TCA cycle or other metabolic pathways can enter the electron transport chain via complex I, the NADH: coenzyme Q reductase, an FMN-dependent enzyme. Each molecule of NADH oxidized back to NAD^+ is responsible for the synthesis of three ATP molecules. NADPH is also necessary for regeneration of GSH and thus sustaining the antioxidant balance of the cell.

4.3. Niacin deficiency

Niacin was discovered in the early 1900s during an investigation of the association between pellagra, a chronic wasting disease typically associated with dermatitis, dementia and diarrhea, and cornmeal-based diets [3]. The disorders and symptoms associated with niacin deficiency are described in Tables 2 and 3. Most proteins contain at least 1% tryptophan, which is a precursor of nicotinic acid. Niacin deficiency is thus rare except where the diet is deficient in tryptophan. Niacin status is commonly assessed by the measure of urinary excretion, plasma concentration or erythrocyte pyridine nucleotides. Increased urinary niacin metabolite endproducts could provide a biomarker for peroxisomal proliferation induced by drugs, such as cholesterol lowering drugs fenofibrate and simvastatin [51]. It could also be useful as an assay for mitochondrial toxins.

NAD^+ and NAD-dependent enzymes are affected by niacin deficiency. A niacin deficient diet administered to rats depleted liver and erythrocyte NAD levels whereas brain NAD levels were not affected. In Japanese quails, niacin metabolites and dependent enzymes were reduced only in the pectoral muscle tissue [52]. KGDH and respiratory chain dehydrogenases activities were the most affected. NAD(P)H can also act as a direct antioxidant by scavenging various free radicals [53]. NAD^+ deficiency leads to decreased mitochondrial membrane potential, decreased ATP synthesis, decreased glycolysis and increased inflammation signaling [53].

DNA repair is affected by niacin deficiency. NAD^+ is used by poly(ADP-ribose) polymerase (PARP) to synthesize poly(ADP ribose) which is used to repair DNA. DNA single strand breaks induced by carcinogens activate PARP. This increases the formation of poly(ADP)ribose that is then degraded by PAR glycohydrolase during the base excision repair reactions. As a consequence, NAD^+ pools and ATP production are reduced [54]. Niacin deficiency thus decreases DNA repair, DNA methylation and increases DNA alkylation and oxidation [54]. In bone marrow, niacin deficiency decreased NAD^+ and poly(ADP)ribose levels and increased DNA damage, spontaneous micronuclei formation and sister chromatide exchange [55]. Niacin deficiency thus appears to lead to an impaired DNA repair system as NAD^+ is necessary for a poly(ADP)ribose response to DNA damage. Niacin deficiency decreases NAD^+ levels required for the poly(ADP)ribose protective response to DNA damage and decreases the repair of DNA nicks and breaks [56]. If associated with folate or cobalamin deficiency it leads to an increase risk of DNA damage and chromosome breaks. Both cellular and animal studies suggest that niacin deficiency can be genotoxic and result in point mutations and chromosomal instability, which may be the origin of cancers and leukemia [57]. A genotoxic stress, such as irradiation increases (ADP)ribose polymer synthesis and increases free (ADP)ribose through the turnover of polymers. (ADP)ribose is a glycating agent that can contribute to the formation of advanced glycation endproducts (AGE)

in nuclear proteins, the formation of reactive carbonyl and oxygen species, and ultimately, to protein and DNA damage [53].

4.4. Prevention of oxidative stress and mitochondrial toxicity by niacin

In dogs and rats, consumption of niacin deficient diets results in pellagra-like conditions, "black tongue" in dogs and a severe dermatitis in rats. Both conditions were reversed with liver concentrates, nicotinic acid or nicotinamide [58].

Niacin has antioxidant properties. The increased cellular NADPH supply appears to increase the ability of GSH reductase to maintain GSH in the reduced state and thus to enhance the cells ability to detoxify H_2O_2 [59]. Nicotinamide, tryptophan and isonicotinic acid were each more effective than α-tocopherol at inhibiting both rat brain mitochondrial ROS formation and protein oxidative damage, and lipid peroxidation induced by ascorbate-Fe^{2+} [60]. Nicotinamide administration to rats 6–10 h after carbon tetrachloride also prevented hepatotoxicity attributed to radical-induced mitochondrial toxicity [61].

The activity of NAD-dependent enzymes can be affected by niacin. Supplementation with niacin and riboflavin decreased lipid peroxidation and glutathione peroxidase, while it increased superoxide dismutase, catalase (NADPH independent) and increased reduced glutathione levels [45]. Nicotinamide given intraperitoneally prevented the global ischemia in the brain that decreased NAD^+ and ATP levels. This was attributed to increased NAD^+ supply and PARP inhibition [62]. Ischemia reperfusion injury results in the loss of mitochondrial potential and apoptosis of cerebral vascular endothelial cells. This effect was reduced by niacin [63]. Niacin deficiency reduced plasma niacin and reduced hepatic and mammary gland mitochondrial dehydrogenases (particularly α-ketoglutarate and respiratory chain dehydrogenases) by 30–50%. The enzyme activity was restored by niacin [46].

High intakes of niacin may pose a risk. Niacin provided at pharmacological doses requires high levels of methyl groups for catabolism. This can reduce the *S*-adenosylmethionine (SAM) pool and increase homocysteine levels in the plasma [64]. High niacin intake can also result in niacin-induced insulin resistance [64].

5. Pantothenic acid (Vitamin B5)

Pantothenic acid is the precursor of coenzyme A (CoA), a molecule essential for 4% of known enzymatic reactions. In the interest of this review it is important to note the role of CoA in heme synthesis, lipid metabolism or as a prosthetic group in the TCA cycle.

5.1. Pantothenic acid and CoA delivery from the diet to mitochondrial enzymes

Pantothenic is widely distributed in foods as coenzyme A, particularly in animal organs, egg yolks, peanuts and broad beans with smaller amounts in lean meat, milk, potatoes and green leafy vegetables. Pantothenic acid is found in all plant and animal cells. Levels, however, are low in highly processed foods including refined grains, fruit products and meat or fish with added fat or cereal extenders.

