Methionine Transamination in Patients With Homocystinuria Due to Cystathionine β-Synthase Deficiency

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To assess the ability of patients with homocystinuria due to cystathionine β-synthase (CBS) deficiency to perform the reactions of the methionine transamination pathway, the concentrations of the products of this pathway were measured in plasma and urine. The results clearly demonstrate that CBS-deficient patients develop elevations of these metabolites once a threshold near 350 μmol/L for the concurrent plasma methionine concentration is exceeded. The absence of elevated methionine transamination products previously reported among 16 CBS-deficient B6-responsive patients may now be attributed to the fact that in those patients the plasma methionine concentrations were below this threshold. The observed elevations of transamination products were similar to those observed among patients with isolated hypermethioninemia. Plasma homocyst(e)ine did not exert a consistent effect on transamination metabolites, and betaine appeared to effect transamination chiefly by its tendency to elevate methionine. Even during betaine administration, the transamination pathway does not appear to be a quantitatively major route for the disposal of methionine.

THE MAJOR METABOLIC ROUTE for the catabolism of methionine in humans involves the sequential formation of *S*-adenosylmethionine, *S*-adenosylhomocysteine, homocysteine, cystathionine, and cysteine.[1] However, extensive evidence has been provided establishing the existence of an alternative, *S*-adenosylmethionine–independent pathway initiated by the transamination of methionine to the corresponding keto-acid, 4-methylthio-2-oxo-butyrate.[2,3] The keto-acid is then oxidatively decarboxylated to form 3-methylthiopropionate,[4] which in turn gives rise to CO_2, methanethiol, hydrogen sulfide, and sulfate.[5] The methanethiol may be further metabolized to form dimethylsulfide and mixed disulfides containing methanethiol (protein-S-S-CH_3 and X-S-S-CH_3, where X is yet unknown).[6]

To provide relative estimates of the importance of methionine degradation by this transamination pathway in humans, the serum concentration of protein-S-S-CH_3 and X-S-S-CH_3, the urinary excretion of 4-methylthio-2-oxo-butyrate, 3-methylthiopropionate, and X-S-S-CH_3, and the concentration of methanethiol and dimethylsulfide in breath were measured.[6] The results indicated that in normal human subjects, the transamination of methionine did occur but was not quantitatively important in methionine catabolism. After administration of an oral dose of L-methionine sufficient to increase the serum methionine concentration rapidly to peak 1 hour later at close to 800 μmol/L, about 30-fold above normal, all of these metabolites increased significantly, but the quantitative importance of methionine transamination to methionine catabolism appeared to remain small.[6]

The indication that methionine transamination might increase as tissue and serum or plasma methionine increases above normal prompted the investigation of methionine transamination metabolites in 2 groups of patients with genetic abnormalities leading to hypermethioninemia. The first group included 26 patients with isolated persistent hypermethioninemia (ie, hypermethioninemia without abnormalities of homocyst(e)ine, without tyrosinemia type I, and without primary hepatocellular disease) were investigated.[6-8] In all such patients in whom the underlying genetic abnormality has been established, inactivating mutations have been demonstrated in *MAT1A*,[9-14] the gene encoding the catalytic subunit that composes the two isozymes of methionine adenosyltransferase (MAT I and MAT III) expressed solely in adult liver.[15] Methionine transamination products in the urine and/or plasma of these patients were consistently abnormally elevated, but only when a threshold at a plasma methionine concentration of 300 to 350 μmol/L was exceeded.[7]

Conversely, in a prior study among a group of 17 patients with hypermethioninemia associated with homocystinuria due to cystathionine β-synthase (CBS) deficiency, only 1 subject had a moderate elevation of serum methionine transamination metabolites. At that time, it was concluded that "methionine degradation via the transamination pathway is very limited in patients with CBS deficiency.[16] However, the subsequent discovery of the threshold for plasma methionine among the patients with isolated hypermethioninemia (as already mentioned) raised the possibility that the general failure of methionine transamination metabolites to achieve supernormal levels in CBS-deficient patients[16] might have been due not to a generalized inability of such patients to use the transamination pathway, but rather to the fact that in most of the patients studied the plasma methionine levels had not exceeded this threshold.

