Thiamine Response in Maple Syrup Urine Disease

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ABSTRACT. We measured the biochemical response for four patients with maple syrup disease to pharmacologic doses of thiamine, and correlated their response to their branched chain α -ketoacid dehydrogenase activity. We observed a linear correlation between the concentrations of each plasma branched-chain amino acid and its corresponding ketoacid analogue. In addition, the renal tubular reabsorption of branched-chain amino and ketoacids was nearly complete within these physiologic concentrations. Three children responded to thiamine therapy with a reduction in concentration of plasma and urinary branched-chain amino and ketoacids. Each responder had at least 5% activity for branched chain α -ketoacid dehydrogenase in their mononuclear blood cells and in whole cell fibroblasts from cultured skin when compared to the activity in normal control cells. We propose that each child with maple syrup urine disease be assessed for their response to thiamine by quantifying the concentration of branched-chain amino acids in plasma before and after vitamin supplementation. (*Pediatr Res* 19: 1011-1016, 1985)

Abbreviations

BCKA, Branched-chain α -ketoacidemia
MSUD, Maple syrup urine disease
BCAA, branched chain amino acids
BCKA, branched chain α -ketoacids
BCKAD, branched chain α -ketoacid dehydrogenase
KIC, α -ketoisocaproic acid
KIV, α -ketoisovaleric acid

More than 25 yr ago, Menkes *et al.* (1) described four siblings with progressive neurologic disorders and sugary-smelling urine. The fragrant urinary compounds have since been identified as BCKA which accumulated as the result of impairment in the mitochondrial enzyme complex, BCKAD (E.C. 1.2.4.1.). All three BCKA accumulated: KIC; KVM; and KIV (2, 3). In patients with MSUD, BCKA results from this impaired catabolic pathway. By restricting dietary BCAA early in infancy, the clinical manifestations of MSUD can be ameliorated or prevented (4, 5).

Several patients have been reported with thiamine responsive

forms of MSUD (6-10). Elsas *et al.* (8) described the responses of two brothers with MSUD to increasing oral doses of thiamine. While taking 150 mg/day of thiamine, the brothers decreased their urinary excretion of BCKA and had increased activity of BCKAD as measured in peripheral blood lymphocytes. Based on these and additional *in vivo* and *in vitro* studies, Danner and coworkers (7, 8, 11-13) proposed that after its conversion to intracellular thiamine pyrophosphate, binding of this active co-factor to the multienzyme complex induced a conformational change which stabilized normal and mutant enzyme. This conformational change decreased the rate of biologic degradation of the complex and over time, increased overall enzyme activity (7, 8, 11-13).

Because of differences in reported doses and rapidity with which thiamine altered the patient's sensitivity to protein, it has remained unclear which patients would respond and which analysis could predict the response to thiamine therapy. The present study controlled for several variables, including the dietary intake of BCAA and the dose of thiamine. Plasma concentrations and renal transport of BCAA and BCKA were compared in four children affected with MSUD to determine whether pharmacologic doses of thiamine affect renal clearance of BCAA and BCKA. Each patient's biochemical response was correlated with residual activity of BCKAD in freshly isolated blood mononuclear cells and in their cultured skin fibroblasts.

METHODS

Clinical protocol. Four patients (Table 1), whose parents gave their informed consent, were studied.

J.P., a 17-month-old black male, was diagnosed at 13 days of age. His developmental landmarks were moderately delayed.

T.Mc., a 2½-yr-old white female, was diagnosed at 19 days of age. Despite normal development, she had recurrent bouts of otitis, seizures, and ataxia.

C.M., a 3½-yr-old white female, was treated from 14 days of age. She had previous episodes of ketoacidosis and seizures. Her development was moderately delayed.

V.H., a 9½-yr-old white male was diagnosed at 2 yr. His development was delayed, but he never developed ketoacidosis. He was the only child in this study whose diet was restricted in protein without specific restriction of the BCAA. He had taken thiamine (100 mg/day) for 7 yr before discontinuing one month prior to this study.

