

Biochemical and medical aspects of vitamin B₁ research

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ABSTRACT

Vitamin B₁ is an indispensable food factor for the human and animal body. In animals, vitamin B₁ is found in the form of thiamine and its phosphate esters – thiamine mono-, di- and triphosphate, as well as an adenylated derivative – adenosine thiamine triphosphate. At present, the only vitamin B₁ form with biochemical functions being elucidated is thiamine diphosphate, which serves as a coenzyme for several important enzymes involved in carbohydrate, amino acid, fatty acid and energy metabolism. Here we review the latest developments in the field of vitamin B₁ research in animals. Transport, metabolism and biological role of thiamine and its derivatives are considered as well as the involvement of vitamin B₁-dependent processes in human diseases and its therapeutic issues, a field that has gained momentum with several important recent developments.

1. Introduction

Vitamin B₁ (thiamine) is an indispensable micronutrient for the human and animal body, the recommended daily allowance for thiamine being 1.0–1.6 mg in adults and 0.7–1.3 mg for children (Bender and Cunningham, 2021). The concept of vitamin B₁ as an essential micronutrient capable of preventing and curing beriberi disease and polyneuropathy was raised at the turn of the 19th and 20th centuries thanks to the works of Eijkman and Grijns (Carpenter, 2012; Eggersdorfer et al., 2012) although the molecular structure of thiamine was identified by Williams only in 1935 (the term « thiamine» was introduced in 1938) (Williams and Cline, 1936; Williams, 1938). This event was preceded by the painstaking work of many researchers, including Casimir Funk, who first isolated a crude precipitate of anti-beriberi factor from rice bran in 1911 and coined the term « vitamin» (Funk, 1911) as well as Jansen & Donath obtaining pure crystals of vitamin B₁ in 1926 (Jansen and Donath, 1926). The first steps towards elucidating biochemical mechanisms of thiamine action were the studies of Peters et al., carried out in the mid-1930s, when the neurological symptoms (the phenomenon of opisthotonus) in vitamin B₁ deficient pigeons were

attributed to a low rate of pyruvate oxidation (Peters, 1936). Soon afterward, in 1937, Lohmann & Schuster found that the active form of thiamine is thiamine diphosphate (ThDP) (we prefer the abbreviation ThDP over TDP still often used, but should be reserved for thymidine diphosphate) serving a coenzyme of yeast pyruvate decarboxylase (Lohmann and Schuster, 1937). Presently, 80 years later, we know approximately 40 ThDP-dependent enzymes, most in prokaryotes (Prajapati et al., 2022) and among which 6 are present in mammals (see Fig. 2). Moreover, the use of thiamine in medicine and nutrition has gone far beyond the frames of the treatment and prevention of nervous system pathologies (Frank, 2015; McGing et al., 2021; Sriram et al., 2012). Nevertheless, many fundamental aspects of the metabolism and biological functions of vitamin B₁ remain poorly understood. The evolution of our knowledge in the area of thiamine biochemistry can be traced in a number of reviews (Aleshin et al., 2019; Bettendorff, 2021; Bettendorff and Wins, 2009; Cooper and Pincus, 1979; Haas, 1988; Leder, 1975; Makarchikov, 2009). The subject of this paper is to cover recent developments in the field of thiamine metabolism in animal cells, its biochemical role and relation to the development of pathologies.

Abbreviations: AD, Alzheimer's disease; AThDP, Adenosine thiamine diphosphate; AThTP, Adenosine thiamine triphosphate; BCKDC, branched chain α -ketoacid dehydrogenase complex; HAC11, 2-hydroxyacyl-CoA-lyase; LMW-PTP, low molecular weight protein tyrosine phosphatase; OGDHC, oxoglutarate dehydrogenase complex; PDHC, pyruvate dehydrogenase complex; OADHC, 2-oxoadipate dehydrogenase complex; OCT, organic cation transporter; RFC, reduced folate transporter; TDD, thiamine deficiency disorders; ThDP, Thiamine diphosphate; ThMP, Thiamine monophosphate; ThTP, Thiamine triphosphate; THTPA, 25-kDa ThTPase; ThTPase, Thiamine triphosphatase; TPK1, Thiamine diphosphokinase; TK, transketolase; WE, Wernicke's encephalopathy; WKS, Wernicke-Korsakoff syndrome.

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2. Thiamine and main derivatives: assay and structure

Currently, various high-performance liquid chromatography (HPLC) techniques are employed to quantify the content of thiamine compounds in biological samples after derivatization of thiamine compounds into fluorescent thiochromes (Bettendorff et al., 1986, 1991; Basiri et al., 2016; Lu and Frank, 2008). It is essential to understand that fluorometric assays can only detect derivatives with an intact thiazol ring. Therefore, several methods based on mass spectrometric detection have been developed (Kim et al., 2019; Verstraete et al., 2020). These have the advantage that they allow the detection of derivatives with an open thiazol ring that cannot be transformed into fluorescent thiochromes (Sambon et al., 2019, 2020).

In animals, as in most other living organisms, vitamin B₁ is found in the form of thiamine and a number of its phosphate esters – thiamine monophosphate (ThMP), ThDP, thiamine triphosphate (ThTP), as well as an adenylated derivative – adenosine thiamine triphosphate (AThTP). All of them are capable of interconverting in the cell under the action of particular enzymes (Fig. 1).

ThDP is the most abundant thiamine derivative (80–90 % of the intracellular content of B₁ vitamers in most organisms (Bettendorff et al., 1987; Egi et al., 1986; Iwata et al., 1988; Makarchikov et al., 2003). It occupies the central place in vitamin B₁ metabolism and in animal cells it is the direct precursor of ThMP, ThTP and AThTP (Fig. 1).

The recent discovery of AThTP raises the question whether there are still some other unknown thiamine derivatives. Indeed, the existence of small amounts of adenosine thiamine diphosphate has been strongly suggested in animal tissues (Frédérich et al., 2009).

Moreover, the group of Y. Parkhomenko, which has greatly contributed to our understanding of thiamine biochemistry, recently suggested the existence of an oxidized form of ThDP in rat brain (Pavlova et al., 2021). As ThDP was obtained after treatment of the samples with dithiothreitol, the oxidized form could be some disulfide compound, either a symmetrical ThDP dimer or a dimer formed by ThDP

and another thiol such as cysteine or glutathione. Reduction of the disulfide bond would yield the open form of ThDP that spontaneously reforms ThDP (Fig. 1) (Bettendorff, 2023). If confirmed, this would be an important step forward in understanding thiamine metabolism. This hypothesis was recently bolstered by the observation that thiamine disulfides can be reduced by reduced glutathione and the thioredoxin system (Folda et al., 2024).

Hence, from a biochemical point of view, we must distinguish between co-enzyme (ThDP)-dependent effects and non-coenzyme effects that can be mediated of other thiamine vitamers, such as ThMP, ThTP or AThTP and even ThDP (Aleshin et al., 2019; Bettendorff, 2021; Bettendorff and Wins, 2009; Mkrtchyan et al., 2015). These so-called non-coenzyme effects are however much less documented, and their physiological significance remains to be established.

3. Cellular thiamine transport and absorption

Ruminants almost entirely rely on thiamine biosynthesis by microbiota in the rumen (Pan et al., 2018). However, humans strictly depend on alimentary thiamine intake. This intake can occur either by capture of thiamine or ThDP (see below). Moreover, dietary thiamine has beneficial effects on the gut microbiota and in particular on some bacteria that lack the *de novo* thiamine biosynthetic pathway. Such bacteria are involved in the production of short-chain fatty acids, essential for a health (Park et al., 2022).

Intestinal thiamine is absorbed by several thiamine transporters. The most well studied are the specific carrier proteins ThTr1 and ThTr2, which are the products of *SLC19A2* and *SLC19A3* genes belonging to the folate transporter family (Dutta et al., 1999; Eudy et al., 2000; Fleming et al., 1999, 2001; Oishi et al., 2001; Rajgopal et al., 2001). 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 trans-membrane domains. Although both genes are functionally active in various organs and tissues, the highest level of hThTr1 mRNA expression

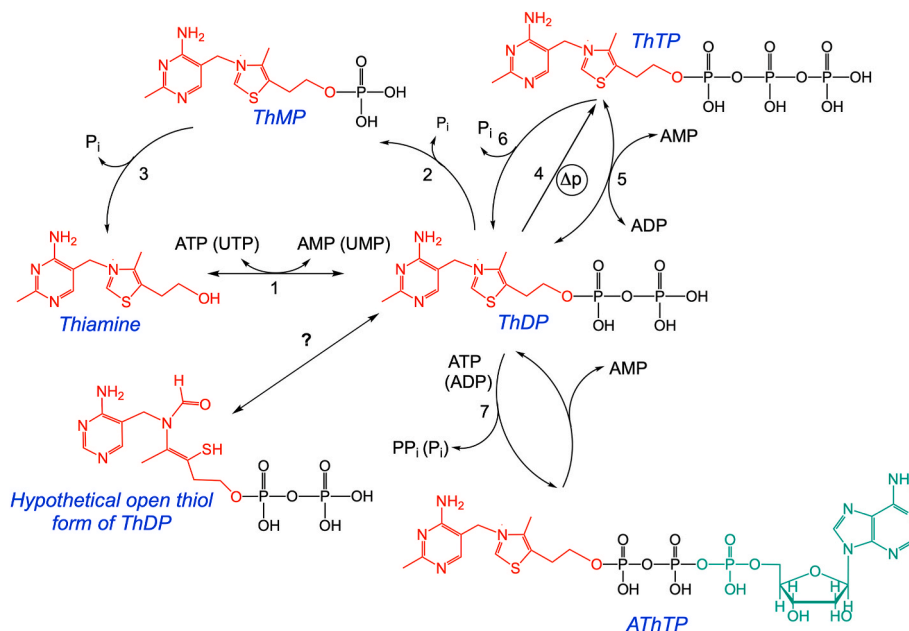


Fig. 1. Chemical structure of thiamine and its naturally occurring derivatives. ThDP is synthesized from thiamine and ATP by thiamine pyrophosphokinase (1). Hydrolysis of ThDP by thiamine pyrophosphatases (2) yields ThMP, which in turn can be hydrolyzed to thiamine by thiamine monophosphatases (3). ThDP 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 adenylate transferase (7). AThTP can be hydrolyzed to ThDP and AMP by a putative AThTP hydrolase (8). Another adenylated thiamine derivative, adenosine thiamine diphosphate (ATHDP), not represented here, has been shown to exist in prokaryotes and eukaryotes, but its mechanism of synthesis has not yet been studied in vitro. The open thiol form of ThDP could be an intermediate between an oxidized form and ThDP. P_i, inorganic phosphate; PP_i, inorganic pyrophosphate; Δp, proton gradient (From (Bettendorff, 2021)).