To clarify this possible metabolic conflict regarding the use of the methionine transamination pathway in different groups of patients, we have now studied transamination metabolites in 26 additional CBS-deficient patients, with special emphasis on those with plasma methionine higher than 350 μmol/L.

SUBJECTS AND METHODS

Patients

For all subjects, the diagnosis of homocystinuria due to CBS deficiency was established pretreatment by the presence of homocystin-

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uria, hyperhomocyst(e)inemia, and hypermethioninemia. Efforts were made to include in the study chiefly patients with a more marked elevation of plasma methionine. Many of the patients in question were patients of one or more of the investigators. Clinical information about and samples from additional patients were kindly sent by other physicians.

Methods

Most analyses of plasma amino acids were performed in the clinical laboratories used by one of the researchers (B.W., G.H.J.B., or H.L.L.), but a few were obtained by the contributing additional physicians. Analyses of plasma methionine were performed with an amino acid analyzer, as were analyses of non–protein-bound (free) homocystine and homocysteine-cysteine mixed disulfide (with care taken to deproteinize the plasma samples quickly after collection). Plasma total free homocyst(e)ine (fHcy) was calculated as twice the molar concentration of homocystine plus the concentration of homocysteine-cysteine mixed disulfide. For assays of plasma total homocyst(e)ine (tHcy), nondeproteinized plasma samples were treated with a reducing agent that cleaves all disulfide bonds, and the resulting homocysteine was either measured directly with an amino acid analyzer[17] or derivatized and measured by high-performance liquid chromatography (HPLC) methods as described previously[18,19] or with minor modifications. Assays of metabolites of the methionine transamination pathway were performed at University Hospital of Nijmegen in the laboratory of one of the investigators (A.T.) as described elsewhere.[6] Transamination metabolite values are expressed as the sum of methanethiol released sequentially into the gas phase at pH 7 (protein-S-S-CH_3), pH 10 (X-S-S-CH_3), and pH 12.5 to 13 (chiefly 4-methylthio-2-oxo-butyrate).[7]

A minor limitation of the present study is that, of necessity, the amino acid column chromatographic analyses of plasma methionine were performed in several different laboratories. Quality-control studies reported by Hommes[20] have shown that when plasma amino acid concentrations are determined by column chromatography by a number of different laboratories, the value reported by a single laboratory may, in a minority of instances, differ from the ''consensus mean'' value by more than 20%. This is true for methionine, an amino acid that was included in the studies in question (F.A. Hommes, personal communication, November 1999). Therefore, for the present purposes, it is advisable not to base conclusions and interpretations on small differences in the measured plasma methionine concentrations. A similar reservation applies to measured tHcy concentrations. A recent report states that these may differ by almost 10% due to interlaboratory and intermethod variations.[21] If an allowance is made for additional variation due to the use of different indices of homocyst(e)ine elevation (tHcy or fHcy), again, the conclusions should not be based on differences of 20% or less. For many of the present samples, plasma tHcy was measured in two of the participating laboratories, and agreement was usually within 20% or less. The values from such duplicate assays were averaged for presentation in the Tables.

Statistics

Linear regression analyses and correlation coefficients were calculated by InStat software (GraphPad Software, San Diego, CA).