Ingested CoA is hydrolyzed by phosphatases of the intestinal lumen to pantetheine and is then split, by pantetheinase secreted from the intestinal mucosa, into pantothenic acid. Pantothenic acid is cotransported across the brush-border membrane of the intestinal enterocyte with sodium via the sodium-dependent multivitamin transporter (SMVT), which transports pantothenate, biotin and lipoate [65]. As shown in Fig. 1, pantothenic acid is transported by the portal vein to the liver where it is again taken up by the SMVT with a K_m of 11 μM. Cysteine, ATP and five enzymes are involved in the synthesis of CoA from pantothenic acid in the hepatocyte. It is first metabolized in the cytosol and mitochondria to 4′-phosphopantetheine (PP), catalyzed by pantothenate kinase (PanK). Then three cytosolic enzymes, PPcysteine synthetase, PPcystine decarboxylase and PPadenytransferase, with cofactors ATP and cysteine catalyze the formation of dephospho-CoA which is then further metabolized to CoA by ATP and dephospho-CoA kinase located on the cytosolic side of the mitochondrial outer membrane [66]. The rate-limiting step for CoA synthesis is believed to be PPadenyltransferase [67]. Of particular interest is the neurodegenerative disease known as Hallenvorden-Spatz syndrome, which was mapped to a PANK2 gene mutation that codes for the mitochondrial kinase involved in initiating intramitochondrial CoA biosynthesis [66].

CoA transport to the mitochondria is initiated by binding to an adenine recognizing site on the mitochondria and then transported by a specific, energy-dependent, uptake process that is sensitive to electrochemical and pH gradients. CoA transport is readily inhibited by uncoupling agents [68].

Pantothenic acid is present in the cell as two active forms, CoA and acyl-carrier proteins. Excess cellular CoA accumulation is prevented by a feedback inhibition by CoA or acetyl-CoA of the first enzyme, pantothenate

kinase. Upon catabolism, acyl-carrier proteins release PP, which is regenerated to CoA, thus sustaining cellular pool levels even during prolonged panthonenic acid deficiency. The cellular CoA is separated into three independent pools, mitochondrial (containing about 2.2 mM CoA), peroxisomal (20–140 μM CoA) and cytosolic (<15 μMCoA) [68]. The mitochondrial pool unlike the other pools is self-regenerating through acetyl-CoA usage in the TCA cycle or ketone body synthesis, fatty acid-CoA and synthesis of free CoA from existing intermediates. The peroxisomal CoA pool is smallest and depletes first. CoA is also degraded outside the mitochondria by lysosomal acid phosphatases to form dephospho-CoA, which is degraded, catalyzed by a plasma membrane nucleotide pyrophosphatase to PP. The latter may then be dephosphorylated by lysosomal phosphatase to pantetheine which is degraded outside the cell by membrane associated extracellular pantetheinases called vanins to form the antioxidant cysteamine and taurine. Vanins are also upregulated by oxidative stress [66,67]. Hepatic CoA levels range from 136 to 434 nmol/g tissue and increase with high fat diets, hypolipidemic drugs, fasting and diabetes [66,67].

5.2. Essential role of CoA in mitochondrial energy production and cellular function

CoA functions as an acyl group carrier and carbonyl-activating group which is essential in the mitochondria for mitochondrial PDH and KGDH of the TCA cycle (see Fig. 2), the β-fatty acid oxidation pathway and leucine metabolism. CoA is also required for the oxidation of other fatty acids located in the peroxisomes and is also required for the first step of cholesterol and fatty acid biosynthesis located in the cytosol [68].

Heme synthesis requires CoA to form succinyl-CoA from α-ketoglutarate catalyzed by α-ketoglutarate dehydrogenase of the TCA cycle. In the mitochondrial matrix, succinyl-CoA and glycine are then condensed, catalyzed by aminolevulinate synthase, the rate-limiting step of heme synthesis. The δ-aminolevulinic acid formed is then exported to the cytosol where it is dimerized into porphobillinogen, which goes through a series of carboxylation and oxidations in both the cytosol and mitochondria to form protoporphyrin IX, before the iron is added to complete the structure. One heme unit thus requires 8 moles of glycine and 8 moles of succinyl-CoA. Pantothenic acid deficiency can thus inhibit heme synthesis to the same extent as biotin deficiency [41,69] and can lead to anemia.

5.3. Pantothenic acid deficiency

Pantothenic acid (pantoyl-β-alanine) was first identified in the early 1930s as an acidic substance required as a growth factor for yeast and antidermatitis factor for chicks [2]. The initial identification of this dietary factor was based on the dermatitis in chicks and the loss of Coat color in black and brown rats. Pantothenic acid deficiency is not normally seen in humans. Only a few other disorders have been associated with pantothenic acid deficiency and are listed in Tables 2 and 3. An RDA has not been established but 4–7 mg per day has been suggested (reviewed by Tahiliani and Beinlich [67]). Pantothenic acid status is commonly measured as urinary excretion, blood, erythrocyte or plasmas levels of pantothenic acid. Pantothenate deficiency in monkeys depresses heme biosynthesis and causes anemia. A subsequent decrease in cytochrome oxidase activity would be expected to increase mitochondrial ROS formation and accelerate cellular senescence [70]. Rats deficient in pantothenate develop hypertrophy of the adrenal cortex, with hemorrhage and necrosis [71]. Deficiency in dogs produces hypoglycemia, and convulsions [72]. Pantothenate antagonists (calcium hopantenate or pantoyl-GABA) can result in hepatic encephalopathy associated with hypoglycemia, leukocytosis, hyperammonemia, hyperlactatemia and elevated serum transaminase levels. Pantothenate supplementation rapidly reduces early symptoms of toxicity [68].

5.4. Prevention of oxidative stress and mitochondrial toxicity by pantothenic acid

Pantetheine administered to rats prevented lipid peroxidation and hepatitis induced by carbon tetrachloride and galactosamine. It also protected the heart from toxicity induced by experimental ischemia and reperfusion (reviewed by Wojtczak and Slyshenkov [73]). Preincubation of Ehrlich ascite tumor cells with pantothenic acid inhibited UV irradiation-induced lipid peroxidation but did not inhibit lipid peroxidation induced by the Fenton reagent. No protection occurred at 0 °C. In vitro liposome autoxidation was not affected by pantothenic acid suggesting that pantothenic acid was not an antioxidant. Pantothenate supplementation also increased hepatic GSH levels in Jurkat cells that was attributed to increased ATP production as a result of increased mitochondrial CoA levels [74]. The cytoprotective effect of pantothenic acid therefore likely resulted from increased CoA and GSH levels [73]. Pantothenic supplementation also increased hepatic GSH and liver resistance to irradiation, suggesting that GSH peroxidase and

phospholipid-hydroperoxide GSH peroxidase may have detoxified any hydroperoxides formed. Pantothenic acid also prevented the collapse of mitochondrial membrane potential and restored ATP synthesis levels as well as the activity of antioxidant enzymes, such as catalase, GSH peroxidase, GSH reductase and the NADPH forming malic enzyme in vitro and in vivo [73].