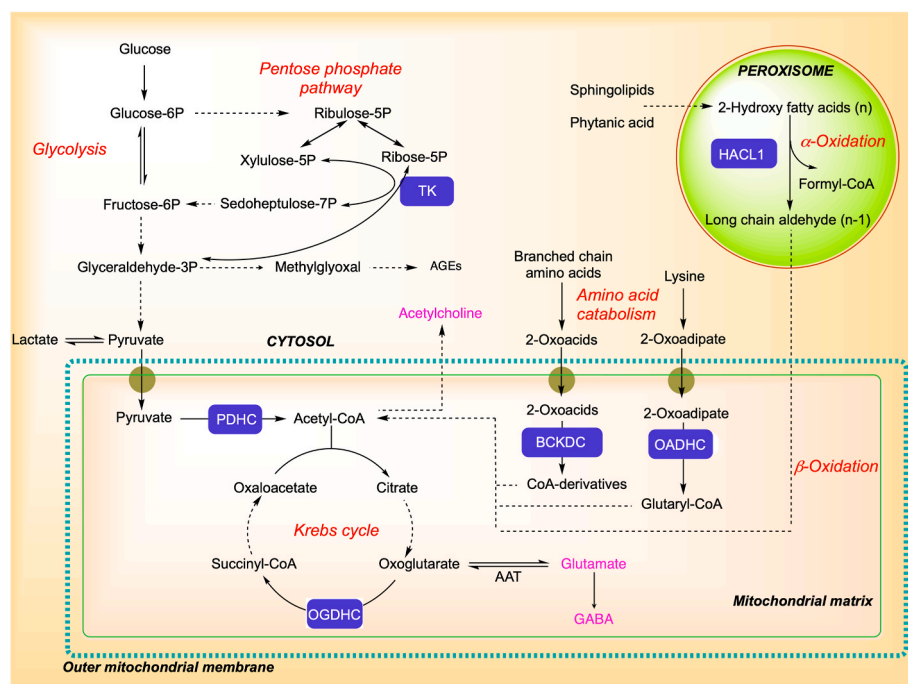


Fig. 2. A general scheme for ThDP-dependent reactions in mammalian cells. Interrupted lines indicate pathways with several steps. (For explanations see text).

was observed in skeletal muscle, followed by placenta, heart, liver, and kidney (Dutta et al., 1999; Labay et al., 1999), whereas mRNA hThTr2 was most abundant in placenta, kidney and liver (Eudy et al., 2000). According to these studies the mRNA expression of both ThTr1 and ThTr2 is rather low in the nervous system, however, according to the Human Brain Atlas, protein expression is relatively high both for ThTr1 (<https://www.proteinatlas.org/ENSG00000117479-SLC19A2/tissue>) and ThTr2 (<https://www.proteinatlas.org/ENSG00000135917-SLC19A3/tissue>) (Stand of January 14th, 2025). Both proteins contribute to the intestinal absorption of thiamine and are involved in its transport in different tissues (Reidling et al., 2010; Said et al., 2004). The thiamine transporters differ significantly in their affinities for substrate: the apparent K_m of ThTr1 for thiamine was evaluated to be around 2.5 μM (Dutta et al., 1999), while ThTr2 appears to function in the nanomolar range ($K_m = 27 \text{ nM}$, Said et al., 2004). The process of thiamine uptake mediated by the thiamine transporters depends on pH but is Na^+ -independent. Recently, the RNAseq-based tissue distribution expression screen identified *SLC19A3* as an adipose-specific/enhanced protein coding gene, its expression seems to be related to obesity and weight loss (Pereira et al., 2021).

Neither ThTr1 nor ThTr2 exhibit absolute specificity; in addition to thiamine, they are capable of transporting metformin and other cationic compounds (1-methyl-4-phenylpyridinium, famotidine, cerdulatinib, fedratinib, trimethoprim) (Giacomini et al., 2017; Liang et al., 2015). This would be the reason why the signs of thiamine deficiency could be occasionally revealed in particular groups of patients, for instance, suffering from diabetes with metformin treatment, much like as in the case of vitamin B_{12} (Wu et al., 2023). Moreover, it has been found that pyridoxine (vitamin B_6) can serve as a substrate for the human thiamine transporters (Yamashiro et al., 2020). In experiments with the expression of hThTr1 and hThTr2 in MDCKII cells, the kinetics of the pyridoxine uptake at pH 5.5 was described by a hyperbolic curve with K_m of 37.8 μM and 18.5 μM for SLC19A2 and SLC19A3, respectively. Interestingly, pyridoxine transport was not detected in mouse and rat SLC19A3 (Yamashiro et al., 2022). The results of site-directed mutagenesis indicate that the differences observed between animal species are due to the specificity of amino acid residues at positions Gln86, Gly87, Ile91, Thr93, Trp94, Ser168 and Asn173, most of which are

conserved in orthologues possessing the ability to transport pyridoxine (SLC19A3 of the monkey, dog, pig, rabbit, guinea pig and frog) (Miyake et al., 2022). Along with thiamine, the absorption of pyridoxine from the intestinal lumen is believed to be a physiological function of SLC19A3.

Genetic defects of thiamine transport (see Table 1) were recently reviewed by Marcé-Grau et al. (2019). Loss-of-function mutations in the *SLC19A2* gene have been found to cause a rare autosomal recessive disease – thiamine-responsive megaloblastic anemia syndrome (TRMA, Rogers syndrome), or thiamine metabolism dysfunction syndrome 1 (THMD1, OMIM 249270) (Diaz et al., 1999; Fleming et al., 1999; Labay et al., 1999). The characteristic manifestations of TRMA include 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 (Sako et al., 2022). Approximately 200 TRMA patients have been described in the medical literature with 89 variants in *SLC19A2* reported until 2022 (Mohsen-Pour et al., 2022). New patients and variants are being discovered regularly.

Mutations in the *SLC19A3* gene lead to an autosomal recessive neurometabolic disorder called biotin-thiamine-responsive basal ganglia disease, also known as thiamine metabolism dysfunction syndrome 2 (THMD2, OMIM 607483) (Debs et al., 2010; Zeng et al., 2005). Although this disease, manifesting already in early childhood by subacute encephalopathy with ataxia, seizures, dysarthria, dystonia and several other symptoms, arises from ThTr2 breakdown, oral administration of high doses of biotin (5–10 mg/kg/day) resolves symptoms within a few days, often no relapses occurring if therapy is continued. In some cases, a positive effect can be achieved only with the combined administration of biotin and thiamine (up to 40 mg/kg/day) (Alfadhel et al., 2013; Tabarki et al., 2020). Thiamine treatment alone was found to be effective as well (Tabarki et al., 2015). The reason of such a dramatic improvement in biotin-thiamine-responsive basal ganglia disease patients on biotin treatment remains unclear.

The level of expression of thiamine transporters ThTr1 and ThTr2 is significantly decreased in brain samples of Alzheimer's disease (AD) patients and in an animal model of the disease (Ramamoorthy et al., 2022), paving the way to understanding the reason for low brain thiamine levels in post-mortem Alzheimer's brains (Mastrogriacomo et al.,

Table 1

Inherited human diseases due to mutations in thiamine transporters, TPK1 or ThDP-dependent enzymes.

Component involved	Disease	Phenotype
Folate:Anion Antiporter (SLC19A1) (also transports ThDP (Dang et al., 2022; Zhao et al., 2001)	Genetic variations may contribute to Wernicke's encephalopathy (O'Brien et al., 2022)	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) (Alfadhel and Tabarki, 2018)	Subacute encephalopathy, fatal ^b
Polyspecific Organic cation transporters (SLC22A, OCT) (OCT1 and possibly OCT3) (Chen et al., 2014; Jensen et al., 2019; Zeng et al., 2023)	Various liver diseases (Koepsell, 2020)	Confusion, ophthalmoplegia, ataxia, fatal ^b
Mitochondrial ThDP transporter (SLC25A19)	Reduced oral glucose tolerance and reduced hepatic uptake of metformin (Liang et al., 2018; Shu et al., 2008)	
Thiamine pyrophosphokinase 1 (TPK1)	Amish lethal microcephaly (THMD3) (Lindhurst et al., 2006; Siu et al., 2010)	Congenital microcephaly, 2-oxoglutaric aciduria, fatal ^c
	Homozygous or heterozygous mutations with bilateral striatal degeneration and progressive polyneuropathy (THMD4) (Leigh-like)	Milder phenotype than THMD3. Episodic encephalopathy—full recovery possible
	encephalopathy (THMD5) (Au et al., 2020; Fraser et al., 2014; Rüsche et al., 2021)	Encephalopathies with various degrees, α-ketoglutaric aciduria and lactic acidosis, Movement disorders, fatal ^a
Pyruvate dehydrogenase (thiamine-responding form)	Leigh's disease (Barnerias et al., 2010; Lee et al., 2020) (Subacute necrotizing encephalomyelopathy)	Encephalopathy, lactic acidosis, fatal ^c
		May in some cases respond to thiamine administration (Kowalska et al., 2024)
Branched chain 2-oxoacid dehydrogenase	Maple syrup urine disease (Harris et al., 1990) (Branched-chain ketoaciduria)	Organic acidemia, sweet-smelling urine, fatal ^d
Transketolase variants	Predisposition to Wernicke-Korsakoff syndrome? (Alexander-Kaufman and Harper, 2009)	Confusion, ophthalmoplegia, ataxia
2-Hydroxyacyl-CoA lyase	Refsum-like disease? (Mezzar et al., 2017)	Metabolic impairment with liver damage
2-Oxoacid dehydrogenase (DHTKD1 gene)	α-aminoacidic and α-ketoacidic aciduria (Danhauser et al., 2012)	Metabolic disorder with neurological abnormalities
	Charcot-Marie-Tooth disease (Xu et al., 2012)	

^a 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.

