Alcohol Abuse: An Important Cause of Severe Hyperhomocysteinemia
Ralph Carmel, M.D., and S. Jill James, Ph.D.

Alcohol has complex direct effects on homocysteine metabolism, which are incompletely understood, and indirect effects mediated by interactions with vitamin metabolism and other factors. Both transmethylation and transsulfuration pathways are affected. Alcohol abuse is a common cause of hyperhomocysteinemia that often fluctuates and is sometimes severe. The causative role of alcohol in hyperhomocysteinemia is often overlooked by clinicians when evaluating patients and by investigators when conducting surveys. A married couple with severe hyperhomocysteinemia owing to surreptitious alcohol abuse, a case study illustrating many of these issues, is presented. A steep rise in S-adenosylhomocysteine as well as homocysteine levels was demonstrated with increased alcohol ingestion, with a decreased S-adenosylmethionine:S-adenosylhomocysteine ratio. Both patients had severe neurologic symptoms as well as macrocytic red blood cells, which, along with the high homocysteine levels, were misattributed to cobalamin deficiency, in one case despite serum cobalamin levels that were normal.

Key Words: alcohol, homocysteine metabolism, transmethylation, transsulfuration, hyperhomocysteinemia, S-adenosylhomocysteine

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Introduction

Despite the strong overall correlations between homocysteine status and folate, the discovery and appropriate treatment of hyperhomocysteinemia in a patient always requires that its cause be identified. This goal is especially pressing when hyperhomocysteinemia is severe. The causes of severe hyperhomocysteinemia that are usually mentioned are hereditary disorders such as cystathionine β-synthase deficiency and disorders of folate or cobalamin metabolism, and acquired disorders such as cobalamin deficiency, folate deficiency, renal failure, and acute lymphoblastic leukemia. This article presents the case of two patients, a married couple, whose puzzling and initially misdiagnosed severe hyperhomocysteinemia turned out to be due to a clinically important cause that is probably as common as the others but is not yet as widely recognized.

Patient 1

A 51-year-old woman was referred from another city in May 2000 for evaluation of severe hyperhomocysteinemia that was attributed to cobalamin deficiency and that resembled the condition her husband (patient 2) had a year earlier. The possibility of an underlying metabolic disorder was now suspected because her condition, unlike his, recurred despite continued vitamin therapy.

Pernicious anemia had been diagnosed initially in July 1997 on the basis of macrocytosis (MCV was 103 fL, but hemoglobin was normal at 13.4 g/dL) and a low cobalamin level (195 ng/L). Gastric biopsy had shown erosive gastritis 5 years earlier. Cobalamin therapy was discontinued in 1998 when her serum cobalamin level became normal, although the blood count did not improve. Treatment was restarted in 1999 when a low serum cobalamin level was found again (172 ng/L; RBC folate level was normal).

Other medical problems included hypertriglyceridemia for which she received fenofibrate and later gemfibrozil; she also took omeprazole for nonspecific gastrointestinal symptoms. Other past history included nephrolithiasis, panic attacks during menopause, and migraine headaches.

In the beginning of 2000, severe hypertriglyceridemia recurred, along with mild elevation of aspartate aminotransferase levels. More troubling was the onset of neuropsychiatric symptoms that included ataxia, tremors, behavior changes, and lethargy. Neurologic evaluation showed decreased vibratory sense in the feet, hyperactive deep tendon reflexes, poor motor coordination, a positive
Romberg sign, anxiety, sleep disturbances, and slurred speech. She still had mild macrocytosis (MCV 101 fl). Despite ongoing cobalamin therapy and normal serum cobalamin, methylmalonic acid (MMA), and folate levels, her total homocysteine level (tHcy) was found to be 68 μmol/L (normal: 5–15).

Oral folic acid was added to her regimen and by the following week her tHcy had fallen to 8.6 μmol/L. Over the next few months, hyperhomocysteinemia nevertheless recurred, with levels reaching 133 μmol/L. Despite a change to weekly cobalamin injections and 6 mg of oral folic acid that further elevated her serum cobalamin and folate levels, tHcy levels subsequently rose again from 6.4 to 94.5 μmol/L. Her neurologic symptoms also progressed and tended to be worst when tHcy levels rose.