Before and during the study patients were fed a diet restricted in BCAA determined as optimal by the referring metabolic center. Patients were admitted to the Emory University Clinical Research Facility for 2 days of diet equilibration followed by 1 wk of baseline studies. These included three plasma samples after overnight fasting and three 24-h urine collections for quantitation of amino acids and organic acids. Mononuclear cells were iso-

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Table 1. Clinical profile of patients

Patient	Age at this study	Estimated developmental age at this study	Dietary prescription (units/kg/day)				Supplemental oral thiamine (mg/day)	
			Protein (g)	Leucine (mg)	Isoleucine (mg)	Valine (mg)	Total	per kg
J.P.	17 mo	10–12 mo	1.72	95	61	67	150	16
T.Mc.	2– ⁴ / ₁₂ yr	2–2 ¹ / ₂ yr	1.28	30	33	26	200	19
C.M.	3– ¹ / ₁₂ yr	2–2 ¹ / ₂ yr	1.24	21	14	19	200	16
V.H.	9– ² / ₁₂ yr	3–4 yr	0.97	70	45	48	200	8

Table 2. Effect of thiamine on fasting plasma branched chain amino acid concentration

Patient/condition	(n)	BCAA (μ M)*			% change in total plasma BCAA during thiamine
		Leucine	Isoleucine and alloisoleucine	Valine	
J.P./prethiamine	(2)	412.9 $\pm$ 86.1†	186.0 $\pm$ 30.2†	360.5 $\pm$ 89.0†	
J.P./during thiamine	(3)	196.2 $\pm$ 42.6	77.4 $\pm$ 9.9	189.6 $\pm$ 30.0	–51.7
T.Mc./prethiamine	(3)	494.6 $\pm$ 78.3†	186.7 $\pm$ 9.7†	143.0 $\pm$ 50.1	
T.Mc./during thiamine	(3)	116.7 $\pm$ 22.6	73.9 $\pm$ 23.7	129.5 $\pm$ 20.3	–61.2
C.M./prethiamine	(3)	240.9 $\pm$ 61.6	129.0 $\pm$ 42.5	134.4 $\pm$ 45.2	
C.M./during thiamine	(3)	316.8 $\pm$ 68.3	159.4 $\pm$ 17.9	102.5 $\pm$ 26.7	+14.8
V.H./prethiamine	(3)	435.0 $\pm$ 214.2	221.5 $\pm$ 109.4	481.5 $\pm$ 270.0	
V.H./during thiamine	(3)	269.3 $\pm$ 59.0	168.7 $\pm$ 48.6	270.2 $\pm$ 50.3	–37.8

* Plasma concentration is the mean $\pm$ 1 SD. When only two samples were available, concentration is the average and range.

† Plasma amino acid concentration was significantly less during thiamine therapy with $p < 0.05$ using a one-tailed t test for independent mean probability.

Table 3. Effect of thiamine on fasting plasma BCKA concentration

Patient/condition	(n)	BCKA (μ M)*			Percent change in total plasma bcka during thiamine
		KIC	KMV	KIV	
J.P./prethiamine	(2)	338.1 $\pm$ 6.7†	192.9 $\pm$ 2.4†	61.0 $\pm$ 1.5†	
J.P./during thiamine	(3)	190.0 $\pm$ 42.1	110.5 $\pm$ 22.2	35.7 $\pm$ 8.1	–43.2
T.Mc./prethiamine	(3)	714.3 $\pm$ 240.5†	239.5 $\pm$ 76.6	51.9 $\pm$ 22.3	
T.Mc./during thiamine	(2)	113.1 $\pm$ 39.2	126.1 $\pm$ 8.8	30.0 $\pm$ 5.9	–73.2
C.M./prethiamine	(3)	218.8 $\pm$ 42.3	117.9 $\pm$ 15.6	19.3 $\pm$ 7.1	
C.M./during thiamine	(3)	308.0 $\pm$ 30.6	148.6 $\pm$ 4.4	24.8 $\pm$ 8.5	+35.2
V.H./prethiamine	(3)	271.5 $\pm$ 12.2	166.7 $\pm$ 27.1	54.4 $\pm$ 4.7	
V.H./during thiamine	(3)	225.4 $\pm$ 36.1	143.2 $\pm$ 5.5	45.7 $\pm$ 4.7	–15.9

* Plasma concentration is the mean $\pm$ 1 SD. When only two samples were available, concentration is the average and range.

