DOI 10.1515/cclm-2012-0308 — Clin Chem Lab Med 2013; 51(3): 457–465

Review

Charles H. Halsted*

B-Vitamin dependent methionine metabolism and alcoholic liver disease

Abstract

Convincing evidence links aberrant B-vitamin dependent hepatic methionine metabolism to the pathogenesis of alcoholic liver disease (ALD). This review focuses on the essential roles of folate and vitamins B6 and B12 in hepatic methionine metabolism, the causes of their deficiencies among chronic alcoholic persons, and how their deficiencies together with chronic alcohol exposure impact on aberrant methionine metabolism in the pathogenesis of ALD. Folate is the dietary transmethylation donor for the production of S-adenosylmethionine (SAM), which is the substrate for all methyltransferases that regulate gene expressions in pathways of liver injury, as well as a regulator of the transsulfuration pathway that is essential for production of glutathione (GSH), the principal antioxidant for defense against oxidative liver injury. Vitamin B12 regulates transmethylation reactions for SAM production and vitamin B6 regulates transsulfuration reactions for GSH production. Folate deficiency accelerates the experimental development of ALD in ethanol-fed animals while reducing liver SAM levels with resultant abnormal gene expression and decreased production of antioxidant GSH. Through its effects on folate metabolism, reduced SAM also impairs nucleotide balance with resultant increased DNA strand breaks, oxidation, hepatocellular apoptosis, and risk of carcinogenesis. The review encompasses referenced studies on mechanisms for perturbations of methionine metabolism in ALD, evidence for altered gene expressions and their epigenetic regulation in the pathogenesis of ALD, and clinical studies on potential prevention and treatment of ALD by correction of methionine metabolism with SAM.

Keywords: alcohol; folate; S-adenosylmethionine; vitamin B6; vitamin B12.

***Corresponding author: Charles H. Halsted,** MD, Department of Internal Medicine, University of California Davis, Davis, CA 95616, USA, E-mail: chhalsted@ucdavis.edu

Introduction

Chronic alcoholism accounts for nearly 5% of all deaths worldwide, mainly as a consequence of alcoholic liver disease (ALD) that occurs in about 30% of active alcohol consumers [1]. Although ALD was originally considered to be a nutritional disorder resulting from poor diet in chronic alcohol addicts, experiments that originated over 40 years ago [2] have established that alcohol is a specific toxin to the liver which triggers an inflammatory response through a complex pattern of molecular signaling that results in lipid accumulation, inflammation, cell death, and collagen deposition leading to diffuse scarring of the liver known as cirrhosis [3]. Recent extensive and ongoing work has established that alcohol has profound effects on the metabolism of methionine in the liver that ultimately regulate antioxidant defense and the expressions of genes related to liver injury [4, 5]. Furthermore, the methionine metabolic cycle is tightly regulated by the supply of three essential B-vitamins. These include folate which is the original methyl donor for the production of S-adenosylmethionine (SAM), the substrate for all DNA and histone methyltransferases including those involved in activation of liver injury genes; vitamin B12 or cobalamin which is the co-factor for methionine synthase (MS); and vitamin B6 or pyridoxine, which is the cofactor for cystathionine-β-synthase (CβS) and cystathionase, which are the initial transsulfuration reactions in the production of the antioxidant glutathione (GSH) (Figure 1).

Methionine cycle and effects of ethanol

Relevant pathways of folate metabolism and the transmethylation and transsulfuration pathways of methionine metabolism are detailed in Figure 1. As shown, the original methyl donor 5-methyltetrahydrofolate (5-MTHF) is derived both from dietary folate and endogenously from tetrahydrofolate (THF) by way of 5,10-methylene

