

# Molecular Mechanisms of Thiamine Utilization

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**Abstract:** Thiamine is required for all tissues and is found in high concentrations in skeletal muscle, heart, liver, kidneys and brain. A state of severe depletion is seen in patients on a strict thiamine-deficient diet in 18 days, but the most common cause of thiamine deficiency in affluent countries is alcoholism. Thiamine diphosphate is the active form of thiamine, and it serves as a cofactor for several enzymes involved primarily in carbohydrate catabolism. The enzymes are important in the biosynthesis of a number of cell constituents, including neurotransmitters, and for the production of reducing equivalents used in oxidant stress defenses and in biosyntheses and for synthesis of pentoses used as nucleic acid precursors. Because of the latter fact, thiamine utilization is increased in tumor cells. Thiamine uptake by the small intestines and by cells within various organs is mediated by a saturable, high affinity transport system. Alcohol affects thiamine uptake and other aspects of thiamine utilization, and these effects may contribute to the prevalence of thiamine deficiency in alcoholics. The major manifestations of thiamine deficiency in humans involve the cardiovascular (wet beriberi) and nervous (dry beriberi, or neuropathy and/or Wernicke-Korsakoff syndrome) systems. A number of inborn errors of metabolism have been described in which clinical improvements can be documented following administration of pharmacological doses of thiamine, such as thiamine-responsive megaloblastic anemia. Substantial efforts are being made to understand the genetic and biochemical determinants of inter-individual differences in susceptibility to development of thiamine deficiency-related disorders and of the differential vulnerabilities of tissues and cell types to thiamine deficiency.

## THIAMINE DEFICIENCY-RELATED DISORDERS

In affluent countries, vitamin B1 or thiamine deficiency (TD) is a clinically significant problem in individuals with chronic alcoholism or other disorders that interfere with normal ingestion of food [1-6]. For example, TD has been reported in up to 80% of alcoholic patients due to inadequate nutritional intake, reduced absorption, and impaired utilization of thiamine [4, 7, 8]. The findings of Wernicke-Korsakoff syndrome (WKS) and cerebellar degeneration, both consequences of severe TD, are present at autopsy in approximately 13% and 42% of alcoholics, respectively [9, 10]. Thiamine administration is reported to have beneficial effects on brain functioning in abstinent cocaine dependent patients [11], suggesting that chronic cocaine use may also cause TD due to the striking anorexia caused by this drug. As many as 12.5% of a population of critically ill children were demonstrated to have significant TD [12]. Recently, the Center for Disease Control has published a series of case reports of lactic acidosis traced to TD resulting from a nationwide shortage of multivitamins for total parenteral nutrition [13].

Moreover, the prevalence of moderate TD may be underestimated in certain clinical populations in which abnormal biochemical thiamine status is a contributing factor to considerable and diverse morbidity, including reduced growth in the young, chronic illness in young or middle-aged adults, and depression, dementia, falls, and fractures in old age [1, 14-17]. In addition, a number of inborn errors of metabolism have been described in which clinical improvements can be documented following administration of pharmacologic doses of thiamine, including thiamine-responsive megaloblastic anemia (TRMA), lactic acidosis, branched chain ketoaciduria, and intermittent cerebellar ataxia [6]. Finally, severe TD is still endemic in underdeveloped countries where the diet is low in thiamine, and malabsorption, due to subclinical intestinal disorders, is prevalent [14]. Recently seasonal ataxia in Nigerians was reported to be due to eating the pupae of an African silkworm that is available at a particular time of the year [18]. The pupae were found to possess a high activity of a heat resistant thiaminase, an enzyme that inactivates thiamine.

The major clinical manifestations of TD in humans involve the cardiovascular (wet beriberi) and nervous (dry beriberi, or neuropathy and/or WKS) systems [5, 6, 19]. Common findings in beriberi heart disease include peripheral vasodilation, biventricular myocardial failure, and sodium and water retention resulting in a high-output state, edema, and potentially acute

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fulminant cardiovascular collapse (soshin beriberi). Administration of thiamine rapidly restores peripheral vascular resistance, but improvement in myocardial function may be delayed. The predominant features of cerebral beriberi are confusion, disordered ocular motility, ataxia of gait, and neuropathy. Treatment with thiamine can rapidly reverse some of these acute signs while leaving significant clinical sequelae including memory and other cognitive deficits and residual neurologic signs (called Korsakoff's syndrome, or the chronic phase of WKS) with characteristic neuropathologic findings [19-22].

