*Metabolic Brain Disease, Vol. 10 No. 1 1995*

# Molecular Genetics of Transketolase in the Pathogenesis of the Wernicke-Korsakoff Syndrome

**Peter R. Martin[1,3], Brian A. McCool[2], Charles K. Singleton[2]**

*Received July 7 1994; Accepted September 5, 1994*

Thiamine deficiency, a frequent complication of alcoholism, plays an important role in the pathogenesis of the Wernicke-Korsakoff syndrome [WKS]. Previous work by a number of investigators has implicated the thiamine-utilizing enzyme transketolase [Tk] as being involved mechanistically in the genetic predisposition to WKS. In particular, Tk derived from fibroblasts has been found to have an increased $K_m$ app for its cofactor thiamine pyrophosphate [TPP] and/or exist in different isoelectric forms in alcoholic patients with WKS as compared with unaffected individuals. We have demonstrated that these differences are not due to different Tk alleles, tissue-specific Tk isozymes, or differential mRNA splicing. These findings point to other mechanisms to explain the biochemical Tk variants, such as differences in assembly of the functional holoenzyme or differences in modification of the primary translation product. Tk assembly or modification, once biochemically characterized, may be found to be subject to genetic variation.

**Key words:** Hysteresis, Molecular genetics, Thiamine deficiency, Thiamine pyrophosphate, Transketolase, Wernicke-Korsakoff syndrome

## INTRODUCTION

Thiamine deficiency has been reported in up to 80% of alcoholics (Hoyumpa, 1980; Morgan, 1982; Tallaksen *et al.*, 1992). It is well established that thiamine deficiency can cause the life-threatening neuropsychiatric syndrome Wernicke's encephalopathy in the alcoholic patient, and that thiamine treatment rapidly reverses most of the acute signs of this disorder, leaving significant clinical sequelae (Wernicke-Korsakoff syndrome [WKS]) and characteristic neuropathologic findings (Victor *et al.*, 1989). Since only a subset of chronic alcoholics (approximately 13 percent) show the autopsy findings of WKS (Torvik

1 Departments of Psychiatry and Pharmacology, Vanderbilt University School of Medicine
2 Department of Molecular Biology, Vanderbilt University
3 To whom correspondence should be addressed at the Department of Psychiatry, A-2205 MCN, Vanderbilt University Medical Center, Nashville, Tennessee 37232, U.S.A.



0885-7490/95/0300-0045$07.50/0 

*et al.*, 1982), and the concordance rate for alcoholic organic brain disease is greater for monozygotic than dizygotic twins (Hrubec and Omenn, 1981), a genetic susceptibility to thiamine deficiency predisposing certain individuals to development of WKS has been proposed (Blass and Gibson, 1979; Leigh *et al.*, 1981).

## TRANSKETOLASE AND THIAMINE DEFICIENCY

To elucidate the biochemical changes during thiamine deficiency, investigators have focused on enzymes which utilize thiamine pyrophosphate [TPP] as a cofactor, transketolase [Tk], α-ketoglutarate dehydrogenase [αKGDH], and the pyruvate dehydrogenase [PDH] complex. The activities of all three enzymes are diminished in prolonged hypothiaminic states, and each may play a partial role in the etiology of the "biochemical lesion" that precedes neuroanatomic damage resulting from thiamine deprivation (Butterworth, 1989; Gibson *et al.*, 1989; Pratt *et al.*, 1990; Butterworth *et al.*, 1993).

Tk activity has been considered the most sensitive measure of dietary thiamine deficiency, and the time course of ataxia onset parallels the fall in cerebral Tk activity (Thomson *et al.*, 1983; Victor *et al.*, 1989). During thiamine deficiency, up to a 90% reduction in Tk activity can occur in the histopathologically most vulnerable midline structures of the rat brain (Gibson *et al.*, 1984). Chronic alcohol administration has been reported (Jung *et al.*, 1991) to decrease the stability of Tk in the brain, possibly by chemical interactions between acetaldehyde and thiamine (Pratt *et al.*, 1990), compounding the destabilizing effects of thiamine deficiency *per se* on apo-Tk (Jeyasingham *et al.*, 1986). Furthermore, many of the biochemical effects and neuropathological changes in the brain due to the thiamine antagonist pyrithiamine or in thiamine-deficient animals are similar to inhibition of the oxidative portion of the pentose phosphate pathway (Gaitonde and Evans, 1982).