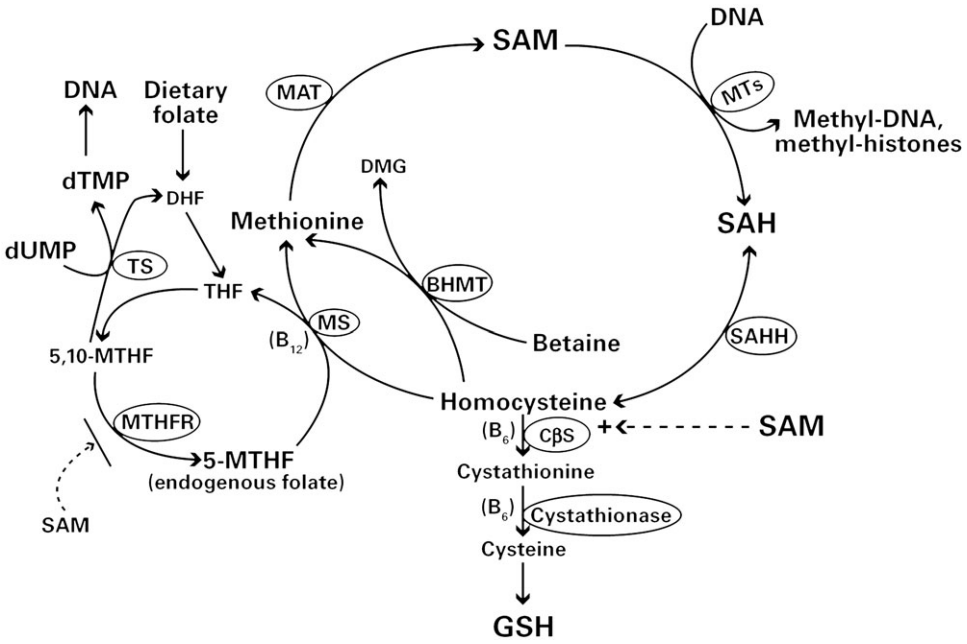

Figure 1 Folate and methionine metabolism in the liver.
Folate metabolism includes DHF (dihydrofolate), THF (tetrahydrofolate); 5,10-MTHF, 5,10 methylene tetrahydrofolate; MTHFR (methylene tetrahydrofolate reductase) and 5-MTHF (5-methyltetrahydrofolate), which is the original methyl donor for transmethylation reactions. DNA synthesis and stability is regulated by TS (thymidylate synthase) and the balance of its substrate dUMP (deoxyuridine monophosphate) and product dTMP (deoxythymidine monophosphate). In transmethylation reactions, 5-MTHF and homocysteine are substrates for vitamin B12 dependent MS (methionine synthase) in production of methionine, which is metabolized to SAM (S-adenosylmethionine) by MAT (methionine adenosyl transferase). Methionine also can be produced from betaine and homocysteine by BHMT (betaine homocysteine methyl transferase) with additional product DMG (di-methylglycine). SAM is the methyl donor for all MTs (methyltransferase reactions), producing methylated DNA and histones as well as other methylated compounds. SAH (S-adenosylhomocysteine) is both product and inhibitor of MT reactions, and substrate for the bidirectional enzyme SAHH (SAH hydrolase) which produces homocysteine when SAH is in excess or SAH when homocysteine is in excess. In transsulfuration reactions, homocysteine is metabolized by vitamin B6 dependent and SAM up-regulated CβS (cystathionine beta synthase) and cystathionase to produce cysteine and the antioxidant GSH (glutathione). Note also that SAM down-regulates MTHFR and up-regulates CβS. Hence SAM deficiency increases production of 5-MTHF while reducing 5,10-MTHF, but reduces production of GSH.

tetrahydrofolate (5,10 MTHF) and methylene tetrahydrofolate reductase (MTHFR). In addition to its role as substrate for MTHFR, 5,10-MTHF is substrate for thymidylate synthase (TS) which maintains DNA nucleotide balance by regulating the levels of deoxyuridine monophosphate (dUMP) and deoxythymidine monophosphate (dTMP). DNA nucleotide imbalance is secondary to decreased availability of 5,10-MTHF and is associated with apoptosis, or hepatocyte death secondary to DNA strand breaks and oxidation, as was observed in ethanol-fed micropigs and rats [6–8]. The level of 5,10-MTHF is controlled in part by SAM which provides negative regulation of MTHFR activity. Hence, when SAM is low, as may occur with folate deficiency in chronic alcoholism, MTHFR activity is augmented, thereby reducing 5,10-MTHF and increasing the risk of nucleotide imbalance, apoptosis, and carcinogenesis [6–8].

