

Thiamine: properties, physiology, and health effects

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Abstract

Thiamine (vitamin B1) is an essential molecule for all living organisms. It is the precursor for several phosphorylated derivatives, the most important being the coenzyme thiamine diphosphate (ThDP). Thiamine is transported into cells by specific transporters and pyrophosphorylated to ThDP in the cytosol. ThDP is an essential cofactor for cellular oxidative energy metabolism. Thiamine deficiency disorders, which lead to severe nervous system lesions and cardiac failure, are still common either as a consequence of chronic and severe alcoholism, nutritional thiamine deficiency. Recent research suggests that synthetic thiamine precursor with high bioavailability could become interesting therapeutic tools in neurodegenerative diseases. The aim of the present chapter is to summarize recent developments in the field with special focus on nutrition.

Keywords

Alzheimer's disease, benfotiamine, oxidative metabolism, thiamine deficiency disorders, thiamine diphosphate, oxidative metabolism, Wernicke-Korsakoff syndrome,

Key points

- Thiamine is probably the most pleiotropic of the B vitamins and is involved in many cellular functions.
- Thiamine diphosphate is an essential coenzyme in several reactions, essential for brain energy metabolism.
- Thiamine deficiency, either as a consequence of chronic alcoholism, medical treatments, or malnourishment (in developing countries) is still relatively common and at the origin of severe disorders, especially in children.
- Genetic modification of enzymes of thiamine transport, metabolism or thiamine diphosphate-dependent enzymes leads to severe, often fatal, conditions.
- Present research efforts are oriented towards introducing food fortification in developing countries and establishing methods for the quick estimation of thiamine status in humans

- The use of thiamine prodrugs of high bioavailability are useful for restoring normal thiamine status, and, at high doses, have antioxidant and anti-inflammatory properties beneficial in patients with neurodegenerative diseases and depressive disorders.

1 Introduction

In this review, we focus on vitamin B1 or thiamine (Fig. 1), maybe, the most pleiotropic of the B vitamins as it is involved in many cellular processes in bacteria, plants and mammals (energy metabolism, cell survival, and antioxidant activity, among others). Several phosphorylated thiamine derivatives have been identified in practically all organisms, but a clear role has only been assigned to the coenzyme thiamine diphosphate (ThDP). ThDP is a coenzyme for approximately 40 enzymes (among which are 6 enzymes and enzyme complexes in mammalian cells) involved in cell energy metabolism as well as other metabolic functions (Makarchikov et al., 2025). Other derivatives, among which thiamine triphosphate (ThTP) and adenosine thiamine triphosphate (AThTP), are thought to be involved in so-called non-coenzyme functions but so far there is only evidence for such mechanisms in bacteria and plants (Bettendorff, 2021). In addition, high pharmacological doses of thiamine or thiamine prodrugs with high bioavailability seem to have antioxidant and anti-inflammatory effects in mammals. These effects are difficult to understand solely by activation of ThDP-dependent enzymes and are probably mediated by activation of some cellular signaling pathways (Bettendorff, 2023). In this review, we focus on biomedical and nutritional aspects of thiamine with special focus on humans.

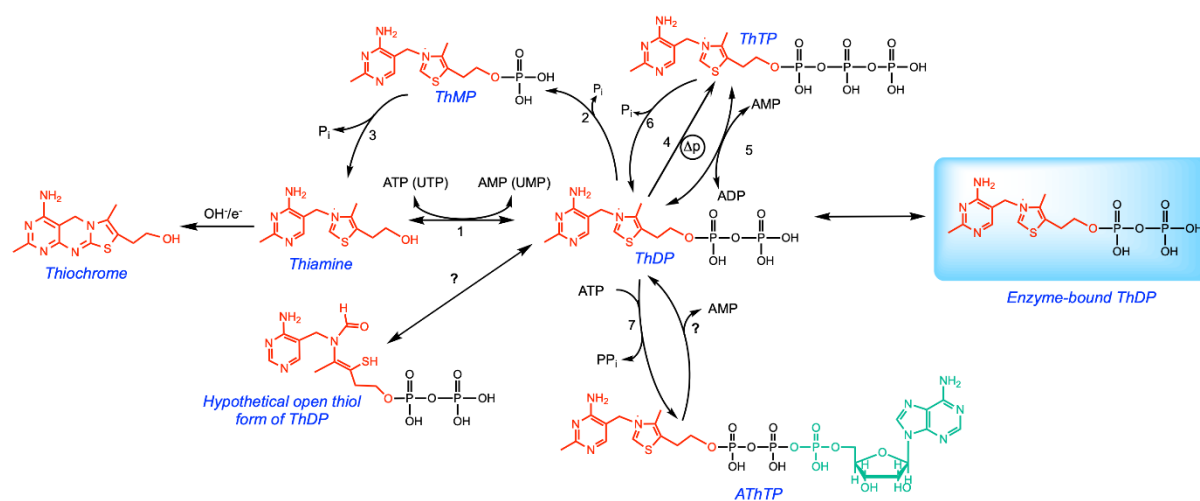


Figure 1. Chemical structure and metabolism of thiamine and its naturally occurring derivatives. ThDP is synthesized from thiamine and ATP by thiamine pyrophosphokinase (1). Hydrolysis of ThDP by unspecific phosphatases (2) yields ThMP, which, in turn, can be hydrolyzed to thiamine by thiamine several phosphatases (3). ThDP can be phosphorylated to ThTP by two mechanisms: mitochondrial ATP synthase (4) and cytosolic adenylate kinase (5). ThTP can be hydrolyzed to ThDP by a very specific cytosolic 25-kDa thiamine triphosphatase (THTPA) but also by other hydrolases (6). ThDP can also be converted to AThTP by a ThDP adenylyl transferase (7). AThTP can be hydrolyzed to ThDP and AMP by a putative AThTP hydrolase (8). The open thiol form of ThDP could be an intermediate between an oxidized form and ThDP. Thiochrome can be formed in vitro by oxidation in very alkaline medium, but its mechanism of formation in vivo remains unknown. (Modified and updated from Bettendorff, 2021). Abbreviations: AThTP, adenosine thiamine triphosphate; P_i , inorganic phosphate; PP_i , inorganic pyrophosphate; ThDP, thiamine diphosphate; ThMP, thiamine monophosphate; ThTP, thiamine triphosphate; THTPA, 25-kDa thiamine triphosphatase; Δp , proton gradient.

2. Natural thiamine derivatives and their physiological roles

The thiamine molecule consists of a pyrimidine moiety and a thiazolium moiety connected through a methylene bridge. The aromatic heterocycle thiazole is rarely found in biological molecules and its synthesis poses a particular challenge for the organisms (bacteria, fungi, and plants) able to synthesize this vitamin. The thiazole ring is substituted by a hydroxyethyl group that can be esterified to form phosphorylated derivatives, the most widespread being the coenzyme ThDP (Fig. 1).

The unphosphorylated thiamine molecule (the only one that should be called vitamin B1) is a minor intracellular form, but it is the main form circulating in the blood stream and transported or eliminated by the cells. Inside the cells, thiamine is pyrophosphorylated to thiamine diphosphate (ThDP) by the enzyme thiamine pyrophosphokinase 1 (TPK1, EC 2.7.6.2). ThDP is the major B1 vitamers in human cells and a coenzyme for several essential enzymes and enzyme complexes (see below). In contrast to many bacteria that can form thiamine monophosphate (ThMP) directly by phosphorylation of thiamine, in animals, ThMP can only be formed by hydrolysis of ThDP. ThMP, in turn can then be recycled to thiamine by hydrolysis.

In the 1950's the existence of the thiamine triphosphorylated compound ThTP was strongly suggested, however the absence of reliable analytical tools makes this claim subject to caution, especially as the amounts reported were generally much higher than with recent methods. The refinement of high performance liquid chromatography (HPLC) in the 1980's and later, clearly proved the existence of ThTP in mammalian, including human, cells (Gangolf et al., 2010). Recent data suggest that ThTP synthesized from ThDP and inorganic phosphate (P_i) through F_0F_1 -ATP synthases by a chemiosmotic mechanisms bacteria, chloroplast, and neuronal mitochondria (Wang et al., 2025). ThTP may also be synthesized by cytosolic adenylate kinase 1. This reaction is only significant in tissues with very high adenylate kinase activity such as skeletal muscle. Animal cells contain a highly efficient and specific soluble thiamine triphosphatase (THTPA, EC 3.6.1.28). The role of this enzyme may be to prevent accumulation of cytosolic ThTP.

It came as a surprise, at a time when all thiamine vitamers were supposed to be known, a new adenylated thiamine compound, adenosine thiamine triphosphate (AThTP), was discovered in *E. coli* where it might play a role as alarmone during energy stress. While this compound also exists in human tissues its possible role remains enigmatic (Gangolf et al., 2010).

Finally, thiamine chemistry is complex at $pH \geq 7.4$. The closed form can be in equilibrium with an open thiazole ring form (Fig. 1). The chemistry of this transformation is complex and has not been investigated recently, but might have unsuspected physiological implications (Bettendorff, 2023; Makarchikov et al., 2025).

