The death of a control subject after an oral load of methionine for a study of the possible relationship between methionine load elevation and Alzheimer’s disease is reported. The subject developed a very high plasma total homocysteine (tHcy) concentration. She was generally in good health, except for known hypertension. Her blood pressure on the day of the test was 186/77 mm Hg. She was taking the following medications: furosemide, potassium, aspirin, and rotecoxib. She was also a concurrent participant in the Women’s Antioxidant Cardiovascular Study (WAS). She was generally in good health, except for known hypertension. Her blood pressure on the day of the test was 186/77 mm Hg. She was taking the following medications: furosemide, potassium, aspirin, and rotecoxib. She was also a concurrent participant in the Women’s Antioxidant Cardiovascular Study (WAS).

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results

Methionine, a constituent of proteins, is a normal and essential dietary component for humans. Most adult western diets contain \( \approx 2 \) g/d. As mentioned above, methionine loading tests carried out with the use of the customary dose of 100 mg/kg body wt have proven to be extremely safe. A review published in 1998 mentions that by 1994 such tests had been performed in 750 vascular patients and 200 control subjects. Adverse effects of these tests were not mentioned.8 More recently, the safety of such tests in 296 vascular patients and 591 control subjects in the Czech Republic was carefully reviewed. Although in 23% of the subjects, methionine administration was followed by \( \approx 1 \) transitory complication (impaired perception, dizziness, nausea, decreased vigilance, or minor drops in diastolic blood pressure), there were no serious adverse effects in the vasculature, and it was concluded that the test “may be considered a safe procedure.”17 Thus, the outcome in the present case must be considered to be most unusual. Several possibilities were considered as potential contributory factors, as follows.

Was There an Impurity in the Methionine Administered?
The lot of methionine that had been used for the loading dose was subsequently tested for purity by the vendor, Spectrum Laboratory Products, Inc, and found to meet “all designated specifications established by the United States Pharmacopeia.” A sample from the bottle used for the study was sent to AAA Service Laboratory, Inc, Boring, Oregon. This sample after hydrolysis at 110°C for 20 hours in 6N HCl plus 2% phenol contained no impurities detected by amino acid chromatography with ninhydrin detection.

Was There a Preexisting Disease State or Genetic Abnormality Affecting Methionine Metabolism That Would Predispose the Subject to the Adverse Outcome?
The subject met the inclusion/exclusion criteria for the study as described in the institutional review board–approved protocol. She had been initially screened under the Alzheimer Center Patient Registry protocol through a medical history and cognitive and physical exams and was determined to be appropriate as a normal control.

To search for metabolic abnormalities, methionine and related metabolites were assayed in plasma samples (Table). A major finding was that in the baseline sample, all metabolites related to methionine metabolism were normal, or virtually normal. An exception was AdoHcy, which was elevated \( \approx 2 \)-fold. However, the fact that in the 2-hour postload sample the AdoHcy was lower and within the nonloaded reference range suggests that the apparent elevation of this metabolite was not constant and probably was of little pathophysiological significance. The absence of elevation of either methionine or tHcy and normal cystathionine rule out cystathionine \( \beta \)-synthase (CBS) deficiency.18 The absence of elevated methionine rules out methionine adenosyltransferase (MAT) I/III deficiency.18 The normal methionine, together with normal AdoMet, rules out glycine \( N \)-methyltransferase deficiency.10 Thus, the known major genetic diseases of methionine metabolism that might contribute to the abnormally high postload concentrations of methionine documented in the next paragraph have been eliminated as contributing to the adverse outcome. The normality of the baseline tHcy and methylmalonate, nM