1996). A very recent report demonstrates the association of SLC19A2 and the ion channel Tmem63b in a membrane complex regulating phospholipid scrambling in mammalian cells. The relation of Tmem63b to the thiamine transport function of SLC19A2 however remains enigmatic (Niu et al., 2024). Another point of possible importance is the observation that thiamine mono- and di-phosphatase activities are increased in the blood of patients with AD compared to control patients,

which may contribute to reduced blood ThDP levels in AD (Pan et al., 2017). A high blood ThDP concentration has indeed been found protective against AD (Wang et al., 2018). It is however not clear how increased blood phosphatase activities can affect brain ThDP levels, especially as brain ThDPase activities do not seem to be increased in AD patients (Mastrogiovanni et al., 1996).

An alternative route also appears to exist for the flow of vitamin B₁ into/out of mammalian cells through the reduced folate transporter (RFC1), the product of the *SLC19A1* gene, capable of transporting ThMP along with 5-methyltetrahydrofolate (Zhao et al., 2002). At least, that could account for the high content of ThMP in extracellular body fluids, amounting to about 60 % of total thiamine in the blood plasma of rats (Gangolf et al., 2010a; Rindi et al., 1968) and cerebrospinal fluid (Gangolf et al., 2010a; Rindi et al., 1981). Expression of RFC1, which operates via an anion exchange mechanism, occurs in all examined organs and tissues of humans and mice (Zhao and Goldman, 2013). O'Brien et al. supposed that genetic variations of RFC1 leading to altered ThDP efflux are associated with Wernicke-Korsakoff Syndrome (O'Brien et al., 2022). However, until now it has not yet been proven that RFC1 effectively transports ThDP.

There is evidence that transporters from the organic cation transporter (OCT) family may participate in the absorption of thiamine in the small intestine along with ThTr1 and ThTr2 (Lemos et al., 2012). In experiments with mice and HEK293 cell culture, Chen et al. showed that thiamine is a natural substrate for the OCT1 transporter (SLC22A1) in the liver, which presumably is involved in the hepatic uptake of large amounts of dietary thiamine (Chen et al., 2014). This transporter has a low affinity for thiamine, but high throughput. Kinetic parameters of thiamine uptake by HEK293 cells expressing the human hOCT1 were estimated to be $K_m = 0.78$ mM, $V_{max} = 2.77$ nmol/min/mg protein. It is likely that the low-affinity component of thiamine transport in rat liver basolateral membrane preparations is due to mOCT1 activity (Moseley et al., 1992). According to Kato et al., OCT1 is involved in the overall plasma clearance and secretion of thiamine in milk (Kato et al., 2015). Also, the OCT1 and OCT2 transporters, together with the MATE1 protein, may be involved in the secretion of thiamine in the renal tubules (Kato et al., 2014, 2015).

The affinity for thiamine is much higher for mouse than for human OCT1 (Meyer et al., 2020). This may explain that clear effects of OCT1 deficiency on thiamine levels were reported in mice but not in humans. It is therefore likely that the activity of OCT1 plays a lesser role in thiamine homeostasis in humans.

Another ThDP transporter was discovered and identified at the molecular level more recently as a product of the *SLC44A4* gene in humans (Nabokina et al., 2014; Nabokina and Said, 2012). Expression of two transcript variants (hTPPT-1, 710 amino acid residues, and hTPPT-3, 634 amino acid residues) of this protein in the gastrointestinal tract is restricted to the large intestine, where they are believed to contribute to the overall host thiamine nutrition by absorption of microbiota-generated ThDP (Nabokina et al., 2014). One may assume that ThDP, available to colonocytes, could be released from bacteria into intestinal environment after cell dying. Indeed, in a recent study, it has been estimated that 28 % of bacterial operational taxonomic units found in human fecal samples were dead and/or injured (Bellali et al., 2021). SLC44A4 is probably the only colonic ThDP transporter and, besides playing an important role in colonic physiology, its deletion in mice increases susceptibility to inflammation (Sabui et al., 2024).

In addition to the well recognized thiamine/ThDP transporters discussed above, another transporter, SLC35F3, has been shown to transport thiamine, and some of its variants are associated with cardiovascular traits leading to hypertension (Zhang et al., 2014).

4. Thiamine monophosphate: simply an intermediate?

After entering the cell or obtained from ThDP hydrolysis, ThMP can be dephosphorylated to thiamine (Fig. 1 and see below). For decades,

the molecular nature of the enzymes catalyzing this reaction in vivo remained obscure, but recently an enzyme with ThPase activity was purified from chicken liver (Kolas and Makarchikov, 2014) and identified by mass spectrometry as cytosolic acid phosphatase (EC 3.1.3.2) – the product of *acp1* gene in humans (Kolas et al., 2018). This enzyme, also called low molecular weight phosphotyrosine protein phosphatase (LMW-PTP, EC 3.1.3.48), is highly conserved across almost all living beings (Caselli et al., 2016). LMW-PTP catalyzes the dephosphorylation of various protein substrates, such as platelet-derived growth factor, ephrin and insulin receptors, *Src* kinase, etc. implying its involvement in important signaling pathways (Bottini et al., 2002; Raugei et al., 2002; Zambuzzi et al., 2008). The relation of LMW-PTP to a variety of pathological processes including tumor progression was proposed and it had drawn attention as a potential target for pharmacological intervention (Alho et al., 2013; Bottini et al., 2002; Maccari and Ottanà, 2012).

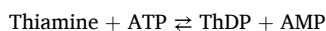
Interestingly, knockout of LMW-PTP in mice protects against stress-induced cardiomyopathy (Wade et al., 2015). In this respect, one may speculate that the efficiency of high dose thiamine treatment of heart diseases in clinics (Vinogradov, 2007) could be due to the competition of ThMP, produced by hydrolysis of excess ThDP, with endogenous substrates of LMW-PTP.

At least in chicken liver, LMW-PTP seems to be the only intracellular protein capable of hydrolyzing ThMP. The enzyme possesses a rather high K_m for ThMP around 0.9 mM with ThMPase activity being as low as 18 % the rate of *p*-nitrophenyl phosphate hydrolysis (Kolas and Makarchikov, 2014). In addition, ThMP can be hydrolyzed by alkaline phosphatase (EC 3.1.3.1) (Kolas and Makarchikov, 2014; Ogawa et al., 1982; Vovk et al., 2002), and fluoride-resistant alkaline phosphatase, a histochemical marker for small sensory neurons of dorsal root ganglia (Knyihar-Csillik et al., 1986). The latter one was shown to be identical to the transmembrane isoform of prostatic acid phosphatase (TM-PAP) (Zylka et al., 2008) widely expressed in mammalian tissues (Quintero et al., 2007). Both alkaline phosphatase and TM-PAP belong to ecto-nucleotidases, i. e. their active sites are exposed outside the cell (Zimmermann, 2009; Zimmermann et al., 2012). Another known natural substrate for LMW-PTP is flavin mononucleotide, suggesting that this enzyme may be involved in the degradation of this substrate (Granjeiro et al., 1997). This also may imply some role for LMW-PTP in linking vitamin B₁ and B₂ metabolism. Recently, it was suggested that mammalian DING phosphatases also exhibit ThMPase activity (Aleshin et al., 2019). Hence, enzyme having ThMPase activity are numerous but there does not seem to exist a specific ThMPase.

5. The central player: thiamine diphosphate

5.1. Mechanism and regulation of thiamine diphosphate synthesis in animals

In animal cells, thiamine taken up through specific transporters is pyrophosphorylated to ThDP by cytosolic thiamine diphosphokinase 1 (pyrophosphokinase, TPK1, EC 2.7.6.2) according to the reaction generally indicated as follows:



TPK1 (another isoform, TPK2 is present only in plants) is the best studied enzyme involved in thiamine metabolism. TPK was first partially purified and characterized from rat brain (Mano, 1960). The enzyme from mammalian brain was later studied in more detail by Johnson & Gubler (Johnson and Gubler, 1968) and Peterson et al. (1975). These authors showed that the enzyme has a high affinity for thiamine ($K_m \approx 0.12 \mu\text{M}$) and a much lower affinity for ATP and other nucleotide diphosphates. There is an absolute requirement for Mg^{2+} (or some other divalent cation) as activator.

Peterson et al. claimed that the equilibrium for the reaction thiamine + ATP $\rightleftharpoons$ ThDP + AMP was strongly in favor of thiamine and ATP

formation ($K_{eq} \approx 10^{-5}$) (Peterson et al., 1975). To explain that appreciable amounts of ThDP are nevertheless formed in vivo, it was assumed that the equilibrium was displaced through tight binding of ThDP to apoenzymes, i. e. transketolase in the cytosol and pyruvate and oxoglutarate dehydrogenases in mitochondria. The question was reexamined in a recent study (Sambon et al., 2022). Using a purified mouse recombinant TPK, the authors showed that the equilibrium is strongly in favor of ThDP formation ($K_{eq} \approx 5 \cdot 10^4$), in agreement with thermodynamic calculations ($\Delta G \approx -30 \text{ kJ mol}^{-1}$). The discrepancy with earlier studies was shown to be due to a strong inhibition of the enzyme by the product ThDP, preventing its accumulation.