Pantothenate treatment could be useful in the treatment of liver failure. Sodium benzoate is a common treatment for hyperammonemia and non-ketonic hyperglycinemia. In HepG2 cells, sodium benzoate is activated by an ATP-dependent reaction with coenzyme-A to form benzoyl-CoA, which then binds glycine to form hippurate that can be excreted in the urine [75]. Pantothenic acid supplementation increased the cellular pool of CoA thus allowing increased hippurate formation for lower, non-toxic doses of sodium benzoate. A protective effect of pantothenic acid was also demonstrated in a mouse model of muscular dystrophy in which pantothenate supplementation improved muscular response [76]. The observed effect was associated with an indirect stimulation of glucose oxidation by increased levels of cytoplasmic CoA synthesis, and mitochondrial CoA utilization. This suggests that some enzyme complex of the TCA cycle or mitochondrial CoA transporter is impaired in muscular dystrophy.

6. Biotin (Vitamin B7)

Biotin is a prosthetic group for five cellular carboxylases and plays a role mostly in lipid metabolism. An essential role for biotin is also as a keeper of genome expression through biotinylation.

6.1. Biotin delivery from the diet to mitochondrial enzymes

Biotin is found in natural food stuff and is at a high concentration in liver, egg yolk, soybeans and yeast but is low in vegetables, fruits and most meat products. Moderate food sources are cereals, legumes and nuts. Biotin deficiency can be induced in individuals with the consumption of large amounts of raw egg white. Biotin deficiency is also observed in some epileptic patients treated with several commonly used anticonvulsant drugs [77] and in individuals receiving broad-range antibiotics.

As shown in Fig. 1, biotin is taken up by enterocytes and hepatocytes by the sodium dependent multivitamin transport (SMVT) system that also transports lipoic acid and pantothenate. In lymphoid tissues, biotin may also be transported by the monocarboxylate transporter, MCT1 that has been located in the plasma and mitochondrial membranes. Once in the cell, biotin distributes mostly to the mitochondria and cytosol fractions with a small amount in the nuclei (histones) and microsomal fractions. Biotin is covalently attached to carboxylases catalyzed by the holocarboxylase synthetase (HCS) in the cytosol and mitochondria. This reaction is dependent on an ATP supply to form a biotinyl-AMP intermediate. When carboxylases turnover by proteolysis the biotin-ε-aminolysine (biocytin) is released and biotin is regenerated by hydrolysis catalyzed by biotinidase. The biotin released is used for biotinylation of newly synthesized carboxylases [78]. A regulatory role has also been proposed for HCS as part of a signaling cascade involving guanylate cyclase and cGMP-dependent protein kinase that triggers mitochondrial carboxylase gene transcription and histone biotinylation [78].

6.2. Essential role of biotin in mitochondrial energy production and cellular function

This water-soluble vitamin, like folic acid and *S*-adenosylmethionine, transfers one carbon units but with the most oxidized form that is CO_2. Biotin is a coenzyme for five mitochondrial carboxylases and is essential for growth, development and normal mitochondrial and cellular functions.

The carboxylases play an important part in energy production and cellular function. Mitochondrial pyruvate carboxylase catalyzes the first step in gluconeogenesis, in which pyruvate, CO_2 and ATP form oxaloacetate. The oxaloacetate is reduced to malate, which leaves the mitochondria to regenerate oxaloacetate in the cytosol. Here, it forms glucose by gluconeogenesis. Cytosolic and mitochondrial acetyl-CoA carboxylases I and II, respectively catalyze malonyl-CoA formation from acetyl-CoA and the control enzyme modulated by hormones for regulating fatty acid synthesis. The mitochondrial malonyl-CoA formed acts by inhibiting carnitine palmitoyltransferase I, and thus fatty acid oxidation. Mitochondrial propionyl-CoA carboxylase participates in the last step of the β-oxidation pathway for odd numbered carbon atoms by catalyzing the reaction of propionyl-CoA, CO_2 and ATP to form methylmalonyl-CoA and succinyl-CoA, part of the TCA cycle. Propionyl CoA is also formed by the mitochondrial degradation of isoleucine, methionine, threonine and valine, and feeds into the carboxylase for further metabolism. Mitochondrial methylcrotonyl-CoA carboxylase catalyzes the last-but-one step in the leucine degradation pathway, by carboxylating methylcrotonyl-CoA to form methylglutaconyl-CoA. This molecule is then metabolized to form acetoacetate and acetyl-CoA.

During biotin deficiency methylcrotonyl-CoA accumulates in the mitochondria and depletes glycine and succinyl-CoA. This prevents heme/cytochrome synthesis and results in mitochondrial ROS formation and premature cellular senescence [69].

Biotinylation is also affected by biotin deficiency. Biotin has an important role in regulating gene expression by biotinylation of histones [79]. This has an impact on gene silencing, cell proliferation, DNA repair pathways and cell death by apoptosis. Genes that are modulated in response to biotin include glucokinase and phosphoenolpyruvate carboxykinase, involved in glucose response.