The neuropathology consists of bilaterally symmetrical midline hemorrhagic and/or necrotic lesions of the mammillary bodies, thalamus (medial dorsal, anterior medial, and pulvinar), periaqueductal region and floor of the fourth ventricle, hypothalamus, and cerebellar vermis [19, 23]. Characteristic pathological changes can vary considerably, from circumscribed lesions of the mammillary bodies to extensive damage throughout the brainstem, diencephalon, and cerebellum [24]. Active (acute) Wernicke's encephalopathy shows changes indicating ongoing thiamine deficiency; inactive (chronic) cases have no vascular endothelial swelling, but there is evidence of previous neuropil destruction [10, 19]. The pathological processes in the thalamus and inferior olives appear to be different from those in the mammillary bodies and subependymal structures [24]. Acute cases show thalamic involvement more often (92%) than those with chronic (44%) lesions. Alling and Bostrom [25] reported that even in chronic alcoholics without pathological evidence of Wernicke's encephalopathy, the mammillary bodies showed loss of myelinated fibers and decreased concentrations of cerebroside, cholesterol, and phospholipids. Nevertheless, the majority of cases of Wernicke-Korsakoff syndrome examined in recent autopsy studies remain clinically unrecognized [26]. These observations suggest that damage to the mammillary bodies may be a relatively early and common complication of alcoholism. Because 30-50% of patients with the neuroanatomical lesions of Wernicke-Korsakoff syndrome have midline cerebellar degeneration, the two disorders are thought to represent different aspects of the same nutritionally determined disease [27, 28]. The anterior and superior part of the vermis and the anterior lobes of the cerebellum are primarily affected. [23, 27]

An estimated 10% of detoxified alcoholics have severe clinical brain dysfunction (WKS or alcoholic dementia) suggesting inadequate treatment or an incomplete response to thiamine [4, 19, 29, 30]. This may be a significant underestimate of the actual prevalence of TD-related brain injury when the neuropathological data are considered [31]. The discrepancy may be partially explained by the fact that less severe cognitive deficits which

qualitatively resemble those of WKS patients are demonstrable with careful neuropsychological testing in 50-70% of detoxified alcoholics without WKS. Also, some of the neuropathological findings that characterize WKS are identified at autopsy in non-WKS alcoholics [31].

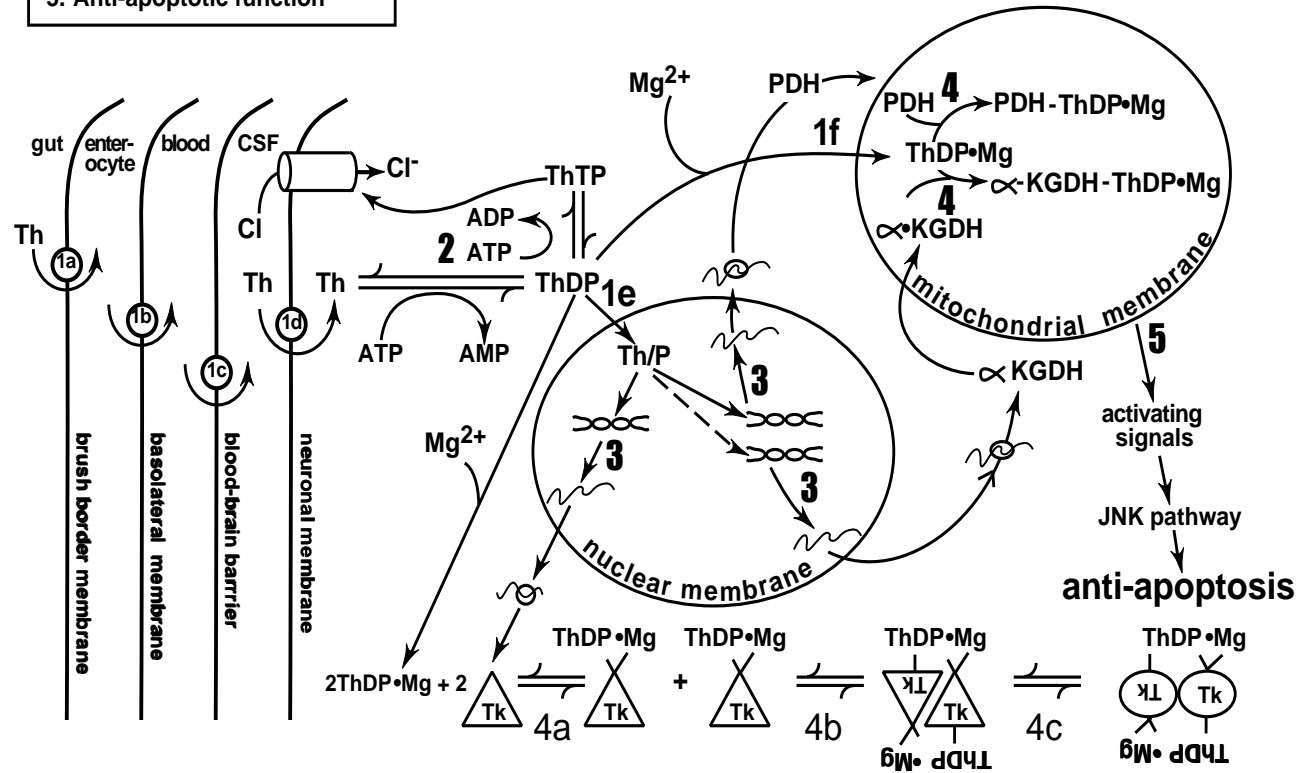
Over the past two decades, numerous biochemical, genetic, and molecular studies have been carried out in order to understand the molecular mechanisms involved in normal thiamine utilization as well as the pathophysiology of TD disorders, alone or in conjunction with alcohol consumption. Obviously, such an understanding has important implications for the prevention, identification, and treatment of TD-related disorders. Two fundamental questions that have been the focus of these investigations are why there are inter-individual differences in susceptibility to development of TD-related disorders and in response to thiamine treatment and what leads to the differential vulnerabilities to TD of certain tissues and cell types [6, 19, 29, 32, 33]. Figure 1 outlines the typical cellular activities and processes associated with thiamine utilization. The current understanding of each of these will be discussed with an emphasis on any known or potential relation to TD-disorders, including contributions to differential susceptibility to TD of individuals and tissues.