† Plasma amino acid concentration was significantly less during thiamine therapy with $p < 0.05$ using a one-tailed t tested for independent mean probability.

lated from heparinized blood for enzyme assays. After baseline studies were completed, the children were given thiamine orally to continue daily at home with their usual diet for 3 wk. During the 4th wk, while continuing thiamine, they were readmitted for fibroblast culture.

Quantitation of amino acids, organic acids, and renal tubular transport. Concentrations of amino acids were determined on protein-free filtrates of urine or plasma using lithium buffers in a Beckman 119 CL amino acid analyzer. Organic acids were quantified using gas chromatography of their *O*-trimethylsilyl quinoxalinols (14). Urinary BCKA were determined by gas chromatography of either their methylesters using benzoic acid and [$1\text{-}^{14}\text{C}$] pyruvate as an internal standard and recovery equivalent, respectively (15), or from their silylated oximes (14).

Renal filtration, excretion, and reabsorption of BCAA and

BCKA were calculated according to the formula (16):

1. $F = \text{GFR} \times P$
2. $E = U \times V$
3. $T = F - E$
4. $\%T = 100 \times T/F$

where F = filtered load in $\mu\text{mol}/24\text{ h}$, GFR = creatinine clearance $\text{ml}/24\text{ h}$, P = plasma concentration in $\mu\text{mol}/\text{ml}$, E = urinary excretion in $\mu\text{mol}/24\text{ h}$, U = urinary concentration in $\mu\text{mol}/\text{ml}$, V = urinary rate in $\text{ml}/24\text{ h}$, T = net tubular reabsorption in $\mu\text{mol}/24\text{ h}$, and $\%T$ = percent reabsorption.

Isolation of mononuclear white cells and culture of skin fibroblast. Granulocyte-free, mononuclear, white blood cells were prepared from 10 ml of heparinized blood using LSM solution (Litton Bionetics, Inc., Kensington, MD), a commercially ob-

tained density gradient according to a modification of a previously described method (7, 8). BCKAD activity from the mononuclear cell fraction of each patient was compared with activity in cells from two controls.

Skin biopsies were obtained and cultured from explants in 75 cm² plastic tissue culture flasks using Dulbecco-Vogt medium supplemented with 15% fetal bovine serum and 4 mM glutamine. Cells from control and MSUD patients were matched for culture age. Monolayer cultures were harvested by treatment with 0.27% trypsin for 30 min at 37° C.

Enzyme assays. Decarboxylation of [1-¹⁴C] labeled BCAA by mononuclear cells from peripheral blood and by intact cultured fibroblasts was performed using previously described methods (3). Mitochondrial inner membranes were isolated from fibroblasts (0.75 to 1.5 g wet weight) using Protease VI, homogenization, and differential centrifugation. Mitochondrial pellets were further treated with 0.5 µg digitonin per ng mitochondrial protein and the inner membrane and matrix were isolated (7, 17). Branched-chain [1-¹⁴C] α-ketoacids were prepared as previously described (15). Enzyme activity was monitored by ¹⁴CO₂ released.

Fig. 1. Relationship between plasma leucine and KIC, effect of thiamine. Symbols represent patients in Table 1 (○ = T.Mc.; □ = J.P.; △ = C.M.; ◇ = V.H.). Open symbols were obtained before thiamine and closed symbols were obtained after 3 wk of thiamine. The prethiamine values (open symbols) are seen predominantly in the higher concentrations.