6.3. Biotin deficiency

Biotin was first recognized as an essential nutrient factor in mid 1930s, 10 years after the first reports of egg-white injury syndrome, which was later recognized as a model of biotin deficiency. A number of other conditions have since been associated with biotin deficiency and are listed in Table 2. Some inborn errors of metabolism have been described that are linked to biotin deficiency and are listed in Table 3. Humans and mammals have lost their ability to synthesize biotin and only microorganisms synthesize biotin including intestinal microorganisms. Clinical findings of frank biotin deficiency are rare although biotin deficiency has been found in 40% of pregnancies [80]. Biotin status is commonly assessed by biotin and 3-hydroxyvalerate excretion, plasma biotin and the concentration of odd-chain fatty acids in the plasma lipids. Rats become biotin deficient if provided with a diet consisting of raw egg-white, which contains avidin, a glycoprotein that specifically forms a tight complex with biotin which is not dissociated in the GI tract. Such rats developed neuromuscular disorders, severe dermatitis and loss of hair around the eyes (spectacle eyes). The syndrome was inhibited by heating the egg white, by the addition of a yeast extract, or by the addition of biotin to the diet [81]. More recently, it has been shown that low biotin levels, induced by dietary egg white diets, can result in teratologic effects in mice and hamsters in the absence of the biotin deficiency syndrome [82].

Carboxylases activity are decreased in biotin deficiency. Mitochondrial pyruvate carboxylase (PC) and propionyl-CoA carboxylase activities, but not their mRNAs, were markedly decreased in liver, kidney and muscle of biotin deficient rats. Brain was not affected. Liver and kidney holocarboxylase mRNA, however, was decreased [83]. Pyruvate carboxylase deficiency is associated with fasting hypoglycemia, which is thought to be linked to increased risk of sudden infant death syndrome (SIDS) as reported in Table 2. The enzyme deficiency also leads more directly to increased pyruvate, and upstream molecules, such as lactate and alanine [84]. These biomarkers, however, cannot be used for detecting biotin deficiency as they appear in thiamin deficiency due to inactivation of pyruvate dehydrogenase complex. Acetyl-CoA deficiency causes an impairment of lipid metabolism and decreased fatty acid elongation. Lower propionyl-CoA decarboxylase activity increases plasma propionate levels, leading to propionate acidemia [85]. There is also an increased urinary excretion of propionate and upstream metabolites, such as isobutyrate, isovalerate, 2-methylbutyrate, 3-methylcrotonate, hydroxypropionate, propionylglycine and methylcitrate. With propionyl-CoA and acetyl-CoA competing for the early steps of odd-chain fatty acid synthesis, biotin deficiency results in increased plasma 15:0 and 17:0 fatty acids levels [86]. The increased plasma odd-chain fatty acids levels is associated with increased poly-unsaturated fatty acids, and decreased palmitate and stearate fatty acid (16:0 and 18:0) levels [87] Finally, biotin deficiency inhibits methylcrotonyl-CoA carboxylase and increases the urinary excretion of methylcrotonate, methylcrotonylglycine and 3-hydroxyisovalerate [88]. The increased methylcrotonoyl-CoA reacts with cellular glycine [89], thus reducing the glycine pool. The combination of decreased succinyl-CoA, due to deficiency in propionyl-CoA carboxylase and glycine leads to inhibition of heme synthesis in biotin deficiency [41].

6.4. Prevention of oxidative stress and mitochondrial toxicity by biotin

There have been no reports of biotin having antioxidant properties or preventing cellular oxidative stress or mitochondrial toxicity. However, biotin deficiency in human lung fibroblasts caused a 40–50% decrease in cytochrome heme associated with premature senescence, likely as a result of ROS formation resulting from impaired mitochondrial respiration. Mitochondrial toxicity resulting from inactivation of essential carboxylases has been reported [83].

Biotin may be important in the development of diabetes. Biotin deficiency has been linked to impaired glucose tolerance, associated with fasting hyperglycemia and decreased glucose oxidation [90]. Biotin deficiency can also affect gene expression other than carboxylases. For instance, liver glucokinase activity is inhibited. There was also limited evidence that marginal biotin deficiency in rats can affect plasma glucose, and perhaps result in insulin resistance [90]. Increased biotin has been shown to improve glucose tolerance in

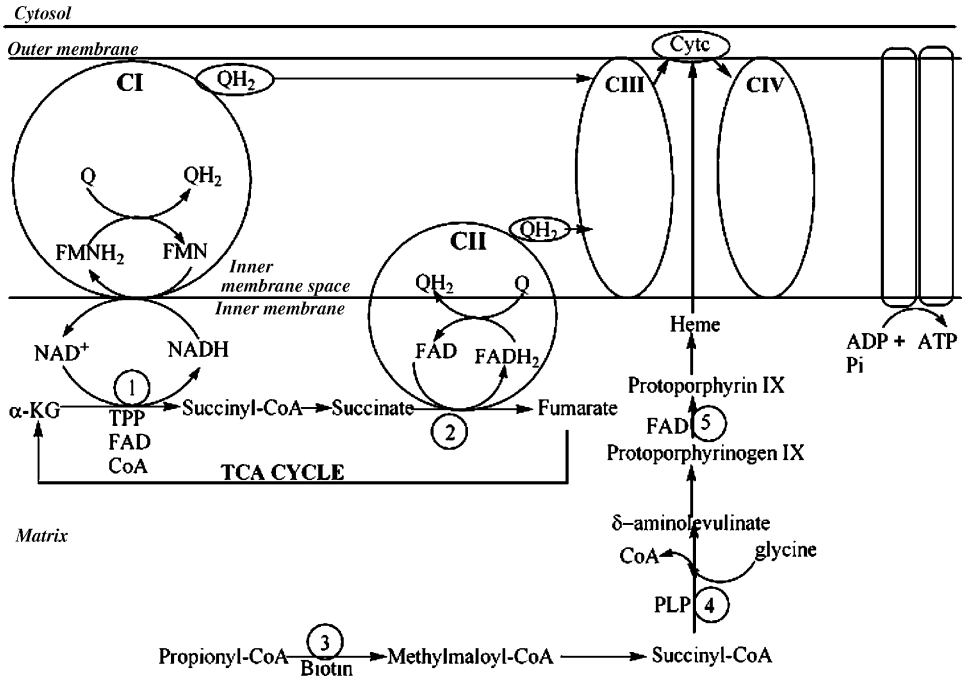


Fig. 4. B vitamins and mitochondrial energy metabolism. This is a schematic and simplified view of the oxidative phosphorylation pathway (electron transport chain (ETC) and ATP synthase) and the role of B vitamins in energy production. Only the reactions and enzymes relevant to the review are detailed here. The electron transport chain contains the following component: (C_I) NADH: ubiquitone oxidoreductase; (C_{II}) succinate: ubiquitone oxidoreductase; (C_{III}) ubiquitone: cytochrome *c* oxidoreductase; (C_{IV}) cytochrome oxidase. Reactions: (1) α-ketoglutarate dehydrogenase, TPP and FAD dependent, substrate CoA and NAD^+; (2) succinate dehydrogenase, part of C_{III}, the only membrane-bound reaction of the TCA cycle; (3) propionyl-CoA carboxylase, biotin dependent; (4) aminolevunilate synthase; pyridoxal-5-phosphate (PLP) dependent; (5) protoporphyrinogen oxidase, FAD dependent; α-KG: α-ketoglutarate. As shown, TPP, CoA, FMN, FAD and NAD are involved in the TCA cycle and complex I and II of the ETC. Biotin, CoA and FAD are involved in heme biosynthesis and thus in later part of the ETC. Succinyl-CoA can feed into either pathway depending on the needs of the cell.