## THIAMINE UPTAKE

Being positively charged, thiamine movement across cellular membranes requires assisted transport. Once thiamine has been imported, it is diphosphorylated by thiamine diphosphokinase (ThDPK) to give the active cofactor thiamine diphosphate (ThDP) that assists in the catalytic mechanisms of several enzymes. The transport and diphosphorylation are together considered below as thiamine uptake. For the ThDP cofactor to be available for intracellular ThDP-utilizing enzymes, thiamine derived from the diet must undergo at least four transport steps (**Figure 1, steps 1a, 1b, 1c, 1d**). This initially involves two transport processes to gain access to the blood, the first being uptake by the brush border membrane followed by export out of the enterocyte by the basolateral membrane [34]. From the blood, thiamine is taken up by cells within liver, heart, and various other tissues except neuronal tissue. Prior to transport into neuronal cells, thiamine must be transported from blood into cerebrospinal fluid via the blood-brain barrier [30]. Once within the cell, further transport must occur (e.g., mitochondrial and nuclear membranes) as the enzymes which utilize thiamine (as ThDP) are distributed in different organelles. Whether organellar transport involves thiamine or ThDP has not been thoroughly addressed. One report has found that mitochondria can "actively" take up thiamine [35], and ThDPK activity has been

# STEPS IN THIAMINE UTILIZATION

1. Transport
2. Diphosphorylation
3. Gene expression
4. Enzyme assembly
5. Anti-apoptotic function



## ORIGINAL FIGURE 1 WILL BE PASTE HERE

**Figure 1.** A diagram of the various steps of thiamine utilization by neuronal and other cells. Variations in or alterations during thiamine deficiency of any of the steps represent possible contributions to inter-individual differences and/or cell type-specific differences in sensitivity to thiamine deficiency. Thiamine uptake is composed of transport (1) and diphosphorylation (2) by thiamine diphosphokinase. There are several sites of transport: 1a and 1b, transport from the gut to the blood stream; 1c, passage from blood to cerebrospinal fluid (CSF); 1d, transport into neurons (or other cell types); 1e, possible transport into the nucleus; and 1f, transport into mitochondria. In the latter two instances, it is presumed that transport is of thiamine, followed by diphosphorylation within the organelles; however thiamine or ThDP transport into organelles (Th/P) is not well characterized. Thiamine triphosphate (ThTP) can be generated from thiamine diphosphate (ThDP) and may modulate ion channel regulation. Thiamine can alter gene expression (3) for the ThDP-utilizing enzymes. 4a, 4b, and 4c represent the assembly of functional transketolase (TK), a process that requires magnesium (Mg<sup>2+</sup>) and proceeds through a ThDP/Mg/monomer intermediate and an inactive dimer. Similar assembly occurs within the mitochondria (4) for pyruvate dehydrogenase complex (PDH) and alpha-ketoglutarate dehydrogenase complex (α-KGDH). Finally, during thiamine deficiency, metabolic imbalances occur and trigger by unknown mechanisms apoptosis (5), thought to be mediated in part by alterations in cJun kinases (JNK) and loss of activating signals that normally maintain anti-apoptotic processes when thiamine is sufficient.

detected in mitochondria and microsomes [36]. Also, thiamine phosphatases exists within cells, with levels varying from tissue to tissue [37].

Thiamine uptake by human tissues has been investigated [34, 38-42] but to a much lesser degree than in rats. In these studies, there was a saturable, high affinity component that accounts for transport at physiological concentrations of thiamine and which demonstrates similar biochemical properties as the saturable component of rats and yeast. Thiamine uptake is indirectly active, not because of the transporter but due to its immediate diphosphorylation by ThDPK in which the diphosphate group is derived from ATP. Indeed, studies using rats have established that phosphorylation of thiamine upon entry into the cell

is the main driving force behind the transport [43-45].