Folate in the form of 5-MTHF is the original methyl donor through its subsequent entry into the transmethylation reaction as substrate with homocysteine for the vitamin B12-dependent reaction MS in the production of methionine and subsequently SAM. When MS expression and activity are inhibited by alcohol exposure, betaine becomes an alternate substrate for betaine homocysteine methyltransferase (BHMT) that converts homocysteine to methionine and produces dimethylglycine (DMG) [9]. Recent work in ethanol-fed rats showed that the co-administration of betaine specifically prevented the development of oxidative injury processes through reduction in homocysteine and preservation of normal SAM levels [10]. Methionine, the product of either MS or BHMT, is the substrate for hepatic methionine adenosyl transferase (MAT) which exists in gene form predominantly as *MAT1A* in progressive alcoholic liver injury and as *MAT2A* after malignant transformation [8]. Whereas *MAT1A* knockout mice develop progressive liver injury [11], similar findings occur in animal models where the expression of *MAT1A* is reduced in part due to the

effects of nitrosylation of one of the cysteine residues in the translated form of the enzyme [12].

SAM, the product of MAT, is the major methyl donor for all methyltransferase (MT) reactions, including those for DNA and histones and many other compounds, which are inhibited by their product S-adenosylhomocysteine (SAH). The ratio of SAM to SAH is a convenient index of gene methylation capacity since the Km values for most methyltransferases are similar to the Ki of inhibitory SAH [13]. In addition to its down-regulation of the MTHFR reaction, SAM promotes the transsulfuration pathway by up-regulation of the activity of CβS [14]. SAH levels are also regulated by SAH hydrolase (SAHH), a bidirectional enzyme that reduces SAH in production of homocysteine in the forward direction or increases SAH in the reverse direction when homocysteine levels are elevated. Summarizing, homocysteine levels are regulated by three different enzymatic reactions including vitamin B12 dependent MS, bidirectional SAHH, and CβS, which is the initial reaction in the transsulfuration pathway for the production of the antioxidant GSH. Both CβS and the subsequent rate limiting enzyme cystathionase are regulated by vitamin B6.

Hepatic methionine metabolism is profoundly affected by chronic alcoholism and experimental exposure to ethanol feeding. A clinical study comparing gene expressions in liver biopsies from ALD patients with those in control normal liver biopsies found decreased transcripts of *MS*, *MAT1A* and *CβS* which could account for observed reduced levels of hepatic SAM and GSH [15]. A subsequent study of livers from ethanol-fed micropigs that developed ALD with a folate deficient diet found decreased transcript expressions of *MTHFR*, *MS*, *MAT1A*, and *SAHH* [16], which accounted for reduced hepatic levels of SAM and the SAM/SAH ratio with increased levels of homocysteine and SAH in this animal model [7]. Whereas two studies found excellent correlations of liver SAM and GSH levels in experimental ALD [7, 17], others recently linked ethanol induced oxidative liver injury in a rat model to reduced SAM and GSH levels that were corrected by inhibition of the ethanol oxidizing enzyme cytochrome P4502E1 (CYP2E1), which also induces reactive oxygen species (ROS) [18]. Summarizing, the data from all these studies provide a strong case for the concept that clinical and experimental ethanol-induced ALD is promoted by alterations in hepatic methionine metabolism, with three potential mechanisms of reduced production of antioxidant GSH secondary to reduced levels of SAM and its regulatory effect on the transsulfuration pathway, reduced levels of SAM and the SAM/SAH methylation ratio that carries potential for profound epigenetic dysregulation of genes relevant to alcoholic liver injury, and the effect of reduced SAM on folate and DNA metabolism by decreasing the availability of substrate 5,10-MTHF for accurate nucleotide balance in DNA synthesis. Each of these mechanisms that involve reduced SAM can be aggravated by B-vitamin deficiency, while reduced vitamin B6 would predictably decrease GSH production through decreased activity of CβS and cystathionase (Figure 1).