Table 1. Methionine Transamination Metabolites in CBS-Deficient Patients

Patient No.	Age (yr)/ Sex	B6 Response*	Treatment†					Plasma Methionine (μmol/L)‡	Plasma tHcy (μmol/L)§	Plasma fHcy (μmol/L)‖	Methionine Transamination Metabolites	
			MR	B6	Fol	Bet	Bl2				Plasma (μmol/L)¶	Urine (mmol/mol creatinine)#
1	21/F	Yes	−	+	−	−	−	58	152		0.7	
2	50/M	Partial	+	+	+	+	+	69		10	0.2	0.9
3	36/F	No	+	+	+	+	+	306		37	1.1	3.9
4	49/M	No	+	+	+	+	+	365		38	0.8	
5	34/M	Partial	−	+	+	+	−	413	81		1.2	
6	38/M	Partial	−	+	+	+	−	428	74		1.4	
7	25/F	No	+	+	+	+	+	444		15	1.7	10.3
8	13/F	No	+	+	+	+	+	456		24	11.8	
9	66/F	Yes	−	−	+	+	−	629	41		1.2	
10	8/M	No	+	+	+	+	+	632	95		7.2	23.7
11	17/F	No	+	+	+	+	+	655		43	19.5	
12	46/M	No	+	+	+	+	+	674		13	19.5	107
13	9/F	No	+	+	+	+	+	782		35	14.2	
14	17/M	No	−	−	−	+	−	833	312	93	3.4	6.1
15	29/M	No	+	+	+	+	+	928		13	18.0	128
16	8/F	No	+	+	+	+	−	981		35	19.4	
17	26/M	No	+	+	+	+	+	1,109		49	3.1	7.6
18	5/F	No	+	+	+	+	−	1,142		35	2.1	2.4
19	19/F	No	+	+	+	+	+	1,224		16	6.7	
20	32/M	No	+	+	+	+	+	1,449		35	7.1	29.5
21	1.5/M	No	−	−	−	−	−	1,569	194		7.5	26.8
22	0.7/F	Unknown	−	−	−	−	−	1,891	180		5.2	52.6

*Pyridoxine responsiveness.

†Treatment: MR, methionine restriction ranging from moderate to stringent; B6, pyridoxine; Fol, folic acid; Bet, betaine; B12, vitamin B12; +, used at the time the sample was obtained; −, not used at the time the sample was obtained.

‡Reference range, 13-45 μmol/L.

§Amount of homocysteine obtained by treatment with a reagent that cleaves disulfide bonds: reference range, 8-12 μmol/L.

‖Total of 2 × (free homocystine) plus homocysteine-cysteine mixed disulfide: reference range, 1-3 μmol/L.

¶Reference range, 0.20-0.54 μmol/L.

#Reference range, 1.2-4.6 mmol/mol creatinine (mean ± SD, 2.2 ± 1.0).

RESULTS

Methionine Transamination Metabolites in CBS-Deficient Patients

Table 1 summarizes data for patients with CBS deficiency for whom only single samples of plasma and (where available for analysis) urine were studied. These data include the B6 responsiveness of the patient and the treatment regimen at the times the samples were collected. Of 21 patients described in Table 1, 14 are currently or were previously under the care of one of the researchers (B.W.). For these patients, B6 responsiveness was tested by administering pyridoxine (200 mg/d) for 1 week, and at the same time ascertaining that the patient was not folate-depleted. B6-responsive patients were defined as those who maintained a plasma total fHcy of 20 µmol/L or less on this regimen. Patients who had a clear-cut but lesser biochemical response to B6 were termed partially responsive.[22] The B6 responsiveness of patients under the care of other co-investigators or cooperating physicians was judged by similar but not necessarily identical criteria. Table 1 also includes concurrent plasma values for methionine, tHcy, and/or fHcy and

Table 2. Sequential Determinations of Methionine Transamination Metabolites in Individual CBS-Deficient Patients