Administration of thiamine and synthetic thiamine prodrugs have strong antioxidant and sometimes anti-inflammatory properties (Sambon et al., 2020; Strekalova et al., 2025). The mechanisms by which these effects are mediated are presently unknown but they are most probably not mediated by activation of ThDP-dependent enzymes. Indeed, many recent observations suggest that there are

several non-coenzyme actions of thiamine derivatives, some of which may involve ThTP and AThTP, but also other mechanisms.

3. Thiamine extraction and assay procedures

Many procedures for the extraction and assay of thiamine and its derivatives have been described. Here we will focuss only on the most recent developments.

3.1. Thiamine extraction

Fig. 2 presents a general procedure for the extraction of thiamine and its derivatives from most tissues (whole tissues, cultured cells, or plant material) (Gangolf et al., 2010; Sambon et al., 2020). Thiamine as well as all its natural vitamers are watersoluble. Extraction can easily be realized by acid precipitation of the tissues. Most studies use a final concentration of trichloroacetic acid (TCA) of 10 - 12% (with less, thiamine extraction may only be partial and with more acid, its elimination becomes challenging). TCA can easily be extracted with water-saturated diethylether (preferably HPLC grade without stabilizer, which may interfere with fluorometric determination). Some authors use perchloric acid instead of TCA.

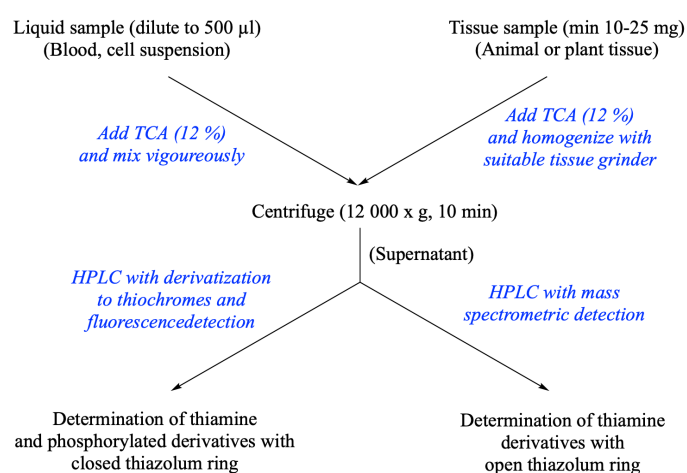


Figure 2. Strategy for the selection of a method for the determination of thiamine in biological samples. Abbreviations: HPLC, high-performance liquid chromatography; TCA, trichloroacetic acid.

3.2. Detection of thiamine and its derivatives

Thiamine vitamers in biological samples can either be detected as fluorescent thiochrome derivatives using HPLC followed by fluorescence detection or by HPLC separation of thiamine derivatives followed by mass spectrometric detection. UV detection of thiamine is neither sensitive nor selective enough and is only useful in relatively pure pharmacological preparations.

Thiochromes are formed from thiamine derivatives by oxidation with potassium ferricyanide in strongly alkaline medium, hence the importance of neutralization to pH > 5 of the sample extract. Moreover, formation of thiochromes requires an intact thiazolium ring. Thiochromes can either be obtained by precolumn or postcolumn derivatization. Precolumn determination is easy but in this case thiochrome derivatives are separated on the column. They are fluorescent only under alkaline conditions and an alkali-resistant column is required. For postcolumn derivatization, thiamine

derivatives are separated on the column, but a further pump is required for injecting the oxidant into a mixing chamber with the column efflux. We found that postcolumn derivatization was more cumbersome and more subject to system failure than precolumn derivatization, mainly because of the high content of sodium hydroxide in the oxidant solution. A spectrofluorometer is to be preferred over a filter fluorometer because of a much better selectivity. Hence, some fluorescence-based HPLC methods are able to simultaneously determine thiamine, ThMP, ThDP, ThTP, and AThTP in human tissues (Gangolf et al., 2010).

Derivatives with an open ring thiol form (Fig. 1), either natural or derived from synthetic thiamine prodrugs such as benfotiamine, dibenzoylthiamine, sulbutiamine, or fursultiamine, cannot be detected with this method. During the last one or two decades, powerful mass spectrometric methods have been developed that allow the detection of all thiamine derivatives and provitamins (Kim et al., 2019; Sambon et al., 2020). These methods are however more sophisticated, require skilled technicians and the instrumentation is highly expensive.

Many variations of the general methods described here have been developed for a rapid, cost-effective, and routine determination of specific vitamers for the estimation of the thiamine status in human populations for instance (Vachali et al., 2025). Many methods have also been developed for the simultaneous determination of several water-soluble vitamins, but they do not allow the determination of vitamers, and hence their usefulness for biological samples is limited.

Thiamine derivatives are present in sample solution at submicromolar concentrations, hence suitable standard solutions must be prepared in the same concentration range and particular attention must be paid for the material used for preparing the sample and further processing in order to avoid absorption of the material. We found that polypropylene was neutral in that sense, but glass should be avoided.

4. ThDP-dependent enzymes : catalytic mechanisms and role in metabolism

ThDP is the central thiamine metabolite and the only one for which we know the precise role and also, most probably, the one that is responsible, when deficient, for the clinical symptoms of thiamine deficiency disorders. In mammals, we know 6 ThDP-dependent enzymes, and all are in central positions of the cellular metabolism, such as the TCA cycle or the pentose phosphate shunt (Fig. 3): cytosolic transketolase (TK, EC 2.2.1.1), 2-hydroxyacyl-CoA-lyase (HACL1, EC 4.1.2.63) in peroxisomes and four 2-oxoacid dehydrogenases being constituents of mitochondrial multienzyme complexes - pyruvate dehydrogenase (PDH, EC 1.2.4.1), oxoglutarate dehydrogenase (OGDH, EC 1.2.4.2), branched-chain 2-keto (2-oxo) acid dehydrogenase (BCKD, EC 1.2.4.4), and, by similarity, 2-oxoadipate dehydrogenase (OADH, EC 1.2.4.2) (Makarchikov et al., 2025).

Some of the ThDP-dependent enzymes are also in strategic positions for the synthesis of neurotransmitters. (Fig. 3).

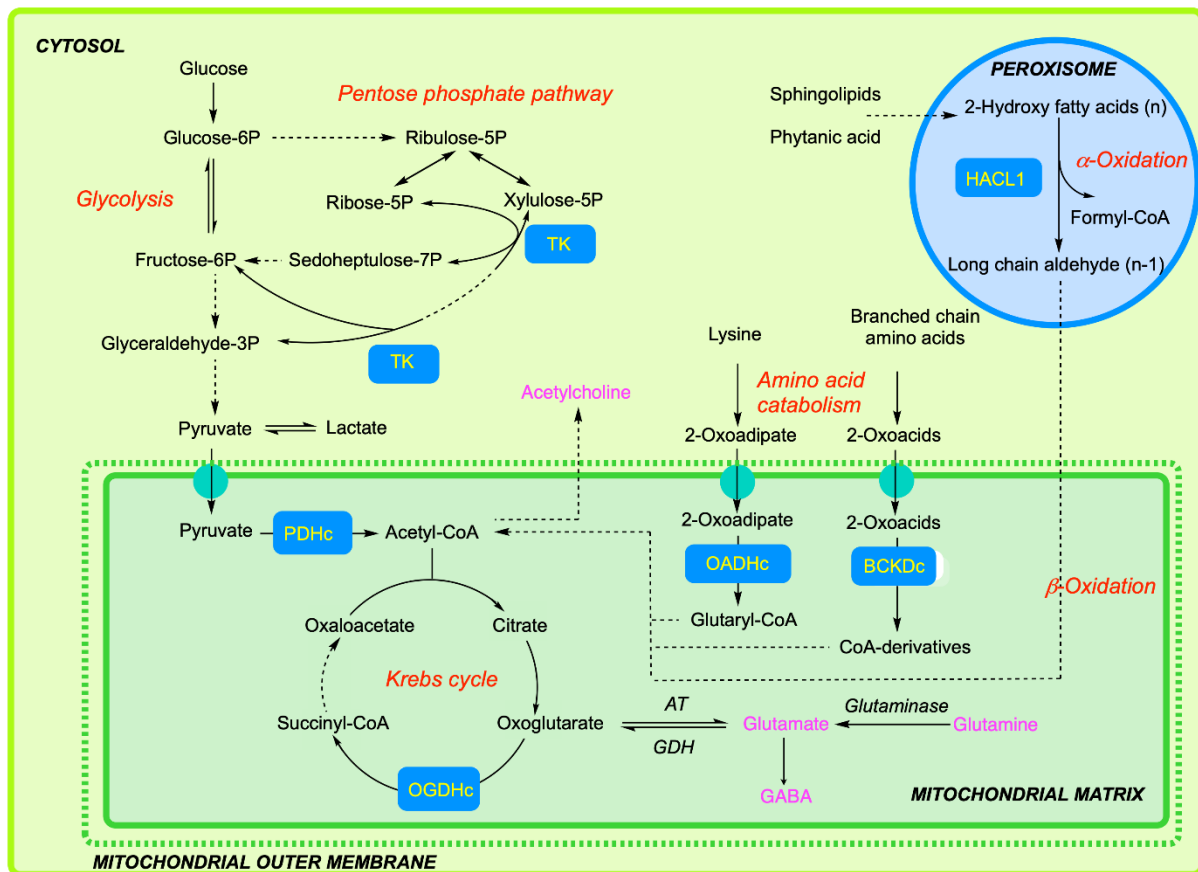


Figure 3. ThDP-dependent reactions in mammalian cells. Interrupted lines indicate pathways with several steps. Note that TK catalyzes two reactions in the pentose phosphate pathway. Neurotransmitters are indicated in Magneta. Abbreviations: AT; aminotransferase, BCKDc, branched chain α -ketoacid dehydrogenase complex; GDH; glutamate dehydrogenase; HACL1, 2-hydroxyacyl-CoA-lyase; PDHc, pyruvate dehydrogenase complex; OADHc, 2-oxoacid dehydrogenase complex; OGDHc, 2-oxoglutarate dehydrogenase complex; TK, transketolase;

4.1. ThDP-dependent enzymes in cell metabolism

Here, we will only discuss ThDP-dependent enzymes present in animals, where we can distinguish three groups: TKs, oxoacid dehydrogenases, and HACL1 (Makarchikov et al., 2025).