Tk catalyzes two (the first and last) of the three steps of the nonoxidative branch of the pentose phosphate pathway, thus serving a critical role in providing a reversible link between glycolysis and the pentose phosphate pathway, essential when the cell must modify requirements for NADPH reducing equivalents and pentoses (Stryer, 1988). The pentose phosphate pathway shows localized regions of high activity in heavily myelinated tracts in adult brain (Buell *et al.*, 1958) and its contribution to carbohydrate metabolism is increased by electrical stimulation (McIlwain and Bachelard, 1985). One can reason that suboptimal functioning of Tk would at the least impair cellular capacity to produce sufficient quantities of biosynthetic reducing equivalents, thus affecting lipid and other syntheses, reduced glutathione availability, and perhaps amino acid production and transport in the brain (Thompson and McGeer, 1985; Heroux and Butterworth, 1988; Langlais and Mair, 1990; Berthon *et al.*, 1992; Martin *et al.*, 1993).

## TRANSKETOLASE VARIANTS IN WKS

Blass and Gibson (1977) reported that partially purified Tk derived from cultured skin fibroblasts of patients with WKS bound its cofactor TPP with lower affinity (had a significantly higher $K_m$ app for TPP) than the enzyme from control fibroblasts; however,

no abnormalities were reported for αKGDH or the PDH complex in these patients. Because this characteristic of Tk persisted in tissue culture cells through serial passages and was also present when cells were cultured in medium containing an excess of thiamine and no ethanol, they suggested that the Tk abnormality in this syndrome was genetically, not environmentally, determined. This was an important observation because individuals with such a Tk abnormality may be prone to develop the complications of thiamine deficiency, including WKS, during periods of malnutrition which often accompany chronic alcoholism.

Mukherjee et al. (1987) replicated the findings of Blass and Gibson and reported that fibroblast Tk from non-WKS men with familial alcoholism and from their alcohol-naive sons also bound TPP less avidly than the enzyme derived from normal volunteers and their sons. However, Nixon et al. (1984) reported that the $K_m$ app for TPP of Tk derived from erythrocytes was not statistically different in patients with WKS than in controls. Nevertheless, using isoelectric focusing, they identified an isoform pattern for Tk which was more prevalent in patients with WKS relative to healthy controls.

Significantly, neither Kaufmann et al. (1987) nor Blansjaar et al. (1991) could corroborate these findings. Greenwood et al. (1984) separated two distinct fractions of Tk apoenzyme from erythrocytes of nonalcoholics which differed in their affinities for recombination with TPP. In addition, damaged variants of Tk found in alcoholics are possibly formed by acetaldehyde adducts or free radical reactions (Pratt *et al.*, 1990). More recently, Nixon et al. (1990) described an abnormal relationship between erythrocyte Tk activity and the magnitude of increase in Tk *in vitro* with excess TPP (TPP effect) in WKS patients consistent with a Tk variant having an altered interaction with its cofactor. Modifications during manipulation and storage (Takeuchi *et al.*, 1986; Jeyasingham and Pratt, 1988) together with different purification procedures and tissue sources (Jung *et al.*, 1991) of Tk used for $K_m$ determinations have been invoked as possible explanations for the discrepancies among these various studies.

## MOLECULAR GENETICS OF TRANSKETOLASE

The most direct way to address the above-mentioned discrepancies reported in the literature is to clone the entire coding region of Tk and investigate whether Tk variants correspond to specific alterations within the nucleic acid sequence of the Tk gene (McCool *et al.*, 1993).

### Transketolase cDNA, mRNA, and Chromosomal Assignment

Screening of libraries via conventional methods resulted in a cDNA clone from human frontal cortex that was about 95% full length (McCool *et al.*, 1993). The remaining portion of the coding region proved difficult to clone and eventually had to be obtained through direct sequencing of Tk mRNA using primers corresponding to the 5' end of the longest cDNA clone. Such sequencing and primer extension indicated that about 240 residues were missing. An oligonucleotide was made corresponding to the sequence 22 nucleotides from the terminus of the mRNA and was used with the primers mentioned above in polymerase chain reactions [PCR] to generate the missing cDNA. This fragment was fused with the longest cDNA clone to give a full length cDNA clone of the Tk gene.

Excluding the poly(A) tail, the Tk mRNA is 2050 nucleotides in length, comparable to the estimated length from northern analysis of 2100-2200 nucleotides. We have only detected a single mRNA species on Northerns using mRNA isolated from HeLa cells, fibroblasts, and lymphoblasts. Furthermore, Southern analysis using a number of different enzymes has led us to conclude that the Tk gene exists in a single copy per haploid genome. In collaboration with Dr. David Goldman and coworkers from the NIAAA Intramural Research Program, PCR-based analysis of hybrid cell lines have shown that the gene is located on chromosome 3p, in agreement with recent findings of Lapsys *et al.* (1992).