Molecular cloning yielded the cDNA sequence and predicted a 243 amino acid (27 kDa) product for mouse TPK (Nosaka et al., 1999). The three-dimensional structure was obtained for the *S. cerevisiae* (Baker et al., 2001), mouse (Timm et al., 2001) and human (PDBe 3SDY) enzymes. In all cases, the enzymes crystallized as homodimers. A tetrameric crystalline form for mouse TPK was also obtained (Liu et al., 2006) indicating the ability of the enzyme to form oligomers of a higher order. Along with thiamine, TPK is able to phosphorylate its structural analogs, including the well-known antagonists pyriothiamine and oxythiamine, while various nucleoside-5'-triphosphates can be used as a pyrophosphate donor (Liu et al., 2006; Onozuka and Nosaka, 2003; Voskoboev and Chernikevich, 1987).

TPK1 is expressed in all animal tissues, but both mRNA and protein expression levels are lowest in the brain, making it, more than the expression of thiamine transporters, responsible for the susceptibility of brain to thiamine deficiency (Xia et al., 2024). Very recently a report showing that low expression of TPK1 drives Alzheimer's-like pathology was deposited on the preprint server medRxiv (Sang et al., 2024).

Concerning the mechanism of the reaction, steady-state kinetic studies (Onozuka and Nosaka, 2003; Peterson et al., 1975) suggested a ping-pong mechanism involving a pyrophosphorylated enzyme intermediate. Alternatively, an ordered sequential mechanism was proposed (Voskoboev and Chernikevich, 1987; Voskoboev and Ostrovsky, 1982) where ATP binds first, followed by thiamine, resulting in a catalytically competent ternary complex. More recent structural data (Liu et al., 2006) are consistent with this mechanism as they suggest a one-step pyrophosphate transfer from ATP to thiamine. Mutational studies of human recombinant TPK1 (Onozuka and Nosaka, 2003) revealed the presence of three aspartate residues essential for catalysis. Structural data suggest that a Mg^{2+} ion is coordinated to these carboxylates and to the β -phosphoryl group of ATP in the enzyme-substrate complex (Liu et al., 2006).

It has been shown that for both mammalian and yeast enzymes the preferred pyrophosphate donor is UTP rather than ATP (Artsukevich, 1979; Onozuka and Nosaka, 2003; Voskoboev and Chernikevich, 1987). As compared to ATP, UTP has a lower K_m for TPK1 and the k_{cat} is higher. Recently, Sahu et al. confirmed that in vivo, the pyrophosphate donor in the TPK reaction is UTP rather than ATP (Sahu et al., 2024). When intracellular UTP concentrations are limiting, the rate of ThDP synthesis may become very low and the activities of ThDP-dependent enzymes (especially PDHC, see Fig. 2) are decreased. Hence, the levels of UTP and pyrimidines in general might play a major role in regulating the citric acid cycle and NADH availability.

Genetic depletion of TPK1 has widespread consequences: decreased ThDP levels, decreased pyruvate utilization and decreased amounts of the transketolase product sedoheptulose 7-phosphate (Sahu et al., 2024). Therefore, mutations in the human *tpk1* gene are severe and often underdiagnosed. These mutations lead to thiamine metabolism dysfunction syndrome 5 (OMIM 614458), also known as TPK1 deficiency, a rare neurodegenerative disorder of inborn error of metabolism with variable clinical manifestations (including acute encephalopathy, seizures and elevated lactate plasma concentrations (Fraser et al., 2014; Rostami et al., 2024; Rüsche et al., 2021) and variable response to thiamine treatment (Bugiardini et al., 2019; Mayr et al., 2011; Thompson et al., 2023). Until 2023, only about 28 patients suffering from

TPK1-related diseases were known (Zhao et al., 2023).

Finally, it should be emphasized that the feedback inhibition of TPK1 by its product ThDP (Sambon et al., 2022; Voskoboev and Chernikevich, 1987; Voskoboyev and Ostrovsky, 1982) is an important mechanism for the regulation of thiamine and ThDP levels in animal tissues. It has been consistently observed that, when cultured mammalian cells are incubated with lipophilic thiamine precursors, the thiamine content rapidly increases in the cells and remains high for several hours, while ThDP levels remain low. It must be recalled that most intracellular ThDP is bound to apoenzymes (especially in mitochondria), while the level of free ThDP in the cytosol always remains very low (Bettendorff, 1994; Sambon et al., 2019). Feedback inhibition of TPK by low concentrations of ThDP may also explain why in mice, rats and humans the administration of high doses of thiamine (or precursors with higher bioavailability such as benfotiamine) induces a strong increase in blood and liver thiamine without parallel increase in ThDP (Sambon et al., 2019, 2020, 2022; Tapias et al., 2023; Volvert et al., 2008).

What could be the physiological significance of such a TPK feedback inhibition. As TPK has a high affinity for thiamine and since the equilibrium is strongly in favor of ThDP formation, it would be expected that, in the absence of a feedback inhibition by ThDP, virtually all the thiamine would be accumulated as intracellular ThDP in erythrocytes and liver. We previously suggested that this could lead to depletion of circulatory thiamine levels putting the brain in a virtual situation of thiamine deficiency (Sambon et al., 2022). As there is no evidence that a high concentration of ThDP would be toxic for cells, the advantage of the feedback inhibition of TPK would possibly be to maintain a significant level of free circulating thiamine in animal cells. Moreover, unphosphorylated thiamine might have some unknown physiological role, so that its absence in cells might be harmful. Last but not least, a TPK feedback inhibition by ThDP will contribute to minimizing variations in intracellular ThDP concentrations preventing the overactivation of coenzyme independent processes by excess ThDP (Mkrtchyan et al., 2015).

5.2. ThDP metabolism and transport into mitochondria

An important finding was that after administration of radioactively labeled thiamine, the specific radioactivity was higher in ThTP and ThMP than in ThDP, both in rats (Berman and Fishman, 1975; Bettendorff et al., 1994) and in cultured neuroblastoma cells (Bettendorff, 1994). As ThDP is the precursor of ThMP and ThTP (Fig. 1), it was suggested that there exist two different cellular ThDP pools, the major low turnover, enzyme-associated pool and a smaller high turnover (metabolic) pool, precursor of ThMP and ThTP.

Depending on the cell type, a more or less important fraction of ThDP is bound to apoenzymes. In neurons, most ThDP is bound and the free cytosolic pool represents less than 10% of total ThDP (Bettendorff et al., 1994). However, the fraction of free ThDP may be higher in hepatocytes, enterocytes and skeletal muscles fibers. This protein-bound coenzyme is believed to form a slow turnover pool, while the rapidly turning over pool of free (unbound to proteins) ThDP, present both in the cytosol and mitochondria, is the precursor of ThMP, ThTP and AThTP (Bettendorff, 1994; Bettendorff et al., 1994; Rindi et al., 1984). In neuronal cells, the protein-bound coenzyme represents 90–95 % of the total ThDP content, the bulk of which (about 85 %) has mitochondrial localization (Bettendorff and Wins, 1999). However, in the mitochondria of rat liver, the amount of protein-bound and free ThDP was estimated to be 89 pmol/mg protein and 531 pmol/mg protein, respectively (Voskoboev and Chernikevich, 1987). The much higher free ThDP levels in liver compared to neuronal cells is probably the result of a very high ThDP content of rat liver (see section 10.3). Peroxisomal ThDP is reported to account for 2–3 % (144 pmol/mg protein) of its total content in the liver (Fracascia et al., 2007). In the cytosol of brain cells, ThDP probably represents 15–20 % of its total cellular amount, with 30–40% of this cytosolic ThDP being bound to transketolase (Bettendorff et al., 1994). The same ratio of protein-bound to free ThDP was observed in the

cytosolic fraction of rat liver (Voskoboev and Chernikevich, 1987).

The rapidly turning over cellular pool of free ThDP is a target for hydrolytic enzymes. A few phosphatases with broad substrate specificity are known to possess ThDPase activity. In rat brain ThDP can serve as a substrate for membrane-associated L-(liver) and B-(brain) type nucleoside diphosphatases (NDPase, EC 3.6.1.6) (Sano et al., 1984). Purified to homogeneity by Sano et al., type B NDPase was capable of hydrolyzing ThDP, GDP, UDP, and IDP without any preference (Sano et al., 1988). In the presence of Mg^{2+} ions the enzyme exhibited maximum activity at pH 6.5–7.0 and the K_m for ThDP was 1.06 mM. The molecular mass of type B NDPase from rat brain was estimated by SDS-PAGE to be 75 kDa. Type L NDPase was purified from bovine and rat liver microsomes (Sano et al., 1984; Yamazaki and Hayaishi, 1968). This enzyme had a pH optimum of 9.0 with ThDP, while its NDPase activity was maximal at pH 6.5–7.5. Just a little ThDPase activity of type L NDPase could be observed in the neutral reaction medium, especially in the absence of ATP which was a potent activator. The apparent K_m of the enzyme for ThDP was evaluated to be 17–20.7 mM (Sano et al., 1984; Yamazaki and Hayaishi, 1968). According to Ohkubo et al., the NDPase of L-type from rat liver is a dimeric 130 kDa protein composed of identical subunits (Ohkubo et al., 1980). Both histochemical and biochemical studies indicate the localization of type B NDPase in the Golgi apparatus, while the enzyme of L-type is in the endoplasmic reticulum (Goldfischer et al., 1971; Sano et al., 1988). To our knowledge, no information concerning the molecular identity of these proteins is available. Another well-known phosphatase possessing ThDPase activity is alkaline phosphatase (Eaton and Moss, 1967; Vovk et al., 2002) widely distributed in animal tissues. As alkaline phosphatase belongs to ecto-nucleotidases (Zimmermann et al., 2012), it is unlikely that this enzyme participates in intracellular thiamine metabolism. A specific enzyme for ThDP hydrolysis in biological objects has not yet been identified, but such an enzyme might not be necessary as ThDP seems to be regulated by retro-inhibition of TPK (Sambon et al., 2022).