The ubiquitous cytosolic TK catalyzes the reversible transfer of a two-carbon fragment from a ketose phosphate donor substrate to an aldose phosphate acceptor substrate. It is a key enzyme of the pentose phosphate shunt, a major source of reduced nicotinamide adenine dinucleotide phosphate (NADPH) for reductive biosyntheses (fatty acids for instance) and production of ribose for nucleic acid synthesis. It consists of a homodimer of 75 kDa each. In humans, there are three isoforms probably emerged by gene duplication: transketolase, transketolase-like 1, and transketolase-like 2. The catalytic competence of the transketolase-like proteins remains controversial. TK-null mouse embryos are not viable. In humans, TK-deficiency is an extremely rare condition associated with developmental delay, short stature and congenital heart defects. TK deficiency is an inborn condition inherited from both parents in an autosomal recessive manner. Probably the mutations in the TK gene (*TKT*) only leads to partial inactivation of TK activity, which, otherwise, would be lethal.

A second group of ThDP-dependent enzymes comprises four 2-oxoacid dehydrogenases (PDH, OGDH, OADH, and BCKDH). All four are part of large multienzyme complexes composed of multiple copies of three core-enzymes, a decarboxylating dehydrogenase referred to as the E1 component, a dihydrolipoyl acyltransferase (E2) and a dihydrolipoyl dehydrogenase (E3) (Fig. 4). E1 and E2 are specific for each complex (except for OGDHc and OADHc that share the same E2 component), while E3 is common to the four complexes.

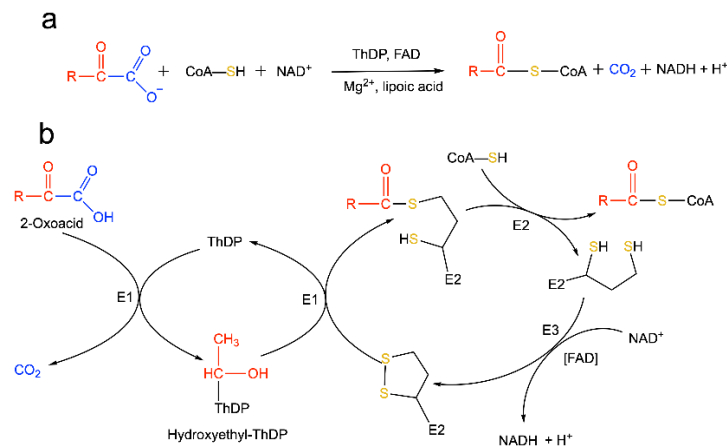


Figure 4. General mechanisms of 2-oxoacid dehydrogenases. a) Global reaction. b) Reaction mechanism. The oxoacid residues are colored in red and the leaving carboxyl residue in blue. Abbreviations: CoA, coenzyme A; FAD, flavin adenine dinucleotide; NAD⁺, nicotinamide adenine dinucleotide phosphate; ThDP, thiamine diphosphate.

The PDH complex (PDHc), which occupies a hinge position between glycolysis and the TCA cycle, catalyzes oxidative decarboxylation of pyruvate to produce acetyl-CoA, CO₂ and NADH. This reaction is a major source for mitochondrial acetyl-CoA feeding the TCA cycle and is highly regulated. PDHc deficiency (mainly mutations in the E1 subunit) is a very rare condition with heterogeneous clinical manifestations mainly of the central nervous system. We are not aware of any PDH-null transgenic animals; this condition is probably lethal in view of the strategic position of this enzyme complex.

OGDHc, a key enzyme complex of the TCA cycle, catalyzes the oxidative decarboxylation of 2-oxoglutarate yielding succinyl-CoA, NADH, and CO₂. It is involved in many aspects of cell metabolism and is the primary control site and rate-limiting step of the metabolic flux through the TCA cycle. The substrate oxoglutarate is situated at a branching point of sugar and amino acid metabolism (Fig. 3). The degradation of most amino acids leads to glutamate which by transamination yields 2-oxaloacetate. In addition, in mammals, where NH₄⁺ concentrations are very low, the mitochondrial glutamate dehydrogenase mainly operates towards the formation of 2-oxoglutarate. Impaired function of OGDHc is an autosomal recessive disorder (Online Mendelian Inheritance in Man – OMIM 203740) associated with features of infantile- and pediatric-onset basal ganglia-associated movement disorders, developmental delay, elevated lactate, ataxia, and seizure. Localized at the intersection of sugar and amino acid metabolism plays a role in the synthesis and degradation of two major brain neurotransmitters, glutamate (mainly synthesized from glutamine in neurons) and GABA.

Oxoglutarate dehydrogenase-like (OGDHL), an isozyme of OGDH and probably catalyzing the same reaction, is primarily expressed in brain and liver, but also in many cancer cells where it may regulate tumor cell proliferation. It is not clear whether this function is related to its enzymatic properties. Only a few patients with mutations in the *OGDHL* gene (OMIM 617513) have been

identified, with, probably depending on severity of the mutation, very heterogeneous neurological phenotypes.

Another isoform of OGDH, encoded by the *DHTKD1* gene (DHTKD1, for dehydrogenase E1 and transketolase domain-containing protein 1) is part of the 2-oxoadipate dehydrogenase complex (OADHc) converting 2-oxoadipate to glutaryl-CoA in the catabolic pathway of L-lysine, L-hydroxylysine, and L-tryptophan in mitochondria. While the E2 and E3 components of the complex are identical for OGDGc and OADHc, the E1 components are specific for each complex. Several *DHTKD1* mutations (OMIM 204750) result in an autosomal recessive inborn error of metabolism with a wide variety of symptoms. A nonsense *DHTKD1* mutation has been associated with a form of Charcot-Marie-Tooth disease (OMIM 615025), an inherited neurodegenerative disorder characterized by motor and sensory neuropathies. Another, newly characterized, *DHTKD1* mutation was linked to a sporadic form of amyotrophic lateral sclerosis.

BCKDc catalyzes the oxidative decarboxylation of branched-chain 2-oxo acids derived from the branched-chain amino acids leucine, isoleucine and valine. Defects in the genes encoding the subunits of BCKDc result in maple syrup urine disease (MSUD, OMIM 248600), an inborn error of metabolism, characterized by the accumulation of branched-chain α -ketoacids in urine with severe clinical consequences including neurological and developmental delay, encephalopathy and feeding problems. The treatment usually requires a dietary restriction of branched chain amino acid combined with thiamine supplementation.

Finally, peroxisomal HACL1 is important for the α -oxydation of 3-methyl-branched fatty acids (like phytanic acid) and 2-hydroxy long-chain fatty acids. It catalyzes the cleavage of the C–C bond in a 2-hydroxyacyl-CoA intermediate to give an aldehyde (n-1) and formyl-CoA. There does not seem to exist a peroxisomal ThDP transporter and the coenzyme is imported into peroxisomes together with the apoenzyme. No human mutations in HACL1 are known. HACL1 deficiency per se is not associated with a particular phenotype, but in combination with phytanic acid intake it might lead to phytanic acid elevation and resemble Refsums disease.

From the above considerations, it results that ThDP-dependent enzymes occupy strategic positions in cellular and energy metabolism. Moreover some are indirectly, by the metabolic sequences they control, involved in the synthesis of several important neurotransmitters, among which are acetylcholine, glutamate and GABA (Fig. 3).

4.2. Thiamine diphosphate the catalytic wizard

The catalytic mechanisms of ThDP-dependent enzymes have been intensively studied since the 1950s and a common leitmotiv for these enzymes relies in the cleavage or formation of carbon-carbon bonds of 2-oxo acids or α -hydroxy ketones. Here, we will not discuss the mechanism of each individual ThDP-dependent enzyme which are discussed at length in all biochemistry textbooks but we will only feature the general mechanism of catalysis (Fig. 5). A special coenzyme is required as there is no simple acid-base-catalyzed mechanism for such reactions that could be catalyzed by an existing amino acid side-chain. The thiazolium ring acts both as a nucleophile and an electron sink (Prajapati et al., 2022).