Patient No.	Age (yr)/ Sex	B6 Response*	Day†	Treatment‡					Plasma Methionine (µmol/L)§	Plasma tHcy (µmol/L)‖	Plasma fHcy (µmol/L)¶	Methionine Transamination Metabolites	
				MR	B6	Fol	Bet	Bl2				Plasma (µmol/L)	Urine (mmol/mol creatinine)**
23	3.5/M	No	0	?	+	−	−	−	353	210		1.4	6.1
			5	Start Bet 800 mg twice daily									
			12	?	−	−	+	−	1,039	173		6.2	
			15	Bet increased to 800 mg thrice daily									
			23	?	−	−	+	−	1,107				
			37	?	−	−	+	−	918	114		14.2	170
24	6/F	No	0	−	−	−	−	−	906				
			0	Start B6									
			16	−	+	−	−	−	1,089	147		3.0	13.6
			36	−	+	−	−	−	945				
			37	Discontinue B6									
			78	−	−	−	−	−	1,153	155		7.8	73.4
			78	Start B6, Fol, Bet, B12									
			95	−	+	+	+	+	1,309				
			97	Start MR									
			113	+	+	+	+	+	137	75		0.9	
			182	+	+	+	+	+	266	70		1.1	
			266	?	+	+	+	+	764	75		2.0	
			323	?	+	+	+	+	887	69		1.6	
			357	?	+	+	+	+	596	57		1.3	
25	8/F	No	0	?	−	−	−	−	564	339		2.1	8.1
			2	Start Bet 125 mg/kg/d									
			16	Increase Bet to 250 mg/kg/d									
			44	?	−	−	+	−	918	51		18.2	
26	46/F	No	0	−	−	−	−	−	699	471		2.7	
			0	Start Bet 125 mg/kg/d									
			35	−	−	−	+	−	917	188		17.0	
27††	18/M	No	0	−	−	−	−	−	549		204		
			0	Start B6									
			49	−	+	−	−	−	655		173	2.5	
			49	Start Fol									
			98	−	+	+	−	−	974		117	4.1	
			421	Start MR									
			1,029	Start Bet 6 g/d									
			2,115	+	+	+	+	−	467		32	1.0	1.5

*Pyridoxine responsiveness.

†Time (in days) after day 0.

‡Treatment: MR, methionine restriction (question marks indicate substantial doubt about compliance); B6, pyridoxine; Fol, folic acid; Bet, betaine; B12, vitamin B12; +, used at the time the sample was obtained; −, not used at the time the sample was obtained.

§Reference range, 13-45 µmol/L.

‖Amount of homocysteine obtained by treatment with a reagent that cleaves disulfide bonds: reference range, 8-12 µmol/L.

¶Total of 2 × (free homocystine) plus homocysteine-cysteine mixed disulfide: reference range, 1-3 µmol/L.

#Reference range, 0.20-0.54 µmol/L.

**Reference range, 1.2-4.6 mmol/mol creatinine (mean ± SD, 2.2 ± 1.0).

††Values for patient no. 27 have been previously published. They are shown here so that the appropriate previously unpublished treatment intervals can be indicated.

methionine transamination metabolites, as well as urinary values for transamination metabolites. The patients are listed in ascending order according to plasma methionine concentration. Because patients were selected chiefly on the basis of the expectation that they would have a marked elevation of plasma methionine, all but two of the samples described in Table 1 had a plasma methionine level of 300 μmol/L or higher. All patients with plasma methionine higher than 300 μmol/L had plasma transamination metabolites above 0.54 μmol/L, the upper limit of the reference range. Likewise, with 2 exceptions (patients no. 3 and 18) for the latter patients, the urinary transamination metabolites were also above the upper limit of the reference range of 4.6 mmol/mol creatinine. In some cases, these elevations were marked (for example, patients no. 12, 15, and 22).

Table 2 summarizes the data for patients in whom it was possible to obtain sequential samples during changes in the therapy used to treat CBS deficiency. Patients no. 24 and 27 were clearly nonresponsive to B6. Decreases in plasma methionine, tHcy, and transamination metabolites occurred following the start of methionine restriction in a non–B6-responsive individual (patient no. 24). Betaine administration to B6 nonresponders was followed in all instances by a decrease in plasma tHcy, a further elevation of plasma methionine, and an increase in transamination products (patients no. 23, 25, and 26). In 1 case (patient no. 27), combined methionine restriction and betaine treatment led to a decrease in plasma fHcy, with decreases in both plasma methionine and transamination products.