streptozotocin-induced diabetes in rats [91]. Supraphysiological doses of biotin stimulate cGMP production and induce glucokinase activity as well as glucose-dependent insulin release from beta cells [92]. High doses of biotin decrease fasting glucose levels in type II diabetics that are non-responsive to sulfonylurea treatment, by the cGMP/biotin-induced expression of glucokinase. Biotin has also been shown to both increase glucokinase and decrease phosphoenolpyruvate carboxykinase [93]. It has been suggested that biotin could be used with chromium picolinate as a co-treatment for type II diabetics. No studies, however, have been published on the effects of biotin deficiency on mitochondrial or cellular metabolism of rats.

7. Conclusions

This review of the scientific literature suggests that B vitamins play an essential role in regulating mitochondrial enzymes. We noted furthermore that mitochondrial integrity and functions are compromised by dietary deficiency of B vitamins or an increased dependency resulting from a genetic mutation of a B vitamin-dependent enzyme. Preincubation of cells with thiamin or pyridoxal protected cells against mitochondrial toxins but remains to be demonstrated for other Bs. Although not yet demonstrated for biotin or riboflavin, evidence has also been published that preincubation of cells with thiamin, pyridoxal, folic acid, cobalamin or pantothenic acid increases their resistance to oxidative stress.

B vitamins are varied in structure. Their mode of cellular uptake ranges from active transporters to passive diffusion or endocytosis (Fig. 1). Although their cellular localizations and especially their tissue specificity may sometimes vary, B vitamins are essential for optimal mitochondrial and cellular functioning and form a tight network of interconnected reactions.

Thiamin, riboflavin, niacin and pantothenic acid, through their active metabolites have a direct effect on mitochondrial aerobic respiration (TCA cycle) and energy production (electron transport chain and ATP formation), as summarized in Fig. 4. The role of biotin is,

however, less evident. Secondary by-products of biotin-dependent reactions, such as acetyl-CoA or succinyl-CoA can feed into the TCA cycle and thus participate in energy metabolism. This effect of biotin deficiency would, however, only be detected in association with a more generalized deficiency in all energy vitamins.

Few metabolic pathways are dependent on only one vitamin, and thus a number of biomarkers would be affected more by multiple deficiencies as could occur in severe malnutrition. Specific identification of the original cause of a given symptom is difficult and cannot be based on a single isolated parameter because of the close connections both in the metabolism and activity of the vitamins. We need to identify metabolic pathway deficiencies rather than isolated specific vitamin deficiencies. Once identified, these deficiencies could be overcome or reversed by multiple vitamin combination therapies/supplementations. Currently, the health consequences to those humans with marginal B vitamin deficiencies and the frequency of these deficiencies in subsets of the human population are not known.

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References

[1] F. Depeint, W.R. Bruce, N. Shangari, R. Mehta, P.J. O'Brien, Mitochondrial function and toxicity: role of B vitamins on the one-carbon transfer pathways, Chem. Biol. Interact. 163 (2004) 113–132.

[2] G.F.M. Ball, Vitamins Their Role in the Human Body, Blackwell Publishing Ltd., 2004.

[3] L.J. Machlin, in: O.R. Fennema, M. Karel, G.W. Sanderson, S.R. Tannenbaum, P. Walstra, J.R. Whitaker (Eds.), Handbook of Vitamins, second ed., Marcel Dekker Inc., New York, Basel, 1991.

[4] W.R. Bruce, R. Furrer, N. Shangari, P.J. O'Brien, A. Medline, Y. Wang, Marginal dietary thiamin deficiency induces the formation of colonic aberrant crypt foci (ACF) in rats, Cancer Lett. 202 (2003) 125–129.

[5] H.M. Said, K. Balamurugan, V.S. Subramanian, J.S. Marchant, Expression and functional contribution of hTHTR-2 in thiamin absorption in human intestine, Am. J. Physiol. Gastrointest. Liver Physiol. 286 (2004) G491–G498.

[6] G. Rindi, U. Laforenza, Thiamine intestinal transport and related issues: recent aspects, Proc. Soc. Exp. Biol. Med. 224 (2000) 246–255.

[7] H.M. Said, A. Ortiz, V.S. Subramanian, E.J. Neufeld, M.P. Moyer, P.K. Dudeja, Mechanism of thiamine uptake by human colonocytes: studies with cultured colonic epithelial cell line NCM460, Am. J. Physiol.—Gastrointestinal Liver Physiol. 281 (2001) G144–G150.

[8] M.R. Ariaey-Nejad, M. Balaghi, E.M. Baker, H.E. Sauberlich, Thiamin metabolism in man, Am. J. Clin. Nutr. 23 (1970) 764–778.

[9] R. Zhao, F. Gao, I.D. Goldman, Reduced folate carrier transports thiamine monophosphate: an alternative route for thiamine delivery into mammalian cells, Am. J. Physiol.—Cell Physiol. 282 (2002) C1512–C1517.

[10] Q. Song, C.K. Singleton, Mitochondria from cultured cells derived from normal and thiamine-responsive megaloblastic anemia individuals efficiently import thiamine diphosphate, BMC Biochem. 3 (2002) 8.

[11] M. Barile, S. Passarella, E. Quagliariello, Thiamine pyrophosphate uptake into isolated rat liver mitochondria, Arch. Biochem. Biophys. 280 (1990) 352–357.

