Thiamine Deficiency Decreases Steady-State Transketolase and Pyruvate Dehydrogenase but not α-Ketoglutarate Dehydrogenase mRNA Levels in Three Human Cell Types[1,2]

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ABSTRACT Reductions in the levels and activities of enzymes that utilize thiamine diphosphate (ThDP) as a cofactor are thought to be responsible for the tissue damage suffered during thiamine deficiency. Although loss of cofactor can account in part for loss of enzyme activity, thiamine and its phosphorylated derivatives may also regulate the expression of the genes encoding these proteins. To examine this possibility, steady-state mRNA levels for three ThDP-dependent enzymes were measured in human fibroblasts, lymphoblasts and neuroblastoma cells cultured under conditions of thiamine sufficiency and deficiency. In all three cell types, the mRNA levels of transketolase and the E1β subunit of pyruvate dehydrogenase complex were lower in thiamine-deficient cultures. In contrast, mRNA levels for a ThDP-binding subunit of α-ketoglutarate dehydrogenase, the E1 subunit did not differ. These results indicate that thiamine or a thiamine metabolite regulates the expression in humans of some, but not all, genes encoding ThDP-utilizing enzymes. J. Nutr. 128: 683–687, 1998.

KEY WORDS: • *transketolase* • *α-ketoglutarate dehydrogenase* • *pyruvate dehydrogenase complex* • *gene regulation* • *thiamine* • *humans*

Thiamine is a water-soluble, B-complex vitamin; when thiamine is phosphorylated to thiamine diphosphate (ThDP),[4] it functions as a cofactor for enzymes that catalyze α-keto acid decarboxylation or formation and cleavage of α-hydroxy ketoses (Voet and Voet 1990). In microbes, thiamine is involved in the regulation of the expression of the genes required for its own synthesis and import (Cary and Bhatnagar 1995, Maundrell 1990, Nishimura et al. 1992a and 1992b, Praekelt et al. 1994, Schweingruber et al. 1986, Webb et al. 1996). In *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Salmonella typhimurium* and *Aspergillus parasiticus*, the absence of thiamine within the growth medium results in thiamine biosynthetic genes being expressed, thereby allowing the vitamin to be synthesized and utilized during deficient conditions. When external thiamine is present, however, the thiamine biosynthetic genes are repressed and thiamine is imported from the surrounding environment.

Mammals and other complex eukaryotes do not have thiamine biosynthetic genes and thus thiamine can be obtained only from the environment. This can lead to severe consequences in humans when thiamine is limiting; thiamine deficiency is the cause of diseases such as beriberi and the Wernicke-Korsakoff syndrome. Reductions in the activities of one or more of the ThDP-dependent enzymes, transketolase, α-ketoglutarate dehydrogenase (α-KGDH) and pyruvate dehydrogenase (PDH), are thought to be responsible for the tissue damage and impaired cell function that accompany thiamine deficiency (Butterworth 1989, Martin et al. 1993).

We previously reported that in cultured human lymphoblasts rendered thiamine deficient by pyrithiamine, a thiamine antagonist, transketolase activity was reduced without a substantial accumulation of apo-enzyme (Pekovich et al. 1996). Loss of transketolase protein was demonstrated to be due to a decrease in its synthesis rate and not to an increase in the degradation rate. Thus thiamine may regulate the expression of genes that encode the enzymes that utilize ThDP. In this paper, we examined a potential role of thiamine in gene regulation by measuring the steady-state mRNA levels for transketolase and the E1 subunit of α-KGDH and E1β subunit of PDH, both of which are involved in binding ThDP, in three different human cell types growing under conditions of thiamine sufficiency or deficiency generated by employing pyrithiamine or thiamine-deficient medium.

MATERIALS AND METHODS

Cell culture. Normal lymphoblasts and fibroblasts were obtained as described (Pekovich et al. 1996, Singleton et al. 1995). The neuroblastoma cells were a SH-SY5Y cell line, a thrice cloned subline of SK-N-SH (Ross et al. 1983). Thiamine-responsive megablastic anemia (TRMA) patient P.M.R. has been described (Rindi et al. 1992 and 1994, Poggi et al. 1989). Lymphoblasts were established from

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[4] Abbreviations used: α-KGDH, α-ketoglutarate dehydrogenase; PDH, pyruvate dehydrogenase; ThDP, thiamine diphosphate; RT-PCR, reverse transcriptase–polymerase chain reaction; TDM, thiamine-deficient medium; TRMA, thiamine-responsive megaloblastic anemia.