Transamination Metabolites as a Function of Plasma Methionine in CBS-Deficient Patients Versus Patients With Isolated Hypermethioninemia

When data for the logarithms of plasma or urinary transamination products derived from the Tables are plotted against the logarithms of the concurrent plasma methionine values (Figs 1 and 2), it is apparent that the transamination products tend to become abnormally elevated in approximate proportion to the elevation of plasma methionine, but the increase of transamination products above the reference range occurred only when a threshold at approximately 350 μmol/L plasma methionine was exceeded. To permit a comparison to patients with isolated persistent hypermethioninemia,[7] the areas that would include values for these patients are indicated on each plot by the outlined enclosed area. In general, the points for CBS-deficient patients are within or close to these areas determined by the values for patients with isolated hypermethioninemia.

As an additional way to compare the transamination response of CBS-deficient patients versus patients with isolated hypermethioninemia, the logarithmic values for each set of samples for which concurrent plasma and urinary values were available were plotted against one another for both groups of patients (Fig 3). Linear regression analyses indicated that plasma and urinary transamination products were highly correlated with one another. The regression lines in Fig 3 were calculated with the points for which both plasma and urinary concentrations were above the upper limit of the respective reference range, to remove from consideration the values within the reference range in which experimental errors are proportionally more severe. For CBS-deficient patients, the ''best-fit'' slope

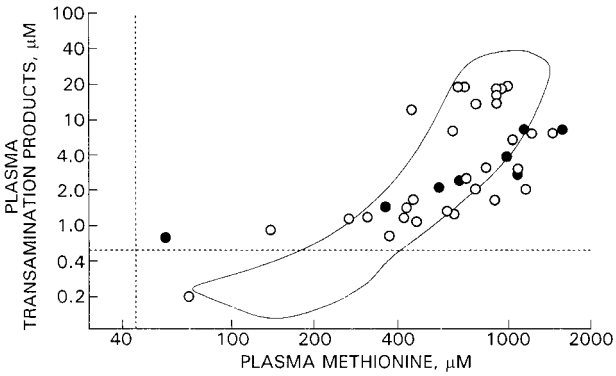

Fig 1. Logarithmic plot of methionine transamination products in the plasma of patients with CBS deficiency as a function of plasma methionine. (●) Patients not on betaine treatment, (○) patients on betaine. The horizontal dashed line indicates the upper level of the reference range for plasma methionine transamination products, and the vertical dashed line, the upper level of the reference range for plasma methionine. No point was plotted for patient no. 22 (Table 1) because the only sample analyzed was obtained at age 0.7 years. Previous experience has shown that there is an age-dependent maturation process affecting methionine transamination such that below an age of approximately 0.9 years, the elevation of transamination metabolites at a given concentration of plasma methionine is far less than would occur with the same plasma methionines at later ages.[7] The enclosed area would include the values previously reported for patients with isolated persistent hypermethioninemia.[7]

(mean ± SE) was 1.24 ± 0.17, with a 95% confidence interval from 0.86 to 1.62 (Spearman $r = .88$, $P < .0001$). For patients with isolated hypermethioninemia, the best-fit slope was 1.50 ± 0.18, with a 95% confidence interval from 1.10 to 1.90 (Spearman $r = .88$, $P < .0001$). As judged by a t test, these slopes were not significantly different. Similarly, the 95% confidence intervals for best-fit y-intercepts overlapped extensively and the 2 intercepts were not significantly different (data not shown).