## TPP-Binding Region

The predicted amino acid sequence of the human Tk has been compared to the sequence of Tk from *Saccharomyces cerevisiae* and *Rhodobacter sphaeroides* and to several other TPP binding proteins. These comparisons (McCool *et al.*, 1993) have led to the suggested role of a region of the human enzyme in substrate binding. The yeast enzyme has recently been crystallized in complexation with TPP (Lindqvist *et al.*, 1992). Thus, we were able to demonstrate, between the two distantly related species, almost perfect conservation of the amino acid residues postulated to interact with the TPP cofactor (Figure 1).

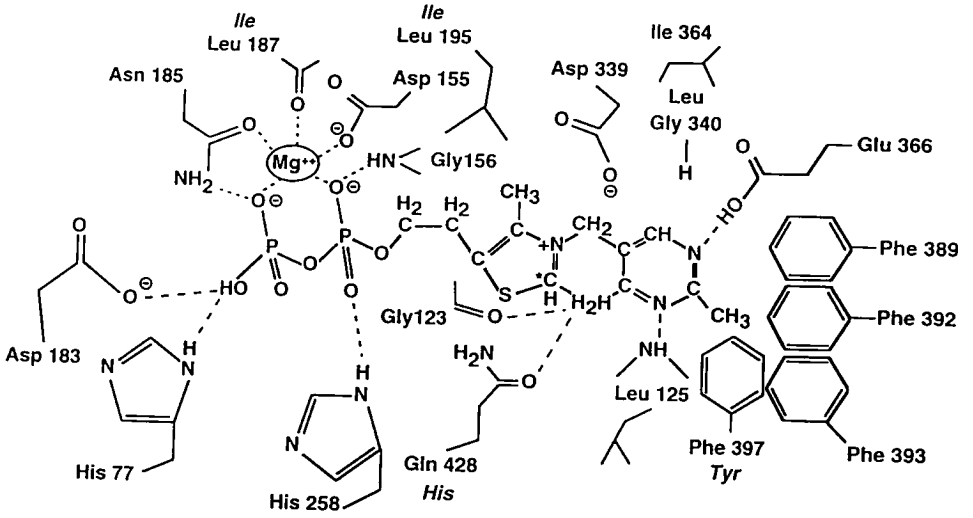


**Figure 1** Postulated interactions between specific amino acids in human transketolase and the TPP cofactor. The amino acids presumed to be involved in TPP binding were determined by sequence comparisons between the human and yeast enzymes, and using the crystal structure of the yeast transketolase (Lindqvist *et al.*, 1992). Amino acids of the human transketolase that are not strictly conserved are indicated by the corresponding amino acid from the yeast enzyme in italics. In nearly all of these instances, conservative substitutions are observed. The amino acid numbering scheme is from McCool *et al.* (1993). The asterisk indicates the reactive carbon atom of TPP. Both subunits of the transketolase dimer contribute to the binding of a single TPP molecule, with two such binding pockets existing symmetrically in the dimer.

Mutations in the nucleic acid sequences encoding the TPP-binding residues, or modifications of the amino acids contributing to its quaternary structure, would be logical candidates to explain putative Tk variants associated with WKS.

### Sequencing Tk Variants

Most importantly, we were able to determine the sequence of the coding region of the Tk gene from four individuals (McCool *et al.*, 1993). These represented the extremes in $K_m$ app values for fibroblast cultures from Mukherjee *et al.* (1987); two (WKS patients) had high $K_m$ app values and two (nonalcoholic controls) had low $K_m$ app values. The sequence was determined by using slightly overlapping primer pairs to generate double stranded DNA regions of about 300 base pairs by PCR. The template used in the PCR reactions was cDNA generated by using one of the primers, mRNA from a particular fibroblast culture, and reverse transcriptase. Negative controls with no added mRNA were always run to check for contamination of solutions with DNA from other sources. The PCR products were sequenced directly to eliminate possible copying errors. Several nucleotide differences between the cloned cDNA sequences and the sequences present in fibroblast cultures were found which resulted in amino acid differences. Although a few nucleotide differences were found among the four individuals, none of these led to amino acid differences. Thus, the amino acid sequence of the Tk from the four individuals was identical. Hence, for at least these four individuals, allelic variations are clearly not responsible for the biochemically distinct forms of the enzyme possessed by them.