[12] L. Bettendorff, F. Mastrogiacomo, J. LaMarche, S. Dozic, S.J. Kish, Brain levels of thiamine and its phosphate esters in Friedreich's ataxia and spinocerebellar ataxia type 1, Movement Disord. 11 (1996) 437–439.

[13] K.J. Carpenter, Acute versus marginal deficiencies of nutrients, Nutr. Rev. 60 (2002) 277–280.

[14] C. Zimitat, P.F. Nixon, Glucose loading precipitates acute encephalopathy in thiamin-deficient rats, Metab. Brain Dis. 14 (1999) 1–20.

[15] P. Munujos, J. Coll-Canti, J. Beleta, F. Gonzalez-Sastre, F.J. Gella, Brain pyruvate oxidation in experimental thiamin-deficiency encephalopathy, Clin. Chim. Acta 255 (1996) 13–25.

[16] R.F. Butterworth, M. Heroux, Effect of pyrithiamine treatment and subsequent thiamine rehabilitation on regional cerebral amino acids and thiamine-dependent enzymes, J. Neurochem. 52 (1989) 1079–1084.

[17] N.Y. Calingasan, G.E. Gibson, Dietary restriction attenuates the neuronal loss, induction of heme oxygenase-1 and blood-brain barrier breakdown induced by impaired oxidative metabolism, Brain Res. 885 (2000) 62–69.

[18] D.D. Mousseau, V.L. Rao, R.F. Butterworth, Vesicular dysfunction during experimental thiamine deficiency is indicated by alterations in dopamine metabolism, Eur. J. Pharmacol. 317 (1996) 263–267.

[19] C. Scharfe, M. Hauschild, T. Klopstock, A.J. Janssen, P.H. Heidemann, T. Meitinger, M. Jaksch, A novel mutation in the thiamine responsive megaloblastic anaemia gene SLC19A2 in a patient with deficiency of respiratory chain complex I, J. Med. Genetics 37 (2000) 669–673.

[20] H.M. Salem, Glyoxalase and methylglyoxal in thiamine-deficient rats, Biochem. J. 57 (1954) 227–230.

[21] N. Shangari, F. Depeint, R. Furrer, W.R. Bruce, P.J. O'Brien, The effects of partial thiamin deficiency and oxidative stress (i.e., glyoxal and methylglyoxal) on the levels of alpha-oxoaldehyde plasma protein adducts in Fischer 344 rats, FEBS Lett. 579 (2005) 5596–5602.

[22] N. Shangari, W.R. Bruce, R. Poon, P.J. O'Brien, Toxicity of glyoxals—role of oxidative stress, metabolic detoxification and thiamine deficiency, Biochem. Soc. Trans. 31 (2003) 1390–1393.

[23] P.I. Lukienko, N.G. Mel'nichenko, I.V. Zverinskii, S.V. Zabrodskaya, Antioxidant properties of thiamine, Bull. Exp. Biol. Med. 130 (2000) 874–876.

[24] I.I. Stepuro, T.P. Piletskaya, V.I. Stepuro, S.A. Maskevich, Thiamine oxidative transformations catalyzed by copper ions and ascorbic acid, Biochem., Russia 62 (1997) 1409–1414.