Family history was not suggestive of hereditary disorders, but her mother, who was diabetic, died with dementia at the age of 80 years. Her father, who was alcoholic, died of colon cancer. She had two children and no miscarriages. Both children were in good health and were later also shown to have normal blood counts and tHcy, cobalamin, and folate levels. The patient was a former smoker and characterized her alcohol intake as social. No exposure to toxins or nitrous oxide was identified.

At the time of her visit to our hospital, her medications included fenofibrate, gemfibrozil, amlodipine, omeprazole, and valsartan in addition to high doses of vitamins. Laboratory data showed the following: tHcy 30.0 μmol/L (normal: 3.3–12.4), cobalamin 1521 ng/L (normal: 250–950), folate 31.0 μg/L (normal: 2.5–20), vitamin B6 3.1 μg/L (normal: 3–20), unremarkable blood count and blood smear except for MCV of 104 fL, and normal lipid and chemistry panels (including renal function and blood smear except for MCV of 104 fL, and normal lipid and chemistry panels (including renal function). Genotyping for 11 common mutations related to methylenetetrahydrofolate reductase (MTHFR), methionine synthase, methionine synthase reductase (MSR), and cystathionine β-synthase was performed by Dr. Viktor Kožich of Charles University, Prague, Czech Republic. The results were unremarkable except for heterozygosity for the 1298A → C MTHFR mutation and the 66A → G MSR mutation.

Although our evaluation proved the presence of food-cobalamin malabsorption, this disorder usually causes only a mild cobalamin deficiency that responds completely to cobalamin therapy. She returned to be followed in her hometown, with 50 mg oral vitamin B6 added to her regimen. She appeared to respond again; tHcy fell to 13.5 μmol/L by her return visit the following month. However, despite continued vitamins, her physician’s monitoring showed that tHcy rose again within a week to 94.5 μmol/L and her symptoms worsened. Results of assays of remethylation and transsulfuration pathway intermediates tested during one of her cycles of hyperhomocysteinemia are shown in Table 1. The cycles continued with varying periodicities of several weeks despite the addition of betaine and riboflavin to her regimen.

Throughout her evaluation and the follow-up period, both the patient and her husband denied alcohol abuse on her part. At one point, her family conducted an unsuccessful search for hidden alcohol when told of continued suspicion of alcohol exposure, bolstered by finding an elevated serum carbohydrate-deficient transferrin (CDT) level, and the absence of any other explanation for her hyperhomocysteinemia. Soon thereafter, the patient and her husband severed medical contact. Several months later, she was hospitalized with acute pancreatitis, at which time she admitted to intermittent alcohol abuse that had increased sharply in late 1999 after her husband’s recovery (see patient 2). She is now abstinent, has no neurologic symptoms, and her tHcy levels have hovered consistently at approximately 4 μmol/L.

<table>
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<tr>
<th>Table 1. Plasma Concentrations of Homocysteine Remethylation and Transsulfuration Pathway Intermediates and Methylmalonic Acid at Low and Peak Homocysteine Levels in Patient 1*</th>
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*Both of the EDTA-anticoagulated samples were obtained while patient 1 was taking high doses of cobalamin and folate and had high serum vitamin levels. Plasma tHcy rose in the week after 8/10/00, presumably owing to heavy alcohol use (CDT levels had risen from 8.7% to 11.7%; normal <6.0%; and, reached a peak on 9/5/00. The patient became symptomatic by 8/30/00, which lasted until 9/10/00, the day before a visit to her neurologist. Her tHcy level fell to 10.6 μmol/L by 9/21/00. §All homocysteine-related analyses were done with HPLC methods using electrochemical detection. MMA assay, using gas chromatography-mass spectrometry, was done by Quest Diagnostics (Teterboro, NJ). AdoMet = S-adenosylmethionine, AdoHcy = S-adenosylhomocysteine, GSH = total glutathione, MMA = methylmalonic acid, tHcy = total homocysteine.
Patient 2

The 51-year-old husband of patient 1 was referred for further evaluation with his wife in May 2000, although he was clinically stable with a diagnosis of treated cobalamin deficiency. In early 1999, he had developed sleep disturbances (diagnosed as sleep apnea by polysomnography), nausea, vomiting, and tremors. These progressed to include ataxia requiring the use of a cane, enuresis, and mental changes that eventually required him to interrupt his work as a clinical psychologist. He was hospitalized in May 1999 when his tHcy level was found to be 76 μmol/L. “Cobalamin deficiency despite a low-normal serum cobalamin level” was diagnosed by his neurologist.

