Inborn Errors of Sulfur-Containing Amino Acid Metabolism

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ABSTRACT Two superimposed metabolic sequences, transsulfuration and the methionine/homocysteine cycle, form the pathway for methionine metabolism in mammalian liver. This combined pathway was formulated first to explain observations in subjects with homocystinuria caused by cystathionine synthase deficiency. Since that time additional inborn errors have been discovered, and currently we know of human subjects with isolated defects in all of the reactions of the combined pathway with only one exception: betaine homocysteine methyltransferase. Studies of these inborn errors have contributed significantly to our knowledge of human methionine metabolism and to the clinical consequences of impaired metabolism. Transsulfuration appears to function primarily for the metabolism of excess methionine, and each of the 5 defects in this pathway results in the accumulation of 1 or more of the normal metabolites. Thus, studies of these disorders may provide insight into both the potential pathological sequelae of nutritional methionine excess as well as whether laboratory testing allows the detection of excess. J. Nutr. 136: 1750S–1754S, 2006.

KEY WORDS: • methionine • homocysteine • hypermethioninemia • inborn errors

Three hundred and fifty years ago, William Harvey, in an extraordinarily prescient statement, noted the value of the "careful investigation of cases of rarer forms of disease" as the means to the "discovery of the usual law of Nature" (1). Certainly, studies of patients with inborn errors of sulfur amino acid metabolism have provided invaluable information concerning both the normal pathways for metabolism and potential mechanisms for pathology when these pathways are impaired. This article provides several examples of these contributions to knowledge before addressing the question of whether such studies provide information relevant to the issue of the toxicity of excess dietary methionine. The specific questions are the nature of the clinical manifestations; the potential toxic agent(s); and whether there is a sensitive and specific test for methionine excess.

The metabolic pathways

As illustrated in Figure 1, 2 metabolic sequences, which are superimposed in liver and kidney, comprise the pathways for methionine metabolism in mammalian tissues (2). The methionine (homocysteine) cycle is found in all tissues. The unique, constituent enzymes are methionine adenosyltransferase (MAT I in liver and MAT II in extrahepatic tissues); any of the numerous AdoMet-dependent methyltransferases; S-adenosylhomocysteine hydrolase (SAHH); and methionine synthase (MFMT). The enzymes of the cycle share several properties including a low $K_m$ for the sulfur-containing substrate; down-regulation by increased dietary methionine; and inhibition by methionine and/or S-adenosylmethionine (AdoMet). The cycle conserves methionine and provides sufficient AdoMet for the essential intracellular transmethylation reactions.

Five enzymes comprise the transsulfuration pathway that converts the sulfur of methionine to that of cysteine. The constituent enzymes are MAT (MAT III in liver and MAT II in other tissues); an AdoMet-dependent methyltransferase; SAHH; cystathionine $\beta$-synthase (CBS); and cystathionase. With the exception of SAHH, which is common to both, the enzymes of transsulfuration differ from those of the methionine cycle in at least 3 properties. The $K_m$ values for the sulfur substrates are significantly higher; the hepatic content of the enzymes is up-regulated by dietary methionine; and AdoMet may be a positive effector (2, 3).

Transsulfuration occurs in a restricted number of mammalian tissues (2, 3). In the rat it occurs in liver, kidney, pancreas, and intestine. Brain possesses CBS but lacks cystathionase. There are other tissue-specific variations. Liver alone contains the high-$K_m$ MAT III and consequently is the only tissue capable of synthesizing additional AdoMet when the concentration of methionine becomes excessive. Transsulfuration may serve both to catabolize excess methionine and homocysteine as well as to provide for the synthesis of cysteine and its derivatives glutathione, taurine, and sulfate.
In order to catabolize excess methionine, transsulfuration requires the presence of an AdoMet-consuming methyltransferase that has a high capacity; utilizes a readily available, nonessential substrate as the methyl receptor; and forms a nontoxic product. Glycine N-methyltransferase (GNMT) fulfills these criteria (2). Indeed, AdoMet is a positive effector of GNMT, which also shares the other properties of the transsulfuration enzymes and should be considered a member of that group.

Betaine-homocysteine methyltransferase (BHMT) shares properties with both the methionine cycle and transsulfuration enzymes (2). The $K_m$ for homocysteine is relatively low, and it is inhibited by methionine. Hepatic BHMT activity is induced by either methionine restriction or methionine excess. The enzyme has a limited distribution and occurs in mammalian liver and in primate and porcine kidney (4). Interestingly, it is the only enzyme of sulfur amino acid metabolism that is not found in the list of inborn errors of metabolism.