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P.M.R. as described (Singleton et al. 1995). All cell types were grown at 37°C in the presence of various concentrations of thiamine-HCl (Sigma Chemical, St. Louis, MO) in RPMI 1640 medium without thiamine (Gibco, Gaithersburg, MD) supplemented with 20% heat-inactivated fetal calf serum (Gibco) and 2 mmol/L L-glutamine (Gibco). The fetal calf serum was dialyzed against four changes of Hanks' balanced salt solution (Gibco) at a total volume 24 times that of the serum by using dialysis tubing with a molecular weight cut-off of 12,000–14,000 Da (Millipore, Bedford MA). On the basis of the serum concentration of thiamine provided from the supplier, estimated thiamine concentration in the growth medium without any additional thiamine added was 0.1 nmol/L; this medium was referred to as thiamine-deficient medium (TDM). In experiments in which pyrithiamine (Sigma Chemical) was used, it was added either to control medium to generate thiamine deficiency or it was added to TDM to further reduce intracellular thiamine concentrations. Control medium contained 10 μmol/L thiamine for all normal cell types and 1 mmol/L for the TRMA lymphoblast cell line.

Cultures were started by adding $\sim 10^6$ cells, grown in control medium, to either 11 mL (lymphoblasts) or 33 mL (fibroblasts and neuroblastoma cells) of experimental medium. After 7 d in control medium, lymphoblasts (which do not attach) were at mid-to-late log phase, whereas neuroblastoma cells and fibroblasts had covered about 90% of the plate surface. Cells were harvested at this time because previous work (Pekovich et al. 1996) and these data (not shown) indicated that enzyme activity levels had equilibrated. The growth medium was changed once after 3–4 d. Effects on cell growth were monitored by counting viable cells stained with trypan blue (Gibco).

Because different cell types respond differently to limiting thiamine (Pekovich et al. 1997), the medium used to generate the thiamine-deficient state varied for each cell type. In each case, the condition that resulted in a maximum or close to maximum reduction in transketolase activity was chosen. For neuroblastoma cells (TDM + 30 nmol/L pyrithiamine) and TRMA lymphoblasts (TDM + 10 nmol/L thiamine), the deficient conditions affected cell growth, whereas the conditions for the fibroblasts (TDM + 3 μmol/L pyrithiamine) and normal lymphoblasts (TDM) did not (Pekovich et al. 1997).

Enzyme assays. The cells were washed three times with a cold isotonic buffer and lysed by resuspending and vortexing in a lysis buffer containing 20 mmol/L Tris-Cl (pH 7.5), 1 mmol/L dithiothreitol, 1 mmol/L potassium EDTA, 0.2 g/L Triton X-100, 0.2 g/L sodium deoxycholate and 0.2 mmol/L phenylmethylsulfonyl fluoride. The protein concentration of the clarified supernatant was determined using the Bio-Rad Protein Assay (Bio-Rad, Hercules CA), and the appropriate amount was added to the assay mixes as described below.

Transketolase activity (EC 2.2.1.1) was measured by using the enzyme-linked method (Smeets et al. 1971) under conditions in which coupling enzymes were not limiting (Tate and Nixon 1987). Reactions were initiated by the addition of 0.36 mg total protein/mL of reaction mix to an otherwise complete reaction mix of 100 mmol/L Tris-Cl (pH 7.5), 10 mmol/L ribose 5-phosphate, 2 mmol/L xylulose 5-phosphate, 1.2 mmol/L $MgCl_2$, 0.1 mmol/L NADH, 2000 U/L glycerol-3-phosphate dehydrogenase and triose phosphate isomerase. In some instances, 1 mmol/L ThDP was added to the reaction mix to activate any apo-transketolase that was present and allow for the determination of the total enzyme levels (apo- plus holo-enzyme) within the cell. Reactions were conducted at 37°C. The oxidation of NADH, which is directly proportional to transketolase activity, was followed by monitoring the decrease in absorbance at 340 nm using a Beckman DU-70 spectrophotometer (Beckman, Palo Alto, CA). Activity was expressed as nmol/(min · mg protein).