The patient had a striking past history of myocardial infarction in 1983, coronary artery bypass graft in 1989, and angioplasty in January 1999. He also had a past history of nephrolithiasis (reportedly non-cysteine), thoracotomy for a benign lung mass, chronic fatigue syndrome, and severe hypertriglyceridemia treated with lovastatin and gemfibrozil. Other medications included tixclopidine, nitrate, and relafen for headaches. The patient had been a heavy smoker until 1994 and considered himself a social drinker.

Family history was remarkable for his father’s myocardial infarction at the age of 33 years, but his father was still alive at the age of 70 years and his tHcy level was normal. Several paternal siblings had Alzheimer’s disease, one uncle having had dementia at an early age.

When hospitalized in 1999, the patient’s neurologic and psychiatric findings were similar to those his wife showed later. Results of extensive neurologic testing were normal although magnetic resonance imaging showed mild cortical atrophy. His blood count included a hemoglobin level of 13.2 g/dL and MCV of 108 fl; these had been 14.2 to 15.4 g/dL and 96 fl, respectively, in 1997. He had moderately abnormal liver function test results that were attributed to lovastatin toxicity or possibly alcohol effect. Other abnormal test results included elevated low-density lipoprotein and cholesterol levels and a slightly high ferritin level. Treatment included oral folate and monthly cobalamin injections. The patient recovered clinically, seemingly confirming the initial diagnosis of cobalamin deficiency, but the cause of cobalamin deficiency was not sought. Two months later his MCV had fallen to 101 fl, tHcy was 9.5 μmol/L, and his liver function test results were normal. However, serum lipid abnormalities persisted.

Evaluation at our hospital in 2000 showed tHcy of 10.7 μmol/L, cobalamin of 678 ng/L, folate >20 μg/L, and normal transcobalamin levels. He was taking 2 mg folic acid and 0.5 mg cobalamin orally daily; cobalamin injections had been stopped 8 months earlier. No abnormalities were found on physical and neurologic examination. Food-cobalamin absorption was normal (3.45% excretion in the egg yolk–cobalamin absorption test), which by extension also ruled out malabsorption of free cobalamin and the diagnosis of pernicious anemia. Genetic testing (see patient 1 description) showed homozygosity for the 677C → T MTHFR mutation; no other mutations were found.

The patient subsequently acknowledged heavy alcohol abuse during most of 1999 until his hospitalization and vitamin therapy. He is now asymptomatic. However, his MCV continues to be minimally elevated, his tHcy fluctuates between 9.1 and 17.6 μmol/L, and retrospective CDT assays of blood samples collected in 2000 showed occasional minimally elevated levels. These findings suggest that he continues occasional use of alcohol.

The Hidden Problem of Alcohol

Although the relationship between moderate alcohol ingestion and homocysteine status is complex and controversial (see references 6 and 7 for review), chronic alcohol abuse is increasingly recognized as a cause of elevated tHcy levels. Given the high frequency of alcohol abuse in surveys of patients, it may well be among the most common causes of both mild and severe hyperhomocysteinemia in patients and in the general population. Nevertheless, alcohol abuse is rarely considered in the assessment of hyperhomocysteinemia by most clinicians or in many studies and is frequently missing as a variable in surveys of homocysteine status.

Given the often episodic nature of alcohol use, the relatively rapid improvement in tHcy levels following its interruption, and the frequent reluctance of people to admit to alcohol abuse and physicians to pursue the possibility—all abundantly illustrated in our two cases—one can speculate further that normalization of tHcy levels owing to interruption or reduction of alcohol abuse may often be misattributed by clinicians to the concurrent interventions that they prescribe and by investigators to the interventions that they study. The misinterpreted “responses” to cobalamin and folate in the two patients presented here illustrate this. The improvement of tHcy noted in study subjects given placebo might have, at least in part, similar explanations.