That thiamine uptake deficiencies exist in humans has been demonstrated for patients with TRMA [40, 46]. Erythrocytes of TRMA individuals lack the saturable, high affinity transport system for thiamine and have only 75% of normal ThDPK activity of normal erythrocytes. The reduction in ThDPK activity may indicate a physical association of the transporter and ThDPK as the defect in TRMA patients, as described below, is the lack of a functional transporter. The concomitant reductions in thiamine uptake capacity are consistent with the genetics of TRMA, best explained as a single mutation within a single gene [40]. The gene for TRMA was localized on the long arm of chromosome 1 by homozygosity mapping [47]. In

cultured cells, TRMA-derived lymphoblasts are 1000 times more sensitive to TD than normal lymphoblasts as defined by loss of ThDP-requiring enzyme activities and by inhibition of cell growth [48]. Furthermore, the effects of TD on mRNA levels of thiamine-utilizing enzymes (see below) differ from that observed in cells with normal thiamine uptake characteristics [49].

Several groups have recently cloned the gene for the human thiamine transporter, using positional cloning based upon the mapping of the TRMA gene [50-52] or its similarity to other vitamin transporters [53]. Interestingly, the predicted protein shares substantial amino acid sequence identity (40%) with reduced folate carrier proteins. Biochemical studies on the encoded gene product conclusively established that the protein transported thiamine. A number of unrelated individuals with TRMA were examined, and all were found to have mutations within the thiamine transporter gene. The majority of the mutations clearly would result in a nonfunctional protein (frameshift and nonsense mutations), while a few were single amino acid changes. These new findings, coupled to the earlier biochemical findings, established that TRMA is indeed the result of the loss of a high affinity, saturable thiamine transporter. Studies are just beginning to examine the tissue distribution and levels of the transporter protein and the possibility of multiple isoforms or even another transporter gene. The variations in the biochemical properties of thiamine transport in different tissues and cell types indicate that different transporter species may exist.

It is possible that more subtle alterations in the transporter protein, leading not to a complete loss of function but to a diminished capability to transport thiamine, either into a particular cell type, across the blood-brain barrier, or from the gut to the blood may contribute to differential sensitivity to TD and/or interfere with thiamine treatment for TD-related disorders. A range in capacity for thiamine uptake may explain differences among TD individuals in response to thiamine treatment (i.e., the expected increase in intracellular thiamine) [4]. Additionally, alcohol is known to affect transport of thiamine in humans [7, 54]. The findings suggest but do not distinguish between direct, biochemical alteration or physical interaction of the transporter and ethanol or its metabolites and alterations in transporter gene expression and/or protein levels.

As mentioned above, ThDPK is also an integral component of thiamine uptake (**figure 1, step 2**). The gene encoding this protein in mice was recently cloned using similarities of sequence to the yeast enzyme [55]. Using the mouse protein sequence, the human ThDPK has been identified (CKS, unpublished results), and it is 90% identical in amino acid sequence to the mouse protein. Very few studies have been carried out on the human ThDPK. Speculation that alterations in the

diphosphokinase possibly contribute to TD-disorders and differential sensitivity to TD remain to be tested. ThDPK from various brain regions and other tissues was found to decrease with both acute and chronic alcohol exposure in the rat to about 70% and 50% of controls, respectively [37]. Although no studies have addressed the potential for a direct effect of ethanol on human ThDPK, several investigators have found a significantly lower ratio of phosphorylated thiamine (most of which is made up of ThDP) to thiamine in alcoholics compared to nonalcoholics [4, 30, 57].

Thiamine must undergo intracellular transport (**figure 1, steps 1e, 1f**) as ThDP-utilizing enzymes are found in the cytosol and mitochondria, and as thiamine has effects on gene expression which suggests it may gain entry into the nucleus. As mentioned above, the transport/uptake systems that mediate intracellular compartmentalization of thiamine are largely uncharacterized and ill-defined. However, studies using cultured cells from rats and humans reveal a capability to regulate in a complex way thiamine and ThDP concentrations in various intracellular pools/compartments [44, 48]. The distribution of ThDP between these pools varies among cell types as thiamine becomes limiting, presumably based on the metabolic needs of a particular cell. As membranes separate these pools, regulation of transport/uptake, both at the plasma membrane and intracellularly, may well underlie this regulatory capability. Alterations during TD or due to altered components of these processes are further possible sites for contributing to TD-related disorders.