α-KGDH activity (EC 1.2.4.2, EC 2.3.1.6, EC 1.6.4.3) was measured as described (Gibson et al. 1988) with minor modifications. Reactions were typically initiated by the addition of 0.36 mg total protein to an otherwise complete reaction mix of 50 mmol/L MOPS (pH 8.0), 1.2 mmol/L $MgCl_2$, 1.2 mmol/L $CaCl_2$, 0.16 mmol/L coenzyme A, 1 mmol/L α-ketoglutarate, 1 mmol/L NAD, 0.5 g/L Triton X-100 and 0.04 mmol/L rotenone. In some instances, 1 mmol/L ThDP was added to the reaction mix to activate any apo-α-KGDH that was present and allow for the determination of the total enzyme levels (apo- plus holo-enzyme) within the cell. The reduction of NAD, which is directly proportional to α-KGDH activity, was monitored as for transketolase activity, only at 30°C. Only the initial portions of the progress curves were used to determine activity because the products of the reaction, NADH and succinyl-CoA, are strong inhibitors of α-KGDH (Voet and Voet 1990). Activity [nmol/(min · mg protein)] for the control condition in each experiment was set at 100% and values are expressed as a percentage of control.

RNA isolation and Northern analysis. Cells were harvested and total RNA was isolated as described (Singleton et al. 1987). After isolation, the RNA preparations were digested with DNase I, ethanol precipitated and stored at −70°C in water. Probe generation and Northern analysis were conducted as described (Singleton et al. 1987). In each lane, 10 μg of total RNA was used. Filters were hybridized to labeled cRNA corresponding to transketolase and to glyceraldehyde-3-phosphate dehydrogenase as an internal control. After hybridization and washing, the filters were dried and the bands were visualized and quantitated using a Molecular Dynamics Phosphorimager (Sunnyvale CA).

Reverse transcriptase–polymerase chain reaction (RT-PCR). DNase-treated total RNA (10 μg) was incubated with 1.5 pmol of the 3′ primer and water to give a total volume of 7.5 μL. This solution was heated for 5 min at 68°C, transferred to 43°C, and 2 μL of 5× RT buffer (0.5 mol/L Tris-Cl, pH 8.6, 0.6 mol/L KCl, 0.1 mol/L $MgCl_2$) and 0.5 μL RNasin (Promega, Madison, WI) were added; 10 μL of prewarmed enzyme mix [1 μL 10× RT buffer, 0.53 μL 25 mmol/L dNTP mix, 3 μL 0.1 mol/L β-mercaptoethanol, 5 μL water, 0.5 μL reverse transcriptase (10 units/μL)] were then added. This solution was incubated at 43°C for 45 min. The following mix (80 μL) was added to each reaction: 15 pmol 3′ primer, 15 pmol 5′ primer, 2 μL of 2 mmol/L KCl, 2 μL of 10 g/L triton X-100, 4 μL of 25 mmol/L $MgCl_2$, 1 μL of 10 mmol/L $HN(CH_3)_3Cl$, 0.6 μL of 25 mmol/L dNTP mix, 2 mBq α-[^{32}P]-dCTP, 0.8 μL Taq polymerase (Fisher Scientific, Pittsburg, PA) and 68 μL water). Amplification was conducted in a Biocycler (BIOS, New Haven, CT) using temperatures of 94°C (15 s)/52°C (15 s)/75°C (25 s) for 18, 20, 22 and 24 cycles. The cycle number for each of the reactions was within the linear range of product generation (not shown). Each reaction set contained primers for one of the ThDP-utilizing enzymes and for glyceraldehyde-3-phosphate dehydrogenase as an internal control. The products were treated with RNase and then fractionated on a 2% agarose gel. The gel was fixed with trichloroacetic acid, dried and exposed by using a phosphorimager screen.