RESULTS

Plasma concentrations of BCAA and BCKA. Two children, J.P. and T.Mc., had significant ($p < 0.05$) decreases in plasma concentration of BCAA and BCKA during thiamine therapy (Tables 2 and 3). During thiamine administration, J.P.'s fasting plasma total BCAA decreased 51.7% and his BCKA decreased 43.2%. T.Mc.'s fasting plasma BCAA concentration decreased 61.2% and her BCKA decreased 73.2%. V.H. also improved. His plasma BCAA decreased 37.8% and his BCKA fell 15.9% during thiamine therapy. However, C.M. showed no improvement. Her BCAA rose 14.8% and her BCKA rose 35.2% during vitamin supplementation.

The relationship between plasma concentrations of leucine and KIC from all patients is illustrated in Figure 1. This relationship remained linear over these moderately elevated plasma values. The slope of their least square ratio is approximately one. Thus for any plasma leucine concentration less than 1.0 mM a corresponding KIC concentration was found. The slopes of the least square ratios for KIV to valine and KMV to isoleucine were 0.25 and 0.7, respectively.

Urinary excretion and renal transport of BCAA and BCKA. The urinary excretion of BCAA and BCKA for 72 h under basal conditions and for 72 h during thiamine therapy are shown in Tables 4 and 5.

J.P. excreted 39.3% less total BCAA and 56.1% less total BCKA during thiamine therapy. T.Mc. excreted 63.3% less BCAA and 91.7% less BCKA after taking thiamine. C.M. increased excretion of total BCAA by 24.4% but had essentially no change in total BCKA excretion during treatment with thiamine. Her increased excretion of BCAA reflected her elevated plasma concentrations of BCAA during her experimental period. Although V.H. excreted 43.3% more BCAA, his excretion of BCKA decreased by 19.8% during thiamine therapy.

The renal tubular transport of BCAA and BCKA was calculated before and during thiamine therapy. The rate of filtration for both amino and ketoacids were generally higher before thiamine and reflected the elevated plasma concentrations. However, the maximum tubular reabsorption capacities were not exceeded and more than 99% of all filtered BCAA and BCKA were reabsorbed during both study periods (Tables 6 and 7).

In vitro BCKAD activity. The ability of freshly isolated mononuclear cells obtained before thiamine therapy to decarboxylate [1-¹⁴C] labeled leucine (20 µM) is shown in Table 8. Compared with control cells, those of J.P., T.Mc., and V.H. showed, respectively, 5.0, 4.9, and 15.7% activity. C.M.'s mononuclear cells had 0.8% of control activity.

BCKAD activity was assayed in whole cell and mitochondrial preparations obtained from cultured skin fibroblasts (Table 8).

Table 4. Effect of thiamine on 24-h urinary excretion of the BCAA

Patient	BCAA (µmol/24 h/g creatinine)*			% change in total excretion of BCAA during thiamine
	Leucine	Isoleucine and alloisoleucine	Valine	
J.P./prethiamine	121.6 ± 6.2†	43.1 ± 5.3†	164.5 ± 29.7†	
J.P./during thiamine	76.2 ± 18.2	22.6 ± 6.3	101.1 ± 7.3	-39.3
T.Mc./prethiamine	232.6 ± 8.9†	39.3 ± 24.0	75.9 ± 50.5	
T.Mc./during thiamine	39.1 ± 11.6	29.8 ± 19.2	58.6 ± 25.6	-63.3
C.M./prethiamine	232.7 ± 18.2†	38.6 ± 2.0†	102.6 ± 2.2	
C.M./during thiamine	311.6 ± 26.6	44.6 ± 0.2	108.8 ± 18.2	+24.4
V.H./prethiamine	124.7 ± 12.3	35.5 ± 11.3†	70.6 ± 22.3	
V.H./during thiamine	149.3 ± 52.8	80.9 ± 13.0	100.6 ± 36.1	+43.3

* Concentration of amino acid is the mean ± 1 SD of three separate 24-h urine collections.

† Urinary concentration of amino acid was significantly less during thiamine therapy with $p < 0.05$ using a one-tailed test t test for independent mean probability.