## THIAMINE AND GENE REGULATION

As discussed below, there are several enzymes that utilize the diphosphorylated derivative of thiamine as a cofactor for catalysis. Is thiamine or its derivatives involved in regulating the expression of the genes that encode these proteins (**figure 1, step 3**), and does TD alter gene expression? In various yeasts, thiamine is known to directly regulate (via repression) the expression of several genes. However, these genes encode enzymes involved in the biosynthesis of thiamine or thiamine repressible phosphatases rather than encoding ThDP-utilizing enzymes [58-60]. In human lymphocytes, fibroblasts, and neuroblastoma cells in culture, TD results in up to a 50% reduction in transketolase (TK) mRNA steady state levels, a more modest reduction of 20% for the mRNA of the ThDP-binding E1 subunit of pyruvate dehydrogenase complex (PDH), and no change in the mRNA levels of the E1 subunit of alpha-ketoglutarate dehydrogenase complex (KGDH) [49]. The degree of reduction in the former two mRNAs was dependent on the cell type, with neuroblastoma cells giving the largest reductions. These findings indicate that thiamine or a thiamine

metabolite regulates the expression in humans of some, but not all, genes encoding ThDP-utilizing enzymes in a manner that gives greater expression with increasing thiamine levels. The physiological reasons for these alterations are unclear, and their potential contribution to TD-disorders remains to be examined. The cell type dependency of the regulation raises the possibility that differences in gene regulation may contribute to differential sensitivity to TD among various tissues and cell types.

Thiamine also has been implicated in regulating the levels of the thiamine transporter. In yeast, expression of the thiamine transporter gene is rapidly repressed with increasing amounts of thiamine available to the yeast cells [61]. Transport in yeast, unlike that in vertebrates, is active, and all of the thiamine in the culture medium is rapidly taken up and stored within the cells [59]. Following the depletion of thiamine from the medium, the transport system is lost by repression of gene expression due to the high intracellular thiamine concentration. In vertebrates, alterations in uptake capacity have been suggested, but the evidence for this is indirect. Laforenza *et al.* [62] studied mucosal biopsy specimens obtained by routine endoscopy in normal subjects and one patient with TD. In the TD patient, the duodenal saturable uptake was increased, suggesting down-regulation of intestinal transporters by thiamine. Preliminary results using cultured cells have found no alteration in the levels of transporter mRNA as the thiamine concentration in the medium is varied (T. Song, CKS, unpublished results). Increases in thiamine uptake capacity could still be accomplished, though, by increased ThDPK activity under a state of TD; such a suggestion remains to be investigated.

## THDP-UTILIZING ENZYMES

To elucidate the biochemical changes and molecular mechanisms responsible for the pathophysiologic consequences of TD, numerous investigations have focused on the enzymes which utilize ThDP as a cofactor (**figure 1, steps 4, 4a, 4b, 4c**). The enzymes include TK, PDH, and KGDH, all of which participate in carbohydrate catabolism and all of which, to one extent or another show reduced activity during TD. The reductions have been demonstrated using cultured cells, experimental models of TD in rats, and autopsied human tissues [29, 32, 48, 49, 63-68]. The degree of the reductions are different for the different enzymes and show a strong cell-type dependency that is also different from one enzyme to another. Although it was expected that the cell type distributions of these enzymes and/or the variations in reductions of their activities during TD might account for differential sensitivity to TD, intriguingly no positive correlation has been found between TD-affected regions of the brain and enzyme

distribution or alterations in activities during TD. Nonetheless, the reductions in ThDP-utilizing enzymes cause multiple biochemical changes within a cell and it is likely that one or more of these alterations leads to other insults that ultimately result in a focal loss of neurons during TD. A number of plausible mechanisms that are based on effects resulting from reductions in one or more of the ThDP-utilizing have been proposed to account for TD-induced neuronal cell death. As detailed below and in a recent, comprehensive review of these mechanisms [69], the neurological damage suffered during TD cannot be accounted for by any single mechanism and instead a number of mechanisms probably contribute to causing the damage.

The biochemical properties of the ThDP-utilizing enzymes have been extensively studied and reviewed elsewhere [70, 71; see issue 2, vol. 1385 of BBA]. The enzymes share a common ThDP-binding motif composed of several completely or highly conserved residues, defined by crystallographic studies and site-directed mutagenesis. The mechanisms of catalysis, including the roles served by the substituents of the thiazole and pyrimidine rings of ThDP and various amino acids of the proteins, have been substantially defined. Binding of thiamine requires a divalent cation associated with the ThDP and results in hysteretic behavior during the assembly and formation of the active holoenzymes (**figure 1, steps 4, 4a, 4b, 4c**) [72, 73]. The ThDP-utilizing enzymes are oligomeric, with PDH and KGDH possessing different protein subunits and associating with other cofactors as well as ThDP. Assembly to give the active enzymes is complex with several rate limiting steps, including the initial association of ThDP [74, 75]. As discussed below variations in several of the assembly and biochemical properties of the enzymes among individuals may contribute to inter-individual differences in susceptibility to TD.