The primers used for glyceraldehyde-3-phosphate dehydrogenase were as follows: 3′ primer, CAGCCTTCTCCATGGTGGTG; 5′ primer, GGTGAAGGTCGGAGTCAACG, which generated a 340-bp product. The primers used for transketolase were as follows: 3′ primer, AATGAGTTTTCTGTCCAGGGGCTTG; 5′ primer, GCTACA-AAGTTGGGGACAAG, which generated a 712-bp product. The primers used for the E1β component of PDH were as follows: 3′ primer, CTTTTGACTGAGCTTCCGGA; 5′ primer, GTGTCTGGCTTG-GTGCGGAG, which generated a 648-bp product. The primers use for the E1 component of α-KGDH were as follows: 3′ primer, AGT-AGGCCATCTCCAGCCGA; 5′ primer-AGGACTTGTGCTGCT-AAGTT, which generated a 630-bp product.

Values in the text are means ± SEM. Paired Student's t test was used to compare values obtained from the same cell type grown in thiamine-sufficient medium or thiamine-deficient medium. In all instances, independent experiments were performed, starting with cells growing under the various conditions.

RESULTS

Northern analysis of transketolase mRNA from human lymphoblasts. Previously, it was found that during thiamine deficiency in human lymphoblasts, loss of transketolase protein occurred as a result of a decrease in the rate of its synthesis (Pekovich et al. 1996). The decreased rate of transketolase production could be due to altered translational efficiency or to decreased mRNA levels. **Figure 1** shows the results of Northern analysis of lymphoblast RNA isolated from cells cultured under conditions of thiamine sufficiency (lane 1) or deficiency (lanes 2, 3) and probed with radioactive transketolase cRNA. Less transketolase mRNA was present in thia-

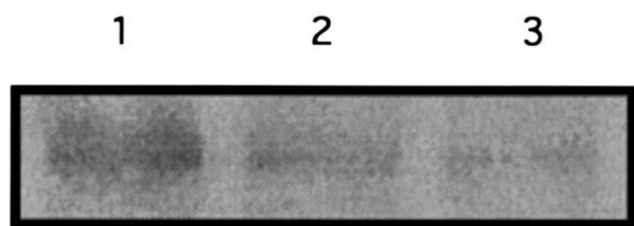

FIGURE 1 Northern analysis of transketolase mRNA derived from thiamine-sufficient and thiamine-deficient human lymphoblasts. A phosphorimage of transketolase mRNA is shown. The mRNA was isolated from cells grown either in control medium (lane 1), in control medium with 91.9 μmol/L pyrithiamine (lane 2) or in thiamine-deficient medium (TDM; lane 3).

mine-deficient lymphoblasts when deficiency was produced by using thiamine-deficient medium or by employing the thiamine analog, pyrithiamine.

Table 1 provides the quantitation of the differences found from two independent experiments, with the transketolase message levels normalized to the mRNA levels of glyceraldehyde-3-phosphate dehydrogenase (not shown). Enzyme activity also was measured under the three conditions and is shown. Transketolase enzyme activity was ~45% of control activity under both thiamine-deficient conditions, consistent with our previous findings (Pekovich et al. 1996 and 1997). Transketolase mRNA levels were correspondingly lower, with the levels in control medium plus pyrithiamine reduced to 51.8 ± 10.3% of control and the levels in TDM reduced to 57.0 ± 2.0%. The difference in mRNA and activity levels can be explained by the previous finding that a 10–15% accumulation of apo-transketolase occurs during thiamine deficiency (Pekovich et al. 1996, and **Fig. 2**). Thus, the decrease in transketolase protein, as opposed to the decrease in activity was ~55–60%, closely corresponding to the decrease in steady-state mRNA levels. The reduction in mRNA was not due to some nonspecific effect of pyrithiamine because the same reduction was seen with the use of TDM. These findings indicate that alterations in the steady-state mRNA level can account for the reduction in transketolase protein during thiamine deficiency and that thiamine can regulate transketolase gene expression.

TABLE 1

Relative levels of transketolase activity and mRNA in thiamine-sufficient and thiamine-deficient human lymphoblasts[1,2]

	Control	Pyrithiamine[3]	TDM
		%	
Enzyme activity[4]	100	44.7 ± 2.2†	45.3 ± 1.4†
mRNA	100	51.8 ± 10.3	57.0 ± 2.0†

[1] Values are means ± SEM, $n = 2$.