In mammals, ThDP transport into mitochondria occurs by a carrier protein, the product of the *SLC25A19* gene, previously known as DNC transporter for deoxynucleotides (Lindhurst et al., 2006). This protein, built up of 320 amino acid residues, is a member of the mitochondrial carrier family of transporters. Kinetic studies have shown that the process of ThDP absorption by rat liver mitochondria is pH-independent and saturable in the micromolar range of coenzyme concentrations ($K_m = 6.79 \mu M$) (Subramanian et al., 2013). The *SLC25A19* mutation G177A is of clinical importance in causing Amish lethal microcephaly, or thiamine metabolism dysfunction syndrome 3 (MCPHA, THMD3, OMIM 607196). In this disease, characterized by malformations of the brain, profoundly delayed psychomotor development and episodic encephalopathy, a 10–100-fold increase of 2-oxoglutarate concentration in the urine and persistent lactate acidemia can be observed (Kelley et al., 2002; Siu et al., 2010). A few other *SLC25A19* mutations are linked to bilateral striatal necrosis with progressive polyneuropathy (thiamine metabolism dysfunction syndrome 4, THMD4, OMIM 613710). Patients suffering from THMD4 display a milder phenotype characterized by childhood onset of episodic encephalopathy and flaccid paralysis, often associated with a febrile illness (Palmieri et al., 2020). Only 14 cases of THMD4 have been reported up to date (Samur et al., 2022).

5.3. ThDP-dependent enzymes

Animal genomes are known to encode 6 proteins that use ThDP as a coenzyme (Fig. 2): cytosolic transketolase (TK, EC 2.2.1.1) (Schenk et al., 1998), 2-hydroxyacyl-CoA-lyase (HACL1, EC 4.1.2.n2) of peroxisomes (Foulon et al., 1999) and four 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-ketoacid dehydrogenase (BCKD, EC 1.2.4.4) and 2-oxoadipate dehydrogenase (OADH, EC 1.2.4.2 by similarity) (Szabo et al., 2024).

TK catalyzes the reversible transfer of a two-carbon fragment from a ketose phosphate donor substrate to an aldose phosphate acceptor substrate, reversibly linking glycolysis to pentose phosphate pathway. This is a dimeric protein with M_r of 140–150 kDa composed of identical subunits, with two ThDP molecules being quite tightly bound in the cleft at the subunit interface (Kochetov and Solovjeva, 2014).

The human genome also encodes two related proteins, which were termed TKTL1 and TKTL2 (transketolase-like proteins 1 and 2) (Coy et al., 1996). It was proposed by Coy et al. that TKTL1 is related to malignant growth providing a high rate of “aerobic glycolysis” (Warburg effect) in tumor cells (Coy et al., 2005). Indeed, overexpression of TKTL1 was reported in human cancers and has been linked to metastasis and poor survival of patients (Furuta et al., 2010). However, in the paper which appeared in 2015, Kämmerer et al. challenge the high expression of TKTL1 in neoplastic tissue attributing it to a low specificity of monoclonal antibody JFC12T10 used in most studies employing antibody-based techniques and published up to that time (Kämmerer et al., 2015). In that study TKTL1 expression both on mRNA and protein level was rare in 17 malign cell lines tested and unrelated to their rate of lactic acid production and resistance against chemo- and radiotherapy. On the other hand, TKTL1 is lacking several amino acid residues important for coenzyme binding and catalysis, as evidenced by the crystal structure of human TK and sequence alignments (Mitschke et al., 2010). It has also been demonstrated that recombinant hTK, which was devoid of the 38-residue fragment absent in TKTL1, lacks enzyme activity and fails to bind ThDP (Meshalkina et al., 2013; Schneider et al., 2012). Yet, in a more recent paper Deshpande et al. based just on computer modeling, insist on the hypothesis that TKTL1 and TKTL2 are functional transketolases (Deshpande et al., 2019). Li et al. reported TKTL1 to be involved in the accumulation (in late G1 and S phases) of ribose-5-phosphate necessary for DNA synthesis during the cell cycle through the formation of TKTL1-TK heterodimers which, being catalytically active, predominantly perform the reaction: glyceraldehyde-3-phosphate + sedoheptulose 7-phosphate $\rightleftharpoons$ xylulose 5-phosphate + ribose 5-phosphate (Li et al., 2019). Further studies should evidently be pursued to clear the issue concerning the functionality of TKTL1 and its relation to oncogenesis.

Peroxisomal ThDP-dependent enzyme, HACL1, is a homotetramer made up of four identical subunits of 63.7 kDa (Foulon et al., 1999). This enzyme has an important role in the degradation of 3-methyl-branched fatty acids (like phytanic acid) and 2-hydroxy long-chain fatty acids catalyzing the cleavage of the C–C bond between the first and second carbon atoms in a 2-hydroxyacyl-CoA intermediate to produce an ($n-1$) aldehyde and formyl-CoA (Casteels et al., 2007). It is assumed that the transport of ThDP into peroxisomes is dependent on HACL1 import, without requirement of a specific solute transporter (Fraccascia et al., 2011). No mutations in HACL1 have yet been described, and whether thiamine deficiency might impact its activity is unknown.

PDH complex (PDHC, EC 1.2.1.104) catalyzes oxidative decarboxylation of pyruvate to produce acetyl-CoA, CO_2 and NADH. This reaction, linking the glycolytic pathway to the tricarboxylic acid cycle, is extremely important for the entire metabolism of aerobic cells, therefore the activity of PDH, is meticulously controlled (Patel and Korotchkina, 2006). Besides pyruvate dehydrogenase (E1p) the complex contains two more catalytic components – dihydrolipoyl acetyltransferase (E2p) (2.3.1.12) and dihydrolipoyl dehydrogenase (E3) (EC 1.8.1.4) (Zdanowicz et al., 2024). Higher eukaryotic PDHCs have an additional structural component, dihydrolipoamide dehydrogenase-binding protein (E3BP), and two regulatory enzymes, pyruvate dehydrogenase kinase (PDK) and pyruvate dehydrogenase phosphatase (PDP). In accordance to the most probable “substitution” model, 48 copies of E2p and 12 copies of E3BP (Hiromasa et al., 2004) or 40 copies of E2p and 20 copies of E3BP (Brautigam et al., 2009) form the E2/E3BP core with icosahedral symmetry. In mammalian PDHC, up to 40 heterotetramers of E1p ($\alpha_2\beta_2$) and 12–20 homodimers of E3 are bound to the core, with 1–3 homodimers of PDK and 2–3 heterodimers of PDP being bound to

the lipoyl domains of E2p and/or E3BP (Patel et al., 2014).

PDHC deficiency is an inborn error of mitochondrial energy metabolism. More than 450 cases have been reported in the literature with heterogeneous clinical manifestations that are limited largely to the central nervous system. About 77 % of patients suffering from PDHC deficiency harbor mutations in the X-linked *PDHA1* gene (OMIM 300502) that encodes the E1 α subunit (containing the E1 active site), the remainder defects are distributed among the other components except PDK (Sperl et al., 2015). No treatment specific to this disease has been evaluated in a randomized controlled trial. The long-term clinical management of patients often includes thiamine administration (from a few milligrams per day to doses exceeding 1 g per day), a ketogenic diet or dichloroacetate (Patel et al., 2012).

Oxoglutarate dehydrogenase complex (OGDHC, EC 1.2.1.105) and branched-chain α -keto acid dehydrogenase complex (BCKDC, EC 1.2.1.25) share the same basic architecture (Zhang et al., 2024). Like PDHC, they consist of multiple copies of E1, E2 and E3 components with specific 2-oxoglutarate dehydrogenase (E1 α , α_2) and dihydrolipoamide succinyltransferase (E2 α , EC 2.3.1.61) for the former complex and 3-methyl-2-oxobutanoate dehydrogenase (E1 β , $\alpha_2\beta_2$) and dihydrolipoyltransacylase (E2 β , EC 2.3.1.168) for the latter, while E3 subunit is common among the three α -ketoacid dehydrogenase complexes. The E2 β of BCKDC and the E2 α of OGDHC exhibit a 24-meric cubic core with octahedral symmetry (Reed, 2001). The intact human OGDHC assembly has been proposed to consist of 24 E1 α , 24 E2 α and 12 E3 chains (stoichiometry of 2:2:1) (Nagy et al., 2021). Eukaryotic BCKDC also contains regulatory kinase and phosphatase binding to the E2 β core (Chuang et al., 2002; Wynn et al., 2012).

OGDHC is a key enzyme in mitochondrial bioenergetics which catalyzes the oxidative decarboxylation of 2-oxoglutarate yielding succinyl-CoA, NADH and CO_2 the rate-limiting step of the Krebs cycle (Hansen and Gibson, 2022). It is involved in many aspects of cell metabolism. Being highly regulated and also being a crucial target for reactive oxygen species, OGDHC represents the primary control site of metabolic flux through the Krebs cycle (Tretter and Adam-Vizi, 2005; Tylicki et al., 2011). An impaired function of this enzyme complex is associated with several neurodegenerative diseases including AD and Parkinson's disease (Chen et al., 2016; Weidinger et al., 2023).