TK participates in and is rate limiting [76] for the nonoxidative portion of the pentose phosphate pathway, a pathway that produces reducing equivalents in the form of NADPH for various cellular biosynthetic reactions, including lipids, and removal of reactive oxygen species and that produces riboses for use in the synthesis of nucleotides, nucleic acids, coenzymes, and polysaccharides. A reduction in such biosynthetic capacity may well lead to irreversible cellular damage, but a direct link to TD-induced cellular damage has yet to be established. Decreases in PDH and KGDH activity result in a reduction in the oxidative decarboxylation of alpha-keto acids, leading to a failure of ATP synthesis. Indeed, TD results in selective decreases in ATP levels within brain regions that are damaged during TD [77], and these alterations in energy levels may contribute to cell death. Loss of KGDH activity is thought to

account for alterations in the intracellular and extracellular levels of several neurotransmitters, including GABA, glutamate, and aspartate, during TD [78-81]. These and other findings have suggested that NMDA-receptor mediated excitotoxicity may play a role in TD-induced neuronal loss. As discussed in detail in [69] several findings are consistent with such a mechanism, but no direct evidence of a such a role has been obtained.

TD results in increased lactic acid concentrations within the brain and an associated acidosis [82, 83]. The acidosis is localized to damaged regions, and is thought to be related to reduced pyruvate entry into the TCA cycle resulting from losses in KGDH and/or PDH activities [84]. Whether acidosis is a byproduct of TD or is a factor in generating neuronal cell death remains to be resolved. However, it is easy to imagine how such metabolic changes can contribute to subsequent and more severe alterations in the ThDP-utilizing enzymes.

## APOPTOSIS AND TD

The alterations in oxidative metabolism, principally due to reductions in KGDH activity, are thought to account for the mitochondrial damage observed during TD, both in TD rat brains and in cultured cells deprived of thiamine [44, 85-87]. Extensive TD-induced mitochondrial damage was observed in primary cultures of rat cerebellar granule cells and mouse hippocampal neurons and in mouse and human neuroblastoma cell lines [44, 85, 86]. Substantial cell death occurred in the TD medium, and in two cases this was attributed to necrosis due to compromised mitochondrial function and acidosis [44, 85]. For human neuroblastoma cells, however, death was attributed to apoptosis (**figure 1, step 5**) [86]. The TD-induced apoptosis paralleled the loss of KGDH activity, but no causal relationship was demonstrated. Known mechanisms of inducing apoptosis following mitochondrial damage were ruled out, and instead induction of apoptosis correlated with the loss of the cJun amino terminal kinase JNK1. It was proposed that thiamine deficiency results in a cellular stress that brings about the loss of Jnk1 activity and the loss of its function of protecting cells from programmed cell death and that focal sensitivity to TD results, in part, from specific neuronal cell types being susceptible to the inactivation of Jnk1 in response to depletion of cellular thiamine [86]. These findings and proposals are consistent with the observation of apoptosis in the thalamus but not in other regions affected during TD in the rat model. Apoptosis has also been found for fibroblasts from TRMA patients if the cells are cultured without abnormally high thiamine concentrations in the medium [88].

## OXIDATIVE STRESS AND TD

Another possible result of TD-induced alterations in oxidative metabolism is the production of oxidative stress [89, 90]. Focusing on one of the earliest TD-damaged regions of the mouse brain, namely the thalamus, Gibson and coworkers found extensive oxidative stress associated with neuronal death [89]. The initial site of neuronal cell death (as is true in the rat brain) was in the submedial thalamic nucleus and with time neuronal loss spread to adjacent ventromedial and ventrolateral nuclei and subsequently produced larger areas of cell loss. This pattern of neuronal loss was paralleled temporally and spatially by the induction of the oxidative stress marker heme oxygenase-1 (HO-1) in microglial cells within the affected foci. HO-1 is thought to serve as a defensive mechanism against oxidative stress, and its induction indicate substantial oxidative stress was occurring very early within the damaged regions during TD. Results also were obtained demonstrating that lipid peroxidation only occurred late during neuronal loss and that nitric oxide synthase induction was not a factor in generating the TD-induced oxidative stress. Gibson and coworkers hypothesize that TD-induced altered oxidative metabolism results in initial neuronal death within the submedial thalamus via an unknown mechanism. This activates microglia and HO-1 induction which contributes to subsequent cell loss in part due to the resultant iron and carbon monoxide production. Eventually, lipid peroxidation contributes to the spread of neuronal loss.

