Elevated Plasma Total Homocysteine in Severe Methionine Adenosyltransferase I/III Deficiency

Sally P. Stabler, Clemens Steegborn, Markus C. Wahl, Jana Oliveriusova, Jan P. Kraus, Robert H. Allen, Conrad Wagner, and S. Harvey Mudd

Abnormal elevation of plasma methionine may result from several different genetic abnormalities, including deficiency of cystathionine β-synthase (CBS) or of the isoenzymes of methionine adenosyltransferase (MAT) I and III expressed solely in nonfetal liver (MAT I/III deficiency). Classically, these conditions have been distinguished most readily by the presence or absence, respectively, of elevated plasma free homocystine, detected by amino acid chromatography in the former condition, but absent in the latter. During the present work, we have assayed methionine, *S*-adenosylmethionine, *S*-adenosylhomocysteine, total homocysteine (tHcy), cystathionine, *N*-methylglycine (sarcosine), and total cysteine (tCys) in groups of both MAT I/III- and CBS-deficient patients to provide more evidence as to their metabolite patterns. Unexpectedly, we found that MAT I/III-deficient patients with the most markedly elevated levels of plasma methionine also had elevations of plasma tHcy and often mildly elevated plasma cystathionine. Evidence is presented that methionine does not inhibit cystathionine β-synthase, but does inhibit cystathionine gamma-lyase. Mechanisms that may possibly underlie the elevations of plasma tHcy and cystathionine are discussed. The combination of elevated methionine plus elevated tHcy may lead to the mistaken conclusion that an MAT I/III-deficient patient is instead CBS-deficient. Less than optimal management is then a real possibility. Measurements of plasma cystathionine, *S*-adenosylmethionine, and sarcosine should permit ready distinction between the 2 conditions in question, as well as be useful in several other situations involving abnormalities of methionine and/or homocysteine derivatives.

ABNORMAL ELEVATION of plasma methionine is well known to result from each of several different genetic defects: homocystinuria due to deficient activity of cystathionine β-synthase (CBS), hepatorenal tyrosinemia (tyrosinemia I) due to deficient activity of fumaryl-acetoacetate hydrolase, and deficient activity of methionine adenosyltransferase (MAT) I/III, the isozymes of methionine adenosyltransferase expressed solely in nonfetal liver. (Figure 1 diagrams the metabolic relationships between methionine, homocysteine, cystathionine, and related metabolites relevant to this report). Hypermethioninemia also occurs in severe liver disease and, in a transient form, in infants fed high-protein diets.[1] The term "persistent isolated hypermethioninemia" has been used to describe hypermethioninemia (elevations 2-fold to 50-fold above normal) that last beyond the first months of life and are not accompanied by abnormal plasma elevations of homocystine detected by amino acid chromatography or tyrosine or by severe liver disease. In almost all such cases in which an underlying genetic abnormality has been identified, inactivating mutations in *MAT1A*, the gene that encodes the catalytically active subunit that composes (tetrameric) MAT I and (dimeric) MAT III, have been implicated,[2] although rare apparent[3,4] or established[5] exceptions are known.

During the present work, we have measured in patients with established MAT I/III deficiencies, an extensive panel of methionine metabolites, including, for the first time in such patients assays of both plasma total homocysteine (tHcy) (a more sensitive indicator of hyperhomocysteinemia than homocystine), and of cystathionine. (In the "Summary" of a previous publication, it was erroneously stated that patients with MAT I/III deficiency do not have elevated homocysteine.[2] More correctly, it should have been stated that such patients do not have elevated plasma homocystine detected by amino acid chromatography with the usual sensitivity.) Because such patients are limited in their ability to synthesize *S*-adenosylmethionine (AdoMet), and, therefore, its metabolic products, *S*-adenosylhomocysteine (AdoHcy) and homocysteine, it was surprising to find that among this group of patients, those with the highest plasma methionine elevations (due to the most severely inactivating lesions of *MAT1A)* also have mild to moderate abnormal elevations of plasma tHcy. This situation constitutes a diagnostic trap because the combination of elevated methionine and elevated tHcy might well be misdiagnosed as being due to CBS deficiency and thereby lead to long-term treatment for this condition by use of methionine-restricted diets or other interventions. Here, we report our recent results to alert clinicians to this trap, and we discuss the utility of assays of plasma AdoMet, cystathionine, and sarcosine (*N*-methylglycine) to rule out CBS deficiency and to establish a more nearly correct diagnosis.