BCKDC catalyzes the oxidative decarboxylation of branched-chain α -ketoacids 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 which is characterized by the accumulation of branched-chain amino acids in plasma and branched-chain α -ketoacids in urine with severe clinical consequences including neurological and developmental delay, encephalopathy and feeding problems (Blackburn et al., 2017). It is interesting that the E1 α and E1 β components of BCKDC can be affected in classic, intermediate or intermittent forms of the disease, while thiamine-responsive MSUD is associated with pathogenic variants in the *DBT* gene that encodes the E2 subunit. In the latter case, the treatment usually requires a combination of branched chain amino acid dietary restriction and thiamine supplementation (10–1000 mg/day) (Chuang et al., 2006).

In addition to the above classical ThDP-dependent enzymes, two isoforms of OGDH (DHTKD1 and OGDHL) were characterized (Bunik and Degtyarev, 2007). The first, the product of *DHTKD1* gene encoding dehydrogenase E1 and transketolase domain-containing protein 1, was later identified as 2-oxoadipate dehydrogenase (OADH, Nemeria et al., 2018). The enzyme is believed to be a part (E1 α component) of 2-oxoadipate dehydrogenase complex (OADHC) converting 2-oxoadipate to glutaryl-CoA within the degradative pathway of L-lysine, L-hydroxylysine, and L-tryptophan in the mitochondria (Nemeria et al., 2022). *In vitro* as well as *in vivo* studies show that both the E1 α and the E1 β proteins exploit the same E2 α and E3 components to assemble into supramolecular structure, thus implying the existence of a hybrid OGDH–OADH complex in the cell (Leandro et al., 2020; Nemeria et al.,

2018). Several *DHDK1* mutations have been revealed to underlie 2-amino adipic and 2-oxoadipic aciduria (OMIM 204750) (Danhauser et al., 2012; Hagen et al., 2015), an inborn error of metabolism with a wide variety of symptoms. A nonsense *DHDK1* mutation also causes Charcot-Marie-Tooth disease type 2Q (OMIM 615025), an inherited neurodegenerative disorder characterized by motor and sensory neuropathies (Xu et al., 2012). A novel *DHDK1* mutation seems to be associated with sporadic form of amyotrophic lateral sclerosis (Menon et al., 2024).

The second, OGDHL (oxoglutarate dehydrogenase-like) is another isoform of E1 α and is similar to OGDH complex. It seems to be brain-specific and could be involved in brain-specific control of 2-oxoglutarate and glutamate metabolism (Lin et al., 2023).

Rare potentially damaging *DHDK1* and *OGDHL* mutations were identified by whole-exome sequencing in patients with eosinophilic esophagitis, implicating *DHDK1* and *OGDHL* in the genetic etiology of this allergic inflammatory esophageal disorder (Sherrill et al., 2018). Pharmacological inhibition of OADHC affects the expression of sirtuin 5 and protein glutarylation, thus providing a link to metabolic diseases such as diabetes in the view of the proposed regulation of PDHC by glutarylation (Boyko et al., 2022). Knock-out of *DHDK1* in cultured HAP-1 cells leads to impaired mitochondrial function and energy metabolism but the relation to its catalytic function, oxidative decarboxylation of 2-oxoadipate, remains unknown (Wang et al., 2021).

6. Seventy years of qualm: thiamine triphosphate

ThTP was chemically synthesized from thiamine in 1948 by L. Velluz et al. (1948), and early reports concerning its occurrence in living systems as well as possible biochemical functions date back to the middle of the last century (Kieślinski, 1953; Rossi-Fanelli et al., 1952). It should be noted however, that it was not until 1980s that reliable results in this compound quantification became possible with the development of HPLC methods (Bettendorff et al., 1991; Bontemps et al., 1984; Egi et al., 1986; Ishii et al., 1979; Iwata et al., 1988). ThTP is a minor thiamine derivative found in mammalian cells at very low concentrations (<0.1 nmol/g tissue) (Gangolf et al., 2010a; Iwata, 1988; Makarchikov et al., 2003). The only exceptions seem to be the electric organ of *E. electricus* and the skeletal muscles of pigs and chicken where ThTP is the major vitamer (Bettendorff et al., 1987; Egi et al., 1986; Miyoshi et al., 1990), which is due to a high adenylate kinase activity combined with the absence or catalytic inactivity of soluble 25 kDa-ThTPase in these species (Bettendorff, 2021). These results suggest that the latter enzyme is essential for the regulation of cytosolic ThTP levels in animal cells.

At the turn of the century, the possible biochemical functions of ThTP were mainly discussed in relation to the non-coenzyme role of vitamin B₁ in excitable tissues (Bettendorff and Wins, 1999). Today, for the most part, this concept is only of historical interest (Bettendorff et al., 2014). Studies carried out over the past 20 years suggest that the role of ThTP may be more fundamental. First of all, the biosynthesis of ThTP occurs in animal cells of different types and specializations. Moreover, ThTP was found in various species – from bacteria to mammals (Makarchikov et al., 2003). In *E. coli*, the concentration of ThTP increases dramatically in response to amino acid starvation, while in plants, its synthesis seems to be dependent on the light period (Hofmann et al., 2020; Lakaye et al., 2004). Summarizing the data available from the literature, one may assume ThTP is involved in the regulation (integration) of metabolic processes in various types of cells (Bettendorff, 2021). Most of our experimental data were obtained from *E. coli*, which is not the subject of this review. However, our results suggest that ThTP may have a regulatory role, possibly at the interface of sugar (Krebs cycle) and amino acid metabolism (pyruvate glutamate axis), in agreement with data obtained by the group of V. Bunik in mammalian brain. These seem to head towards the same direction by showing that glutamate dehydrogenase and pyridoxal kinase are regulated by ThTP (Bunik et al., 2022; Mkrtchyan et al., 2015).

Despite the fact that articles concerning the biosynthesis of ThTP in living organisms have been sporadically published in the literature over the past four decades, there is no complete clarity on this issue. A mechanism of ThTP biosynthesis according to the equation $\text{ThDP} + \text{ATP} \rightleftharpoons \text{ThTP} + \text{ADP}$ was proposed in 1964 by Eckert & Möbus (Eckert and Möbus, 1964), based on the results of experiments on extracts from the spinal cord of pig; the enzyme was named ThDP: ATP-phosphotransferase (ThDP-kinase) with the classification number of 2.7.4.15. In 1982, Cooper et al., followed by Nishino et al. a year later, published the results of the purification of ThDP kinase with M_r 103 kDa from an acetone powder of the bovine brain mitochondrial fraction (Cooper et al., 1982; Nishino et al., 1983). In the presence of a cofactor of unknown nature, the enzyme catalyzed the phosphorylation of protein-bound ThDP according to the scheme: $\text{protein-ThDP} + \text{ATP} \rightleftharpoons \text{protein-ThTP} + \text{ADP}$. The substrate for determining the activity was isolated from the cytosol of rat liver by 5-step purification. To identify the macromolecular substrate of ThDP kinase, Voskoboev and Chernikevich carried out a special study, which indicated the only TDP-binding protein in the cytosol of rat liver cells to be TK (Voskoboev and Chernikevich, 1985). At least in the liver, TK is not capable of serving as a substrate for ThTP biosynthesis. In the early 1990s, the cytosolic AK1 (EC 2.7.4.3) from pig and chicken skeletal muscles was found to catalyze the synthesis of ThTP in accordance with the reaction $\text{ThDP} + \text{ADP} \rightleftharpoons \text{ThTP} + \text{AMP}$ (Shioda et al., 1991). However, based on the kinetic parameters of the purified enzyme at pH 7.5 (pig AK1: K_m for ThDP = 0.83 mM, $k_{cat} \sim 0.2 \text{ min}^{-1}$; recombinant chicken AK1: K_m for ThDP = 2.3 mM, $k_{cat} \sim 0.7 \text{ min}^{-1}$), it is doubtful that this reaction had any physiological significance. At last, a decade ago it was found that in *E. coli* and mitochondria isolated from rat brain, ThTP could be synthesized from ThDP and inorganic phosphate by a chemiosmotic mechanism under the action of ATP synthase (EC 7.1.2.2): $\text{TDP} + \text{Pi} \rightleftharpoons \text{ThTP}$ (Gangolf et al., 2010b; Gigliobianco et al., 2013). This mechanism seems to be responsible for ThTP accumulation in bacteria under specific conditions of nutritional downshift (Gigliobianco et al., 2013). Provided such a mechanism is present in vivo in animal cells, it is unclear how widespread it is, since ThTP-synthesizing activity, for example, was not revealed in mitochondria from rat liver (Gangolf et al., 2010b).

Initially, subacute necrotizing encephalomyelopathy (Leigh's syndrome) was considered to be related to the inhibition of ThTP biosynthesis in the brain (Haas, 1988) but this hypothesis has proven to be erroneous (Bettendorff et al., 2014; Schrijver et al., 1978).

In the cytosol of mammalian cells, the concentration of ThTP is controlled by a soluble ThTPase (EC 3.6.1.28), which was purified to homogeneity from bovine brain and kidneys (Makarchikov, 2001; Makarchikov and Chernikevich, 1992) and characterized at the molecular level 20 years ago (Lakaye et al., 2002). Currently, this enzyme is quite well studied – from its molecular structure to its molecular evolution (Bettendorff and Wins, 2013; Delvaux et al., 2013; Makarchikov, 2009; Song et al., 2008; Szyanirowski et al., 2005). The mammalian soluble ThTPase is Mg^{2+} -dependent protein with $M_r \sim 25 \text{ kDa}$ exhibiting high affinity for the substrate ($K_m \sim 40 \mu\text{M}$) and catalytic efficiency ($k_{cat}/K_m \sim 6 \times 10^6 \text{ s}^{-1}/\text{M}^{-1}$). An outstanding property of the enzyme is its virtually absolute specificity for ThTP. In the absence of additional data, it can only be inferred that this enzyme plays a role in the control of cytoplasmic ThTP levels (Bettendorff et al., 2014). Detailed information on the biochemistry of ThTP has been provided in a recently published review (Bettendorff, 2021).