The first committed step in ThDP-dependent enzyme reactions is the deprotonation of the thiazolium C-2 to form a dipolar thiazolium intermediate (ylide) (Fig. 5). The anionic carbon (carbanion) of the thiazolium ring readily reacts with substrates by addition to a carbonyl group, forming a covalent

adduct. In parallel, the aminopyridinium moiety of ThDP acts as a general base facilitating the formation of the carbanion. This process is facilitated by a strictly conserved glutamate residue of the apoenzyme that forms a hydrogen bond with the weakly basic N-1' of the aminopyridinium.

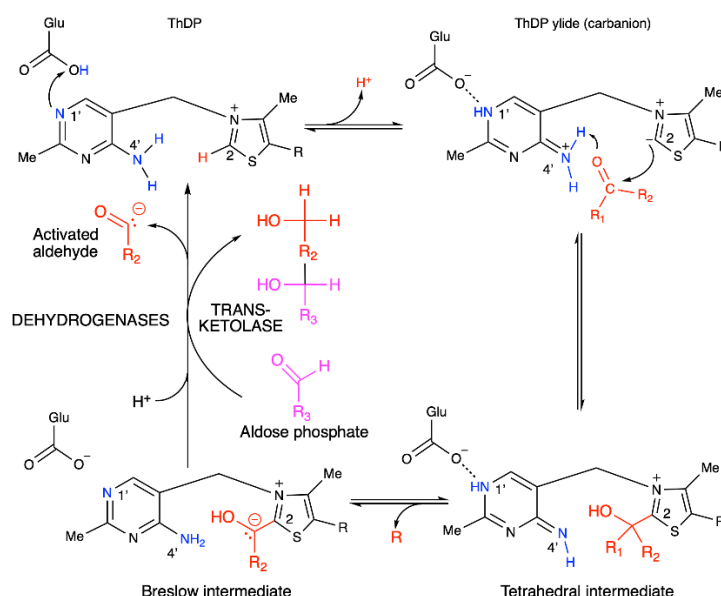


Figure 5. Catalytic mechanism in ThDP catalyzed reactions. In oxoacid dehydrogenases, the activated aldehyde is transferred to lipoic acid by E1. In transketolase, the leaving group combines with an incoming aldose phosphate.

The ylide formed by this deprotonation is the key intermediate in all ThDP-dependent enzyme reactions. Note that the phosphorylated part of the coenzyme plays no role in catalysis and is only required for tight binding of ThDP to the apoenzymes. The various types of reactions catalyzed by ThDP in mammalian cells are summarized in Table 1.

Table 1. Reactions catalyzed by ThDP-dependent enzymes in mammalian cells.

Enzymes	Bond split	Metabolic pathway
Pyruvate dehydrogenase (EC 1.2.4.1) 2-Oxoglutarate dehydrogenase (EC 1.2.4.2) 2-Oxoadipate dehydrogenase (EC:1.2.4.-) Branched chain 2-oxoacid dehydrogenase (EC 1.2.4.4)	$R_1-\overset{\overset{O}{\parallel}}{C}-\overset{\overset{O}{\parallel}}{C}-O^-$	Acetyl-CoA synthesis TCA cycle Lysine catabolism Amino acid catabolism
Transketolase (EC 2.2.1.1)	$R_2-\overset{\overset{O}{\parallel}}{C}-\overset{\overset{OH}{\mid}}{C}-R_3$	Pentose phosphate shunt
2-Hydroxyacyl-CoA lyase 1 (EC 4.1.2.63)	$R_4-\overset{\overset{OH}{\mid}}{C}-\overset{\overset{O}{\parallel}}{C}-S-CoA$	α -oxidation of branched fatty acids with shortening by one carbon atom

5. Thiamine compartmentation and body homeostasis

Thiamine homeostasis is maintained by a subtle equilibrium between intestinal absorption, uptake by cells and excretion.

Understanding the compartmentation of thiamine derivatives is essential for understanding how nutritional thiamine enters the body and how it diffuses through cells and tissues. Therefore, understanding thiamine transport mechanism is particularly important.

In humans, thiamine has a short half-life of only about 12 h, so it is important to consume it regularly in the diet (Mrowicka et al., 2023). The total thiamine store of a human body is about 30-50 mg with a daily consumption of about 2 mg per day. Therefore, without nutritional compensation, the thiamine stores are depleted within 2–3 weeks.

5.1. Thiamine absorption

Animals, which are unable to synthesise thiamine, totally rely on dietary sources. While a contribution of thiamine produced by gut bacteria cannot be excluded in ruminants and other herbivores, it is negligible in humans which rapidly become thiamine-deficient in case of insufficient thiamine intake.

While thiamine produced by the microbiome is mainly absorbed by the colon and sustains colon health, the absorption of dietary thiamine mainly takes place in the jejunum.

When entering the digestive tract, the intestinal epithelium represents the first hurdle for thiamine assimilation. ThDP is the major vitamin present in our alimentation, it is largely hydrolyzed by intestinal phosphatases. Thiamine is transported across the intestinal epithelium by two thiamine transporters, the specific carrier proteins ThTr1 and ThTr2, which are the products of *SLC19A2* and *SLC19A3* genes belonging to the solute carrier protein super family involved in thiamine and folate transport.

In addition to *SLC19A1* and *SLC19A2*, which carry thiamine (a cation), the reduced folate carrier (RFC, *SLC19A1*), an anion exchanger carrying ThMP in addition to 5-methyltetrahydrofolate, universally expressed in animal tissues. It is possibly involved in the extrusion of ThMP and its activity could be responsible for the high content of ThMP in extracellular body fluids, in particular blood plasma, cerebrospinal fluid and maternal milk (Bettendorff, 2020).

The open reading frames of human ThTr1 and ThTr2 code for proteins of 497 and 496 amino acids ($M_r \sim 55$ kDa) respectively, with 12 putative transmembrane domains. ThTr1 is expressed in both the apical brush border membrane and basolateral membranes of the intestinal epithelium for transport through the intestinal epithelium and exit to the blood stream. ThTr2 is mainly found in the apical membrane of the enterocytes where it is probably essential for the capture of intestinal thiamine. Both transporters function as pH dependent thiamine⁺/H⁺ exchangers with a high affinity for thiamine ($K_m < 0.1 \mu\text{M}$). Hence these transporters are rapidly saturated and when thiamine is administered at pharmacological doses, a low-affinity process predominates. While some studies suggest that, at high concentrations, the vitamin may diffuse through biological membranes this is unlikely for a cationic molecule such as thiamine. Rather, it seems that low affinity, but saturable, transporters may take over.

5.2. Transport of thiamine by the blood and distribution to the tissues

Thiamine and ThMP are the main forms of thiamine transported in blood plasma. In animals, ThMP can only come from the hydrolysis of ThDP as there is no enzyme catalyzing the phosphorylation of thiamine to ThMP. As ThDP only exists inside the cells, ThMP can only come from ThMP transported outside cells after hydrolysis of ThDP. Whether some tissue are preferentially involved in this phenomenon is unknown. The potential ThMP transporter involved could be RFC1 (see section 5.3.)

5.2.1. Blood transport

After uptake through the intestinal epithelium, plasmatic thiamine is distributed throughout the body, but a large part is transported into erythrocytes and hepatocytes where it is sequestered as ThDP. There are of course no data available for all organs and most data available are from rats and mice, but it has been unambiguously shown that after high dose thiamine administration, thiamine and ThDP content rapidly increases in red blood cells and liver, but not in brain. Unfortunately, no data are available on muscles which make up a significant part of the body mass (about 40 %), but as far as we know, excess thiamine is stored as ThDP in red blood cells and hepatocytes which act as a buffer reservoir for the vitamin. Erythrocytes contain a high-affinity thiamine transport system, probably ThTR1, as well as a low-affinity system. Inside erythrocytes, thiamine is pyrophosphorylated to ThDP by TPK1. ThDP cannot exit erythrocytes as such but is hydrolyzed to ThMP and thiamine which can exit erythrocytes and represent the major thiamine forms in blood plasma.

5.2.2. Cellular transport

In hepatocytes thiamine is transported by the high capacity and highly expressed Na⁺-dependent organic cation transporter (OCT) -1. Though having a low affinity for thiamine ($K_m = 0.78$ mM), it allows the liver to store significant amounts of the vitamin when circulating thiamine concentrations are very high. The liver must however not be understood as a thiamine-storage organ, but rather as a buffer: thiamine exits the liver as soon as circulating thiamine concentrations have normalized. Other tissues, such as brain for instance, that have low expression levels of OCT1, do not have this capacity. Hence, although OCT1 is present in the apical membrane of the blood brain barrier, it does not seem to be involved in thiamine transport, at least at physiological concentrations. The high-affinity ThTR2 seem to be the major thiamine transporter involved in the entry of the vitamin into the brain parenchyma (Gabriel et al., 2024).