In collaboration with Dr. Goldman, we have determined the genomic organization (introns/exons) of the Tk gene and identified six sequence variants at the Tk locus (one nonconservative amino acid substitution). Comparison of Caucasian patients with WKS (n=29), Caucasian alcoholics without neurologic dysfunction (n=35), and Caucasian controls (n=74) revealed no statistically significant differences in Tk allele frequencies.

The significance of these molecular genetic findings are worth summarizing. The finding that Tk is a single copy gene effectively rules out different encoded isozymes being responsible for the biochemical and/or chromatographic differences for this enzyme that have been reported in the literature over the last decade. We have also observed that the Tk gene produces a single mRNA species, and we find no evidence for differential or alternative splicing of this transcript. Taken together, these findings indicate that the nascent, primary translation product of the Tk gene is the same within all cell types. Finally, our findings indicate that for at least the four individuals examined, nucleotide variations within the Tk coding sequence cannot represent genetic predisposition for the development of WKS. Likewise, allelic variants of the Tk gene cannot account for the biochemically distinct forms of the enzyme found in these individuals. Nonetheless, since most genetic disorders are polymorphic, our findings do not fully exclude the possibility that some cases of WKS are actually due to mutation(s) in the Tk gene. Furthermore, genetic variation in the other TPP-utilizing enzymes, α-KGDH or PDH complex, is also a distinct possibility which has not yet been investigated.

## BIOCHEMICAL CHARACTERIZATION OF TRANSKETOLASE

As described above, conflicting results have been obtained with regard to the association with WKS of a Tk variant having high $K_m$ app for TPP. We were able to replicate the findings of Mukherjee *et al.* (1987) with respect to the $K_m$ app of Tk from the cultures used in our sequencing comparisons. Thus, it is clear that even though the amino acid sequence of Tk from these four individuals is identical, two of them have high, and two have low $K_m$ app's. In addition, we have found that many (9 of 11) of the WKS patients, more recently diagnosed, also have a high $K_m$ app. However, not *all* WKS patients have the high $K_m$ app form, and we have identified non-WKS individuals (including one patient with dementia associated with alcoholism [DAA]) who have the high $K_m$ app form. The molecular genetic findings reviewed above point to other mechanisms than allelic variation to explain the biochemical Tk variants derived from different individuals, such as differences in assembly of the functional holoenzyme or differences in modification of the primary translation product. Tk assembly or modification, once biochemically characterized, may in turn be found to be subject to genetic variation.

### Assembly of Functional Transketolase Holoenzyme

As with other TPP-utilizing enzymes (Egan and Sable, 1981), a lag period is observed in the Tk reaction progress curves before steady state velocity is obtained (Figure 2). This lag (hysteresis) is seen when the reaction is started by the addition of either the cofactor or apoenzyme to an otherwise complete reaction mixture. Both the lag period and the apparent rate constant for the transition between the initial and the final steady state velocities (Neet and Ainslie, 1980) were found to be highly dependent on TPP, with the lag time decreasing (rate constant increasing) with increasing TPP concentration (Singleton *et al.*, 1995). Significantly, the relation was not linear but the lag period reached a limiting, constant value at high TPP levels. This demonstrates that slow binding of TPP is not the only factor responsible for the observed lag. The constant, limiting value reached at high TPP levels was found to be independent of the enzyme concentration, indicating that a slow isomerization step during Tk holoenzyme formation is responsible for part of the lag. Our results also demonstrated a negative cooperativity with respect to TPP binding (Hill constant of 0.6 - 0.7).

In contrast to the human enzyme, for yeast Tk the limiting lag time at high TPP is due to slow dimerization during holoenzyme formation (Egan and Sable, 1981). The cause of this distinct difference between the human and yeast Tk's is unclear, but may pertain to a postulated (Martin *et al.*, 1993) role of Tk as a thiamine storage protein in the human, a function which may not be necessary in single cell organisms which can synthesize thiamine *de novo*. There is ca. 25% overall identity between the yeast and human Tk and most of the residues involved in interactions with TPP in the yeast enzyme seem to be conserved in the human enzyme (Figure 1). Therefore, our ongoing collaboration with Dr. Lindqvist and coworkers, who have solved the structure of Tk from *Saccharomyces cerevisiae* in complexation with the TPP cofactor (Lindqvist *et al.*, 1992), should help elucidate the structural basis for the difference in dynamics of Tk assembly in humans and yeasts.