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Baseline</th>
<th>2 Hours</th>
<th>4 Hours</th>
<th>2 Days</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methionine, ( \mu M^* )</td>
<td>19</td>
<td>4640</td>
<td>5760</td>
<td>202</td>
<td>10–30</td>
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<tr>
<td>Methionine, ( \mu M )</td>
<td>18</td>
<td>3540</td>
<td>5280</td>
<td>310</td>
<td>13–43</td>
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<tr>
<td>AdoMet, nM</td>
<td>79</td>
<td>740</td>
<td>1089</td>
<td>6716</td>
<td>104–21</td>
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<tr>
<td>AdoHcy, nM</td>
<td>66</td>
<td>31</td>
<td>59</td>
<td>358</td>
<td>27–7</td>
</tr>
<tr>
<td>tHcy, ( \mu M )</td>
<td>9</td>
<td>25</td>
<td>43</td>
<td>18</td>
<td>5–14</td>
</tr>
<tr>
<td>Cystathionine, nM</td>
<td>164</td>
<td>541</td>
<td>1499</td>
<td>3344</td>
<td>44–342</td>
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<tr>
<td>tCys, ( \mu M )</td>
<td>374</td>
<td>340</td>
<td>329</td>
<td>356</td>
<td>203–369</td>
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<tr>
<td>PtdCho, ( \mu M )</td>
<td>6210</td>
<td>2600</td>
<td>2760</td>
<td>2820</td>
<td>2170–340</td>
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<tr>
<td>Choline, ( \mu M )</td>
<td>0.42</td>
<td>154</td>
<td>308</td>
<td>nd</td>
<td>0.20–0.54</td>
</tr>
<tr>
<td>Sarcosine, ( \mu M )</td>
<td>2.1</td>
<td>2.7</td>
<td>3.5</td>
<td>3.1</td>
<td>0.6–2.7</td>
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<tr>
<td>Betaine, ( \mu M )</td>
<td>27</td>
<td>36</td>
<td>59</td>
<td>44</td>
<td>52±7</td>
</tr>
<tr>
<td>Dimethylglycine, ( \mu M )</td>
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<td>2.0</td>
<td>2.9</td>
<td>2.4</td>
<td>1.4–5.3</td>
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<tr>
<td>Methylmalonate, nM</td>
<td>245</td>
<td>261</td>
<td>181</td>
<td>253</td>
<td>73–271</td>
</tr>
</tbody>
</table>

Reference values are range or mean±SD.
*Values determined by the Associated Regional and University Pathologists Laboratories, Salt Lake City, Utah. All other values reported in this table were determined in the laboratory of one of the coauthors.

References

1. Cottington et al Death Associated With Methionine Loading Test.
served, severe renal disease would be expected to be accompanied by an elevated, not normal, baseline plasma tHcy but (see paragraph below) no change in the concentration or timing of the peak postload methionine.

In the postload samples, there were acute elevations in AdoMet, tHcy, and cystathionine, intermediates in the major pathway for methionine disposal whereby methionine is converted to cysteine. However, more striking was the unexpected extent of the elevations that occurred in methionine itself—to some 4640 μmol/L at 2 hours and 5760 μmol/L at 4 hours after load (Table). For comparison with these values, after methionine loads of 100 mg/kg body wt, in 10 normal postmenopausal women, the peak concentrations attained ranged from 774 to 1406 μmol/L, with a mean±SEM of 1107±53 μmol/L; 22 in 2 female obligate heterozygotes for CBS deficiency, peak values after similar loads were 618 and 1258 μmol/L; 23 after somewhat higher loads of 200 mg/kg body wt, the peak mean±SEM in 10 normal women was 854±101 μmol/L; and even among 8 women with CBS deficiency not being treated with pyridoxine, the peak concentrations ranged from 849 to 1627 μmol/L, with a mean±SEM of 1205±86 μmol/L. 24 The extremely abnormally elevated postload methionine concentrations in the subject were initially found during analyses carried out by the Associated Regional and University Pathologists Laboratories, Salt Lake City, Utah. Because they were so remarkable, repeat methionine analyses were performed in the laboratory of one of the coauthors (S.P.S.). The resulting values were somewhat lower (eg, the concentration in the repeat analysis of the 4-hour postload sample was 92% of that obtained in the initial analysis). These differences may probably be attributed to some oxidative loss of methionine during storage between analyses, although calibration differences between the laboratories may also have played a role. In any case, the postload elevations in methionine concentrations in this subject were very much higher than those previously reported. The time course of postload methionine concentrations in the subject was also different from that expected on the basis of previous experience: in normal individuals, plasma methionine usually peaks at 1 hour after the customary oral methionine load, 24 whereas in the present subject, the methionine level continued to rise between 2 and 4 hours (and presumably peaked at an even higher level at a later time when the agitated state of the subject made it impossible to obtain further blood samples).