MATERIALS AND METHODS

Plasma samples were obtained from 13 MAT I/III-deficient patients. Clinical and metabolic details about all patients except 32 and 33 have been published previously.[2,6-14] These details, as well as specification

From the Departments of Medicine, Pediatrics, and Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, CO; Abteilung Strukturforschung, Max Planck Institut für Biochemie, Planegg-Martinsried, Germany; Department of Biochemistry and the Clinical Nutrition Research Unit, Vanderbilt University, Nashville; Department of Veterans Affairs Medical Center, Nashville, TN; and the Laboratory of Molecular Biology, National Institute of Mental Health, Bethesda, MD.

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Address reprint requests to Sally P. Stabler, MD, Department of Medicine, University of Colorado Health Sciences Center, Campus Box B-170, 4200 E. 9th Ave, Denver, CO 80262.

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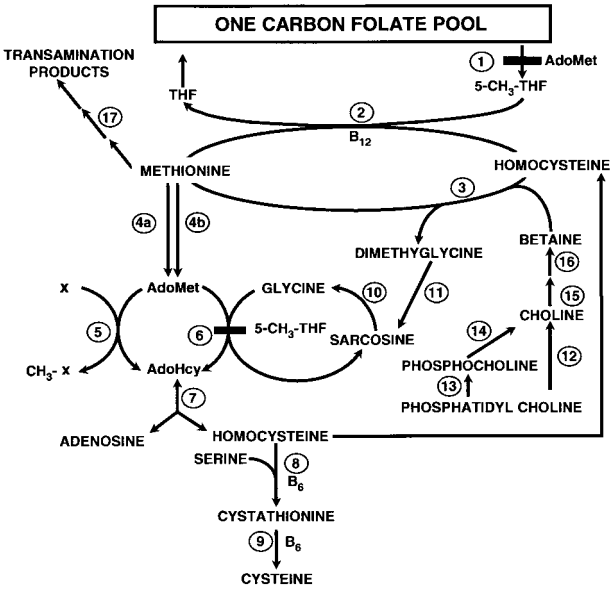

Fig 1. Metabolism of methionine, *S*-adenosylmethionine (AdoMet), homocysteine, cystathionine and related compounds. The circled numbers refer to the following enzymes: 1, methylenetetrahydrofolate reductase (MTHFR); 2, methionine synthase (E.C. 2.1.1.13); 3, betaine homocysteine methyltransferase (E.C. 2.1.1.5); 4a, methionine adenosyltransferase I/III (MAT I/III); expressed in adult liver; 4b, methionine adenosyltransferase II (MAT II); expressed in nonhepatic tissues, fetal liver, and (slightly) in adult liver; 5, a variety of AdoMet-dependent methyltransferases; 6, glycine *N*-methyltransferase (GNMT); 7, *S*-adenosylhomocysteine (AdoHcy) hydrolase (E.C. 3.3.1.1); 8, cystathionine β-synthase (CBS); this reaction is allosterically stimulated by AdoMet; 9, cystathionine gamma-lyase (E.C. 4.4.1.1); 10, sarcosine (*N*-methylglycine) dehydrogenase (E.C. 1.5.99.1); 11, *N,N*-dimethylglycine dehydrogenase (E.C. 1.5.99.2); 12, phospholipase D (E.C. 3.1.4.4); 13, phospholipase C (E.C. 3.1.4.3); 14, alkaline phosphatase (E.C. 3.1.3.1); 15, choline dehydrogenase (E.C. 1.1.99.1); 16, betaine aldehyde dehydrogenase (1.2.1.8); 17, methionine transamination pathway. Inhibitions of reaction 1 by AdoMet and reaction 6 by 5-methyltetrahydrofolate (5-CH_3-THF; chiefly in the pentaglutamyl form) are indicated by heavy dark horizontal bars.