Table 5. Effect of thiamine on 24-h urinary excretion of the BCKA

Patient/condition	BCKA ($\mu\text{mol}/24 \text{ h/g creatinine}$)*			% change in excretion of total BCKA during thiamine
	KIC	KMV	KIV	
J.P./prethiamine	67.9 $\pm$ 24.7	87.9 $\pm$ 32.2	18.6 $\pm$ 9.9	-56.1
J.P./during thiamine	32.6 $\pm$ 16.8	56.7 $\pm$ 25.3	8.5 $\pm$ 3.0	
T.Mc./prethiamine	210.9 $\pm$ 96.0†	294.4 $\pm$ 156.4†	76.4 $\pm$ 42.6†	-91.7
T.Mc./during thiamine	16.3 $\pm$ 10.6	31.0 $\pm$ 16.1	1.0 $\pm$ 1.0	
C.M./prethiamine	34.1 $\pm$ 6.1	23.2 $\pm$ 1.5	5.7 $\pm$ 0.5†	-1.4
C.M./during thiamine	34.5 $\pm$ 4.	23.9 $\pm$ 5.0	3.7 $\pm$ 0.9	
V.H./prethiamine	33.1 $\pm$ 5.8	150.4 $\pm$ 12.2	30.8 $\pm$ 3.0†	-19.8
V.H./during thiamine	31.1 $\pm$ 0.1	128.3 $\pm$ 9.4	12.4 $\pm$ 1.2	

* Concentration of α -ketoacid is the mean $\pm$ 1 SD of three separate 24-h urine collections.

† Urinary concentration of α -ketoacid was significantly less during thiamine therapy with $p < 0.05$ using a one-tailed t test for independent mean probability.

Table 6. Rates of BCAA filtration (F_{AA}), excretion (E_{AA}), and net tubular reabsorption (T_{AA}) expressed in μmol of amino acid/24 h and % of filtered amino acid reabsorbed (% T_{AA}) before and during thiamine therapy*

Patient	Amino acid	Study period	F_{AA}	E_{AA}	T_{AA}	% T_{AA}
J.P.	Leucine	Prethiamine	5,097.6	9.1	5,088.5	99.8
		During thiamine	2,889.0	5.6	2,883.4	99.8
	Isoleucine	Prethiamine	2,298.0	3.2	2,294.8	99.9
		During thiamine	1,179.2	1.6	1,177.6	99.9
	Valine	Prethiamine	4,454.1	12.6	4,441.5	99.7
		During thiamine	2,888.6	7.5	2,881.1	99.7
T.Mc.	Leucine	Prethiamine	9,558.0	21.2	9,536.8	99.8
		During thiamine	2,478.7	4.2	2,474.5	99.8
	Isoleucine	Prethiamine	3,607.9	3.8	3,604.1	99.9
		During thiamine	1,569.6	2.6	1,567.0	99.8
	Valine	Prethiamine	2,763.4	7.4	2,756.0	99.7
		During thiamine	2,750.6	5.8	2,744.8	99.8
C.M.	Leucine	Prethiamine	8,596.0	35.6	8,560.5	99.6
		During thiamine	12,522.5	52.9	12,469.6	99.6
	Isoleucine	Prethiamine	4,603.1	5.9	4,597.2	99.9
		During thiamine	6,300.8	7.6	6,293.2	99.9
	Valine	Prethiamine	4,795.8	16.0	4,779.8	99.7
		During thiamine	4,051.6	18.4	4,032.2	99.5
V.H.	Leucine	Prethiamine	31,476.6	61.5	31,415.1	99.8
		During thiamine	26,517.2	76.1	26,441.1	99.7
	Isoleucine	Prethiamine	16,027.7	17.5	16,010.2	99.9
		During thiamine	16,611.4	41.5	16,569.9	99.8
	Valine	Prethiamine	34,841.3	34.9	34,806.4	99.9
		During thiamine	26,605.8	51.3	26,554.5	99.8

* Values for filtration and excretion were derived from the mean plasma and urinary concentrations of BCAA from two or three collections during each study period. Calculations are described in "Results."