In contrast to the elevations in methionine, the elevations during the first 4 hours in plasma AdoMet were within the range for control subjects reported by Loehrer et al 25; those for tHcy, within or very close to the range for women aged between 61 and 75 years specified by Silberberg et al 26; and those for cystathionine, within the control range found by Ubbink et al. 27 Among the other metabolites reported in the Table, sarcosine and phosphatidylcholine, each formed by AdoMet-dependent transmethylation reactions, did not change dramatically, and at most, there were small rises in the products normally formed from phosphatidylcholine: free choline, betaine, and dimethylglycine. In the 2-day postload sample, the concentrations of plasma methionine and tHcy had decreased markedly, although each remained above normal. In contrast, there were further elevations in AdoMet, AdoHcy, and cystathionine. Not enough is known about the kinetics of the turnovers of grossly elevated tissue and plasma quantities of these metabolites or about the possible effects of the acute arrest on these kinetics to permit a detailed explanation of these observations.

The possibility was considered that the subject might have had a defect in the TAM pathway, which serves as an alternative, albeit relatively minor, pathway for the catabolism of methionine, especially at higher methionine concentrations. 14 However, assay of the products of this pathway (TAM in the Table) yielded post–methionine load values among the highest ever observed. Among 6 control individuals after methionine loads of 100 mg/kg body wt, the maximum concentration of TAM attained in serum was 3 μmol/L, 15 whereas the 2- and 4-hour postload values in the present patient were ~50 and 100 times that concentration. The postload excretion of TAM in the urine of this patient, 186 mmol/mole creatinine, was also highly elevated compared with the postload excretions of normal control subjects. 15 The patient also developed an unpleasant breath and body odor that was apparent to the nurses caring for her. It seems likely that this was due to dimethyl sulfide, a volatile product of the TAM pathway. 15 Together, these findings convincingly rule out a defect in the TAM pathway as a cause of the unusually high postload concentrations of methionine.

Was an Overdose of Methionine Administered?

Given the exceptionally high concentrations of methionine attained after the methionine load and the fact that the concentrations probably rose even higher after the 4-hour postload sample, the possibility arises that she received a substantial overdose of methionine. According to the institutional review board–approved protocol, the subject should have received 8.39 g l-methionine dissolved in 250 mL orange juice (100 mg/kg body wt). The research nurse conveyed this order orally on the phone to the nutrition services attendant by stating that the subject was to receive 8390 mg methionine. The nutrition services attendant weighed the methionine, dissolved it in 100 mL warm water, added 250 mL orange juice in a blender, blended it, placed it in a cup with a lid, and delivered it to the research nurse. Interviews with both individuals indicated that there was some confusion about the conversion of milligrams to grams. However, both stated during their interviews that they believe the subject did receive ~8.39 g. The attendant said that after the adverse event, she weighed out another 8 g to visually confirm that she had given the proper amount and showed this to another nurse at the research site. The attendant stated also that she subsequently weighed out 80 g of the methionine to again visually confirm that she did not mix such a quantity for the subject. She said that she discarded this sample without showing it to anyone, although afterward she informed research site personnel that she had done so. The amount of methionine remaining in the bottle was consistent with the removal of ~96 g. The committee investigating the adverse outcome in this case satisfied themselves that as much as 80 g of methionine could be dissolved/suspended in a glass of orange juice so as to be ingested when the juice was...
Drunk. Thus, although definitive proof is not available, it is certainly a possibility that the subject received as much as 80 g of methionine. Indeed, no other tenable explanation of the extraordinary postload elevations in plasma methionine concentrations occurs to us. The exceptional postload elevations of TAM in the subject support this supposition.