Genetic defects of thiamine transport (see section 6.3) were recently reviewed by Marcé-Grau et al. (2019). The first loss-of-function mutations in thiamine transporters were reported in the *SLC19A2* gene and lead to a rare autosomal recessive disease – thiamine-responsive megaloblastic anemia syndrome (TRMA, Rogers syndrome), or thiamine metabolism dysfunction syndrome 1 (THMD1, OMIM 249270). In 2025, only about 200 TRMA patients have been described in the medical literature but new patients and variants are being discovered regularly. TRMA is characterized by a triad of early onset megaloblastic anemia, progressive sensorineural hearing loss and diabetes mellitus. Lifelong oral thiamine therapy of patients at pharmacological doses (25-100 mg/day) usually helps to alleviate the symptoms of the disease.

In contrast, mutations in the *SLC19A3* gene lead to a much more severe affection, an autosomal recessive neurometabolic disorder called biotin-thiamine-responsive basal ganglia disease, also known as thiamine metabolism dysfunction syndrome 2 (THMD2, OMIM 607483). This disease

manifests already in early childhood by various neurological symptoms such as subacute encephalopathy with ataxia, seizures, dysarthria, and dystonia. Oral administration of high doses of thiamine combined with biotin resolves symptoms within a few days, often no relapses occurring if therapy is continued. The reason of such a dramatic improvement in biotin-thiamine-responsive basal ganglia disease patients on biotin treatment remains unclear. An important challenge is to recognize the condition as thiamine/biotin dependent as, untreated, the outcome may be fatal.

5.3. Thiamine excretion

Thiamine excretion has been poorly studied (Bettendorff, 2020). It seems, that under normal conditions part of thiamine present in the renal filtrate is reabsorbed at the level of glomerular tubules so as to minimize loss. Human urine thiamine concentrations are about 0.5 μmol per day. However, after administration of high doses of thiamine, when plasma thiamine concentrations are high, the process is reversed and thiamine is excreted. Several thiamine degradation products have also been found in the urine, but the metabolic pathways leading to these products remain unknown. Most probably they are byproducts of detoxication enzymes present in liver and kidneys. Elimination of thiamine through transpiration and feces (mainly thiamine produced by colonic bacteria) remains negligible. Finally, human milk contains relatively high concentrations (compared to blood plasma) of the vitamin (0.5 $\mu\text{mol/L}$) mostly in the form of ThMP, suggesting that RFC1 may be involved in the excretion, of the vitamin. This might explain why lactating women are at risk of thiamine deficiency in regions where thiamine intake is already limit (Kareem et al., 2023). Indeed, lactating women transfer about 0.2 mg of thiamine to their infants through the milk, and an additional 0.1 mg is needed to compensate for the increased energy cost of lactation.

5.3. Intracellular distribution of thiamine derivatives

The intracellular distribution of thiamine derivatives is highly variable among cell types. Presently, most of our knowledge comes from brain cells, cultured neuroblastoma cells, or hepatocytes.

5.3.1. Mammalian cells contain a low and a high turnover ThDP pool

In the cytosol of mammalian cells, only a small fraction of the vitamin remains as free (unphosphorylated) thiamine. Most of it is rapidly pyrophosphorylated and only when the intake is very important (for instance when lipophilic provitamins are used) does the influx exceed the capacity of pyrophosphorylation and thiamine may accumulate. Excess cytosolic thiamine may be released from the cells, provided extracellular concentrations are low. In some cells, a very small fraction can be adenylated to AThTP. ThDP can also be hydrolyzed to ThMP, probably by several unspecific phosphatases. ThMP in turn may be hydrolyzed to thiamine by various phosphatases among which cytosolic acid phosphatase may play a primal role. Hence, in animal cells, ThMP can only be formed from hydrolysis of ThDP, but not by synthesis from thiamine. This metabolic ThDP corresponds to the high turnover pool (turnover time 1-2 hours).

In addition to the metabolic ThDP pool, a significant part of ThDP formed is either bound to cytosolic transketolase or transported into mitochondria, where it is mostly bound to mitochondrial dehydrogenases (Fig. 3). The sum of bound ThDP represents the low turnover pool (~17 hours). In addition to binding to various apoenzymes, mitochondrial ThDP may be phosphorylated to ThTP by mitochondrial ATP synthase.

5.3.2. Thiamine pyrophosphorylation

Thiamine pyrophosphokinase 1 (TPK1, EC 2.7.6.2), the enzyme responsible for the pyrophosphorylation of thiamine to ThDP is one of the best characterized enzymes of thiamine metabolism. TPK1 is a 27 kDa protein expressed in all animal tissues, able to pyrophosphorylate thiamine and many of its structural analogs. The affinity for thiamine is high ($K_m \leq 0.1 \mu\text{M}$), but the rate of reaction is low ($k_{\text{cat}} = 0.03 \text{ s}^{-1}$) and the equilibrium is strongly in favor of ThDP synthesis ($\Delta G^\circ = -27 \text{ kJ/mol}$). But excess ThDP inhibits the enzyme, preventing its accumulation (Sambon et al., 2022). The specificity for the nucleoside 3-phosphate is poor, but the preferred donor seems to be UTP (Fig. 1).

TPK1 is expressed in all animal tissues, but both mRNA and protein expression levels are lowest in the brain, suggesting that limited TPK1 expression rather than the expression of thiamine transporters, is responsible for the susceptibility of brain to thiamine deficiency. A few cases of genetic depletion of TPK1 were described with severe consequences: decreased ThDP levels, decreased pyruvate utilization and decreased amounts of the TK product sedoheptulose 7-phosphate. Therefore, mutations in the human *tpk1* gene (thiamine metabolism dysfunction syndrome 5, OMIM 614458, also known as TPK1 deficiency) are severe, with variable clinical manifestations (including acute encephalopathy, seizures and elevated lactate plasma concentrations) and often fatal. In some cases the patients respond to high-dose thiamine administration (Bettendorff, 2020).

5.3.3. ThDP transport into mitochondria

ThDP transport into mitochondria is mediated by a carrier protein, the product of the *SLC25A19* gene, previously known as deoxynucleotide carrier protein. This 320 amino acid protein is a member of the mitochondrial carrier transporter family. The process is pH-dependent with a K_m of approximately $6.8 \mu\text{M}$. Mutations in *SLC25A19* are rare but severe and often lethal with important brain malformations and microcephaly in its most severe forms. Peroxisomal ThDP is imported in association with the apoenzyme HACL1.

5.3.4. Synthesis of thiamine triphosphate and adenosine thiamine triphosphate

ThTP and AThTP exist in most human tissues but their role remains unknown (Gangolf et al., 2010). ThTP seems to be synthesized by F_0F_1 -ATP synthase in brain mitochondria from free mitochondrial ThDP (high turnover pool, see below). It could be involved in the regulation of ATP synthesis by this enzyme. AThTP, on the other hand, is present only sporadically in adult human tissues but is consistently found in fetal tissues and cell lines. It is synthesized by a cytosolic ThDP adenyllyl transferase that is not yet identified.

6. Nutritional importance of thiamine: requirements and deficiencies

Thiamine deficiency in humans can result either from toxic factors (chronic alcohol abuse, thiaminase containing food, or specific medical conditions) or from a poor nutritional status (nutritional deficiency). The first category is mainly limited to developed countries, while nutritional thiamine deficiency is still a very underestimated condition in many developing countries. A third, but very rare

indirect cause of thiamine deficiency results from genetic factors, *e.g.*, mutations in thiamine transporters, TPK1, or ThDP-dependent enzymes.

6.1. Thiamine requirements and occurrence

The daily requirement of an adult human is in the range of 1.1–1.5 mg/day, but it is dependent on age, sex (pregnancy and lactation), physiological condition (sedentary or active lifestyle) calorie intake (Table 2). The reader should however be aware that there are no clearly and universally established reference values concerning thiamine requirements. This is mainly due to the absence of adequate cutoff values for estimation of thiamine deficiency (Whitfield et al., 2018).

Table 2. Recommended dietary allowances (RDA, mg/day) for thiamine in humans (Data compiled from (Bettendorff, 2020; Johnson et al., 2019)).

	Males	Females
Adults		
19+ years	1.1	1.2
Pregnant woman	1.4	-
Lactating woman	1.5	-
Adolescents		
10 - 18 years	1.2	1.1
Infants and Children		
0 – 6 months	0.2	0.2
7 – 12 months	0.3	0.3
1 – 3 years	0.5	0.5
4 – 6 years	0.6	0.6
7 – 9 years	0.9	0.9

Thiamine is present in all kinds of food, but meat (especially pork) is particularly rich in thiamine (mainly in the form of ThDP). Vegetables contain much less thiamine than meat, but whole grain cereals or nuts are rich in the vitamin.

Conservation may decrease the thiamine content of food as thiamine is light- and temperature-sensitive. The decrease may be important in canned food where processing may lead to 20 to 70% loss of the vitamin.

Regions with low dietary thiamine intake are obviously at risk of thiamine deficiency. These are those with 50% of calories are derived from low-thiamine staple crops like cassava (mainly West-African countries) and polished rice (mainly South-East Asian countries) (Whitfield et al., 2018). History shows that it is very difficult to change food habits (for instance switching from polished-rice to whole grain rice) in most populations. Therefore, food fortification strategies may be of outmost importance (Fitzpatrick et al., 2024).

6.2. Thiamine deficiency disorders

As seen above, all diseases resulting from mutations in ThDP-dependent enzymes or enzymes of thiamine metabolism are rare but mostly severe. The most common problem in humans results from nutritional thiamine deficiency.