Evidence and mechanisms for B-vitamin deficiencies in chronic alcoholism

The associations of B-vitamin deficiencies with chronic alcoholism has been known for over 45 years, when a survey of 140 consecutive patients with ALD admitted to a large municipal hospital in the USA found low serum levels of folate in 78%, of vitamin B6 in 60%, and of vitamin B12 in 25% [19]. A contemporary publication described low serum folate in 80% of chronic alcoholic patients admitted to the Boston City Hospital, which was ascribed mainly to poor diet and was unrelated to the presence or absence of ALD [20]. More recently, chronic alcoholics in Portugal were found to have significantly lower red blood cell and serum vitamin B6 levels, but, paradoxically, higher serum vitamin B12 levels together with higher serum homocysteine levels than found in healthy control subjects [21]. Since the onset of folic acid fortification of the US diet in 1998, the population incidence of low serum and red blood cell folate levels has decreased from 24% and 3% to <1% for each [22]. A recent post-folic acid fortification study of 77 intoxicated patients in an emergency room setting found none with low serum folate or vitamin B12 levels [23]. There is no reported evidence to date that folic acid fortification of the diet has reduced the incidence of ALD. However, our recent controlled study found significantly lower levels of serum folate and B6 but higher B12 levels in 40 ALD patients compared to matched healthy control subjects, suggesting a significant effect of associated liver disease on the status of these vitamins [24].

Normal folate physiology

Dietary folate consists of folic acid that may be oxidized or reduced and methylated as 5-MTHF and is bound in gamma peptide linkage with up to seven glutamate residues [25]. As depicted in Figure 2, the metabolism