of the *MAT1A* mutations can be cross-referenced by use of the identifiers shown in the tables. Patients 32 and 33 have been described by Kim et al (Kim SZ, personal communication). Plasma or serum tHcy, methylmalonic acid, sarcosine, cystathionine, total cysteine (tCys), serine, and glycine were quantitated as previously described using capillary gas chromatography-mass spectrometry.[15-19] These abovementioned metabolites have been shown to be stable in stored samples.[15-19] Reference ranges had been determined previously for normal blood donors. Previously reported coefficients of variation for tHcy were 2% to 5%,[19] cystathionine 3.5% to 6.8%,[16] and sarcosine 5%.[17] Because the undeproteinized sample is initially treated with a reducing agent that cleaves all disulfide bonds, and therefore releasing the amino acids from disulfides, tHcy and tCys are measured in this assay.[15-20] Methionine was measured by column chromatography. AdoMet and AdoHcy were assayed by high-performance liquid chromatography (HPLC).[21] Because AdoMet, AdoHcy, and methionine may deteriorate during prolonged frozen storage, relatively recent plasma samples were studied. A second group of subjects, those with well-documented CBS deficiency also had the same metabolites measured, and the results were compared with those obtained for the patients with MAT I/III deficiency. Recombinant human CBS was expressed in *Escherichia coli* XL1-Blue MR (Stratagene, La Jolla, CA).[22] Forms of high purity, both 63 kd (full length) and 45 kda (truncated with the C-terminal 138 amino acid residues deleted), were assayed,[22] using 5 mmol/L homocysteine as substrate in the presence of varying concentrations of L-methionine (Sigma Chemical, St Louis, MO) from 0 up to 150 mmol/L. Stimulation by AdoMet was measured by addition of 1 mmol/L AdoMet. Recombinant human cystathionine gamma-lyase (hCGL) was expressed in *E coli* strain BL21(DE3) under control of a T7-promoter and purified in soluble form as described previously.[23] The recombinant enzyme behaves similarly to native preparations from various sources (see Steegborn et al[23] and references therein). Time courses for hCGL catalysis were monitored at 30°C using a colorimetric assay for formation of free sulfhydryl groups. The 0.5-mL reaction mixtures contained 39.3 mmol/L borate buffer, pH 8.2, 1 μmol/L 5,5'-dithio-bis(2-nitrobenzoic acid), 0.01% ethanol, and varying concentrations of L-cystathionine and L-methionine. Pyridoxal phosphate was not added because the enzyme is purified in holoenzyme form and dissociation of the cofactor is so slow as to be irrelevant at the time scale of the reaction as studied. After addition of 15 μg of hCGL and rapid mixing, development of 412 nm absorption was followed for 2 minutes with a Beckman (München, Germany) DU 7500 diode array spectrometer. The kinetic data were analyzed with the program KaleidaGraph (Albeck Software, University of California, San Francisco, CA) in reciprocal form according to Lineweaver and Burk.[24]

RESULTS

Methionine Metabolites in MAT I/III-Deficient Patients

Table 1 reports plasma metabolite concentrations for 11 patients with proven MAT I/III defects and for 2 additional patients, 32 and 33, with presumptive MAT I/III deficiency (Kim SZ, personal communication, May 2001). The patients with proven defects are arranged in descending order according to plasma methionine levels. The 6 patients with the highest plasma methionine values each had mild to moderate elevations of plasma tHcy. Five of these patients also had plasma cystathionine concentrations that were slightly elevated (patients 7 and 9) or very near the top of the reference range (patients Mr C, patient C, and patient 3). In plasma samples with methionine concentrations comparable to the highest 2 or 3 listed in Table 1, patients 32 and 33 likewise had elevated tHcy and cystathionine concentrations. Despite the hypermethioninemia, plasma AdoMet was normal to low in every subject.[14] AdoMet was not totally absent, even in those patients with no residual activity of MAT I/III, presumably because of the presence of MAT II, an isoform of MAT, the catalytic unit of which is encoded by MAT2A, a gene distinct from MAT1A (the gene mutated in MAT I/III-deficient patients). MAT II is known to be functional in fibroblasts, red blood cells, and lymphoid cells of such patients.[4] In accord with the lack of elevation of AdoMet, sarcosine levels were not elevated. tCys was normal in almost all of the subjects. Serine, glycine, and methylmalonate were generally within the normal ranges (data not shown). One subject, Mr C, had a mild elevation of methylmalonate (301 nmol/L) on 1 occasion, but 4 years later, it had fallen to normal.

Methionine Metabolites in CBS-Deficient Patients

For comparison, also shown in Table 1 are mean and range values for plasma concentrations of the same metabolites in patients with CBS deficiency either on methionine-restricted or normal diets. To avoid confounding the sarcosine data with the

Table 1. Clinical Variables and Plasma Metabolite Concentrations for Subjects With MAT I/III or CBS Deficiency