Except for V.H. there was close agreement between the enzyme activity in freshly prepared mononuclear cells and fibroblasts grown *in vitro*.

DISCUSSION

Since responsivity to thiamine may not correlate with the clinical course, we sought to determine how to predict which patients with MSUD would respond to thiamine therapy. Three of four children in this study responded after 4 wk of thiamine, as demonstrated by improved biochemical status.

Three of these four patients were classified as having classical disease because of their presentation in infancy and recurrent episodes of ketoacidosis (18). Two of these children, J.P. and T.Mc., had a significant decrease in their fasting plasma concen-

trations and their 24 h urinary excretion of total BCAA and BCKA during thiamine treatment. V.H. who was classified as having an intermediate form, *i.e.* developmental delay without bouts of symptomatic ketoacidosis, also had a biochemical response to thiamine. His response might have been blunted as he probably had residual effects of his previous 7 yr of thiamine treatment.

Each patient's response to thiamine correlated with the enzyme activity in their peripheral blood monocytes. The three patients with residual enzyme activity of approximately 5% or more, showed an *in vivo* response to thiamine. C.M. with less than 1% activity in her white cells, did not respond. She subsequently died during an episode of severe ketoacidosis.

The higher enzyme activity in V.H.'s freshly isolated peripheral blood mononuclear cells as compared to that in his skin fibro-

Table 7. Rates of BCKA filtration (F_{KA}), excretion (E_{KA}), and net tubular reabsorption (T_{KA}) expressed in μ moles of α -ketoacid/24 h and % of filtered α -ketoacid reabsorbed (% T_{KA}) before and during thiamine therapy*

Patient	α -Keto acid	Study period	F_{KA}	E_{KA}	T_{KA}	% T_{KA}
J.P.	KIC	Prethiamine	4,177.2	5.3	4,171.9	99.9
		During thiamine	2,894.7	2.3	2,892.2	99.9
	KMV	Prethiamine	3,383.3	6.9	2,376.4	99.7
		During thiamine	1,683.5	3.7	1,679.8	99.8
	KIV	Prethiamine	733.7	1.5	732.2	99.8
		During thiamine	543.9	0.6	543.3	99.9
T.Mc.	KIC	Prethiamine	13,803.7	19.6	13,784.1	99.9
		During thiamine	2,402.2	1.5	2,400.7	99.9
	KMV	Prethiamine	4,628.3	27.8	4,600.5	99.4
		During thiamine	2,678.4	2.9	2,675.5	99.9
	KIV	Prethiamine	1,003.0	7.3	955.7	99.3
		During thiamine	637.2	0.2	637.0	99.9
C.M.	KIC	Prethiamine	7,807.5	5.1	7,802.4	99.9
		During thiamine	12,174.6	5.8	12,168.8	99.9
	KMV	Prethiamine	4,207.0	3.6	4,203.4	99.9
		During thiamine	5,873.9	4.0	5,869.9	99.9
	KIV	Prethiamine	688.7	0.9	687.8	99.9
		During thiamine	980.3	0.6	979.7	99.9
V.H.	KIC	Prethiamine	19,645.7	15.4	19,630.3	99.9
		During thiamine	22,194.5	16.9	22,177.6	99.9
	KMV	Prethiamine	12,062.4	63.6	11,998.8	99.5
		During thiamine	14,100.5	76.9	14,023.6	99.5
	KIV	Prethiamine	3,936.4	15.3	3,921.1	99.6
		During thiamine	4,500.0	6.4	4,493.6	99.9

* Values for filtration and excretion were derived from the mean plasma and urinary concentration of BCKA from two or three collections during each study period. Calculations are described in "Results."