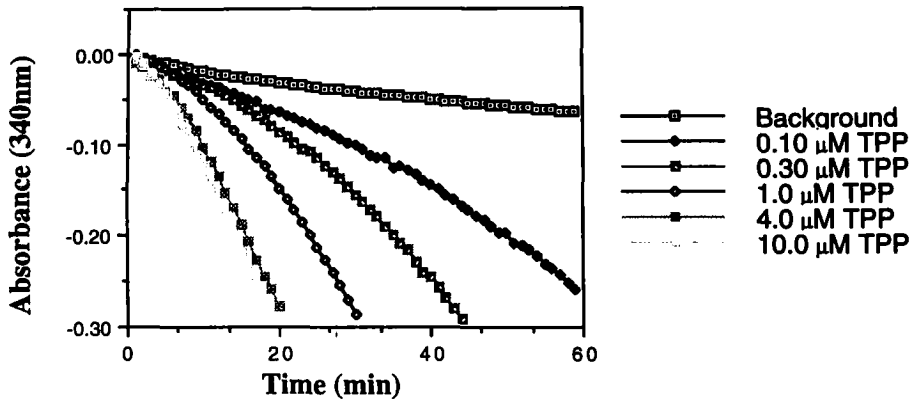


**Figure 2** The progress of the transketolase reaction is revealed by the decrease in absorbance at 340 nm. Background represents the reaction in the presence of no exogenously added TPP to ensure that the cofactor had been removed and apo-transketolase was being added to the reaction mix. The significant, TPP-dependent lag prior to reaching linearity (steady state) can be seen.

## Reinterpretation of $K_m$ app

The characterizations of the lag period in the progress curves has led to a reevaluation of the interpretation of the $K_m$ app. This hysteretic behavior makes calculation of the $K_m$ for TPP problematic, as activities derived from the initial velocities clearly do not meet the steady state assumption, and conversely, those derived from the linear velocities clearly are not initial velocities (Table I). In general, a relatively higher $K_m$ app is found using initial velocities whereas a relatively lower $K_m$ app results when steady state values are used. The magnitude of the difference between the two values for a given individual was found to be dependent on whether the individual had an overall high $K_m$ app form, or a low $K_m$ app form of Tk, relative to other individuals. Since using the progress curve within an early time window gave a greater difference in $K_m$ app values for high and low $K_m$ individuals than using later time windows of the reaction, this suggests that the difference in $K_m$ app between individuals may be a function of a difference in the assembly of functional holoenzyme rather than a difference in affinity for the TPP cofactor *per se*. This work also clarifies some of the contradictory findings in the literature, as different researchers have used different portions of the progress curves to calculate $K_m$ app.

**Table I.** Effect of progress curve window used to determine $K_m$ app of transketolase for TPP for individuals with high $K_m$ app and low $K_m$ app enzyme variants.

| | Observed $K_m$ app (μM) | |
|---|---|---|
| | Early time block | Late time block |
| High $K_m$ Tk | 2.51 | 0.62 |
| Low $K_m$ Tk | 0.58 | 0.25 |

## Interindividual Differences in the Transient Lag for Transketolase

We have found substantial variation in the lag periods at the low TPP concentrations for Tk derived from different individuals (Singleton *et al.*, 1994). At TPP concentrations below 1 μM the rate constant for the transition from the initial velocity to steady state velocity differed by as much as 2-fold, and these differences were not dependent on $K_m$ app differences. This suggests that for different individuals, significantly different times are required for the formation of functional and active holo-Tk. Furthermore, we also have found that substantial variation in the lag periods at low TPP exists for Tk derived from either fibroblasts or lymphoblasts of a given individual. Tk from lymphoblasts consistently required longer times for functional holoenzyme formation relative to fibroblast-derived enzyme (2.5-fold at the lowest TPP concentration analyzed).

## Transketolase Modifications

Although no specific modification(s) of human Tk have been identified to date, there are several results indicating that Tk can be modified (Paoletti and Mocali, 1991). Isoelectric focusing results in 4 to 6 isoelectric forms of Tk in all cell types examined (Martin *et al.*, 1994) even though as discussed above, there is a single nascent, primary translation product produced in each cell type. In addition, we have found that Tk synthesized *in vitro* using rabbit reticulocyte lysate reproducibly migrates faster under certain gel conditions than Tk from fibroblasts, lymphoblasts, or brain cells (Figure 3). Appropriate controls have ruled out trivial explanations such as proteolysis of the *in vitro* synthesized Tk. Thus, Tk made *in vivo* possesses modifications not found on Tk made *in vitro*. Finally, our preliminary studies suggest that phosphorylation is not the source of the *in vivo* modification.