Patient No.*	Age/Sex (yr/M/F)	Methionine (μmol/L)	tHcy (μmol/L)	Cystathionine (nmol/L)	Sarcosine† (μmol/L)	tCys (μmol/L)	AdoMet (nmol/L)	AdoHcy (nmol/L)	Mutation‡
7[2,9]	6.3/M	1,394	21.9	401	1.7	206	94	ND	spl/spl
9[9,11]	6.0/M	1,265	19.3	591	1.8	250	87	15	tr/pt
5[2,9]	12/F	1,199	15.1	230	1.5	219	65	ND	pt/pt
Mr C[6,7,13]	43/M	968	26.1	325	1.1	277	81	12	tr/tr
Patient C[8,11]	19/F	795	23.5	340	0.8	297	71	ND	tr/tr
3[9,11]	6.5/F	618	24.9	332	1.5	282	48	ND	tr/tr
10[9,11]	11/M	439	7.7	240	1.1	218	120	11	pt/pt
2[9,10]	24/F	349	5.0	154	0.4	156	85	ND	pt/pt
11[9,11]	15/F	307	8.6	268	0.8	257	85	14	pt/pt
14[2,9]	6.4/F	206	7.9	378	1.8	313	89	ND	pt/pt
C 11-5[12]	38/F	64	10.1	140	1.0	385	112	ND	wt/pt
33	2.8/F	1,243	17.4	401	2.5	179	62	20	ND
32	0.2/F	1,089	17.0	475	1.3	187	99	36	ND
Mean	17	764	15.7	329	1.3	248	85	18	
Reference range§		13-45	5.4-13.9	50-342	0.6-2.7	200-361	92.8 ± 16.2	15-45	
CBS-deficient patients on methionine-restricted diets (n = 6)									
Mean	8.4	56	102	11	3.4	175	368	126	
Range	0.17-20	15-160	5.4-312	0-66	1.6-5.7	61-245	111-1,130	20-519	
CBS-deficient patients on normal diets (n = 7)									
Mean	11.5	920	245	11	8.8	76	1,380	636	
Range	0.01-46	353-1,891	155-471	0-79	3.6-15.1	40-140	888-2,030	147-1,700	

NOTE. Serum or plasma samples, usually drawn in the nonfasting state, were assayed. Many of the methionine and AdoMet values have been reported previously.[14]

Abbreviation: ND, not determined.

*The number listed has been used to identify the patient in previous reports. References are listed as superscripts.

†Sarcosine is an alternative term for *N*-methylglycine.

‡spl, splicing; tr, truncating; pt, point mutation.

§Normal range ± SD determined among 60 blood donors age 18 to 65 years.

high values of this compound found in patients receiving betaine (a metabolic precursor of sarcosine), no samples were included from subjects receiving betaine supplements when the plasmas were obtained. Patients with CBS deficiency had: (1) generally higher tHcy concentrations, with values overlapping the range for those among the MAT I/III-deficient group only in those on methionine restriction; (2) elevated AdoMet values with only a single value (again from the methionine-restricted group) overlapping the range for the MAT I/III deficient group; (3) usually undetectable (11/13), occasionally low, cystathionine concentrations; (4) higher AdoHcy concentrations; (5) a tendency to have elevated sarcosine concentrations; and (6) frequently low tCys values, as contrasted to normal levels in MAT I/III-deficient subjects. There was no difference in the methionine concentrations between the whole group of MAT I/III deficients as compared with the whole group of CBS-deficient subjects (691 ± 461 *v* 521 ± 572 μmol/L, $P = .44$). As expected, tHcy was lower (15.5 ± 7.9 *v* 179.2 ± 133.1 μmol/L, $P = .001$), AdoMet was lower (85 ± 20 *v* 912 ± 673 nmol/L, $P = .001$), cystathionine was higher (309 ± 126 *v* 11 ± 27 nmol/L, $P < .001$), and sarcosine was lower (1.2 ± 0.5 *v* 6.3 ± 4.0 μmol/L, $P = .001$). The mean age for the MAT I/III deficients was 17.0 ± 13.0 as compared with the CBS-deficient group (10.1 ± 12.4 years), which was not different ($P = .20$).

To provide statistical comparisons between the 2 groups most likely to be diagnostically confused, the means for the first 6 MAT I/III-deficient patients (ie, those with proven *MAT1A* mutations and elevated tHcy; Table 1) and for the CBS-deficient patients on methionine restriction were calculated. Mean cystathionine was strikingly lower in the CBS-deficients than in the MAT I/III-deficient subjects (11 ± 27 *v* 370 ± 121 nmol/L, $P < .0001$). Cystathionine was detected in only 1 of the former group. Methionine also was significantly lower in the methionine-restricted CBS deficients (56 ± 53 *v* 1,040 ± 298 μmol/L, $P < .001$). Both tHcy (102 ± 114 *v* 21.8 ± 4.0 μmol/L, $P = .14$) and AdoMet (368 ± 381 *v* 74 ± 16 nmol/L, $P = .12$) were higher in the CBS-deficient group and, although the differences between the means were not significant at the .05 level, there was little overlap in the ranges. tCys was significantly lower in the CBS deficients (175 ± 70 *v* 255 ± 37 μmol/L, $P < .05$). Sarcosine was higher in the CBS deficients (3.4 ± 1.6 μmol/L *v* 1.4 ± 0.4, $P = .026$).