Table 8. Activity of BCKAD as measured in mononuclear peripheral blood cells and cultured skin fibroblasts*

Patient	Enzyme activity expressed as mean % control activity		
	Peripheral blood mononuclear cells†	Cultured Skin Fibroblasts	
		Whole cell‡	Mitochondria§
J.P.	5.0	9.4	7.5
T.Mc.	4.9	2.7	2.6
C.M.	0.8	0.5	0.6
V.H.	15.7	5.5	6.0

* % activity derived from mean of triplicate observations.

† 1-[14 C] leucine 20 μ M as substrate.

‡ 1-[14 C] leucine 20 μ M as substrate. Control activity = 2.56 pmol 14 CO₂ released/min/mg protein.

§ 1-[14 C] KIC 0.1 μ M as substrate. Control activity = 45 pmol 14 CO₂ released/min/mg protein.

blasts may have also reflected his previous thiamine therapy. Unlike the mononuclear cell and whole cell fibroblast assay, the mitochondrial assays required addition of all soluble cofactors and contained saturating amounts of thiamine pyrophosphate. This assay may more accurately define the maximal activity of normal and mutant enzymes, but does not define the functional activity in the intact cell.

While we, as have others (19), found a linear correlation between plasma concentrations of each BCAA and its ketoacid analogue, an unexpected finding was the efficient renal tubular reabsorption of BCAA and BCKA within these physiologic conditions. Reflecting elevated plasma concentrations, the rates of filtration of BCAA and BCKA were higher before thiamine therapy. However, all BCAA and BCKA were maximally reabsorbed in all children during both study periods. Thus the reduced plasma concentrations of BCAA and BCKA in three

responders did not result from increased urinary excretion. These observations differ from Lancaster, et al. (20) who found a higher ratio of BCKA to BCAA in the urine compared to that in the blood, and concluded that the BCKA were preferentially excreted by urinary tubular secretion. There are at least two possible explanations for these different observations. Lancaster *et al.* (20) made their observations in one newborn female. Her serum concentrations of BCKA were five to 10 times greater than in our patients. Additionally, renal transport of amino acids in older children is more efficient than in a newborn infant (21). If both BCAA and BCKA are maximally reabsorbed, an additional treatment of the acute ketoacidosis of MSUD would be to enhance excretion of BCKA.

Several mechanisms could explain thiamine responsivity. We proposed one such model (7, 8, 11, 12). By increasing thiamine intake, intracellular concentrations of thiamine pyrophosphate increase and saturate binding sites on the decarboxylase moiety of the BCKAD complex. The complex undergoes a conformational change, rendering it more resistant to biologic degradation and thus increasing its total activity by prolonging its biologic half-life. Assuming that patients with MSUD have normal mechanisms for absorption of thiamine, and conversion to thiamine pyrophosphate, the differences in clinical responsivity reflect the ability of thiamine pyrophosphate to bind and stabilize the mutant complex.

Chuang *et al.* (22) have proposed an alternate model by finding a thiamine pyrophosphate mediated increase in affinity of BCKAD for the BCKA in cells cultured from a patient originally described by Scriver *et al.* (6). The data from this current study are compatible with either model. If thiamine pyrophosphate saturation produced stabilization or increased affinity of a mutant enzyme complex to BCKA then those mutations with at least a critical amount of residual activity would most likely respond with an increase in the level of enzyme activity. Such a response to excess thiamine would not be expected if there were

a major conformational change in the protein, or if the protein were absent because of a regulatory mutation. Further explanations for vitamin responsivity await *in vitro* confirmation by complementation studies of various cell lines with mutant BCKAD, and better resolution at the molecular level.

With increased newborn screening for MSUD and other metabolic disorders we must define clinical and biochemical criteria for responsivity to dietary and vitamin therapies. Control studies are rarely performed on the newly diagnosed neonate. Therefore, experiences gained from the study of older children must be used in the neonate. Based on our experiences with these and other patients, we believe that after the diagnosis of MSUD has been established, all infants should be started on thiamine (10 to 20 mg/kg/day) and a diet restricted in BCAA. A biochemical response to thiamine may require 3 to 4 wk.

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