Indications of the mechanisms inducing oxidative stress during TD may come from studies in yeast. Yeast strains that are overly sensitive to oxidative stress, including those with defective superoxide dismutase, were found to "revert" to normal resistance to oxidative stress by elevating their expression of TK [91, 92]. As discussed above, TK participates in producing NADPH, which is used in combating oxygen radicals. These studies concluded that, at least in yeast, the pentose phosphate pathway in general, and TK in particular, was critical for maintenance of the cellular redox state.

Misonou *et al.* recently demonstrated that oxidative stress induced accumulation of amyloid protein [93] in human neuroblastoma cells. This may account for the observations of amyloid precursor protein in some TD-damaged regions of rat brain [94] if TD-induced oxidative stress has a similar effect.

## INTER-INDIVIDUAL SENSITIVITY TO TD

While the studies described above address and shed some light on the mechanisms that underlie the cell specificity of TD-related damage, only

limited investigations have given insight into inter-individual differences in susceptibility to TD. Variations among individuals in ThDP-utilizing enzymes have been looked for and assessed as possible predisposing factors for TD-related disorders [32, 33, 95]. No correlation of variants and sensitivity to TD has been found to date. However, during work on the assembly of functional TK, it was found that a required "assembly" factor was defective in at least one WKS individual whose cells in culture showed an enhanced sensitivity to TD [75]. Normally, the dimerization step (**figure 1, step 4b**) during TK holoenzyme assembly is not rate limiting because of a posttranslational modification brought about by an as yet unidentified assembly factor [96]. However, if this factor is defective, dimerization becomes rate limiting and the dimeric state is unstable [75]. Indirect evidence strongly suggests that the factor is involved in modifying other ThDP-requiring proteins. This finding indicates one molecular mechanism that may account for differential sensitivity to TD among individuals is a defect in the assembly of ThDP-utilizing enzymes. Based on the above discussions, differential sensitivity to TD may also be related to individual differences in thiamine uptake capacity, differences in resisting cellular oxidative stress, and differences in anti-apoptotic factors; such possibilities remain to be investigated.

## THIAMINE AND ALCOHOL

As mentioned, the most common cause of TD in developed nations is alcoholism. The effects of alcohol/alcoholism on thiamine utilization have been discussed throughout the review. Alcohol and/or its metabolite acetaldehyde have been demonstrated to interact extensively with thiamine utilization at the molecular level at nearly every step in Figure 1; namely, thiamine transport, diphosphorylation, and modification and turnover of thiamine requiring enzymes. Accordingly, even with seemingly adequate dietary intake, alcoholics may have insufficient thiamine for maintenance of normal brain functions [7, 37]. Furthermore, the effects of repeated episodes of TD during a lifetime of alcohol consumption may contribute significantly to brain dysfunction in alcoholics [29, 97]. It remains to be determined whether alcohol/acetaldehyde significantly influence thiamine effects on gene expression or apoptosis.

As mentioned above, there is a requirement of Mg<sup>2+</sup> for ThDP binding to TK (**figure 1, step 4a**) and the other ThDP-binding enzymes prior to their activation. Mg<sup>2+</sup> is often depleted with chronic alcohol consumption [8, 40], and thus the Mg<sup>2+</sup> requirement for activation of ThDP-utilizing enzymes provides a possible molecular explanation for the recognized refractoriness to thiamine treatment alone of hypomagnesemic alcoholics with Wernicke's encephalopathy [98], the effect of

Mg<sup>2+</sup> deficiency on recovery of liver TK activity upon thiamine administration to TD rats [99], and the exacerbation by Mg<sup>2+</sup> deficiency of the histopathologic brain lesions of TD [100].

As already alluded to, TD is accompanied by diverse changes in brain glucose metabolism. As a result, there is decreased lipid incorporation into myelin; marked alterations in biosynthesis and turnover of several putative neurotransmitters, including acetylcholine (ACh), GABA, and glutamate; and focal regions of metabolic (lactic) acidosis and intracellular calcium accumulation, all of which may contribute to the neurotoxic effects of ethanol. Inactivation of ThDP-requiring enzymes by the ethanol metabolite acetaldehyde exacerbates these metabolic effects of TD. In addition to the importance of the inhibitory neurotransmitter GABA in alcohol withdrawal [101], disturbances during TD in GABA metabolism [80, 102] are of interest. Chronic ethanol administration results in up-regulation of the NMDA receptor system, and reduced intracellular pH can inhibit presynaptic high-affinity re-uptake of glutamate [29, 103]. Therefore, in alcoholism-associated TD, excitotoxicity mediated by increased extracellular glutamate concentrations [81] may go unopposed.