Metabolites in a Subject With Congenital B6-Nonresponsive Cystathioninuria

CBS from rat liver has been shown to be reversible under certain conditions, leading to the production of homocysteine and serine.[25] To ascertain the extent to which abnormal accumulation of cystathionine might lead to elevation of plasma tHcy or to changes in other relevant metabolites, assays were performed on a plasma sample from a 29-year-old, clinically normal patient with B6-nonresponsive congenital cystathioninuria.[26] Cystathionine was markedly elevated to 12,000 nmol/L, 35-fold above the upper limit of the reference range; methionine was normal at 22 μmol/L; tHcy was only slightly elevated to 18.2 μmol/L; and tCys was normal at 246 μmol/L.

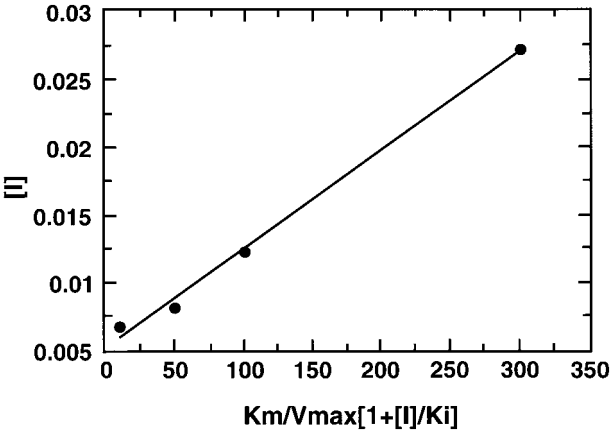

Fig 2. Determination of the inhibition constant K_i for competitive inhibition of hCGL by L-methionine. The slopes from Lineweaver-Burk plots ($[K_m/V_{max}] \cdot [1 + [I]/K_i]$) at different inhibitor concentrations were plotted against the inhibitor concentration [I]. K_m/V_{max} was determined from the intersection on the y-axis and subsequently used for the determination of K_i from the slope $K_m/[V_{max} \cdot K_i]$.

Studies of Possible Methionine Inhibition of CBS and hCGL

The CBS activity of neither the full-length recombinant human enzyme nor the truncated form lacking the C-terminal 138 amino acids was inhibited by methionine in concentrations up to 150 mmol/L, some 30 times the 5 mmol/L homocysteine used in the assays. Nor was the extent of stimulation of the full-length enzyme by AdoMet affected by this range of methionine concentrations.

hCGL activity was assayed with L-cystathionine concentrations between 0.01 and 0.1 mmol/L and varying concentrations of L-methionine (0, 1, 5, 10, 50, 100, or 300 mmol/L). Kinetics were linear under the standard assay conditions and throughout these concentration ranges. Methionine inhibition was observed (Fig 2). It seemed to follow a competitive mechanism in a Lineweaver-Burk[24] plot, although minor measurement inaccuracies made it impossible to rule out a mixed-type inhibition due to allosteric communication between the active sites within the hCGL homotetramer.[23] The calculated inhibition constant was 72 mmol/L ± 10 mmol/L.

Intervention Efforts in a Severely MAT I/III-Deficient Patient

To gain information as to the possible cause of the elevated tHcy concentrations observed in patients with the most severe MAT I/III deficiencies, several studies were performed with Mr C (Table 2): In an effort to stimulate reconversion of homocysteine to methionine, a month-long trial of oral folate, 1.0 mg/d, plus vitamin B12, 500 μg/d, was given. This regimen did not lead to major changes in the relevant plasma metabolite concentrations. A month of pyridoxine administration, 200 mg daily, to ascertain if CBS activity could be stimulated sufficiently to lower the tHcy led to similarly negative results. However, at the conclusion of this regimen, the concentration of plasma cystathionine was lower than the concentrations measured at any other time during these studies. Finally, oral AdoMet (1,4-butanedisulphonate), 400 mg, 4 times daily was administered. The concentration of this compound in the liver of Mr C had been shown by direct assay to be somewhat low, 18 μmol/kg wet weight, compared with values for control human livers of 35 to 70 μmol/kg.[6] AdoMet is known to allosterically stimulate CBS activity.[27-29] This dose of AdoMet was sufficient to increase the plasma concentration some 4 hours after ingestion of 400 mg by 3-fold to 4-fold to a value well above the reference range and similar to reported values.[30] However, the plasma tHcy concentration remained slightly elevated at approximately the same level as it had been in the sample of 10/31/00 at the beginning of AdoMet administration.