Clinical Comments on the Two Cases

The severe hyperhomocysteinemia in both patients was originally attributed to cobalamin deficiency because they had severe neurologic dysfunction and macrocytosis, and patient 1 also had low cobalamin levels. In fact, however, the evidence for deficiency was weak in patient 1 (who may have had, at best, subclinical deficiency caused by food-cobalamin malabsorption) and nearly nonexistent in patient 2.
Several cautionary diagnostic notes are illustrated by the cases. One is the nonspecificity of elevated tHcy levels and, in patient 2, the hazards of overdiagnosing cobalamin deficiency on the basis of "low-normal" cobalamin levels. Another is the unfortunate trend for physicians not to search for the cause of deficiency when evaluating cobalamin-deficient patients. Malabsorptive disease underlies cobalamin deficiency in the vast majority of patients who have anemia or neurologic symptoms, and pursuing the gastroenterologic diagnosis serves many useful purposes.14

Ready evidence against cobalamin deficiency in our two patients was that neither one had megaloblastic changes, their macrocytosis persisted after therapy, the neurologic picture, although mostly consistent with cobalamin deficiency, included a few abnormalities atypical for that diagnosis, such as tremor and slurred speech, and MMA levels were not elevated. When the hyperhomocysteinemia and symptoms recurred in patient 1, alternative metabolic explanations were sought. Special concern was raised by the alarming neurologic dysfunction in both patients and by the premature cardiovascular disease and family histories of both dementia and heart disease in patient 2.

The neurologic symptoms were entirely due to alcohol toxicity, including withdrawal symptoms. Indeed, many laboratory findings that are sensitive although not specific for alcohol abuse were also present but were attributed to other reasons, especially in view of both patients' denials. These laboratory markers included the macrocytosis, abnormal transaminase and γ-glutamyl transferase levels, and perhaps even the hyperlipidemia. It is unclear to what extent, if any, the heterozygosity for an MTHFR mutation and an MSR mutation in patient 1, her use of fenofibrate, which is reported to elevate tHcy levels,15,16 or the homozygosity of patient 2 for the thermolabile MTHFR mutation influenced the magnitude of the hyperhomocysteinemia that the alcohol abuse produced.

**Mechanisms**

Elevated tHcy levels are well documented in experimental and clinical studies of chronic alcohol consumption and arise from direct and indirect effects of alcohol on one-carbon metabolism, including secondary deficiencies of folate and vitamin B6. Most information on the direct mechanisms of alcohol effects is derived from studies of animals fed ethanol while protected from vitamin deficiency (see references 7 and 17 for reviews).

Ethanol, or perhaps more specifically its immediate metabolic product, acetaldehyde, inhibits methionine synthase activity directly (Figure 1),18 thus providing a mechanism to produce hyperhomocysteinemia independent of vitamin status. In rats, there is a compensatory increase in betaine:homocysteine methyltransferase to generate methionine from homocysteine, although this might be more prominent in rodents than humans. Methionine levels vary but methionine adenosyltransferase (also called AdoMet synthetase) activity has been decreased in most but not all19 studies. As a result, hepatic AdoMet concentrations are diminished in most but not all20 animal studies. AdoHcy levels rise, and thus the AdoMet:AdoHcy ratio, an important determinant of methylation activity, has been consistently decreased.

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**Figure 1.** Diagram of the transmethylation and transsulfuration pathways involved in one-carbon metabolism. Vertical arrows indicate increased or decreased enzyme activities and metabolite concentrations resulting from independent effects of alcohol. THF = tetrahydrofolate, B12 = cobalamin, MS = methionine synthase, BHMT = betaine-homocysteine methyltransferase, AdoMet = S-adenosylmethionine, MTase = methyltransferase, AdoHcy = S-adenosylhomocysteine, SAHH = S-adenosylhomocysteine hydrolyase, CBS = cystathionine β-synthase, PL = phospholipid, DMG = N,N-dimethylglycine.
Acetaldehyde also accelerates the intracellular degradation of pyridoxal-5’-phosphate (PLP), resulting in decreased hepatic PLP content despite adequate dietary intake of vitamin B6.21 Because both cystathionine β-synthase and cystathionase lyase are PLP-dependent enzymes, alcohol metabolism can compromise homocysteine transsulfuration. Furthermore, nitric oxide production, secondary to elevated homocysteine, also inhibits methionine synthase activity, most likely by inactivating cob(II)alamin;22,23 this would further disrupt methionine synthase activity despite cobalamin supplementation. The methionine synthase inhibition also raises the possibility that “trapping” of folate as 5-methyltetrahydrofolate results, as suggested by studies in ethanol-fed rats.24–26

Human data are relatively sparse, and the picture in chronic alcoholics often is complicated by liver disease and/or dietary insufficiencies. Therefore, metabolic data obtained during exacerbation of hyperhomocysteinemia despite high-dose supplementation with cobalamin, folate, and vitamin B6 in patient 1 are of interest (Table 1), even though they are limited. The findings support those described in animals in suggesting that patient 1’s acute elevation of tHcy level was mediated by alcohol-induced inhibition of both transmethylation and transsulfuration pathways.