**Inborn errors of metabolism**

Several excellent and comprehensive review articles are available that provide the detailed information not present in this paper (5,6).

**Disorders of the methionine cycle.** All of the reported defects in the methionine cycle derive from impairment of the methionine synthase reaction by disorders of the synthesis of the essential cobalamin coenzyme; by failure of synthesis of the cosubstrate, methyltetrahydrofolate (methylene tetrahydrofolate reductase deficiency); by abnormalities in the formation of the apoenzyme; or by failure of the necessary methionine synthase reductase (5). In terms of methionine metabolism, all of these defects result in homocysteine accumulation, decreased methionine, decreased AdoMet, and increased AdoHcy. Cystathionine may be increased as a result of the increased flow through the transsulfuration pathway, but the increase in homocysteine proves that transsulfuration alone is insufficient to remove this metabolite.

Because the cycle functions to conserve methionine, disruption does not provide a pattern of abnormalities that is relevant to considerations of the consequences of methionine excess.

**Disorders of transsulfuration.** Cystathionine synthase deficiency. Homocystinuria was described first in 1962, and the defect in cystathionine synthase was reported in 1964 (7). Increases in homocyst(e)ine and methionine were the originally described metabolic abnormalities. Subsequently, these were extended to include increases in AdoMet, AdoHcy, and sarcosine with decreases in cystathionine and cysteine. The clinical abnormalities include major cardiovascular thromboembolic episodes, mental retardation, musculoskeletal abnormalities, and subluxation of the ocular lenses. As the process of ascertainment changed from the initial screening of symptomatic individuals and their relatives to a more general survey of the population, the range and diversity of the pathology have become apparent (6).

Studies of patients with this abnormality have been very significant in establishing the patterns for normal sulfur amino acid metabolism in humans. Because the pathway connecting...
them is irreversible, the finding of increases in both methionine and homocysteine required a reformulation of thinking regarding the metabolic sequences. Homocysteine remethylation could provide for functional reversibility. Indeed, the metabolic chart that combined the methionine cycle with the transsulfuration pathway appears in the paper that described the enzyme defect (7). Other insights that derived from studies of CBS-deficient patients include the following: 1) CBS deficiency compromises the conversion of methionine to both cysteine and sulfate, thus establishing the unique role of the transsulfuration sequence (6). 2) Homocysteine methylation alone, by MFMFT and/or BHMT, was insufficient to clear the homocysteine formed. Subsequently it became apparent that the demands of homocysteine methylation can deplete both betaine and methyltetrahydrofolate. 3) The increased AdoHcy is evidence that the SAHH reaction is reversible in vivo as well as in vitro and that removal of the products is essential to drive the reaction in the direction of hydrolysis.

Cystathionase deficiency. Primary or congenital cystathioninuria results from a defect in cystathionase. There is no apparent pathology despite the marked accumulation of cystathionine in liver, kidney, brain, and cerebrospinal fluid. Plasma cysteine concentrations remain within the normal range, possibly indicating sufficient residual enzyme or adequate dietary intake. Lessons from studies of this metabolic defect include (6) 1) the discovery of alternate pathways for cystathionine metabolism that begin with either oxidation or transamination of the metabolite. Clearly these pathways are not sufficient to clear cystathionine synthesized during normal metabolism. 2) Patients with cystathioninuria may have mild to moderate hyperhomocysteinemia. This inconsistent finding may be caused by a reversal of the CBS reaction, a property that can be demonstrated in vitro under extreme conditions.

MAT I/III deficiency. That a defect in MAT was the cause of idiopathic, persistent hypermethioninemia was first described in 1974 (6). The observation that the defect was more apparent when the hepatic enzyme was assayed at high concentrations of substrate was an early indication of the possible existence of isoenzymic forms of MAT (8). Subsequent studies demonstrated that the defect involved only the high-K_m, hepatic enzyme MAT I/III. Tissue levels of MAT II, the extrahepatic isoenzyme, remain normal.

The only consistent metabolic consequence of the enzyme defect is the marked hypermethioninemia with plasma values as high as 30 times the upper limit of the reference range. Plasma levels of AdoMet and AdoHcy are in the low to low-normal range. Slight increases in both plasma homocysteine and cystathionine have been noted, but these have been inconsistent findings (9).