An Example of Incorrectly Diagnosing MAT I/III Deficiency as CBS Deficiency and Its Adverse Consequences

Patient 7 provides a specific example of the sort of misdiagnosis that may occur in MAT I/III-deficient patients with elevations of plasma tHcy. This child was found at an early age to have markedly elevated plasma methionine and a plasma tHcy level of 47.2 μmol/L (Table 3). Pending assay of CBS activity in cultured fibroblasts, a tentative diagnosis of CBS deficiency was made and the patient was treated initially with pyridoxine. Plasma concentrations of neither methionine nor tHcy were normalized (with the exception of a value of tHcy of

Table 2. Serial Plasma Metabolite Concentrations in a Subject With Proven Severe MAT I/III Deficiency: Effects of Oral Vitamin or Adomet Treatments

Date	Methionine (μmol/L)	tHcy (μmol/L)	Cystathionine (nmol/L)	Sarcosine (μmol/L)	AdoMet (nmol/L)	AdoHcy (nmol/L)
9/10/96	836	40.2	384	3.6	ND	ND
4/3/00	968	26.1	325	1.1	81	12
Administered folic acid, 1.0 mg and vitamin B12, 500 μg, each daily by mouth for 30 days						
5/31/00	1,199	29.4	339	1.2	83	11
Administered pyridoxine, 200 mg, daily by mouth for 30 days						
7/31/00	1,045	29.5	209	1.0	112	24
10/31/00	710	20.9	269	0.6	83	9
Administered AdoMet, 400 mg, 4 times daily, by mouth for 30 days; last pill 4 hours prior to sample						
11/30/00	680	21.1	312	0.8	330	22
Reference ranges	13-45	5.4-13.9	50-342	0.6-2.7	92.8 ± 16.2	15-45

NOTE. The subject was Mr C (see Table 1 and Gahl et al[6,7] and Hazelwood et al[13]).
Abbreviation: ND, not determined.

Table 3. Serial Plasma Metabolite Concentrations in Patient 7

Age	Methionine (μmol/L)	tHcy (μmol/L)	Cystathionine (nmol/L)	Methylmalonate (nmol/L)	2-Methylcitrate (nmol/L)
16 days	1,664	47.2	776	146	128
16 days	Start pyridoxine, 500 mg/d orally				
27 days	Admitted to hospital for respiratory failure requiring ventilator support				
31 days	970				
34 days	ND	5.0	398	899	ND
38 days	1433				
41 days	ND	30.3	429	144	41
44 days	Methionine restriction begun				
46 days	Pyridoxine discontinued				
50 days	Respiratory function returned				
61 days	395	25.1	248	155	96
118 days	281 (lowest methionine level attained while on methionine restriction)				
244 days	1,451	20.7	312	71	30
11 months (approximately)	Methionine restriction discontinued about this time				
6.3 year	1,394	21.9	401	ND	ND
Reference ranges	13-45	5.4-13.9	50-342	71-271	60-228

NOTE. Patient 7 is described also in Chamberlin et al[2] and Mudd et al.[9]
Abbreviation: ND, not determined.

5 μmol/L at a time the patient was hospitalized and on ventilator support). Methionine restriction was begun subsequently. Compliance was thought to be questionable, and plasma methionine did not decrease below 281 μmol/L, and tHcy remained elevated. Ultimately, CBS activity was found to be normal in cultured fibroblasts, methionine restriction was discontinued, and, at age 7 years, the boy was demonstrated to be homozygous for a splicing defect in his *MAT1A* gene.[2]

DISCUSSION

We have assayed several methionine metabolites for the first time in patients with MAT I/III deficiency. Many of these subjects were clinically normal, although there is a clustering of abnormalities of the central nervous system in those with the most severe loss of MAT I/III activity as judged by the mutations in question or the extent of plasma methionine elevations.[2] The more severely affected patients are now shown also to tend to have abnormal elevations of plasma tHcy and, often, of plasma cystathionine (Table 1). In agreement with our findings, Lagler et al[31] recently reported (in abstract form) that 2 individuals with homozygous mutations in *MAT1A* had elevations of plasma tHcy to 30 to 45 μmol/L or 37 to 59 μmol/L. Judged by their plasma methionines (as high as 1,100 μmol/L and 2,069 μmol/L), the *MAT1A* mutations in these patients must also be severely inactivating. Cystathionine values were not reported for these patients.[31]