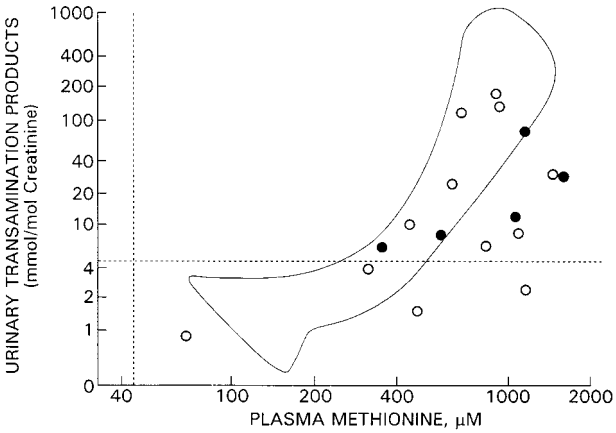

Fig 2. Logarithmic plot of methionine transamination products in the urine of patients with CBS deficiency as a function of plasma methionine. Symbols and other indicators are as in Fig 1, except that the horizontal dashed line indicates the upper level of the reference range for urinary methionine transamination products.

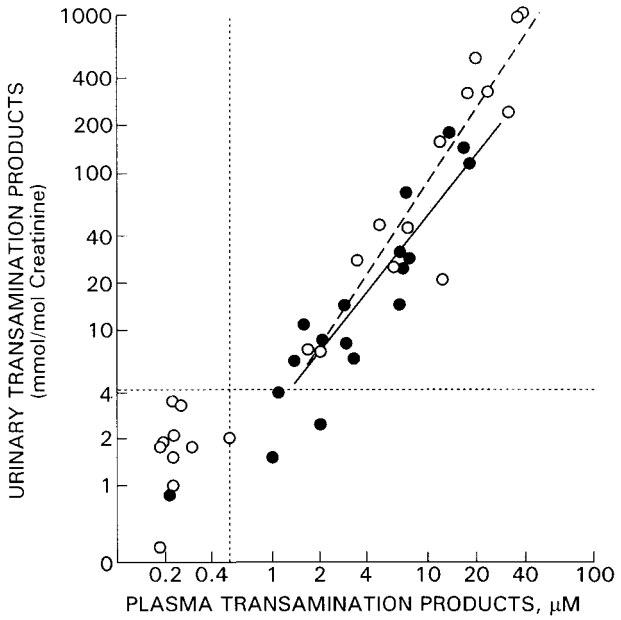

Fig 3. Plot of logarithms of concurrent values for plasma and urinary methionine transamination products in patients with either CBS deficiency (●) or isolated hypermethioninemia (data from Mudd et al,[2] ○). The vertical dashed line indicates the upper level of the reference range for plasma methionine transamination products, and the horizontal dashed line, the upper level of the reference range for urinary methionine transamination products. The best-fit linear regression lines (calculated using points that are above both of the upper limits of the reference ranges) are indicated by the continuous line for CBS-deficient patients and the dashed line for patients with isolated hypermethioninemia.

Factors Other Than Plasma Methionine That Might Affect Transamination Metabolites

Betaine. There appears to be no clear difference in Figs 1 and 2 between the sample sets obtained either while the patient was not receiving betaine therapy or while the patient was on betaine therapy. Such a difference might have been observed if betaine exerted an effect on methionine transamination other than through its capacity to increase plasma and (presumably) tissue methionine.

Homocyst(e)ine. To evaluate the possibility that the plasma homocyst(e)ine concentration exerts an effect on transamination, plasma concentrations of methionine transamination metabolites were compared within groups selected such that the concurrent plasma methionine concentrations were similar, but the plasma concentrations of homocyst(e)ine were markedly dissimilar (Table 3). As homocyst(e)ine increased (and methionine remained relatively constant), plasma transamination metabolites either changed little (patient groups 3, 4, and 23, 24 and 25, and 25, 23, and 26), increased 2-fold or more (groups 9 and 10 and 24 and 14), or decreased severalfold (groups 11 and 27, 12 and 26, and 16 and 27). Clearly, changes in homocyst(e)ine were not accompanied by any consistent effect on transamination metabolites.