[25] L. Rossi, M.F. Lombardo, M.R. Ciriolo, G. Rotilio, Mitochondrial dysfunction in neurodegenerative diseases associated with copper imbalance, Neurochem. Res. 29 (2004) 493–504.
[26] M. Arciello, G. Rotilio, L. Rossi, Copper-dependent toxicity in SH-SY5Y neuroblastoma cells involves mitochondrial damage, Biochem. Biophys. Res. Commun. 327 (2005) 454–459.
[27] C.T. Sheline, D.W. Choi, Cu^{2+} toxicity inhibition of mitochondrial dehydrogenases in vitro and in vivo, Ann. Neurol. 55 (2004) 645–653.
[28] K. Rokutan, K. Kawai, K. Asada, Inactivation of 2-oxoglutarate dehydrogenase in rat liver mitochondria by its substrate and *t*-butyl hydroperoxide, J. Biochem. 101 (1987) 415–422.
[29] R. Mehta, N. Shangari, P.J. O'Brien, Therapeutic targets for preventing cell death induced by mitochondrial toxins or Maillard Reaction Intermediates with B vitamins, Mol. Nutr. Food Res., submitted for publication.
[30] R. Bais, A.M. Rofe, R.A. Conyers, Investigations into the effect of glyoxylate decarboxylation and transamination on oxalate formation in the rat, Nephron 57 (1991) 460–469.
[31] V. Poldelski, A. Johnson, S. Wright, V.D. Rosa, R.A. Zager, Ethylene glycol-mediated tubular injury: identification of critical metabolites and injury pathways, Am. J. Kidney Dis. 38 (2001) 339–348.
[32] E.S. Yang, J.H. Yang, J.E. Park, J.W. Park, Oxalomalate, a competitive inhibitor of NADP+-dependent isocitrate dehydrogenase, regulates lipid peroxidation-mediated apoptosis in U937 cells, Free Radic. Res. 39 (2005) 89–94.
[33] E. Beltramo, E. Berrone, S. Buttiglieri, M. Porta, Thiamine and benfotiamine prevent increased apoptosis in endothelial cells and pericytes cultured in high glucose, Diab. Metab. Res. Rev. 20 (2004) 330–336.
[34] R. Babaei-Jadidi, N. Karachalias, C. Kupich, N. Ahmed, P.J. Thornalley, High-dose thiamine therapy counters dyslipidemia in streptozotocin-induced diabetic rats, Diabetologia 47 (2004) 2235–2246.
[35] A.A. Booth, R.G. Khalifah, P. Todd, B.G. Hudson, In vitro kinetic studies of formation of antigenic advanced glycation end products (AGEs). Novel inhibition of post-Amadori glycation pathways, J. Biol. Chem. 272 (1997) 5430–5437.
[36] M. Barile, S. Passarella, A. Bertoldi, E. Quagliariello, Flavin adenine dinucleotide synthesis in isolated rat liver mitochondria caused by imported flavin mononucleotide, Arch. Biochem. Biophys. 305 (1993) 442–447.
[37] P.S. Brady, C.L. Hoppel, Hepatic peroxisomal and mitochondrial fatty acid oxidation in the riboflavin-deficient rat, Biochem. J. 229 (1985) 717–721.
[38] M. Nagao, K. Tanaka, FAD-dependent regulation of transcription, translation, post-translational processing, and post-processing stability of various mitochondrial acyl-CoA dehydrogenases and of electron transfer flavoprotein and the site of holoenzyme formation, J. Biol. Chem. 267 (1992) 17925–17932.
[39] H.J. Powers, Riboflavin (Vitamin B-2) and health, Am. J. Clin. Nutr. 77 (2003) 1352–1360.
[40] C.G. Coimbra, V.B.C. Junquiera, High doses of riboflavin and the elimination of dietary red meat promote the recovery of some motor functions in Parkinson's disease patients, Braz. J. Med. Biol. Res. 36 (2003) 1409–1417.
[41] H. Atamna, Heme iron, and the mitochondrial decay of ageing, Ageing Res. Rev. 3 (2004) 303–318.
[42] C.J. Bates, Glutathione and reduced indices in rat lenses, liver and red cells during riboflavin deficiency and its correction, Exp. Eye Res. 53 (1991) 123–130.
[43] D.A. Adelekan, D.I. Thurnham, Glutathione peroxidase (EC 111.1.9) and superoxide dismutase (EC 1.15.1.1) activities in riboflavin-deficient rats infected with *Plasmodium berghei* malaria, Br. J. Nutr. 79 (1998) 305–309.
[44] Z. Zaman, R.L. Verwilghen, Effect of riboflavin deficiency on oxidative phosphorylation, flavin enzymes and coenzymes in rat liver, Biochem. Biophys. Res. Commun. 67 (1975) 1192–1198.
[45] S.S. Perumal, P. Shanthi, P. Sachdanandam, Augmented efficacy of tamoxifen in rat breast tumorigenesis when gavaged along with riboflavin, niacin, and CoQ10: effects on lipid peroxidation and antioxidants in mitochondria, Chem. Biol. Interact. 152 (2005) 49–58.
[46] S.S. Perumal, P. Shanthi, P. Sachdanandam, Energy-modulating vitamins—a new combinatorial therapy prevents cancer cachexia in rat mammary carcinoma, Br. J. Nutr. 93 (2005) 901–909.
[47] L. Vergani, M. Barile, C. Angelini, A.B. Burlina, L. Nijtmans, M.P. Freda, C. Brizio, E. Zerbetto, F. Dabbeni-Sala, Riboflavin therapy—biochemical heterogeneity in two adult lipid storage myopathies, Brain 122 (1999) 2401–2411.
[48] E. Gianazza, L. Vergani, R. Wait, C. Brizio, D. Brambilla, S. Begum, T.A. Giancaspero, F. Conserva, I. Eberini, D. Bufano, et al., Coordinated and reversible reduction of enzymes involved in terminal oxidative metabolism in skeletal muscle mitochondria from a riboflavin-responsive, multiple acyl-CoA dehydrogenase deficiency patient, Electrophoresis 27 (2006) 1182–1198.
[49] B. Marriage, M.T. Clandinin, M. Glerum, Nutritional cofactor treatment in mitochondrial disorders, J. Am. Diet. Assoc. 103 (2003) 1029–1038.
[50] E.A. Carrey, R.T. Smolenski, S.M. Edbury, A. Laurence, A.M. Marinaki, J.A. Duley, L. Zhu, D.J. Goldsmith, H.A. Simmonds, Origin and characteristics of an unusual pyridine nucleotide accumulating in erythrocytes: positive correlation with degree of renal failure, Clin. Chim. Acta 335 (2003) 117–129.
[51] J. Delaney, M.P. Hodson, H. Thakkar, S.C. Connor, B.C. Sweatman, S.P. Kenny, P.J. McGill, J.C. Holder, K.A. Hutton, J.N. Haselden, et al., Tryptophan-NAD^+ pathway metabolites as putative biomarkers and predictors of peroxisome proliferation, Arch. Toxicol. 79 (2005) 208–223.
[52] Y.H. Koh, K.H. Choi, Y.H. Park, Effects of niacin deficiency on the levels of soluble proteins and enzyme activities in various tissues of Japanese quail, Int. J. Biochem. Cell Biol. 30 (1998) 943–953.
[53] E.L. Jacobson, P.U. Giacomoni, M.J. Roberts, G.T. Wondrak, M.K. Jacobson, Optimizing the energy status of skin cells during solar radiation, J. Photochem. Photobiol. B—Biol. 63 (2001) 141–147.
[54] G.J. Hageman, R.H. Stierum, Niacin, poly(ADP-ribose) polymerase-1 and genomic stability, Mut. Res. 475 (2001) 45–56.
[55] J.C. Spronck, J.B. Kirkland, Niacin deficiency increases spontaneous and etoposide-induced chromosomal instability in rat bone marrow cells in vitro, Mut. Res. 508 (2002) 83–97.
[56] B.N. Ames, DNA damage from micronutrient deficiencies is likely to be a major cause of cancer, Mut. Res. 475 (2001) 7–20.
[57] J.B. Kirkland, Niacin and carcinogenesis, Nutr. Cancer 46 (2003) 110–118.
[58] C.A. Elvehjem, R.J. Madden, F.M. Strong, D.W. Woolley, The isolation and identification of the anti-black tongue factor, J. Biol. Chem. 123 (1938) 137–149.
[59] P. Arun, K.G. Padmakumaran-Nair, V. Manojkumar, K.V. Deepadevi, L.R. Lakshmi, P.A. Kurup, Decreased hemolysis and lipid peroxidation in blood during storage in the presence of nicotinic acid, Vox Sang. 76 (1999) 220–225.