The 3.6-fold increase in AdoHcy levels that accompanied alcohol relapse in Table 1 is a new observation in alcoholic patients. Interestingly, the increase in AdoHcy parallels the patient’s fivefold increase in tHcy levels. Most likely, this reflects the equilibrium dynamics of the AdoHcy hydrolyase (or SAH hydrolase) reaction, which strongly favors AdoHcy synthesis over hydrolysis under conditions of elevated homocysteine.27 This interpretation is consistent with recent studies showing parallel increases in tHcy and AdoHcy in human plasma that can negatively affect essential cellular methylation reactions.28–30 Although unexpected, the increase in AdoMet levels in patient 1 (Table 1) is consistent with reduced AdoMet utilization secondary to AdoHcy product inhibition of most cellular methyltransferases (Figure 1), as previously described.31

Another unexpected observation was patient 1’s 50% reduction in plasma glutathione despite the fact that cystathionine β-synthase activity is upregulated by both oxidative stress32 and elevated AdoMet and AdoHcy.27 The depletion of glutathione despite presumed upregulation of cystathionine β-synthase suggests that alcohol-induced inactivation of vitamin B6 and/or oxidative stress diminished the capacity for glutathione regeneration in this patient.

The neurologic symptoms in both patients, which progressed in patient 1 despite high doses of cobalamin and folate and tended to peak during her hyperhomocysteinemia, also merit comment. Accumulating evidence suggests that high levels of homocysteine and/or its breakdown product, homocysteic acid, can induce neuronal cell damage and cell death by excitotoxic stimulation of N-methyl-D-aspartate receptors.33–35 Severe hyperhomocysteinemia has been associated with the withdrawal syndrome.10 Alcohol may also induce oxidative neuronal damage by promoting the generation of reactive oxygen species via stimulation of CYP2E1 and iNOS pathways in the brain.36,37 The expression of microsomal cytochrome p450 CYP2E1 is induced with chronic alcohol intake that exceeds the capacity of alcohol dehydrogenase and results in the release of hydroxethyl free radicals. The alcohol-induced increases in tHcy and CYP2E1 despite vitamin supplementation were likely contributors to the progression of neurologic symptoms in our two patients.

Mild Versus Severe Hyperhomocysteinemia
It is not known if the usually mild but occasionally severe hyperhomocysteinemia that accompanies chronic alcoholism represents simply a quantitative variation on the effect of modest alcohol use on homocysteine metabolism or a qualitative difference. This question cannot be resolved at present because the nature of the interaction between “normal” alcohol ingestion and homocysteine is poorly understood. Direct, inverse, and U-shaped correlations between alcohol intake and tHcy levels have been described, and they may be modified by the type of alcoholic drink, vitamin status, and genetic or other factors.6,7,38–40

The circumstances that convert mild into severe hyperhomocysteinemia in some alcoholics also remain unknown. The observations in patient 1 suggest that cobalamin, folate, or vitamin B6 deficiencies are unlikely explanations. Severity of liver disease does not seem to be a factor either, as noncirrhotic patients can have severe hyperhomocysteinemia.8,10 However, it cannot be excluded that the type of liver dysfunction might play a role (e.g., fatty liver or alcoholic hepatitis). It may also be relevant that most reports of severe hyperhomocysteinemia have been in studies conducted in detoxification wards.8,10 It has been suggested that severe hyperhomocysteinemia correlates with serum ethanol levels and is associated with alcohol withdrawal.10 As discussed in the earlier section, homocysteic acid and other homocysteine derivatives may have a potential neuroexcitatory role. Future studies will need to determine whether the perceived association between severe hyperhomocysteinemia and alcohol withdrawal is simply a temporal one or mechanistic.

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