Compared to other mammals, humans have relatively low plasma and cellular thiamine concentrations which may explain why humans are particularly sensitive to thiamine deficiency (Gangolf et al., 2010). Another factor is our digestive system. Our microbiota, which is essential for good health, contributes to a significant production and absorption of the vitamin. Thiamine is essential for the gut microbiota by favoring the growth of butyrate and other short chain carboxylic acid producing bacteria. However there is no evidence that the gut microbiota contribute to a significant transfer of the vitamin to its host in humans. This is in contrast to ruminants, or even rodents, that are extremely difficult to render thiamine-deficient by lowering thiamine intake. In humans a few weeks of limited thiamine intake can already lead to thiamine deficiency symptoms.

In adults, severe chronic or acute deficiency may lead to two main diseases, beriberi (cardiac and neurological forms) and Wernicke-Korsakoff syndrome, the latter most often in relation to high and chronic alcohol consumption. Infantile beriberi has a large spectrum of nonspecific clinical features with neurological abnormalities culminating in death by heart failure. There is no single symptom that by itself can direct the clinician towards thiamine deficiency diagnosis, except for the fact that thiamine deficiency, as long as there is no irreversible neurological damage, rapidly responds to thiamine administration. Hence, the importance of early diagnosis of thiamine deficiency. Because of the various and overlapping manifestations of thiamine deficiency, the Gates Foundation-Sackler Institute Thiamine Deficiency Consultation group suggested to use the umbrella term “Thiamine Deficiency Disorders” (TDD) (Whitfield et al., 2018).

6.2.1. Beriberi

Beriberi is a polyneuropathic syndrome. Major symptoms of “dry” beriberi include weakness and pain mainly in the lower limbs, weight loss and partial paralysis. In contrast, the so-called “wet” beriberi is characterized by cardiac symptoms such as cardiac insufficiency and enlargement, with tendency to edema. Wet and dry beriberi are the classical manifestations of chronic dietary thiamine deficiency. Beriberi used to be a major health problem in East Asian countries, where polished rice (thiamine is mainly present in the husk) is the staple food. Presently, food fortification programs, underway in several countries, may play an essential role in the prevention of thiamine deficiency (Whitfield et al., 2021).

6.2.2. Wernicke-Korsakoff syndrome

In developed countries where nutritional thiamine deficiency has become rare, except for some high-risk groups (see below), another form of thiamine deficiency is associated with chronic alcoholism (Batarfi, 2025). Wernicke’s encephalopathy manifests as a state of confusion accompanied by ophthalmoplegia, nystagmus and ataxia. It may appear without prewarning and, in the absence of high-dose thiamine therapy, coma and death occur rapidly. Recovery is often not complete and irreversible sequelae, due to specific brain lesions, will persist. The appearance of neurological lesions may result in a state of dementia called Korsakoff’s psychosis, an amnesic syndrome, with loss of anterograde memory. From a clinical point of view, Wernicke’s encephalopathy and Korsakoff psychosis are different diseases but with apparently the same underlying causes (alcohol-induced thiamine deficiency). However, it is not clear why some patients develop only the first, others the second and for other Korsakoff psychosis appears

as a consequence of Wernicke's encephalopathy. Therefore, they are often referred to as Wernicke-Korsakoff syndrome. From a histological point of view, it is characterized by rather specific brain lesions mainly affecting thalamic nuclei and mammillary bodies. The cortex (in contrast to Alzheimer's disease) is largely spared. The reason for the selective vulnerability of midbrain ganglia in Wernicke-Korsakoff syndrome remains elusive.

These lesions are probably the result of impairment of oxidative energy metabolism leading to neuronal death by a combination of excitotoxicity and oxidative stress. Inflammatory processes that primarily affect endothelial cells of the microvessels rather than neurons are probably an early event in the progression of the disease. The relation between thiamine deficiency and alcoholism is not clear and several factors may be responsible: on one hand there is a poor nutritional status in many alcoholics, but alcohol may also interfere with intestinal thiamine absorption and its intracellular conversion to ThDP. Moreover, only a fraction of alcoholics develop Wernicke-Korsakoff syndrome suggesting that other factors, possibly inherited (TK variants), may be of importance.

While sex-based differences in the neuropathological presentation of Wernicke-Korsakoff syndrome have been reported, they remain inconclusive.

While Wernicke-Korsakoff syndrome is traditionally linked to chronic alcoholism, non-alcoholic forms have also been increasingly reported, linked to nutritional deficiencies due to eating disorders, bariatric surgery, hyperemesis gravidarum and gastrointestinal disorders. Non-alcoholic Wernicke-Korsakoff syndrome seems to be slightly more frequent in females compared to males.

6.2.3. Thiamine deficiency in infants (infant beriberi)

Recent research in developing countries has shown that newborn infants are particularly at risk of thiamine deficiency, mainly as a result of maternal thiamine deficiency or malnutrition (Whitfield et al., 2018). It is of interest to deal with infant beriberi separately. Indeed, the symptoms are very unspecific in the first months of age (irritability, tachycardia, vomiting, and crying, among others) and the leading cause is often overlooked. The progression is often rapid with fatal outcome due to congestive heart failure as observed in adult cardiac beriberi. In addition infant thiamine deficiency may be rather common in some parts of Africa and South East Asia. In Cambodia for instance, the prevalence of thiamine deficiency in infants (aged 6-59 months) was estimated as high as 15 - 58%. The large interval is the result of different cutoff values used. One of the difficulties detecting presymptomatic thiamine is the lack of reliable biomarkers of thiamine deficiency and cutoff values. Among the hallmarks of nutritional thiamine deficiency is its rapid response to thiamine administration and reversal of the symptoms. Hence, the importance of early diagnosis of TDDs. In case of doubt, administration of thiamine can be life-saving at a low cost and without danger.

6.2.4. Marginal thiamine deficiency

As mentioned above, in the absence of biomarkers and cutoff values, the individual thiamine status is difficult to establish. Moreover, there is probably no clear limit below which thiamine deficiency can be ascertained as nutritional (a diet rich in sugars increases the need for thiamine) and hereditary factors are important. Here, we define marginal thiamine deficiency as a low thiamine presymptomatic status, meaning that the body is still able to compensate for the metabolic stress induced. Particular groups at high-risk of thiamine deficiency are elderly people, lactating women, diabetic and AIDS patients.

Strangely, people suffering from Alzheimer’s disease also have about 20% lower blood and cerebral ThDP levels compared with matched controls, possibly as a result of pro-inflammatory cytokines that inhibit thiamine uptake (Ramamoorthy et al., 2022).

6.2.5. Genetically-induced thiamine deficiency

In addition to nutritional or toxin-induced thiamine deficiency, mutations in the genes coding for thiamine transporters, TPK1 or ThDP-dependent enzymes may be responsible for specific diseases in humans. They are very rare and generally lead to only a partial inactivation of the mutated gene. A complete functional knockout would be lethal considering the important cellular functions impaired. Nevertheless these mutations lead to severe disabilities. In some cases, the biochemical lesions can be overcome by the administration of high doses of thiamine or thiamine prodrugs with high bioavailability.

Table 3. Inherited human diseases due to mutations in thiamine transporters, TPK1 or ThDP-dependent enzymes (Modified from Bettendorff, 2020; Makarchikov et al., 2025).

Component involved	Disease	Phenotype
Folate:Anion Antiporter (SLC19A1) (also transports ThDP)	Genetic variations may contribute to Wernicke’s encephalopathy	Megaloblastic anemia resulting from decreased folate transport into erythrocytes
Thiamine transporter 1 (SLC19A2, THTR1)	Thiamine-responsive megaloblastic anemia (THMD1) ^a	Diabetes mellitus and sensorineural deafness
Thiamine transporter 2 (SLC19A3, THTR2)	Biotin-thiamine-responsive basal ganglia disease (THMD2) Wernicke-like encephalopathy	Subacute encephalopathy, fatal ^b Confusion, ophthalmoplegia, ataxia, and fatal ^b
Polyspecific Organic cation transporters (SLC22A, OCT1 and possibly OCT3)	Various liver diseases	Reduced oral glucose tolerance and reduced hepatic uptake of metformin
Mitochondrial ThDP transporter (SLC25A19)	Amish lethal microcephaly (THMD3) Homozygous or heterozygous mutations with bilateral striatal degeneration and progressive polyneuropathy (THMD4)	Congenital microcephaly, 2-oxoglutaric aciduria, and fatal ^c Milder phenotype than THMD3. Episodic encephalopathy, full recovery possible
Thiamine pyrophosphokinase 1 (TPK1)	(Leigh-like) encephalopathy (THMD5)	Encephalopathies with various degrees, α -ketoglutaric aciduria and lactic acidosis, movement disorders, and fatal ^a
Pyruvate dehydrogenase E1 component (thiamine-responding form)	Leigh’s disease (Subacute necrotizing encephalomyelopathy)	Encephalopathy, lactic acidosis, and fatal ^c May in some cases respond to thiamine
2-Oxoglutarate dehydrogenase E1 component	2-oxoglutarate (alpha-ketoglutarate) dehydrogenase deficiency	Acidosis, encephalopathy, brain atrophy, basal ganglia abnormalities, early childhood onset, and fatal
Oxoglutarate dehydrogenase-like (OGDHL)	Overexpressed in cancer cells	Heterogeneous neurological abnormalities

2-Oxoacid dehydrogenase E1 component (<i>DHDKD1</i> gene)	α -aminoacidipic and α -ketoadipic aciduria Charcot-Marie-Tooth disease	Metabolic disorder with neurological abnormalities
Branched chain α -ketoacid dehydrogenase complex (BCKDC)	Maple syrup urine disease	Accumulation of branched-chain α -ketoacids in urine with severe neurological consequences and encephalopathy
Transketolase variants	Predisposition to Wernicke-Korsakoff syndrome?	Confusion, ophthalmoplegia, and ataxia
2-Hydroxyacyl-CoA lyase	Refsum-like disease?	Metabolic impairment with liver damage

^a THMD, thiamine metabolism dysfunction (or deficiency) syndrome

^b Fatal outcome only if not treated.