DISCUSSION

The data reported herein establish the following: (1) CBS-deficient patients may manifest abnormal elevations of methionine transamination metabolites in both plasma and urine, at least when plasma methionine exceeds an apparent threshhold near 300 to 350 μmol/L (Tables 1 and 2 and Figs 1 to 3). Above this threshold, transamination products tend to increase as plasma methionine increases, but the relationship is not always straightforward, especially for methionine concentrations above 900 to 1,000 μmol/L. For example, patient no. 23 on day 12 had a plasma methionine of 1,039 μmol/L and plasma transamination metabolites of 6.2 μmol/L. On day 37, plasma methionine was slightly lower at 918 μmol/L, but plasma transamination metabolites were 14.2 μmol/L. A second possibly anomalous pair of values are those for patient no. 24 on days 16 and 78, when a minor change of uncertain significance in plasma methionine was accompanied by a larger percentage increase in plasma and urinary transamination products. The observations for patient no. 23 may indicate a slow increment in the transamination capacity as a given high methionine level is maintained, but no explanation for the observations in patient no. 24 is apparent, unless it is an unexpected effect of the discontinuation of B6 administration; and (2) The elevation of transamination metabolites at a given elevation of plasma methionine is generally within or close to the range of the elevation previously found in patients with isolated hypermethioninemia, at least for methionine concentrations less than 900 to 1,000 μmol/L. At methionine concentrations above 900 to 1,000 μmol/L, transamination metabolites often appeared to be less elevated than might have been expected (Figs 1 and 2). The patients in whom this occurred were usually those judged, on

Table 3. Lack of a Consistent Effect of tHcy or fHcy on Plasma Methionine Transamination Metabolites When Plasma Methionine Is Relatively Constant

Patient No.	Plasma Concentration (μmol/L)			
	Methionine	tHcy	fHcy	Methionine Transamination Metabolites
3	306	37		1.1
4	365	38		0.8
23	353	210	88	1.4
24	596	57		1.3
25	564	339		2.1
9	629	41		1.2
10	632	95		7.2
11	655		43	19.5
27	655		173	2.5
12	674		13	19.5
26	699	471		2.7
24	887	69		1.6
14	833	312	93	3.4
25	918	51		18.2
23	918	114		14.2
26	917	188		17.0
16	981		35	19.4
27	974		117	4.1

the basis of the time of onset and the variety and extent of clinical manifestations, to be among the most severely affected subjects included in this study. Although this finding may be a hint that a robust capacity for methionine transamination can sometimes protect against the adverse clinical consequences of CBS deficiency, convincing proof of such a conclusion would require more extensive data.

The availability of data on a number of different patients receiving different treatments for CBS deficiency (Table 1), and especially the serial measurements on individual patients as their therapies were altered (Table 2), permit some insight into the variables that may affect the elevations in transamination metabolites. As noted before, the plasma methionine concentration, presumably reflecting the intracellular methionine concentration, is clearly a major determinant. However, even large differences in the extent of accumulation of homocyst(e)ine, well outside of any uncertainties in the relevant assays, do not seem to play a consistent role (Table 3). Nor do the data suggest a role for betaine other than the tendency of this compound to increase methionine concentrations.

The fact that abnormal elevations of transamination metabolites were demonstrated in most CBS-deficient patients in the present study but in only 1 of 17 such patients reported previously[16] can now be confidently attributed to the fact that in the previous patients the elevations of plasma methionine did not exceed the threshold above which transamination metabolites begin to increase abnormally.[7] Of the previously studied CBS-deficient patients, 16 were responsive to pyridoxine (B6). In the fasting state and while not receiving pyridoxine, their mean serum methionine concentration was 107 μmol/L, about 3.7-fold above the mean reference value but far below the threshold at 300 to 350 μmol/L plasma methionine. The single non–B6-responsive patient had a serum methionine level 15- to 31-fold above the mean control value, and was exceptional in that his transamination metabolites were significantly elevated.[16]

The conclusion that in humans methionine transamination is of minor importance below the plasma threshold at 350 μmol/L contrasts with findings in rats by Benevenga,[23] who suggested that transamination is important at normal methionine concentrations. However, from a consideration of the effects of excess methionine in rats, Finkelstein and Martin[24] later suggested that ''transamination may be relevant only at extremely high concentrations of methionine.''