Possible Mechanisms Underlying the Elevations of Plasma tHcy and Cystathionine

The elevations of tHcy and cystathionine observed in the subjects with the most severe MAT I/III deficiencies were unexpected, because the reaction catalyzed by this enzyme, conversion of methionine to AdoMet, is a necessary step in the metabolic production of both homocysteine and cystathionine. Balance studies of 1 typical such patient, Mr C,[7] showed that on a normal diet, he forms homocysteine at a barely normal rate. In this man, homozygous for a truncating mutation in *MAT1A*,[13] the synthesis of the AdoMet required for this homocysteine production is necessarily being catalyzed by MAT II. However, this man lacks the normal reserve capacity to form more homocysteine when more methionine is provided and forms cystathionine at only a subnormal rate.[7] Evidently, the abnormal elevations of plasma tHcy and cystathionine under discussion are not due to overproduction of either of these compounds. Retardation in the rates of their removals must then be considered.

Homocysteine removal. Homocysteine is metabolized either to methionine or to cystathionine (Fig 1). Methionine is known to inhibit both betaine-homocysteine methyltransferase[32] and N^5-methyl-THF-homocysteine methyltransferase,[33] so that the abnormally elevated methionine in the MAT I/III-deficient patients might retard conversion of homocysteine to methionine. However, any such inhibition is not sufficiently strong to reduce homocysteine recycling below normal, as indicated by the fact that the balance studies of Mr C showed he was recycling homocysteine at a more-than-normal rate.[7] As shown in the present report, any stimulation of N^5-methyl-THF-dependent homocysteine methylation that may have occurred during administration of B12 and folate failed to normalize Mr C's plasma tHcy.

Could the elevated tHcy concentrations be due to retarded conversion of homocysteine to cystathionine? During the present studies, it was shown that methionine does not inhibit CBS activity. Previous studies have failed to show inhibition of CBS by concentrations of cystathionine up to 5 mmol/L (Kraus et al, unpublished observations). In agreement, the plasma tHcy of merely 18.2 μmol/L in the present cystathioninuric subject with a 35-fold elevated plasma cystathionine of 12,000 nmol/L indicates that inhibition of CBS by the very much lower concentrations of cystathionine found in the MAT I/III-deficient subjects is not sufficient to explain the elevations of plasma tHcy observed in these patients. A much more likely cause of retarded flux of homocysteine into cystathionine is abnormally low stimulation of CBS activity by AdoMet. The importance of

AdoMet regulation in homocysteine homeostasis was recently emphasized by Kluijtmans et al,[29] who identified a woman with markedly elevated plasma non–protein-bound Hcy due to a D444N mutation in the *CBS* gene. Although CBS activity in her fibroblast extracts was in the heterozygous range, that activity was not susceptible to stimulation by AdoMet, as is normal CBS.[29] Likewise, marked elevations of plasma tHcy have been observed in additional patients in whom CBS activities are comparable to normal in the absence of AdoMet, but fail to show AdoMet-dependent stimulation (Kraus et al, unpublished observations). In agreement, Finkelstein and Martin,[34] using a system approximating the in vivo conditions in rat liver, found that AdoMet enhanced the flux into cystathionine to as much as 720%. Because the hepatic concentration of homocysteine[35] is far below the K_m of CBS for this compound,[22,36] the change in k_{cat}/K_m upon addition of AdoMet provides the most relevant estimate of the potential stimulation it causes. AdoMet increases the k_{cat}/K_m ratio for CBS by approximately 12-fold (Kraus et al, unpublished observations). The apparent K_{act} for AdoMet stimulation of human CBS activity is near 15 μmol/L.[28,29] AdoMet is present at 35 to 70 μmol/L in normal human liver, whereas the hepatic concentration in Mr C was 18 μmol/L.[6] Therefore, his CBS activity and that of other MAT I/III-deficient patients, may be stimulated by AdoMet less than occurs normally, and this lower-than-normal stimulation would inhibit cystathionine formation and contribute to homocysteine accumulation. AdoMet administration did not restore Mr C's plasma tHcy concentration to normal (Table 2). However, in view of the uncertainty that sufficient exogenous AdoMet enters liver, this observation does not prove that such lack of stimulation is not a major contributor to his elevated plasma tHcy.

Homocysteine is normally removed from human plasma into tissues.[37] Inhibition of this process by methionine is therefore another possible cause of elevated plasma tHcy. Homocysteine uptake by human endothelial cells has been reported, but the transport appeared to be of homocysteine, not of its disulfide derivatives[38] that constitute the major non–protein-bound homocysteine-containing compounds in normal human plasma.[20] Uptake of homocystine by what appeared to be 2 transport systems into rat renal cortical cells has been described.[39] At concentrations of homocystine below 250 μmol/L, transport by a low Km system predominated. Only cystine and dibasic amino acids inhibited this system. However, the effect of methionine was not tested. Thus, not enough is known about the uptake of the relevant homocysteine derivative(s) from plasma to permit an informed suggestion as to any effect of methionine on this process.