^c Does not generally respond to thiamine therapy

^d Does not generally respond to thiamine therapy, but requires a diet poor in branched-chain amino acids

6.3. Estimation of the thiamine status in humans

As is apparent from above, thiamine deficiency syndromes can manifest in many different ways, making an assessment of the thiamine status in human patients of primary importance. Evidently, only blood and urine are easily available for such an assessment.

Urine thiamine levels may depend on many factors and are not representative of the thiamine status of the patient. When measured in 24-hour urine collections thiamine levels primarily reflect dietary intake in individuals rather than the thiamine status of the patient.

Thiamine and ThMP levels in plasma or serum can be subject to important variations depending on the thiamine intake and are thus not a reliable indicator of the thiamine status. On the other hand, ThDP which is present in erythrocytes (the proportion of ThDP present in leucocytes is generally negligible compared to red blood cells) is a better indicator as its variability is less than for plasma thiamine. Finally another indicator of the thiamine status is the degree of saturation by ThDP of erythrocyte transketolase (ETK).

6.3.1. Blood ThDP levels

Unfortunately, there exists a high variability of basal thiamine concentrations in human blood between laboratories and there is no standardization of thiamine assays (Table 4). Hence it is not possible to derive any biochemically significant cutoff values indicating marginal or at risk of thiamine deficiency. Values are only meaningful in case of severe thiamine deficiency, when blood ThDP level are less than 50% of a control sample for instance.

Table 4: Potential cutoff values for the evaluation of thiamine status (For more details see Whitfield et al., 2018 and Johnson et al., 2019).

Evaluation	Cutoff	Status	Advantages	Disadvantages
Plasma thiamine	Unknown	Only when strongly decreased	Indicates recent intake	Not a good indicator of thiamine status
Plasma ThMP	Unknown	Unknown	Indicates recent intake	Not a good indicator of thiamine status
Blood ThDP	< 80 nmol/L	Deficiency	Biologically active vitamer and indicator of thiamine status	Unstable, requires freezing for long-term storage and chromatographic

				equipment with fluorescence mass spectrometric detector
Erythrocyte ETKac	≥ 1.2	Deficiency	Functional assay of biological activity	Assay not readily available, requires rapid separation of erythrocytes
Basal ETK	$< 0.47-0.59$ $\mu\text{mol}/\text{min}/\text{g Hb}$	Deficiency	Functional assay of biological activity	Assay not readily available, requires rapid separation of erythrocytes. Important differences in cutoff values between laboratories.

6.3.2 Erythrocyte transketolase (ETK) assay

The ETK assay provides a functional marker of thiamine status. Most commonly, the activity of the transketolase enzyme is measured in both the absence and presence of exogenous ThDP and an activity coefficient (ETKac) or ‘ThPP effect’ is calculated, providing a biomarker of ETK saturation with ThDP. Basal ETK activity provides an alternative measure of thiamine status. Basal ETK activity is reported in absolute units of enzyme activity; additional calculations are required as well as measurement of hemoglobin in the red blood cell lysate prepared in the laboratory. These additional steps have limited the use of this method.

7. Synthetic thiamine prodrugs

As we have discussed above, thiamine uptake requires specific transporters. These have a high affinity for the vitamin but are already saturated at submicromolar concentrations. While low-affinity thiamine transporters seem to exist, they have a low capacity. Therefore, thiamine precursors (prodrugs with high bioavailability) were developed in the 1940s in Japan, where beriberi was still common. These molecules are mainly four, sulbutiamine (SBT), thiamine tetrahydrofurfuryl (TTFD, fursultiamine), benfotiamine (BFT, S-benzoylthiamine O-monophosphate) and O,S-dibenzoylthiamine (DBT) (Fig. 6).

These derivatives are either liposoluble or, in the case of DBT, are rapidly hydrolyzed to the liposoluble S-benzoylthiamine, and rapidly diffuse across the intestinal epithelium, where they are transformed to thiamine leading to blood vitamin concentrations 2-4 orders of magnitude higher than after administration of an equivalent dose of thiamine (Sambon et al., 2021). It is important to realize that all four compounds have a open thiazol ring and, as such, cannot be detected by fluorescence detection and mass spectrometric detection is required (Fig. 2).

Thiamine prodrugs have many neuropharmacological properties with antioxydant and anti-inflammatory effects, being most powerful with BFT and DBT, especially for the latter effects. BFT has recently been shown to have beneficial effects in mouse models of Alzheimer’s disease and has been tested with some success in clinical studies of patients with Alzheimer’s disease (Gibson et al., 2020). BFT shows approximately the same success than treatments with β -amyloid targeted antibodies, but

without the severe side effects (brain swelling and bleeding) attributed to these drugs. These results suggest that BFT and DBT may be interesting candidates for an extension to larger studies. Recently DBT was shown to be effective in a mouse model of amyotrophic lateral sclerosis (Probert et al., 2022).

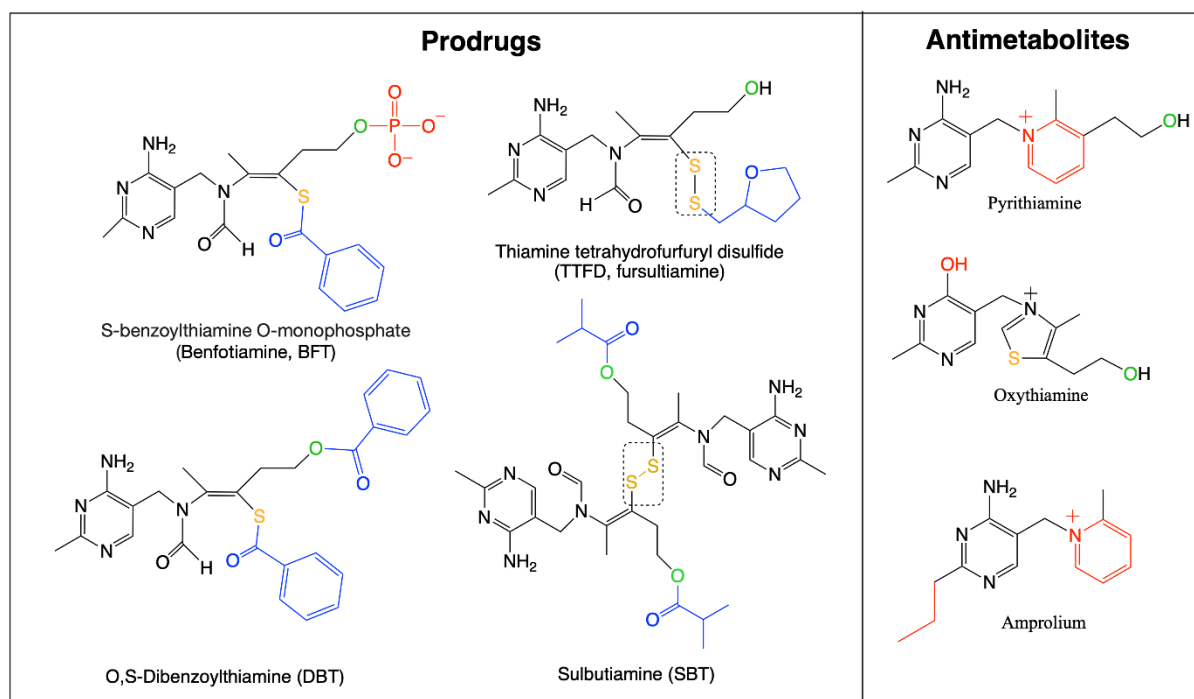


Figure 6. Main thiamine prodrugs of clinical importance and thiamine antimetabolite. BFT and DBT are thioesters, while TTFD and SBT (a symmetric dimer) are disulfides. In all the open thiazole ring derivatives the $-CH_3$ group and the sulfur atom must be in “trans” position to allow thiamine to be formed. The sulfur atom of the thiamine thiazolium ring is shown in yellow. The oxygen atom of the alcohol group susceptible to be phosphorylated in thiamine is shown in green. Organic substituents are in blue and the phosphate group of BFT is in red. Please note that benfotiamine, fursultiamine and sulbutiamine are commercial names and spelled without the letter “h” in thiamine. In thiamine antimetabolites, the structural changes with respect to thiamine are colored in red. Pyrithiamine is the pyridine analogue of thiamine (the thiazole ring is replaced by a pyridine ring). In oxythiamine the amino group of the pyrimidine ring is replaced by an alcohol. Amprolium is also a pyridine analogue of thiamine but lacks the hydroxyethyl group. Source:

BFT and DBT are thioesters and restitution of an intact thiamine molecules requires hydrolases. On the other hand, TTFD and SBT are disulfides requiring an oxidation possibly by glutathione, cystine or cysteine (Sambon et al., 2021). The mode of action of these compounds remains unknown, both as to their molecular target and the active compound involved. Some efforts have been made to detect the intact prodrugs in body fluids, in particular blood, using mass spectrometric detection, but these efforts have remained fruitless: only intact thiamine has been detected. More intriguingly, no significant increase in brain ThDP content has been shown so far after administration of these prodrugs in rodents, suggesting that their mechanisms of action is independent of the coenzyme function of ThDP. Hence, the mechanism of action of these drugs remains unknown: we do not know that they have strong antioxidant and anti-inflammatory properties but we do not know which thiamine derivative is responsible for the effects and we ignore their biochemical target. Hence the

question as to their mode of action is of primary importance and we are left with essentially three hypothesis.