Abstinence-related improvements in brain functioning occur after discontinuing alcohol consumption and improved nutrition [104]. The pathophysiologic underpinnings of brain recovery are incompletely understood. Administration of thiamine reverses several of the behavioral and metabolic changes associated with TD in alcoholic patients and in animal models [19, 21, 105]. How thiamine protects the brain from excitotoxicity associated with combined TD and alcohol withdrawal [106, 107] and sets the stage for CNS recovery during abstinence remain to be determined. The NMDA antagonist MK-801 [107], the calcium channel blocker nimodipine [108], and the acetylcholinesterase inhibitor physostigmine [109], have all been demonstrated to reverse the metabolic and/or neurologic effects of experimental TD in rats. Acetylcholinesterase inhibitor physostigmine inhibition would also be expected to ameliorate the cholinergic deafferentation of chronic ethanol consumption [110], and benefits of both physostigmine [111] and tacrine [112] have been reported in detoxifying alcoholics. Calcium channel blockers [113] and NMDA antagonists [106] have more conclusively been shown effective in treatment of alcohol withdrawal per se, but clinical use of NMDA antagonists is still hazardous [106]. Voltage-dependent blockade of excitatory NMDA receptor channels by Mg<sup>2+</sup> provides a rationale for its use in the alcohol withdrawal syndrome [114], even though it is not routinely administered in clinical practice [115]. Mg<sup>2+</sup> may also influence the effects of anticonvulsant medications (independent of NMDA function) with potential clinical utility in

treatment of alcohol withdrawal [106]. It remains to be determined if the above nutritional and pharmacologic interventions, targeting the pathophysiology of TD and alcohol withdrawal, provide complementary neuroprotective effects in alcoholics during detoxification.

Water-suppressed proton magnetic resonance spectroscopy (MRS) is a noninvasive technique for determining, in anatomically localized volumes of interest, the relative concentrations of brain metabolites relevant to the pathophysiology of brain dysfunction and recovery in chronic alcoholics [116-120]. Studies using brain proton MRS in recently abstinent alcoholics [121] show a relative increase over time in choline (Cho) containing compounds in cerebellar vermis with a significant correlation between low Cho/NAA in cerebellar vermis and brain dysfunction. Furthermore, the human study parallels proton MRS findings in a rat model of TD in which decreases in the brain ratio of Cho/NAA reversed with thiamine administration in a dose-dependent manner [105]. Using high resolution MRS studies of brain extracts from rats, it has recently been demonstrated that glycerophosphocholine (GPC) is the primary component responsible for the observed decrease in the Cho peak during TD (Lee, Price, and P.R. Martin, unpublished). The shared metabolic correlates of brain recovery in abstinent alcoholics and thiamine reversal of experimental TD in the rat also support the role of TD in the pathophysiology of alcoholism-associated brain injury. Possible explanations for these findings include alterations during abstinence in the concentrations of: 1) lipid constituents of myelin [122, 123], 2) the acetylcholine precursor, choline [109, 110], or 3) GPC from degradation of phosphatidylcholine by phospholipase A2 [124] consistent with repair of myelin abnormalities, reversal of partial cholinergic deafferentation, or effects on signal transduction, respectively.

## THIAMINE AND CANCER

In humans, the major reactions of TK occur in the pentose phosphate pathway. Together with transaldolase, TK participates in the nonoxidative portion of the pathway, thereby reversibly linking the pentose phosphate pathway and glycolysis. In human erythrocytes, TK was demonstrated to be the rate-limiting enzyme in the nonoxidative portion of the pentose phosphate pathway [76]. The pathway is active in most tissues, with greater activity found in tissues involved in fatty acid and steroid synthesis and those producing high levels of reactive oxygen species. Various reaction schemes of TK and transaldolase are possible and are used depending on the particular needs for reducing equivalents and/or riboses that a given cell type may have. When cells require the production of excess ribose over reducing equivalents, as would be expected in

rapidly dividing tumor cells, high TK activity is required. This provides a rationale for using thiamine antagonists in anti-cancer treatments. Indeed, antagonists of thiamine were found to significantly decrease tumor cell proliferation, and the effect primarily was thought to be due to the inhibition of TK and the concomitant inability to provide sufficient riboses for nucleic acid synthesis [125, 126]. However, the antagonists used not only inhibit TK but also affect the thiamine transporter and ThDPK. Such findings warrant further studies of anti-thiamine compounds as anti-cancer drugs and raise a concern of the use of thiamine supplementation of cancer patients' diets [125].

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## LIST OF ABBREVIATIONS

Ach	= Acetylcholine
KGDH	= alpha-Ketoglutarate dehydrogenase complex
Cho	= Choline
GPC	= Glycerophosphocholine
NAA	= N-Acetyl-aspartate,
MRS	= Proton magnetic resonance spectroscopy
PDH	= Pyruvate dehydrogenase complex
NMDA	= N-Methyl-D-aspartate
TD	= Thiamine deficiency
ThDP	= Thiamine diphosphate
ThDPK	= Thiamine diphosphokinase
TRMA	= Thiamine-responsive megaloblastic anemia
TK	= Transketolase
WKS	= Wernicke Korsakoff syndrome

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