Most of the affected individuals are asymptomatic, and this provides the major insight derived from studies of these patients. Either chronic hypermethioninemia is not toxic to human subjects or the toxicity requires synthesis of AdoMet. It is important to note that the few symptomatic patients who suffer a demyelinating syndrome may represent the most complete loss of MAT I/III activity. The fact that their pathology occurs despite normal MAT II in neural tissue suggests that normal myelination may require the export of either AdoMet or some other compound from the liver or hepatic detoxification of some neurotoxin. There are additional insights from studies of the MAT I/III–deficient patients. 1) MAT II suffices for the synthesis of AdoMet required for essential AdoMet-dependent reactions, both transmethylation and polyamine synthesis, in liver and extrahepatic tissues (10). 2) Methionine transamination products are found in patients with MAT I/III deficiency, and 2 conclusions derive from this observation. Clearly this alternate pathway for methionine metabolism is insufficient to maintain normal tissue concentrations of the amino acid. Nor does the enhanced transamination result in demonstrable pathology (6). 3) Balance studies indicate that despite the marked increase in tissue methionine, methionine metabolism in MAT I/III-deficient subjects is directed inappropiately to methionine conservation. This supports the regulatory role of AdoMet in the distribution of homocysteine between remethylation and transsulfuration (3,11) 4) The increase in plasma cystathionine led to the demonstration that cystathionine can inhibit cystathionase (9).

Glycine N-methyltransferase deficiency. Recent reports have described this defect, first in 2 Italian siblings and then in a Greek child (12,13). All 3 had modest elevations of the serum transaminases and hepatomegaly. Hepatic biopsy in 1 patient showed centrilobular fibrosis with eosinophilia but neither hepatic necrosis nor cholestasis. Laboratory studies revealed marked increases in the plasma concentrations of both methionine and AdoMet. The concentrations of AdoHcy, tHcy, and cystathionine were moderately elevated. The normal concentration of plasma sarcoicine in the presence of the marked elevations of methionine and AdoMet suggested a defect in GNMT, and molecular genetic studies confirmed this hypothesis. Both the biochemical abnormalities and the evidence of hepatic pathology diminished when the children were fed a methionine-restricted diet. The pathophysiological basis for the hepatic dysfunction must be established.

The most important metabolic insight from the study of these children is the confirmation of both the assignment of GNMT to the transsulfuration pathway and the suggestion that this enzyme is essential for the catabolism of excess methionine. The hypothesis, based on animal studies of both the kinetic properties of the enzyme and the changes in hepatic content in response to dietary methionine, is applicable to human metabolism (14). Additional findings include the following: 1) The elevation of methionine suggests inhibition of MAT II in extrahepatic tissues. AdoMet does inhibit that enzyme, although it is a positive effector of hepatic MAT III. 2) The increased cystathionine probably derives from AdoMet activation of CBS together with methionine inhibition of cystathionase (2,9). 3) The basis for a modest increase in plasma tHcy and AdoHcy remains to be clarified, but it may result from inhibition of homocysteine remethylation in hepatic and extrahepatic tissues because methionine inhibits both homocysteine methylases and AdoMet inhibits the synthesis of methyltetrahydrofolate, the substrate for methionine synthase. 4) The diagnostic value of the plasma sarcoicine determination that showed only normal levels of sarcoicine in the presence of marked excesses of methionine and AdoMet.

S-Adenosylhomocysteine hydrolase (SAHH) deficiency. Baric et al. reported SAHH deficiency in a Croatian boy (15) and subsequently in his younger brother (16). Both children presented with psychomotor delay, severe myopathy, and demyelination. The older child had a mild hepatitis with periporal fibrosis. In the older child, the plasma AdoHcy was markedly elevated (>150 × control), as were AdoMet (30 × control) and methionine (20 × control). The younger child had similar, but quantitatively less marked, abnormalities, perhaps because of diagnosis and treatment at an earlier age. Both children had elevations in plasma cystathionine, sarcosine, and dimethylglycine. SAHH was deficient in erythrocytes, liver, and skin fibroblasts from the older child and in erythrocytes from the younger. Both children showed the same abnormality in the gene for SAHH.