[2] Two independent experiments, starting from growing cells under the indicated conditions, were conducted. In each experiment, the activity or mRNA levels for the control medium were set at 100%, and the values in the other conditions are relative to this. Values obtained under each thiamine-deficient condition were compared with the control values by Student's paired t test; † $P < 0.05$.

[3] Pyrithiamine was added at a concentration of 91.9 μmol/L to control medium.

[4] Enzyme activity was determined in the absence of exogenous thiamine diphosphate.

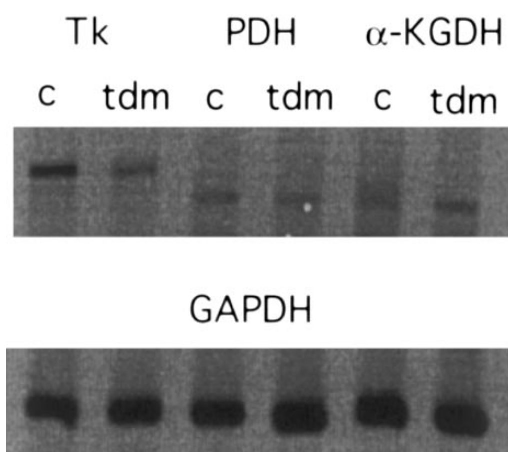

FIGURE 2 Reverse transcriptase–polymerase chain reaction analysis of transketolase (Tk), E1 α-ketoglutarate dehydrogenase (α-KGDH), and E1β pyruvate dehydrogenase (PDH) mRNA from human lymphoblasts grown either in control medium (c) or thiamine-deficient medium (tdm). In each sample, message levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were determined as an internal control.

RT-PCR analysis of mRNA levels of transketolase, PDH, and α-KGDH in human lymphoblasts, fibroblasts and neuroblastomas cells during thiamine deficiency. To examine whether the effect of thiamine on gene regulation is specific for transketolase or is more general for other ThDP-utilizing enzymes, an RT-PCR procedure was employed. Using the published sequences of the E1β subunit of PDH and the E1 subunit of α-KGDH, pairs of primers were made for each of these ThDP binding proteins as well as for transketolase and were used to determine the relative mRNA levels during thiamine sufficiency and deficiency.

Figure 2 shows the results of an RT-PCR experiment for each of the three enzymes in normal lymphoblasts growing in control and TDM media. Relative message levels for transketolase (TDM level of 63.6 ± 6.8% of control) were the same as those found in the Northern analysis above (57.0 ± 2.0%). E1β PDH mRNA levels also showed a reproducible decrease when thiamine was limiting, with a decrease to 78.7 ± 4.5% of control levels. In contrast, E1 α-KGDH mRNA was unaffected by thiamine deficiency.

mRNA levels for each protein were determined in several cell types (**Table 2**) to examine if thiamine effects on gene expression are cell type–dependent. Similar responses to thiamine deficiency with respect to mRNA levels were found for each enzyme in lymphoblasts, fibroblasts and neuroblastoma cells. In each cell type, transketolase mRNA was the most affected of the three mRNAs that were examined. E1β PDH message levels decreased to a lesser extent than transketolase mRNA. As seen in lymphoblasts, the E1 α-KGDH mRNA levels were unaffected in the other two cell types.

Finally, mRNA levels in lymphoblasts derived from patients with TRMA were also evaluated. TRMA is a rare disease associated with diabetes mellitus and sensorineural deafness (Rindi et al. 1992). Erythrocytes from TRMA patients lack the saturable, high affinity component of thiamine transport and have a small but reproducible decrease in thiamine-phosphorylation capability (Poggi et al. 1989, Rindi et al. 1992 and 1994). Thus it was of interest to examine gene expression levels in TRMA lymphoblasts to gain insight into the contri-

TABLE 2

Quantitation of reverse transcriptase–polymerase chain reaction (RT-PCR) analysis and total enzyme activity for thiamine diphosphate–utilizing enzymes in different human cell types cultured with thiamine sufficiency or deficiency[1]