[60] J.P. Kamat, T.P. Devasagayam, Nicotinamide (Vitamin B3) as an effective antioxidant against oxidative damage in rat brain mitochondria, Redox Rep. 4 (1999) 179–184.
[61] E.C. de Ferreyra, A.S. Bernacchi, M.F. San Martin, G.D. Castro, J.A. Castro, Nicotinamide late protective effects against carbon tetrachloride-induced liver necrosis, Exp. Mol. Pathol. 60 (1994) 214–223.
[62] L. Klaidman, M. Morales, S. Kem, J. Yang, M.L. Chang, J.D.J. Adams, Nicotinamide offers multiple protective mechanisms in stroke as a precursor for NAD^+, as a PARP inhibitor and by partial restoration of mitochondrial function, Pharmacology 69 (2003) 150–157.
[63] Z.Z. Chong, S.H. Lin, K. Maiese, Nicotinamide modulates mitochondrial membrane potential and cysteine protease activity during cerebral vascular endothelial cell injury, J. Vasc. Res. 39 (2002) 131–147.
[64] M.F. McCarty, Co-administration of equimolar doses of betaine may alleviate the hepatotoxic risk associated with niacin therapy, Med. Hyp. 5 (2000) 189–194.
[65] K. Balamurugan, A. Ortiz, H.M. Said, Biotin uptake by human intestinal and liver epithelial cells: role of the SMVT system, Am. J. Physiol. Gastrointest. Liver Physiol. 185 (2003) G73–G77.
[66] R. Leonardi, Y.M. Zhang, C.O. Rock, S. Jackowski, Coenzyme A: back in action, Prog. Lipid Res. 44 (2005) 125–153.
[67] A.G. Tahiliani, C.J. Beinlich, Pantothenic acid in health and disease, Vit. Horm. 46 (1991) 165–228.
[68] D.A. Bender, Optimum nutrition: thiamin, biotin and pantothenate, Proc. Nutr. Soc. 58 (1999) 427–433.
[69] B.N. Ames, H. Atamna, D.W. Killilea, Mineral and vitamin deficiency can accelerate the mitochondrial decay of aging, Mol. Aspect Med. 26 (2005) 363–378.
[70] R. Brambl, N. Plesofsky-Vig, Pantothenate is required in Neurospora crassa for assembly of subunit peptides of cytochrome *c* oxidase and ATPase/ATP synthase, Proc. Natl. Acad. Sci. U.S.A 83 (1986) 3644–3648.
[71] H. Baker, O. Frank, Clinical Vitaminology, Interscience Publishers, New York, 1968.
[72] E.A. Robinson, The Vitamin Cofactors of Enzyme Systems, Pergamon Press, Oxford, 1966.
[73] L. Wojtczak, V.S. Slyshenkov, Protection by pantothenic acid against apoptosis and cell damage by oxygen free radicals—the role of glutathione, Biofactors 17 (2003) 61–73.
[74] V.S. Slyshenkov, D. Dymkowska, L. Wojtczak, Pantothenic acid and pantothenol increase biosynthesis of glutathione by boosting cell energetics, FEBS Lett. 569 (2004) 169–172.
[75] A. Palekar, Effect of pantothenic acid on hippurate formation in sodium benzoate-treated HepG2 cells, Pediatric Res. 48 (2000) 357–359.
[76] P.C. Even, A. Decrouy, A. Chinet, Defective regulation of energy metabolism in mdx-mouse skeletal muscles, Biochem. J. 304 (1994) 649–654.
[77] K.H. Krause, J.P. Bonjour, P. Berlit, W. Kochen, Biotin status of epileptics, Ann. N. Y. Acad. Sci. 447 (1985) 297–313.
[78] D. Pacheco-Alvarez, R.S. Solorzano-Vargas, A.L. Del Rio, Biotin in metabolism and its relationship to human disease, Arch. Med. Res. 33 (2002) 439–447.
[79] N. Kothapalli, G. Camporeale, A. Kueh, Y.C. Chew, A.M. Oommen, J.B. Griffin, J. Zempleni, Biological functions of biotinylated histones, J. Nutr. Biochem. 16 (2005) 446–448.
[80] D.M. Mock, J.G. Quirk, N.I. Mock, Marginal biotin deficiency during normal pregnancy, Am. J. Clin. Nutr. 75 (2002) 295–299.
[81] P. Gyorgy, C.S. Rose, K. Hofmann, D.B. Melville, V. duVignaud, A further note on the identity of Vitamin H with biotin, Science 92 (1940) 609.
[82] T. Watanabe, Dietary biotin deficiency affects reproductive function and prenatal development in hamsters, J. Nutr. 123 (1993) 2101–2108.
[83] R. Rodriguez-Melendez, S. Cano, S.T. Mendez, A. Velazquez, Biotin regulates the genetic expression of holocarboxylase synthetase and mitochondrial carboxylases in rats, J. Nutr. 131 (2001) 1909–1913.
[84] J. Hymes, B. Wolf, Biotinidase and its role in biotin metabolism, Clin. Chim. Acta 255 (1996) 1–11.
[85] Y.Y. Liu, Y. Shigematsu, A. Nakai, Y. Kikawa, M. Saito, K. Fukui, K. Hayakawa, J. Oizumi, M. Sudo, The effect of biotin deficiency on organic acid metabolism: increase in propionyl coenzyme A-related organic acids in biotin deficient rats, Metabolism 42 (1993) 1392–1397.
[86] D.M. Mock, C.L. Henrich, N. Carnell, N.I. Mock, L. Swift, Lymphocyte propionyl-coA carboxylase and accumulation of odd-chain fatty acid in plasma and erythrocytes are useful indicators of marginal biotin deficiency, J. Nutr. Biochem. 13 (2002) 462–470.
[87] V.V. Modi, S.P. Mistry, Fatty acids in biotin-deficient rat-liver mitochondria, Biochim. Biophys. Acta 63 (1962) 517–519.
[88] N.I. Mock, D.M. Mock, Biotin deficiency in rats: disturbances of leucine metabolism are detectable early, J. Nutr. 79 (1992) 1039–1043.
[89] D.M. Mock, Biotin, ILSI Press, Washington, DC, 1996.
[90] C Fernandez-Mejia, Pharmacological effects of biotin, J. Nutr. Biochem. 16 (2005) 424–427.
[91] H. Zhang, K. Osada, H. Sone, Y Furukawa, Biotin administration improves impaired glucose tolerance of streptozotocin-induced diabetic Wistar rats, J. Nutr. Sci. Vitaminol. 43 (1997) 271–280.
[92] Y Furukawa, Enhancement of glucose-induced insulin secretion and modification of glucose metabolism by biotin, Nippon Rinsho. 57 (1999) 2261–2269.
[93] M.F. McCarty, High-dose biotin, an inducer of glucokinase expression, may synergize with chromium picolinate to enable a definitive nutritional therapy for type II diabetes, Med. Hyp. 52 (1999) 401–406.