Treatment was based on the hypothesis that the pathophysiology derived in part from inhibition by AdoHcy of 2
essential AdoMet-dependent transmethylases with consequent
deficiency of both phosphatidylcholine and creatine. Consequently,
these 2 compounds were added to a methionine-restricted diet.
The clinical findings improved concurrent with this interven-
tion, although the biochemical markers of muscle disease
persisted. The abnormalities in the concentrations of the
methionine metabolites also improved with treatment, and the
plasma concentrations in the younger child became normal. In
the older child, although lower, the plasma concentrations of
AdoMet and AdoHcy on 1 determination remained elevated at
8 X and 30 X the control values, respectively (16). This may
reflect failure of total compliance with the restricted diet.

**DISCUSSION**

**Patterns of plasma metabolite abnormalities.** There are
obvious limitations inherent in the use of the plasma levels of
metabolites as a measure of intracellular concentrations. The
most apparent difficulty is the definition of the source and the
recipient organ(s). For example, does the plasma level represent
movement to or from the liver? In the case of the transsul-
fuluration metabolites, the need to compare changes in different
metabolites compounds the difficulty because it is unlikely that
membrane transport, both uptake and release, is equal for all
compounds. Methionine and homocysteine appear to equili-
brate, and plasma concentrations (in micromoles per liter) are
of same order of magnitude as the hepatic concentrations
expressed as nanomoles per gram. In contrast, the hepatic con-
centrations of AdoMet, AdoHcy, and cystathionine approximate
120, 20, and 90 nmol/g, respectively. These values are
orders of magnitude greater than the normal plasma concen-
trations of 90, 30, and 200 nmol/L. For this reason, it is appro-
priate to be cautious when extrapolating from changes in the
plasma concentration of a given metabolite to the simultaneous
change in tissue concentration.

That caution is applicable to **Table 1**, which presents the
concentrations of plasma metabolites in patients with the 5
inborn errors of the transsulfuration pathway. Given the few
patients for whom all of the 6 determinations are available, the
table is admittedly anecdotal (9,12,16). Nevertheless, a pattern
is present. MAT I/III deficiency is characterized by a marked
26-fold increase in plasma methionine, far greater than the
changes in other metabolites. Similarly, the major increases in
the 2 patients with GNMT deficiency are in methionine and
AdoMet, whereas the dominant increases in SAHH deficiency
include methionine, AdoMet, and AdoHcy. Last, plasma values of
tHcy as well as of methionine, AdoMet, and AdoHcy are
increased in CBS deficiency. Thus, the pattern of change
identifies the defective enzyme by the breakpoint at which a
marked difference occurs in the magnitude of the changes
between “adjacent” metabolites.

**Applicability of metabolomic analysis to dietary excess.**
Studies of the effects of graded excesses of dietary methionine
on methionine metabolism in rat liver revealed that the first
significant change is an increase in AdoHcy (14). This occurs
after 7 days with a diet containing 1.5 g/100 g methionine.
Signs of toxicity occur at 3.0 g/100 g methionine, at which
point the hepatic concentrations of methionine and AdoMet
are also increased, but not to the extent of the AdoHcy.
Homocysteine was not assayed, but the likely sequence of
metabolic changes can be deduced from the fact that depletion
of betaine and serine preceded the rise in AdoHcy. This
suggests that adaptation to the excess methionine appears to
have failed because of a limitation of available cosubstrates for
the reactions that metabolize homocysteine, CBS, BHMT, and
presumably MFMT, although methyltetrahydrofolate was not
determined. In this construct, the increase in AdoHcy is
secondary to a reversal of the SAHH reaction as a result of the
increase in homocysteine. The next step will be to correlate
plasma changes in the plasma concentrations of the 6 me-
tabolites with the changes in liver. The results would allow us
to gauge the risk in the extrapolation from plasma assays.
Furthermore, in these studies, the rate-limiting reaction might be
expected to vary as a function of the changing availability of
serine, betaine, folate, and possibly glycine. In turn, this might
affect the pattern of abnormalities in plasma.

The feeding of a high-methionine diet preparation to neo-
ates provides the only study of methionine excess in humans
in which the sequential analyses of the plasma metabolites are
available (17). Because this was not a planned and prospective
study, the data are not optimal for purposes of the analysis.
Because hypermethioninemia on routine screening identified
the affected children, that abnormality was invariable. Data
from 7 cases met the criteria that all 6 metabolites were de-
termined in relatively close proximity to the peak methionine.
The following were the results, expressed as multiples of either
the mean reference value or the value in the middle of the
range, whichever was available for the reference
group: Methionine 33 X; AdoMet 14 X; AdoHcy 4 X; tHcy
2 X; cystathionine 16 X; and sarcosine 4 X. Interpretation of
this pattern is difficult. The “breakpoint” between AdoMet and
AdoHcy suggests a relative impairment of GNMT, possibly
caused by a lack of sufficient glycine to catabolize the excessive
AdoMet. The additional abnormality, the increased cystathi-
onine, probably relates to the reduced level of cystathionase in
the immature liver. More studies are needed both in animals
and in humans, where ethically permissible, to validate this type
of metabolomic analysis.