Cell type (Growth condition)[3]	Protein	mRNA	Enzyme activity[2]
		% of control levels[4]	
Neuroblastoma (TDM + 30 nmol/L pyr)	Transketolase	51.4 ± 9.3	17.5
	PDH[5]	77.0 ± 5.3	ND
	α-KGDH[5]	109.0 ± 9.0	3.1
Fibroblast (TDM + 3 μmol/L pyr)	Transketolase	73.0 ± 6.4	50.3
	PDH	82.5 ± 3.1	ND
	α-KGDH	105.1 ± 4.2	89.9
Lymphoblasts (TDM)	Transketolase	63.6 ± 6.8	64.5
	PDH	78.7 ± 4.5	ND
	α-KGDH	95.4 ± 9.6	80.8
TRMA lymphoblasts[6] (TDM + 10 nmol/L thi)	Transketolase	65.8 ± 3.3	20.7
	PDH	100.7 ± 12.3	ND
	α-KGDH	95.6 ± 4.2	13.0

[1] Values are means ± SEM, n = 2 for mRNA and means, n = 1 for enzyme activity.

[2] Total enzyme activity (apo- plus holo-enzyme) was obtained in the presence of exogenous thiamine diphosphate. For mRNA levels, two independent experiments, starting from growing cells under the indicated conditions, were conducted. For enzyme activity, measurements were made for only one of the experiments; ND, not detected.

[3] The thiamine-deficient growth conditions for each cell type are indicated in parentheses; pyr, pyrithiamine; TDM, thiamine-deficient medium; thi, thiamine.

[4] In each experiment, the activity or mRNA levels for the control medium were set at 100%, and the values in the other conditions are relative to this.

[5] For mRNA levels, values are given for the E1β subunit of pyruvate dehydrogenase (PDH) or the E1 subunit of α-ketoglutarate dehydrogenase (α-KGDH).

[6] TRMA, thiamine-responsive megaloblastic anemia.

bution of the transport system and thiamine phosphorylation activity to mRNA production.

The TRMA lymphoblasts responded much like normal lymphoblasts, with transketolase mRNA decreasing to 65.8 ± 3.3% of control levels and E1 α-KGDH remaining unaffected when thiamine was limiting. However, E1β PDH mRNA levels were unaffected in TRMA lymphoblasts, whereas in the normal cell types the message levels for this enzyme were lower when thiamine was limiting.

For all cell lines examined, changes in protein levels for transketolase and α-KGDH also were determined (Table 2). The enzyme assays in this instance were conducted in the presence of exogenous ThDP so that total protein levels (apo- plus holo-enzyme) within the cell were measured as opposed to enzyme activity. In the two cell lines in which the thiamine-deficient conditions did not affect cell growth (fibroblast and normal lymphoblast), the measured total transketolase protein levels were in good agreement with the levels of transketolase mRNA. However, when the total protein levels were examined in the cells in which growth was affected by the deficient conditions (neuroblastoma and TRMA lymphoblast), the decreases in protein levels were substantially greater than the decreases in mRNA levels. Under these conditions, growth of the cells had virtually ceased by 7 d and cells appeared to be dying (Pekovich et al. 1997). Under these conditions, there may well be an enhanced presence of proteases, either real or artifactually upon cell lysis. For α-KGDH, no correlation was seen between total protein levels and mRNA levels in any cell type. This was expected because E1 α-KGDH mRNA levels were not affected by thiamine deficiency.

DISCUSSION

In three different types of cultured human cells, thiamine deficiency induced by using either pyrithiamine, a pyrithiamine analog frequently employed to generate thiamine deficient rats, or thiamine-deficient medium brought about a decrease in the steady-state levels of mRNA for the two ThDP-dependent proteins transketolase and E1β PDH. These findings indicate that thiamine or a thiamine metabolite regulates gene expression in humans either at the level of mRNA synthesis or turnover. Recently, other water-soluble vitamins have been found to be involved in gene regulation. These include ascorbic acid (Tajima and Pinnell 1996), biotin (Maeda et al. 1996), folate (Antony 1996) and pyridoxal 5′-phosphate (Oka et al. 1995). In the last-mentioned case, vitamin B-6 was shown to directly interact with specific transcription factors and altering their activity by this interaction (Oka et al. 1995).