- 1) The increase of blood thiamine is responsible for the beneficial effects. A hypothesis might be an effect of brain capillaries.
- 2) Some unknown compound with pharmacological properties is formed from thiamine prodrugs (the open thiol form for instance).
- 3) They affect the intestinal microbiota with effects on the gut-brain axis.

Another important point is that thiamine prodrugs (especially BFT) have no apparent toxicity, even at very high doses. This is in contrast to monoclonal antibodies directed against β -amyloid, such as Lecanemab or Aduhelm, that have important side effects and are not more efficient. Hence, thiamine prodrugs represent important therapeutic tools that warrant further investigation.

8. Thiamine antimetabolites and toxicity

Sensu stricto, thiamine antimetabolites are organic molecules with structural analogy to thiamine, that act by competitively blocking enzymes of thiamine metabolism or, after pyrophosphorylation, ThDP-dependent enzymes. The definition can however be expanded so as to encompass various compounds that act by destroying the vitamin.

8.1. Synthetic thiamine antagonists (true antimetabolites)

Several synthetic thiamine antagonists have been developed with the most important depicted in Fig. 6. They are mainly used in research to mimic thiamine deficiency or as therapeutic agents, such as to target bacterial or fungal infections or as an anti-cancer agent, by blocking ThDP-dependent enzymes. The most potent is pyrithiamine, a competitive inhibitor of thiamine transport and TPK1. Other analogs such as oxythiamine, amprolium and the chemically unrelated diuretic amiloride also inhibit thiamine transport but with a much lower affinity than pyrithiamine.

Pyrithiamine and oxythiamine can both be pyrophosphorylated by TPK1 to their respective diphosphorylated derivatives. Oxythiamine diphosphate is a potent inhibitor of thiamine-dependent enzymes, while pyrithiamine diphosphate is not. Amprolium cannot be phosphorylated.

Pyrithiamine easily crosses the blood-brain barrier, in contrast to oxythiamine, which does not enter the brain. This property was used in the development of animal models of Wernicke-Korsakoff syndrome. In contrast, oxythiamine-treated animals have no neurological symptoms, but suffer weight loss, anorexia and cardiac enlargement, probably as a result of inhibition of ThDP-dependent enzymes by oxythiamine diphosphate in peripheral tissues.

8.2. Sulfites

Sulfites are added as preservatives to many foodstuff. They are able to cleave the thiamine molecule at the level of the methylene bridge, yielding separate pyrimidine and thiazole moieties. Cases of thiamine deficiency in animals due to feeding on sulfite-preserved meat have been described.

8.3. Thiaminases

Some foodstuff may contain thiaminases (thiamine-destroying) enzymes. Some fish (*e.g.*, carp, eel, baltic herring, or catfish), shellfish, invertebrates (African silk worm) or roots (cassava) may contain thermolabile thiaminase I, a pyrimidine transferase (EC 2.5.1.2). This is also the case of some ferns (*Pteris aquilina*)

that, when consumed by grazing cattle or horses, may result in severe thiamine deficiency. Thiaminase I is destroyed by cooking, but consumption of raw food containing this enzyme may cause beriberi in humans. Another thiaminase found in microorganisms, thiaminase II (EC 3.5.99.2), catalyzes the hydrolysis of thiamine in separate pyrimidine and thiazole moieties. This enzyme, which is involved in thiamine salvation in microorganisms, does not pose health problems in humans.

8.4. Thiamine toxicity

Thiamine and prodrugs have an extremely low toxicity after oral administration. In very rare cases, allergic reactions have been reported after intravenous administration of thiamine.

9. Conclusion

Thiamine was the first vitamin identified about a century ago and this discovery was at the origin of the concept of vitamin *e.g.*, substances that are required in small amounts (at mg quantities per day) for cell survival and that, at least for B vitamins, have a catalytic role. This is in contrast to sugars and essential amino acids or lipids that are either fuels or building blocks for more complex macromolecules.

During this last century the coenzyme function of ThDP has been extensively characterized. ThDP-dependent enzymes occupy strategic positions in catabolytic pathways and energy metabolism. This is particularly important for cells that totally rely on oxidative metabolism such as neurons and cardiocytes, explaining the high sensitivity of these cells to thiamine deficiency. However, while all neurons heavily rely on oxidative metabolism, it is not clear why some midbrain neurons are particularly sensitive to thiamine deficiency. Therefore, other factors, such as anatomical localization (proximity to the choroid plexus) or biochemical specificities might be important. In recent years, the discovery of new enzyme isoforms (TK variants or OGDHL) may contribute to our understanding of the mechanisms involved in thiamine deficiency. The situation is further complicated by the existence of newly discovered thiamine derivatives with coenzyme-independent roles. The thiamine triphosphorylated derivatives ThTP and AThTP exist in practically all organisms.

From the above, it is obvious that thiamine is a highly pleiotropic vitamin. The coenzyme ThDP is involved in virtually all compartments and major metabolic pathways of the cell, including the synthesis of the major neurotransmitters (Fig. 3). This is probably one of the reasons for the very high variability of the phenotype associated with thiamine deficiency disorders or hereditary diseases involving ThDP-dependent enzymes or proteins involved in thiamine metabolism.

Thiamine deficiency, because of alcohol consumption, other medical condition, or malnourishment, remains a major health problem throughout the world. On the other hand, nutritional thiamine deficiency continues to be a major health problem in many developing countries. It is the consequence of either general malnourishment or consumption of a staple food poor in thiamine (polished rice) or containing thiaminases (cassava). As it is difficult to change alimentary habits, thiamine fortification, either by direct addition of the vitamin or genetically modified crops, may be an achievable solution.

In addition to purely nutritiounal aspects, *e.g.*, replenishing thiamine stores, thiamine prodrugs have been shown to be active in various neurological conditions and their mechanism of reation is investigated very actively. Hence thiamine prodrugs could become interesting therapeutic tools at least for slowing down the progression of Alzheimer's disease at early stages. It is now well

documented that human patients with Alzheimer's disease have impaired thiamine homeostasis, and BFT administration correct these abnormalities linked to the suboptimal working of ThDP-dependent enzymes. However, in addition to the coenzyme effects, thiamine prodrugs also exert strong, and probably coenzyme-independent, antioxidant and anti-inflammatory effects.

Hence, a 100 years after its discovery, thiamine, remains a highly investigated field with special emphasis on the molecular mechanisms of ThDP-dependent enzyme complexes and their implications in metabolic pathways, the development of rapid and easy methods and strategies for detecting populations at risk of thiamine deficiency, the prevention of subclinical thiamine deficiency by food fortification and thiamine supplementation and the exploration of thiamine prodrugs as potential therapeutic agents in neurodegenerative diseases.

Abbreviations

A_{Th}TP, adenosine thiamine triphosphate;

BCKDC, branched chain α -ketoacid dehydrogenase complex;

BFT, benfotiamine;

FAD, flavin adenine dinucleotide;

HACL1, 2-hydroxyacyl-CoA-lyase;

HPLC, high performance liquid chromatography;

NAD⁺, oxidized nicotinamide adenine dinucleotide phosphate;

NADPH, reduced nicotinamide adenine dinucleotide phosphate;

OCT, organic cation transporter;

OADHC, 2-oxoadipate dehydrogenase complex;

OGDHC, oxoglutarate dehydrogenase complex;

OMIM, online mendelian inheritance in man;

PDHC, pyruvate dehydrogenase complex;

RFC, reduced folate transporter;

SBT, sulbutiamine;

TCA, trichloroacetic acid;

TDD, thiamine deficiency disorders;

ThDP, thiamine diphosphate

THMD, thiamine metabolism dysfunction (or deficiency) syndrome;

ThMP, thiamine monophosphate;

ThTP, thiamine triphosphate;
THTPA, 25-kDa thiamine triphosphatase;
TK, transketolase;
TPK1, thiamine diphosphokinase;
WKS, Wernicke-Korsakoff syndrome;

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