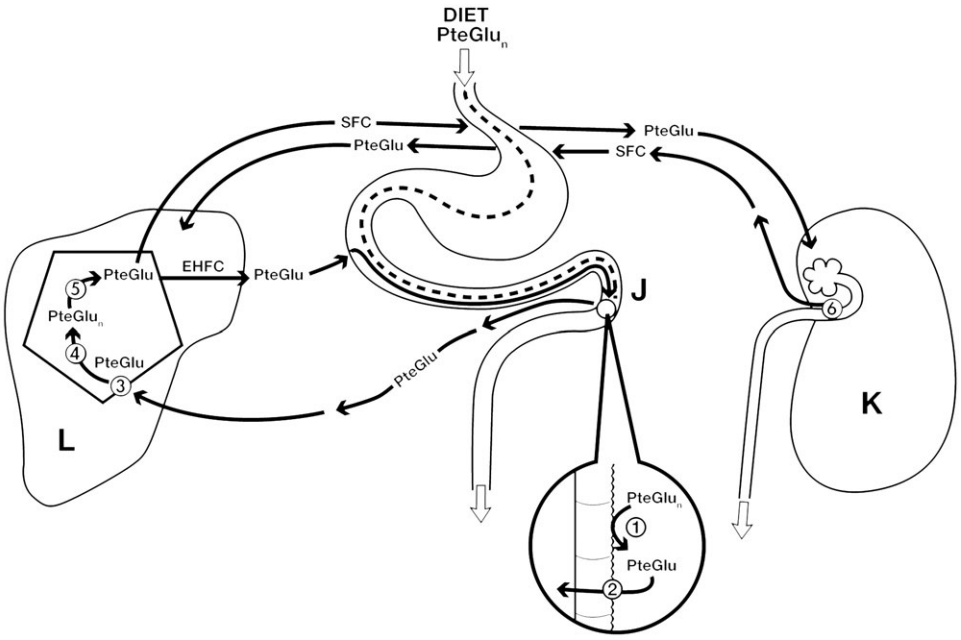

Figure 2 Folate physiology.
Folate is present in the diet as a mixture of oxidized, reduced, and methylated pteroylpolyglutamates (PteGlu$_n$), which are hydrolyzed at the jejunal (J) brush border membrane by glutamate carboxypeptidase II (1). The derivative pteroylmonoglutamates (PteGlu) are transported across the same membrane (2) by the synergy of the reduced folate carrier and proton coupled folate transporter. PteGlu are transported through the portal vein and cross the liver (L) hepatocyte membranes (3) most likely by folate binding protein. Subsequently, PteGlu reacts with PteGlu synthetase (4) to reconstitute PteGlu$_n$ as the storage folate form in the liver. Subsequent hydrolysis of storage PteGlu$_n$ (5) produces methylated PteGlu for transport through the enterohepatic folate circulation (EHFC) or systemic folate circulation (SFC). Whereas all but 1% of folate is re-absorbed back to the liver from the EHFC, 90% of folate circulating through the kidney (K) is reabsorbed by the renal tubular membrane (6) probably through the synergy of reduced folate carrier and proton coupled folate transporter mechanisms.

of dietary folate involves many regulated steps, including the initial digestion of dietary pteroylpolyglutamates (PteGlu$_n$) to their pteroylmonoglutamate (PteGlu) forms of folic acid or 5-MTHF by the intestinal brush border enzyme glutamate carboxypeptidase II (GCPII) [26], which is then transported across the apical brush border and the basolateral membranes of the enterocytes by the reduced folate carrier (RFC) [25]. More recent evidence indicates the additional presence of the proton coupled folate transporter (PCFT) in the intestinal brush border membrane [27]. Since RFC activity is optimal at a neutral pH and PCFT at an acid pH, it is likely that the two transporters act in concert to move monoglutamyl folate from the neutral luminal contents across the acid microclimate of the brush border membrane [28]. Once at the hepatocyte membrane in the liver, the optimal pH for folate transport is 5.0 [29], which is consistent with a folate binding protein (FBP) mechanism known to be present in mammalian liver [30]. A complex sequence of re-synthesis of PteGlu$_n$ forms of folate for hepatic storage is followed by hydrolysis to PteGlu as 5-MTHF for subsequent transport to both bile and systemic circulation [25]. All but about 1% of biliary folate is reabsorbed and taken back up by the liver, then secreted back to the systemic circulation [31]. Within the kidney, 5-MTHF is conserved by active re-absorption by renal tubular cells in an active process that is mediated by both RFC [32] and PCFT [33]. Therefore, there are multiple potential causes of folate deficiency that include dietary lack, intestinal malabsorption, altered hepatic uptake and metabolism, and reduced re-absorption by renal tubular cells. Each one of the processes can be involved in the chronic alcoholic state.

Folate malabsorption in chronic alcoholism

Using methods of measuring radioactivity in the systemic circulation or its uptake from perfused jejunum, our group determined that ^{3}H-labeled folic acid was poorly absorbed in malnourished chronic alcoholic patients, whereas absorption could be normalized by 2 weeks of abstinence with a healthy diet [34, 35]. A subsequent study in chronic ethanol-fed macaque monkeys found increased fecal excretion of radioactivity after oral administration of ^{3}H-labeled folic acid consistent with folate malabsorption [36]. Mechanistic studies found decreased activity of GCPII

in jejunal brush border vesicles isolated from ethanol-fed miniature pigs [37] as well as reduced ^{3}H-folic acid transport by jejunal brush border vesicles and reduced transcript levels of *RFC* in ethanol-fed micropigs compared to control diet fed animals [38]. Summarizing these studies, folate malabsorption was proven in chronic alcoholic patients and in both ethanol-fed monkey and pig models according to a mechanism that includes decreased activity of GCPII and expression and activity of jejunal brush border *RFC*.

Effects of alcohol on folate metabolism in the liver

Whereas the liver is the major storage site for folate in the body [39], the amount of folate stored in the liver is markedly reduced in ALD according to a clinical study in which the time frame for development of megaloblastic anemia while ingesting a folate free diet was reduced by half compared to that found in a healthy subject [40, 41]. Decreased liver folate stores in ALD could result from decreased uptake or from abnormal intrahepatic metabolism of the vitamin. Studies in the chronic ethanol-fed macaque monkeys with liver folate deficiency showed decreased hepatic uptake of ^{3}H following intramuscular ^{3}H-folic acid but a normal distribution pattern of intrahepatic $PteGlu_n$ folates [42].