**Figure 3** Western blot of transketolase from fibroblasts (lanes 1 and 3) and that made *in vitro* (lanes 2 and 4). Lanes 1 and 2 and lanes 3 and 4 represent two independent experiments and gels to illustrate the reproducibility. Fibroblast transketolase migrates slower by the equivalent of ca. 1.5 KDa. The migration difference is very sensitive to the percentage of polyacrylamide, to the ratio of bis- to linear-acrylamide, and to the length of the electrophoretic run. In contrast, lymphoblast and brain cell derived transketolase co-migrate with fibroblast transketolase under all gel conditions.

## ASSEMBLY OF FUNCTIONAL TRANSKETOLASE HOLOENZYME AND PREDISPOSITION TO THIAMINE DEFICIENCY

Our findings of significant interindividual differences in the lag period, as well as similar significant differences between two cultured cell types derived from a given individual, suggest testable mechanisms to account for the recognized differences in sensitivity to thiamine deficiency among individuals and among different tissues (Blass and Gibson, 1979; Victor *et al.*, 1989). Specifically, if loss of activity of one or more of the TPP-utilizing enzymes represents the metabolic basis of tissue injury due to thiamine deficiency, then it is reasonable to postulate that individuals possessing an enzyme with a relatively long lag period may be more susceptible to thiamine deficiency-related disorders. Likewise, cell types which have an enzyme characterized by a longer lag period may be expected to lose enzymatic activity more rapidly and to a greater extent than tissues possessing a shorter lag period enzyme. Indeed, we have found that when synthesis of human Tk is induced in *E. coli* cells in the presence of excess thiamine in the growth medium, abundant active holo-Tk is obtained (Singleton *et al.*, unpublished observations). However, induction in the absence of added thiamine results, not only in no activity, but in no Tk protein, presumably due to proteolysis. Moreover, precedents exist for greatly increased sensitivity to proteolysis *in vivo* for proteins with altered ability to assemble. For example, impairment of dimerization of human glutathione reductase leads to a greater than 30-fold decrease in the half-life of the enzyme within cells (Nordhoff *et al.*, 1993). Also, impaired dimerization leading to a destabilization of a protective protein for lysosomal modification enzymes results in a form of galactosialidosis (Zhou *et al.*, 1992).

Our preliminary findings show a trend towards slower assembly rates (higher tau values) for Tk in patients with alcoholic organic mental disorders (WKS and DAA) than in neuropsychologically unimpaired alcoholic controls, but too few individuals have been studied to make a conclusive statement at this time (Table II). These new biochemical characterizations of Tk shed light on the discrepancies among reports in the literature over the past 15 years (cited above), and focus attention on the molecular interactions during assembly, instead of affinity *per se*, between TPP-utilizing enzymes and their cofactor in the pathogenesis of WKS.

**Table II.** Assembly rates for functional holo-transketolase derived from fibroblasts of individuals with and without alcoholic organic mental disorders

| | Tau (min)* |
|---|---|
| WKS (n=6) | 17.9 ± 2.63 |
| DAA (n=1) | 18.2 |
| Alcoholic control (n=2) | 15.1 ± 0.78 |
| Non-alcoholic control (n=3) | 17.5 ± 5.62 |

*tau values (mean ± SD) are calculated as described in Singleton *et al.*, 1995.

## ACKNOWLEDGEMENTS

This work was supported in part by the National Institute on Alcohol Abuse and Alcoholism (AA084592), the Alcohol Beverage Medical Research Foundation, and Vanderbilt University Research Council.

## REFERENCES

Berthon H.A., Kuchel P.W. and Nixon P.F. (1992). High control coefficient of transketolase in the nonoxidative pentose phosphate pathway of human erythrocyte: NMR, antibody, and computer simulation studies. *Biochem.* **31**:12792-12798.

Blansjaar B.A., Zwang R. and Blijenberg B.G. (1991). No transketolase abnormalities in Wernicke-Korsakoff patients. *J. Neurol. Sci.* **106**:88-90.

Blass J.P. and Gibson G.E. (1977). Abnormality of a thiamine requiring enzyme in patients with Wernicke-Korsakoff syndrome. *N. Engl. J. Med.* **297**:1367-1370.