In summary, the cause, or causes, of the elevation of plasma tHcy in severely MAT I/III-deficient patients is/are not fully established, although the available evidence suggests that less that normal stimulation of CBS by AdoMet is a major contributor.

Cystathionine removal. We report here that L-methionine inhibits hCGL. Although the K_i is relatively high, it is noteworthy that cystathioninuric persons with severe genetically determined impairment of CGL activity have had plasma cystathionine concentrations of 6 to 84 μmol/L,[26,40-48] concentrations many times more elevated than those noted in the present MATI/III-deficient patients. Therefore, only slight inhibition of CGL activity might be sufficient to bring about the minimal elevations of plasma cystathionine found in the latter patients. Methionine inhibition of CGL activity then appears to provide a very plausible explanation for these increases, increases that occurred despite some reduction of the flux of homocysteine into cystathionine. The fact that Mr C's plasma cystathionine was at its lowest concentration after a month's administration of pyridoxine (Table 2) is compatible with a B6-induced increase in CGL activity. CGL activity in extracts of human liver is stimulated by some 24% to 144% by addition of pyridoxal phosphate,[49,50] and (in rat liver) the maximal CGL activity is increased about 3-fold by feeding pyridoxine.[51]

Possible Incorrect Diagnosis of MAT I/III Deficiency as CBS Deficiency

In MAT I/III-deficient patients, diagnostic uncertainty or error could easily arise due to the abnormal elevations of plasma tHcy combined with elevated plasma methionines. Until now, this combination of abnormalities has been taken to be diagnostic of CBS deficiency. Free homocystine was not found to be abnormally high in MAT I/III-deficient patients when, as was formerly the practice, amino acid column chromatography was used to assay this compound. This result is understandable because free homocystine is detected by this means only when tHcy nears 60 μmol/L or higher.[52] The current use of procedures that measure tHcy means that lower elevations are now being detected. A diagnosis of CBS deficiency rather than the correct one of MAT I/III deficiency may lead to prolonged use of methionine-restricted diets, as occurred with patient 7. As has been discussed in detail previously, such methionine restriction may not be advisable in MAT I/III deficiency.[9] The available evidence suggests that in this condition, pathophysiologic consequences are more likely to result from AdoMet deprivation rather than from elevated methionine. Lowering the methionine might decrease the rate of methionine conversion to AdoMet catalyzed by any residual activity of MAT I/III.

Means to decide on the basis of metabolite values whether a given patient with combined hypermethioninemia and hyperhomocysteinemia has CBS deficiency or MAT I/III are established by the present studies. The metabolite patterns in the patients with CBS deficiency were variable, depending upon treatment regimens. The results for these and additional CBS-deficient patients will be reported in more detail separately (Mudd SH, et al, manuscript in preparation). Nevertheless, comparisons of the results for these patients with those for individuals with MAT I/III deficiency (Table 1) show these groups may be readily distinguished. Assay of cystathionine may be the single most useful test. In CBS deficiency, this compound was usually undetected, although it ranged in 2 subjects to the very low end of the reference range. In contrast, in MAT I/III deficiency, cysthationine was normal or elevated. Plasma AdoMet is also very useful, being elevated in CBS-deficient patients (except occasionally in those on stringent methionine restriction) and low or normal in MAT I/III deficiency. Plasma sarcosine followed a pattern similar to that of plasma AdoMet. Finally, elevations, of plasma tHcy were usually much more pronounced in CBS-deficient patients (again

with the exception of those on severe methionine restriction) than in those with MAT I/III deficiency.

Diagnostic confusion may arise also for patients with elevated plasma tHcy found during evaluation for vitamin deficiencies, neurologic diseases, or vascular disease. Most such patients will be adults, and standard diagnostic tests will rarely include methionine (a metabolite commonly assayed in infant screening programs). The rare patient with vitamin-resistant elevated tHcy should also have a determination of plasma methionine. MAT I/III deficiency may be present. Likewise, for subjects with demyelinating illness with elevated tHcy concentrations, once cobalamin deficiency has been ruled out, methionine and AdoMet assays are indicated to test for MAT I/III deficiency.

In summary, measurement of the complete range of metabolites reported here may be useful in several situations in which methionine and/or methionine metabolites are abnormally elevated, and a correct diagnosis is essential for optimal management.

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