The decrease in transketolase mRNA explains our previous observation that transketolase protein decreases during thiamine deficiency without a substantial build-up of apo-enzyme (Pekovich et al. 1996). In contrast, a substantial level of apo-α-KGDH was found during thiamine deficiency; we now find no decrease in the E1 α-KGDH mRNA under these conditions. Together, the present and previous results indicate that during thiamine deficiency, transketolase activity is lost as the essential ThDP cofactor becomes progressively unavailable. A similar thiamine depletion–induced decrease in transketolase mRNA, coupled with the rapid turnover of transketolase protein (Pekovich et al. 1996), prevents a substantial build-up of apo-transketolase. In contrast, for α-KGDH, a concomitant increase in apo-enzyme occurs as activity is lost due to the depletion of the cofactor. This occurs because thiamine depletion has no negative effect on at least the E1 subunit mRNA levels. Once thiamine depletion begins affecting cell growth and viability, both apo-enzymes become unstable, presumably due to an enhanced proteolytic environment.

Recently, Sheu and co-workers (1996) found that transketolase mRNA levels were not altered even though decreases

in transketolase protein did occur in various brain regions of thiamine-deficient rats. The difference between their findings and ours may indicate a species difference in the effect of thiamine on gene expression or may reflect tissue differences in regulation. The latter explanation seems less likely because we found an effect on transketolase mRNA in all human cell types examined, including neuroblastoma cells.

The contribution of thiamine regulation of gene expression to the tissue damage and impaired cell function that accompany thiamine deficiency (Butterworth 1989, Martin et al. 1993) is unclear. Simple loss of the cofactor can account for loss of enzyme activity (Pekovich et al. 1996 and 1997). Several models have been proposed to explain why the loss of activity of transketolase, α-KGDH or PDH leads to neuronal tissue damage during thiamine deficiency (Butterworth 1989, Butterworth et al. 1993, Sheu et al. 1996). Our findings that thiamine alters the expression of these enzymes do not bear on these models because they are independent of how the loss in activity occurs. Nonetheless, our results provide insight into the molecular mechanisms underlying the loss of activity, and they indicate that the cell has more flexibility to deal with thiamine depletion than a simple removal of cofactor. Thus, differential regulation of the genes that encode ThDP-utilizing enzymes may provide mechanisms for preferentially maintaining one enzyme activity over another according to the metabolic needs of the cell. In addition, the reductions in transketolase and E1β PDH mRNA but not in E1 α-KGDH mRNA may influence the differential recoveries of enzymatic activity upon a return to thiamine sufficiency (Gibson et al. 1984).

It was somewhat surprising that the message levels of the thiamine-binding E1β subunit of PDH were decreased during thiamine deficiency. Although we have not measured the activity or protein levels for this enzyme, we expected it to respond similarly to α-KGDH with respect to mRNA levels. Unlike transketolase, both of these enzymes are composed of multiple peptides, are located in the mitochondria and are both key control points in the process of carbohydrate metabolism. It is possible that thiamine depletion negatively affects PDH but not α-KGDH mRNA in an attempt to preferentially maintain the activity of one enzyme over the other, as has been shown to be true for riboflavin and enzymes that use it as a cofactor (Ross and Hansen 1992).

Finally, the absence of a decrease of E1β PDH mRNA levels in TRMA lymphoblasts is interesting. We previously found in TRMA lymphoblasts that both α-KGDH and transketolase activities were much more sensitive to thiamine deficiency than in normal lymphoblasts (Pekovich et al. 1997). However, the increase in sensitivity was significantly greater for transketolase than for α-KGDH. It is possible that in the TRMA cells, the normal distribution of thiamine may be altered such that mitochondrial thiamine concentrations are maintained at the expense of cytosolic levels. Because PDH is also located in the mitochondria, perhaps this enzyme too is not as affected during thiamine deficiency in comparison with transketolase. This may in turn prevent the loss of E1β PDH mRNA in TRMA lymphoblasts.

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