Blass J.P. and Gibson G.E. (1979). Genetic factors in Wernicke-Korsakoff syndrome. *Alcohol. Clin. Exp. Res.* **3**:126-134.

Buell M.V., Lowry O.H., Roberts N.R., Chang M-L.W. and Kapphahn J.I. (1958). The quantitative histochemistry of the brain: enzymes of glucose metabolism. *J. Biol. Chem.* **232**:979-993.

Butterworth R.F. (1989). Effects of thiamine deficiency on brain metabolism: implications for the pathogenesis of the Wernicke-Korsakoff syndrome. *Alcohol & Alcoholism* **24**:271-279.

Butterworth R.F., Kril J. and Harper C. (1993). Thiamine-dependent enzyme changes in the brains of alcoholics: relationship to the Wernicke-Korsakoff syndrome. *Alcohol. Clin. Exp. Res.* **17**:1084-1088.

Egan R.M. and Sable H.Z. (1981). Transketolase kinetics: the slow reconstitution of the holoenzyme is due to rate-limiting dimerization of the subunits. *J. Biol. Chem.* **256**:4877-4883.

Gaitonde M.K. and Evans G.M. (1982). The effect of inhibition of hexosemonophosphate shunt on the metabolism of glucose and function in rat in vivo. *Neurochem. Res.* **7**:1163-1179.

Gibson G.E., Ksiezak-Reding H., Sheu K-F.R., Mykytyn V. and Blass J.P. (1984). Correlation of enzymatic, metabolic, and behavioral deficits in thiamine deficiency and its reversal. *Neurochem. Res.* **9**:803-814.

Gibson G.E., Nielsen P., Mykytyn V., Carlson K. and Blass J.P. (1989). Regionally selective alterations in enzymatic activities and metabolic fluxes during thiamin deficiency. *Neurochem. Res.* **14**:17-24.

Greenwood J., Jeyasingham M., Pratt O.E., Ryle P.R., Shaw G.K. and Thomson A.D. (1984). Heterogeneity of human erythrocyte transketolase: a preliminary report. *Alcohol & Alcoholism* **19**:123-129.

Heroux M. and Butterworth R.F. (1988). Reversible alterations of cerebral gamma-aminobutyric acid in pyrithiamine-treated rats: implications for the pathogenesis of Wernicke's encephalopathy. *J. Neurochem.* **51**:1221-1226.

Hoyumpa A.M. (1980). Mechanisms of thiamine deficiency in chronic alcoholism. *Am. J. Clin. Nutr.* **33**:2750-2761.

Hrubec Z. and Omenn G.S. (1981). Evidence of genetic predisposition to alcoholic cirrhosis and psychosis: Twin concordances for alcoholism and its biological end points by zygosity among male veterans. *Alcohol. Clin. Exp. Res.* **5**:207-215.

Jeyasingham M.D., Pratt O.E., Thomson A.D. and Shaw G.K. (1986). Reduced stability of rat brain transketolase after conversion to the apo form. *J. Neurochem.* **47**:278-281.

Jeyasingham,M.D. and Pratt O.E. (1988). Rat brain apotransketolase: activation and inactivation. *J. Neurochem.* **50**:1537-1541.

Jung E.H., Itokawa Y. and Nishino K. (1991). Effect of chronic alcohol administration on transketolase in the brain and the liver of rats. *Am. J. Clin. Nutr.* **53**:100-105.

Kaufmann A., Uhlhaas S., Friedl W. and Propping P. (1987). Human erythrocyte transketolase: no evidence for variants. *Clinica Chimica Acta* **162**:215-219.

Langlais P.J. and Mair R.G. (1990). Protective effects of the glutamate antagonist MK-801 on pyrithiamine-induced lesions and amino acid changes in rat brain. *Neuroscience* **10**:1664-1674.

Lapsys N.M., Layfield R., Baker E., Callen D.F., Sutherland G.R., Abedinia M. *et al.* (1992). Chromosomal location of the human transketolase gene. *Cytogenet. Cell Genet.* **61**:274-275.

Leigh D., McBurney A. and McIlwain H. (1981). Wernicke-Korsakoff syndrome in monozygotic twins: A biochemical peculiarity. *Brit. J. Psychiatry* **139**:153-159.

Lindqvist Y., Schneider G., Ermler U. and Sundstrom M. (1992). Three-dimensional structure of transketolase, a thiamine diphosphate dependent enzyme, at 2.5 A resolution. *EMBO J.* **11**:2373-2379.