Perry described an infant with hereditary tyrosinemia which, as often happens in tyrosinemia type I,[25] was accompanied by an elevation of plasma methionine, in this case to 1,200 μmol/L. An extreme elevation of 4-methylthio-2-oxo-butyrate to 91 mmol/mol creatinine was detected in the urine of this infant.[26] Thus, an abnormal accumulation of plasma methionine in 3 independent genetic diseases may be accompanied by abnormal elevations of the products of methionine transamination.

A question of some clinical importance is whether an intact capacity of the reactions of the methionine transamination pathway is beneficial to patients with CBS deficiency. On the one hand, on the basis of animal experiments, methionine has been considered the most highly toxic of the dietary amino acids,[27] and the possibility has been considered that metabolites produced by the transamination pathway such as methanethiol and hydrogen sulfide might contribute to this toxicity.[28,29] On the other hand, the transamination pathway offers a means in CBS-deficient patients to bypass the defect in transsulfuration and to decrease not only methionine but also homocyst(e)ine, especially if the conversion of the latter to methionine is enhanced, for example, by the use of betaine.[27,30] In recent years, concern about the toxicity of methionine transamination metabolites to humans has been somewhat mitigated by the observation that patients with isolated hypermethioninemia, some of whom have high concentrations of these metabolites (Figs 1 and 2 and Mudd et al[2]), are often clinically unaffected, and in those who do have clinical problems, these do not resemble the abnormalities prominent in CBS deficiency.[1,7] Therefore, it seems unlikely that a shared tendency for elevated methionine transamination metabolites is responsible for the clinical manifestations in either group. Nevertheless, as mentioned earlier, it seems possible that a robust capacity for methionine transamination, under some circumstances, may be clinically beneficial in CBS deficiency.

The ability of betaine to decrease plasma homocyst(e)ine in CBS-deficient patients has been convincingly established.[27,31,32] Likewise, the efficaciousness of betaine in the prevention of thromboembolic episodes in CBS-deficient patients, especially those who are not responsive to B6 or for whom dietary restriction of methionine is not satisfactory, has received substantial experimental support.[22] A question that remains is the extent to which this clinical benefit is merely due to decreased homocyst(e)ine, on the one hand, or, on the other, depends in some manner on the increased metabolism of methionine via transamination that follows the increases in plasma methionine which also occur—as exemplified in the data reported here (Table 2). A first approximation of the contribution of transamination to overall methionine catabolism in non–B6-responsive patients treated with betaine can be made using the urinary excretion of methionine transamination metabolites (Tables 1 and 2). For example, among the patients for whom an estimate of the dietary methionine intake was available, the highest concentrations of urinary transamination metabolites were observed in patients no. 12 and 15, both adult males, 0.107 and 0.128 mmol/mmol creatinine (Table 1). Using the normative values of Clark et al[33] for 17-year-old males, their daily creatinine excretion may be considered to be 0.233 mmol/kg/d (a maximal estimate, since creatinine excretion decreases slightly with age[34]). The urinary transamination metabolites were then (0.107 to 0.128) $\times$ 0.233 = 0.024 to 0.030 mmol/kg/d. The dietary protein intake of these subjects was at least 1.5 g/kg/d. A conservative estimate is that methionine contributes 2% of the protein weight. Therefore, methionine intake would have been 1,500 mg/kg/d $\times$ 0.02 = 30 mg/kg/d = 0.201 mmol/kg/d. Transamination would then account for [(0.024 to 0.030)/0.201] $\times$ 100% = 12% to 15% of the total daily methionine intake and catabolism at steady state. These values probably modestly underestimate the contribution of methionine transamination, because some products of this pathway are not taken into consideration.[8] Nevertheless, even in the individuals in question, with among the highest concentrations of urinary methionine transamination products, it appears reasonable that such transamination may account, at most, for a

significant but not preponderant portion of overall methionine catabolism. The clinical benefit of betaine is then likely to reside predominately in its ability to decrease homocyst(e)ine.

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