7. Fickle: adenosine thiamine triphosphate

In 2007, a new thiamine derivative, AThTP, was discovered in living organisms. The compound was isolated from bacterial cells and proved to be identical to its chemically synthesized counterpart (Bettendorff et al., 2007). At present, a few methods of AThTP preparation have been described in the literature (Frédérich et al., 2009; Hayat et al., 2022; Hofer et al., 2016; Makarchikov et al., 2022; Möhler et al., 2020).

Biochemical functions of AThTP have not yet been established. In *E. coli*, the concentration of AThTP was found to increase manifold under certain types of stress, in particular after the transfer of bacterial culture from a rich medium to a minimal medium devoid of a carbon source; upon addition of a metabolizable substrate, the AThTP concentration quickly returned to the basal level (Bettendorff et al., 2007; Gigliobianco et al., 2010). According to Tanaka et al., in vitro AThTP inhibits the activity of poly(ADP-ribose) polymerase-1 (PARP-1), a nuclear enzyme that occupies a central place in the system of cellular responses to various stress factors (Tanaka et al., 2011). However, this inhibitory effect was not reproduced in a later study (Hofer et al., 2016). Another study showed the activation of rat brain glutamate dehydrogenase by AThTP (Mkrtchyan et al., 2015).

The list of the cell types and organisms in which the presence of AThTP was documented include *E. coli*, brewer's yeast, plant roots, some cultured cell lines, rat, mouse, bovine, chicken and human tissues (Bettendorff et al., 2007; Gangolf et al., 2010a; Makarchikov et al., 2018). AThTP can be easily determined by HPLC in bacteria, but in animal tissues this is not always the case. As reported by Gangolf et al., AThTP was detected only sporadically in adult human biopsies and more consistently in fetal tissues and cell lines (Gangolf et al., 2010a). In another study (Makarchikov et al., 2018), AThTP was found in two of three bovine kidneys, lungs and spleens as well as in two of three samples of rat skeletal muscle. The reason why this compound appears in samples of a tissue from some individuals but is absent in others is not clear.

In bacteria, AThTP is synthesized from ThDP and ADP (ATP) by a soluble enzyme, which was isolated and partially purified from *E. coli* (Makarchikov et al., 2007). It has also been shown that AThTP-hydrolase activity is present in membranes of prokaryotic and eukaryotic cells (Bettendorff et al., 2007; Makarchikov et al., 2018).

No relation of AThTP to human or animal diseases is known to date. In the only study (Makarchikov et al., 2022), AThTP was administered to alloxan diabetic rats for 15 days with no effect on hyperglycaemia, the level of hepatic thiamine compounds and activities of thiamine metabolizing enzymes in the liver.

An interesting point is that AThTP can be viewed as the thiaminylated 5' end of an RNAmolecule (Ado 5'-P-P-P-T) (Fig. 1) and the possibility that 5'-thiamine-capped RNA molecules are formed. Such forms were obtained in vitro but their existence in vivo has yet to be proven (Möhler et al., 2020).

8. Thiamine deficiency

8.1. Nutritional thiamine deficiency disorders

Cellular ThDP depletion due to thiamine deficiency caused by poor oral intake or other reasons, e. g. eating disorders, bariatric surgery, dependence on parenteral nutrition, renal disease etc. (Sriram et al., 2012), results in several thiamine deficiency disorders (TDDs) with symptoms being primarily localized to nervous and cardiac tissues (McCandless, 2010; Whitfield et al., 2018). Beriberi and Wernicke's encephalopathy (WE) are the typical manifestations of thiamine deficiency in humans (Kril, 1996). While beriberi, mostly a cardiac and peripheral nervous system disease, has rare occurrence in high income societies (Gomes et al., 2021), it remains a concern for developing countries (Johnson et al., 2019). Therefore, efforts are made for vitamin B₁ fortification in the endosperm of rice through thiamine sequestration (Fitzpatrick et al., 2024). WE, characterized with a classic triad of ocular abnormalities, such as nystagmus and ophthalmoplegia, mental changes and ataxic gait (although all three presenting features in combination are only found in 16 % of patients), is the most frequently encountered manifestation of thiamine deficiency in Western societies. It is commonly seen among alcohol abusers and, in severe form, can lapse into Korsakoff's psychosis with amnesia, profound confusion, confabulation and absence of working memory with irreversible lesions in the

midbrain (Wernicke-Korsakoff syndrome, WKS) (Arts et al., 2017). Nonalcoholic WKS cases have also been quite frequently reported in the literature (Oudman et al., 2021). In practice, the dosages of thiamine used to cure TDDs vary widely (50–1500 mg/day) depending on disease manifestation, the patient's condition as well as clinical experience, and the treatment at early stages may result in successful resolution of symptoms within several hours, but it is less effective when irreversible tissue lesions appeared (Smith et al., 2021; Sriram et al., 2012). According to the European Federation of Neurological Societies (EFNS) guidelines, for the treatment of suspected or manifested WE thiamine should be given intravenously 200 mg 3 times daily (Galvin et al., 2010). In a recent study, no clear benefit of high parenteral dose (500 mg thrice daily) thiamine over intermediate (300 mg thrice daily) or lower (100 mg thrice daily) doses for 5 days for the treatment and prevention of cognitive and neurological abnormalities related to Wernicke-Korsakoff syndrome was revealed (Dingwall et al., 2022). Currently, oxidative stress, glutamate-mediated excitotoxicity and inflammation owing to impairment of oxidative metabolism are considered to be the underlying mechanisms of brain cell damage in thiamine deficiency (Hazell and Butterworth, 2009). It is also believed that the low level of energy production in the mitochondria of brain cells due to inadequate ThDP-dependent processes forms a pathogenetic background for the development of neurodegenerative diseases including such a socially significant one as AD (Bettendorff and Wins, 1999; Gibson et al., 2013). Using benfotiamine to increase blood thiamine to pharmacologically high levels has been proposed as a therapeutic approach in early AD (Cardinali et al., 2024; Gibson et al., 2022) and very recently in Parkinson's disease (Bashir et al., 2024; Wang et al., 2024) and amyotrophic lateral sclerosis (Mann, 2023; Probert et al., 2022; Sambon et al., 2020). Pharmacological doses of thiamine were reported to have beneficial effects in patients with Charcot-Marie-Tooth neuropathy, possibly by altering the regulation of transketolase (Artukhovich et al., 2024).

8.2. The particular sensitivity of brain to thiamine deficiency

As evident from Tables 1 and in the genetically induced breakdowns of thiamine metabolism, the phenotype overlaps with mitochondrial diseases, except for TRMA (THMD1) which results in anemia and diabetes, though deafness may be associated.

Thiamine deficiency of any origin leads to an impairment of the oxidative synthesis of ATP (Hernandez-Vazquez et al., 2016; Vernau et al., 2015). Reduced oxidative metabolism results in oxidative stress and causes functional and morphological cell damage (Calingasan et al., 2000; Calingasan and Gibson, 2000a, 2000b; Hernandez-Vazquez et al., 2016; Langlais et al., 1997). Indeed, thiamine deficiency is sometimes considered as equivalent in its consequences as hypoxia (Valle et al., 2022). Therefore, it is not surprising that the thiamine deficiency phenotype overlaps with several mitochondrial diseases (Mani et al., 2020; Scharfe et al., 2000). In this respect, it is obvious that the nervous and the cardiovascular systems are particularly sensitive to thiamine deficiency. Both neurons and cardiomyocytes have a high oxidative energy metabolism but, more importantly, are strictly dependent on oxidative metabolism. In addition, as noted earlier, TPK expression is lower in brain compared to other tissues, contributing to its susceptibility to thiamine deficiency (Xia et al., 2024). Moreover, reduced cerebral oxidative metabolism is an important source of oxidative stress. It is therefore not astonishing that TDD mainly affects the nervous system. Glycolysis and lactate fermentation are not sufficient to sustain the high metabolic activity of these cells, and they will die rapidly. This is in contrast to hepatocytes and red muscle fibers, for instance, that better resist to inhibition of oxidative metabolism (Zoremba et al., 2014), and among brain cells, neurons seem to be the most sensitive to thiamine deficiency (Park et al., 2001). In addition, ThDP-dependent enzymes are also important for producing some of the main neurotransmitters (glutamate, GABA, acetylcholine, Fig. 2).

Hence, high energy-consuming tissues like the brain are especially

vulnerable to thiamine deficiency because of high oxidative stress and its dependence on mitochondria and glucose metabolism (Ames et al., 1995; Dhir et al., 2019; Navarro et al., 2002). However, one additional point deserves attention. While all neurons seem to depend exclusively on oxidative metabolism, they are not all equally sensitive to thiamine deficiency. For instance, in WKS, amnesic syndrome is characterized by anterograde memory loss, accompanied by confusion, apathy, disorientation and fabrications. In this syndrome, the brain lesions are rather specific mid-brain lesions while the cortex is largely spared (Murata et al., 1999). These lesions are assumed to result from impaired oxidative energy metabolism (Vetreno et al., 2011) probably due to decreased activity of the 2-oxoglutarate complex in vulnerable regions (Butterworth et al., 1986, 1993). However, the reasons for the selective vulnerability remain unknown. One of the primary events in the cascade of events leading to neuronal loss appears to be a breakdown of the blood-brain-barrier regions, but this seems to affect both vulnerable and non-vulnerable brain regions (Hazell and Butterworth, 2021; Sarkar et al., 2016). With this respect, it is also important to keep in mind that mutations leading to decreased activity of THTR2 mainly affect basal ganglia. Hence, TDD can display various forms depending on the cause (alcohol consumption, dietary or genetic), but also on the time of onset (prenatal, postnatal or adult).