Discussion

The totality of the findings in this case strongly suggest that a relatively large dose of methionine may bring about severe, potentially lethal, cerebral effects. Support for this possibility is found in several earlier publications: Each of 2 control subjects given 30 g IV l-methionine over 30 minutes in a study reported by Floyd et al28 developed acute nausea and vomiting, accompanied in one of these individuals by increased sweating, chill followed by fever, moderate hypotension, tachycardia, and intermittent disorientation. Arginine, lysine, phenylalanine, leucine, valine, or histidine at the same dose did not cause such effects, although smaller doses of isoleucine, threonine, or tryptophan were accompanied by a variety of manifestations. A control subject given 150 mg l-methionine/kg body wt in a study reported by Perry et al29 experienced nausea and vomiting 2 hours after the administration of the methionine that was severe enough to lead to a reduction of the dose in the 4 control subjects subsequently studied to 100 mg/kg body wt. None of the latter 4 subjects showed similar effects.29 Cohen et al,30 in 1974, reviewed the results of 10 earlier studies in which large doses of methionine (eg, 20 g/d for 5 days) had been administered orally to schizophrenic patients. A number of the subjects studied developed confusion, disorientation, delirium, agitation, listlessness, and/or similar symptoms. For some cases, these abnormalities were interpreted not as being accentuations of the preexisting schizophrenia but as being signs and symptoms of an “organic brain syndrome.”30 An additional example of a possible dangerous cerebral effect of elevated methionine has recently been reported by Yaghmai et al.31 These authors describe the case of a 10-year-old CBS-deficient girl whose plasma methionine rose to concentrations close to 3000 μmol/L while she was being treated with betaine and was noncompliant with dietary methionine restriction. This girl developed massive cerebral edema that responded promptly to strict dietary methionine limitation.

The pathophysiological means whereby elevation of methionine or its metabolites might cause such cerebral effects is not known. Hardwick et al32 have discussed the possibility that utilization of ATP to form AdoMet leads to pathological depletion of hepatic ATP. Methionine loading has been well documented to cause vascular endothelial dysfunction in humans,33–36 including impairment of cerebrovascular reactivity.37 The bulk of,33–36 but perhaps not all of,38 the evidence indicates that such effects are due to Hcy formed from methionine rather than the methionine itself. By 4 hours after load (after the present subject began to vomit and shortly before the onset of her most serious confusion and agitation), her plasma tHcy level had not risen above concentrations that have not been accompanied by such striking adverse cerebral manifestations in other control subjects, although, of course, plasma tHcy may have peaked higher later. The extreme elevations in TAM metabolites that occurred after load are of interest because the feter hepaticus that is due to one of these, dimethyl sulfide, has often been regarded as a component of portal systemic encephalopathy.39 Although published evidence indicates that neither methanethiol metabolites40 nor dimethyl sulfide has a relation to the signs of such encephalopathy,39 these findings do not exclude toxic effects of methanethiol or its derivatives in the present case because the concentrations of these compounds were much higher in her than in patients with portal encephalopathy.

What does seem quite clear from the facts in the present report is that any effort to increase the sensitivity of the methionine loading test as a means of revealing a tendency to develop hyperhomocysteinemia by significantly increasing the dose of methionine administered above the customary 100 mg/kg body wt should be undertaken only with extreme caution and for sufficient reasons. In addition, given this experience, it is recommended that only licensed dieticians or pharmacists be responsible for dispensing methionine for methionine-loading tests.

Acknowledgments

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