Martin P.R., McCool B.A. and Singleton C.K. (1993). Genetic sensitivity to thiamine deficiency and development of alcoholic organic brain disease. *Alcohol. Clin. Exp. Res.* **17**:31-37.

Martin P.R., Pekovich S.R., McCool B.A., Whetsell W.O., and Singleton C.K. (1994). Thiamine utilization in the pathogenesis of alcohol-induced brain damage. *Alcohol & Alcoholism* (in press)

McCool B.A., Plonk S.G., Martin P.R. and Singleton C.K. (1993). Cloning of human transketolase cDNAs and comparison of the nucleotide sequence of the coding regions in Wernicke-Korsakoff and non-Wernicke-Korsakoff individuals. *J. Biol. Chem.* **268**:1397-1404.

McIlwain H. and Bachelard H.S. (1985). *Biochemistry and the Central Nervous System*, Churchill Livingstone, New York, NY.

Morgan M.Y. (1982). Alcohol and nutrition. *Br. Med. Bull.* **38**:21-29.

Mukherjee A.B., Svoronos S., Ghazanfari A., Martin P.R., Fisher A., Roecklein B. *et al.* (1987). Transketolase abnormality in cultured fibroblasts from familial chronic alcoholic men and their male offspring. *J. Clin. Invest.* **79**:1039-1043.

Neet K.E. and Ainslie G.R., Jr. (1980). Hysteretic enzymes. *Meth. Enzym.* **64**:192-226.

Nixon P.F., Kaczmarek M.J., Tate J., Kerr R.A. and Price J. (1984). An erythrocyte transketolase isoenzyme pattern associated with the Wernicke-Korsakoff syndrome. *Eur. J. Clin. Invest.* **14**:278-281.

Nixon P.F., Price J., Norman Hicks M., Williams G.M. and Ker R.A. (1990). The relationship between erythrocyte transketolase activity and the 'TPP effect' in Wernicke's encephalopathy and other thiamine deficiency states. *Clin. Chim. Acta* **192**:89-98.

Nordhoff A., Buckeler U.S., Werner D. and Schirmer R.H. (1993). Folding of the four domains and dimerization are impaired by the Gly446-Gln exchange in human glutathione reductase: implications for the design of antiparasitic drugs. *Biochem.*, **32**:4060-4066.

Paoletti F. and Mocali A. (1991). Enhanced proteolytic activities in cultured fibroblasts of Alzheimer patients are revealed by peculiar transketolase alterations. *J. Neurol. Sci.* **105**:211-216.

Pratt O.E., Rooprai H.K., Shaw G.K. and Thomson A.D. (1990). The genesis of alcoholic brain tissue injury. *Alcohol Alcohol* **25**:217-230.

Singleton C.K., Pekovich S.R., McCool B.A. and Martin P.R. (1995). Hysteretic behavior of human transketolase: Implications for thiamine deficiency. *J. Nutr.* (in press)

Stryer, L. (1988). *Biochemistry*, W.H. Freeman and Company, New York, NY.

Takeuchi T., Nishino K. and Itokawa Y. (1986). Purification and characterization of, and preparation of an antibody to, transketolase from human red blood cells. *Biochim. Biophys. Acta* **872**:24-32.

Tallaksen C.M.E., Bohmer T. and Bell H. (1992). Blood and serum thiamin and thiamin phosphate esters concentrations in patients with alcohol dependence syndrome before and after thiamin treatment. *Alcohol. Clin. Exp. Res.* **16**:320-325.

Thompson S.G. and McGeer E.G. (1985). GABA-transaminase and glutamic acid decarboxylase changes in the brain of rats treated with pyrithiamine. *Neurochem. Res.* **10**:1653-1660.

Thomson A.D., Ryle P.R. and Shaw G.K. (1983). Ethanol, thiamine and brain damage. *Alcohol & Alcoholism* **18**:27-43.

Torvik A., Lindboe C.F. and Rogde S. (1982). Brain lesions in alcoholics: a neuropathological study with clinical correlations. *J. Neurol. Sci.* **56**:233-248.

Victor M, Adams R.D. and Collins G.H. (1989). *The Wernicke-Korsakoff Syndrome and Related Neurologic Disorders Due to Alcoholism and Malnutrition*, F.A. Davis Company, Philadelphia.

Zhou X.Y., Galjart N.J., Willemsen R., Gillemans N., Galgaard H. and d'Azzo A. (1992). A mutation in a mild form of galactosialidosis impairs dimerization of the protective protein and renders it unstable. *EMBO J.* **10**:4041-4048.