9. Inherited human diseases of thiamine metabolism

Mutations in enzymes of thiamine metabolism (thiamine transporters, TPK, or ThDP-dependent enzymes) may lead to inherited diseases that are very often fatal (Table 1). Inborn disorders due to impairment of ThDP-dependent enzymes, thiamine transport or metabolism (mainly TPK1) result in decreased ThDP coenzyme function. The severity depends on whether the mutation leads to complete or only partial loss of protein function. Especially thiamine transporters display a high polymorphism, and mutations can be located either in coding or regulatory regions of the genes (Marcé-Grau et al., 2019). Most often, these diseases start to manifest in the earliest childhood. They have a wide spectrum of primarily neurological symptoms summarized in Table 1 (Alexander-Kaufman and Harper, 2009; Alfadhel et al., 2013; Barnerias et al., 2010; Danhauser et al., 2012; Fraser et al., 2014; Harris et al., 1990; Lindhurst et al., 2006; Mezzar et al., 2017; Siu et al., 2010). In many cases, the outcome is fatal if not treated. Thus, it is essential that the involvement of thiamine (in the widest sense) be recognized as early as possible in order to start a treatment with thiamine supplementation that may help overcome the metabolic impairment (Rüsch et al., 2021).

10. Tissue-specific differences of thiamine metabolism and thiamine homeostasis

This is an important point for understanding body thiamine homeostasis, but difficult to assess. Moreover, there are only a very limited number of studies among which many have been done with cultured cells and not necessarily representative of the in situ tissues. Another point warrants attention. While rodents are the first choice for the study of thiamine metabolism, rodent tissues contain 5–10 times higher total thiamine and ThDP levels than human tissues (Bettendorff et al., 1996; Gangolf et al., 2010a). The reason for these differences is not known. While the fact that animal chow is enriched in vitamins might have some responsibility, other factors must play a role. Finally, while the liver for instance is a relatively homogenous organ, the intestine, kidney and brain are histologically complex organs with different cell types and different transporters. Nevertheless, several conclusions can be drawn, though, to our knowledge, no recent data are available on this important subject.

10.1. Thiamine levels are highly regulated in the mammalian brain

A central question is to understand the distribution of thiamine

derivatives between different organs. Administration of radioactively labeled thiamine in rats leads to a much more rapid and higher accumulation of radioactivity in the blood and the liver than in the brain (Rindi et al., 1980). In the rat, only 1.5% of total thiamine is found in the brain, while 22% are found in the liver (Balaghi and Pearson, 1966). This observation is compatible with the fact that rat liver has about 4-times higher ThDP levels than brain, with an important part of it free in mitochondria (see 5.2).

The most puzzling observation is that even after administration of thiamine prodrugs such as benfotiamine or sulbutiamine, when thiamine plasma concentrations increase to extremely high levels (up to 100–1000 times higher than normal), no significant increase in total brain thiamine content is observed (Pan et al., 2010; Sambon et al., 2019; Tapias et al., 2018; Vignisse et al., 2017; Volvert et al., 2008). This suggests that the brain parenchyma is also protected against thiamine increase.

This can be explained by at least two factors.

- The rate of thiamine transport across the blood-brain barrier is low compared to the rate of uptake by hepatocytes, where thiamine is taken up by the high capacity Na⁺-dependent OCT1 transporter (Chen et al., 2014; Said et al., 2002).
- Thiamine efflux through the blood-brain barrier is activated by *trans*-stimulation (Lockman et al., 2003). Thiamine efflux is also stimulated by extracellular thiamine in cultured rat neuroblastoma cells, possibly by a self-exchange mechanism (Bettendorff, 1995). Such a *trans*-stimulation of thiamine efflux would limit excess thiamine uptake by the brain.

During thiamine deficiency, thiamine depletion is much slower in brain than in liver, heart or kidney (De Caro et al., 1961; Balaghi and Pearson, 1966; Murata et al., 1999; Page et al., 1989), though the brain is more vulnerable. This observation is in agreement with peripheral tissues (especially liver and erythrocytes) acting as a limited thiamine store, buffering plasma thiamine concentrations (Volvert et al., 2008). This is in contrast to brain, where thiamine content is strongly regulated. As long as close to normal plasma thiamine concentrations are maintained by loss of peripheral tissues, brain thiamine levels remain relatively stable. This would suggest that too high thiamine brain levels might be detrimental, possibly by activating processes through non-coenzyme mechanisms.

Finally, TPK, the only enzyme known to form ThDP from thiamine is subject to retroinhibition by its product ThDP, possibly participates in the maintenance of thiamine homeostasis. Indeed, in the absence of retroinhibition, peripheral tissues (and especially liver) would accumulate ThDP and thus sequestering the vitamer, and depleting plasma thiamine, leading to a virtual short come of the vitamin for the brain (Sambon et al., 2022).

The emerging picture is that thiamine, after entering the bloodstream, is mostly captured and phosphorylated by the liver (which is the main short-term thiamine storage organ) and possibly to a lesser extent, by erythrocytes acting as a thiamine buffer. In contrast, thiamine levels in the brain are highly regulated to minimize variations in thiamine and thiamine phosphate levels: mostly unknown regulatory mechanisms control both the influx and efflux of thiamine.

10.2. Thiamine status in humans

Over ten years ago, we published an extensive study on the distribution of thiamine derivatives in humans (Gangolf et al., 2010a). This was the most complete study ever done on the thiamine status in any species. The results of this study show that there are important differences between organs. Total thiamine was highest in liver in agreement with studies done on rats (Makarchikov et al., 2003). The general picture that emerges is that ThDP is the most abundant thiamine compound, followed depending on the tissue, by ThMP or thiamine. ThTP can

account for up to 10% of total thiamine in some tissues, which is higher than in other mammalian species, and likely due to a lower expression of the specific 25-kDa ThTPase.

ThTP is generally a minor compound (less than 1% of total thiamine), but it is found in most organisms, including humans (Makarchikov et al., 2003; Gangolf et al., 2010a). It is always present in brain and skeletal muscle, but the localization and mechanisms of synthesis are probably different: in brain, ThTP is mainly mitochondrial and synthesized by a chemiosmotic mechanism from ThDP and P_i (Gangolf et al., 2010b), while in skeletal muscle it is cytosolic and at least in part synthesized by adenylate kinase according to the reaction ThDP + ADP $\rightleftharpoons$ ThTP + AMP (Shioda et al., 1991).

AThTP was found sporadically in many samples, but very consistently in fetal and fetal-derived tissues, and in cultured cells. It might thus play a role in rapidly dividing cells.

11. Conclusions

In conclusion, one would like to highlight the progress made in the field of vitamin B₁ research over the past three decades. The most striking events were undoubtedly the molecular identification of new TDP-dependent enzymes (peroxisomal 2-hydroxyacyl-CoA-lyase, DHTKD1, OGDHL), the identification of thiamine transporters, the molecular characterization of a soluble specific mammalian ThTPase, as well as the discovery of a new thiamine derivative AThTP.

These advances, along with other results, allowed us to broaden our understanding of the coenzyme functions of vitamin B₁ and its relationship to hereditary diseases, and also to take a fresh look at the possible non-coenzyme role of thiamine. Nonetheless, many aspects of vitamin B₁ metabolism and functions remain poorly understood.

As evident from Fig. 2, ThDP-dependent enzymes play a fundamental role in cellular metabolism and in particular catabolism, oxidative energy metabolism and neurotransmitter synthesis.

The identification of mutations in ThDP-dependent enzymes and thiamine and ThDP transporters has clearly shown the often fatal impact of these mutations on the brain. It is astonishing that the phenotypes of these inborn diseases and the underlying lesions are so diverse (Table 1), while in most cases the unifying principle is a decrease in brain energy metabolism. The heterogeneity of the brain in terms of cell-type, synaptic connection and neurotransmitter is probably one of the reasons for these diverse phenotypes. Nevertheless, a clear mechanism explaining this diversity and the selective vulnerability of certain brain regions towards thiamine deficiency remains unexplained. Also, no systematic information could be found in the literature on intracellular thiamine metabolism in various pathologies, especially those not specifically related to the nervous system.

The data reviewed here substantiate the view that thiamine status is particularly low in humans compared to other mammalian species (especially rodents), justifying the proposal that thiamine supplementation should be envisaged in many pathological states and especially in elderly patients and vulnerable populations. This also poses the problem of the applicability of the conclusions obtained from rodent models to human diseases.

This leads us to consider another interesting and often overlooked observation. While the recommended daily allowance for thiamine is about 1.5 mg for an adult person, much higher doses have pharmacological, in particular antioxidant, effects. These effects are particularly obvious when thiamine precursors with higher bioavailability (such as benfotiamine) are administered. These effects occur even when ThDP-dependent enzymes are close to saturation, raising the possibility that they are mediated by presently unknown metabolites and are coenzyme-independent. The possible pharmacological importance of an open thiamine/ThDP form is a further challenge especially with recent data showing at least a partial protection of benfotiamine in neurodegenerative diseases.

So far, only two specific enzymes involved in thiamine metabolism in

higher animals, TPK and soluble 25 kDa ThTPase, have been identified at the molecular level. ThMP and ThDP are most probably hydrolyzed by unspecific phosphatases, among which LMW-PTP for ThMP, but the question of the existence of a specific ThDPase remains. The very existence of the two triphosphorylated derivatives ThTP and AThTP is a challenge that requires further investigation.

Hence, the biological significance of thiamine and its derivatives goes far beyond the problem of simple deficiency and it is to be hoped that further research is going to bring a lot of exciting findings in coming years.

CRedit authorship contribution statement

Alexander F. Makarchikov: Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Pierre Wins:** Writing – review & editing. **Lucien Bettendorff:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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