**Methionine toxicity in humans.** The potential toxicity
of excess dietary methionine is a function of species, age,
magnitude of methionine excess, duration of exposure, dietary
content of serine, glycine, choline or betaine, and cysteine,
and adequate supply of folate, cobalamin, pyridoxine, and
riboflavin.

Studies of the inborn errors do inform our thinking regarding
the potential toxin. The findings in patients with MAT I/III
deficiency warrant the conclusion that, in chronic exposure,
methionine itself is an unlikely candidate and that formation of
the toxin requires the synthesis of AdoMet. However, if we can
generalize from the 3 known cases of GNMT deficiency, neither
the consumption of ATP to form AdoMet nor some reactivity
of AdoMet is pathogenic. Pathology does occur with deficiency
of SAHH, which causes marked increases in AdoHcy, and
there is reason to suspect that the pathophysiology involves the

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**TABLE 1**

<table>
<thead>
<tr>
<th>Deficiency (n)</th>
<th>Met</th>
<th>AdoMet</th>
<th>AdoHcy</th>
<th>tHcy</th>
<th>Cystathionine</th>
<th>Sarcosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAT I/III (11)</td>
<td>26</td>
<td>1</td>
<td>&lt;1</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>GNMT (2)</td>
<td>14</td>
<td>33</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>“Normal”</td>
</tr>
<tr>
<td>SAH (2)</td>
<td>12</td>
<td>21</td>
<td>90</td>
<td>90</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>CBS (7)</td>
<td>32</td>
<td>15</td>
<td>21</td>
<td>26</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>C-ase (1)</td>
<td>1</td>
<td>1</td>
<td>—</td>
<td>2</td>
<td>&gt;35</td>
<td>—</td>
</tr>
</tbody>
</table>

1 Data is from Stabler et al. (9), Mudd et al. (12), and Baric et al. (16).

Values are expressed as multiples of either the mean value for the
reference group or the middle of the reference value range.
inhibition of essential, AdoMet-dependent transmethylation reactions such as those required for the synthesis of creatine and phosphatidylcholine. The trapping of adenosyl moieties in the accumulated AdoHcy and AdoMet is a less likely possibility in chronic exposure but is a suggested mechanism for acute toxicity. The situation with CBS deficiency is even more complex, and the pathology is more variable. This may relate to the additional abnormality: the marked increase in homocysteine and its derivatives.

The children who were given the excess methionine recovered without complications when the diet was amended. It is interesting that their dominant metabolic abnormalities were the increases in methionine and AdoMet. It is possible that the increased AdoMet, in excess of the increase in AdoHcy, may protect by limiting the inhibition of transmethylation. The same consideration might pertain to some patients with CBS deficiency, particularly those being treated with folate and/or betaine, who often improve despite persistence of the hyperhomocysteinemia.

Concluding comments. Patients with any one of the several inborn errors of the transsulfuration pathway respond both clinically and chemically to dietary methionine restriction. The improvement is not complicated by any apparent deficiency of methionine, cysteine, and their derivatives. This observation supports the suggestion that the transsulfuration pathway exists primarily to metabolize excess methionine. Furthermore, it seems apparent that the capacity of the transsulfuration pathway is far greater than the requirement for catabolizing even the moderate excess of dietary methionine that is the standard diet in many parts of the world. For example, obligatory heterozygotes with CBS content of <50% and homozygotes for MAT I/III deficiency with 8–25% residual activity may show no abnormalities when fed a diet with a limited, but adequate, content of methionine. Thus, there appears to be a predictable capacity for catabolism of methionine supplements, even those that bring total intake to levels substantially above the essential nutritional requirement. In those situations, where the supplement is to an apparently normal intake, monitoring of the metabolic profile would be warranted. However, in these circumstances, the uncertainty of the consequences of long-term administration of excess methionine mandates caution, and it may be that the prudent question is when or why to risk toxicity.

Conversely, the wide margin between essential nutritional requirement and toxicity makes it virtually certain that supplementation will be effective and without harm in populations whose predictable intake of methionine is marginal or less.

LITERATURE CITED