Effects of alcohol on renal excretion of folate

Increased urinary folate excretion contributes to folate deficiency, as documented in chronic alcoholic subjects [43], ethanol-fed rats [44] and macaque monkeys [45]. Whereas these observations can be explained by an effect of acute ethanol on decreasing renal tubular uptake and transport of folate in rats [46], long-term ethanol exposure in micropigs had no effect on the expression of renal *RFC* [38].

Effects of alcoholism on vitamin B6 and B12 status

Vitamin B6 deficiency occurs in about two-thirds of chronic alcoholics with liver disease with overall significantly lower levels than in normal subjects [21, 47]. One of the principal clinical manifestations of vitamin B6 deficiency in alcoholism is sideroblastic anemia as was found by bone marrow examination in 23% of a sequential series of hospitalized anemic chronic alcoholic patients [48]. Vitamin B6 circulates in the form of protein bound pyridoxal phosphate, and the most widely accepted etiology for vitamin B6 deficiency is the degrading effect of the acetaldehyde product of alcohol metabolism on this compound with subsequent urinary wastage of the free vitamin [49, 50]. By contrast, circulating levels of vitamin B12 are often elevated in ALD [21, 24], even though its absorption from the intestine is compromised by chronic alcoholism [51]. This apparent conundrum was resolved by a clinical study of patients with active ALD that showed reduced liver vitamin B12 levels in spite of elevated serum levels of vitamin B12 and its analogs, suggesting an inability of compromised hepatocytes to retain vitamin B12 stores [52]. The deficiency of vitamin B12 in the liver of ALD patients is critical to substantiating its potential role in aberrant methionine metabolism.

Aberrant hepatic methionine metabolism in the pathogenesis of alcoholic liver disease

Whereas B-vitamin deficiencies are common in chronic alcoholism, continuously emerging data indicate that these deficiencies and their impacts on hepatic methionine metabolism may play highly significant roles in the pathogenesis of ALD. For example, a study of intragastric ethanol-fed mice found elevated plasma homocysteine and activations of endoplasmic reticulum (ER) stress pathways for steatosis and apoptosis, all of which could be prevented by co-administration of betaine [53], while the co-administration of SAM to ethanol-fed baboons increased hepatic GSH while attenuating liver pathology of ALD [17]. Studies of hepatocytes from ethanol-fed mice associated elevated SAH levels with increased sensitization of injury pathways to tumor necrosis factor (TNFα) [54]. The inclusion of dietary folate deficiency with ethanol feeding of micropigs accelerated the onset of the pathology of ALD while increasing plasma homocysteine levels and decreasing liver SAM, the SAM to SAH ratio and GSH levels [7], as well as reducing the expressions of relevant transmethylation enzymes [16]. Subsequent studies of livers from the same ethanol and control diet fed micropigs demonstrated augmenting effects of the folate deficient diets on the expressions of ethanol-induced enzymes involved in ER stress pathways of lipogenesis and apoptosis [55].

Additional studies found that the pathology of ALD, abnormalities in hepatic methionine metabolism, and activations of ER stress pathways of lipogenesis and apoptosis and of oxidative liver injury could be prevented by the co-administration of SAM with ethanol in folate deficient diets in micropigs [56, 57]. The positive effects of SAM on ALD prevention could be ascribed to promotion of antioxidative GSH levels [57] and reducing the expressions of genes relevant to steatosis [56] and oxidative liver injury [56, 57].

Aberrant hepatic methionine metabolism in the epigenetic regulation of ALD

Accumulating evidence indicates that pathways of alcoholic liver injury are regulated by epigenetic mechanisms that are influenced by intrahepatic methylation reactions. Since SAM is the principal donor for methylation reactions, ethanol induced alterations in hepatic methionine metabolism and the SAM to SAH methylation ratio are directly germane to these epigenetic phenomena. According to recent reviews, epigenetic regulation in ALD involves both DNA and histone methylation that can be altered by ethanol exposure [58, 59]. For example, chronic ethanol-fed rats demonstrated decreased SAM levels and global DNA methylation that was also associated with DNA strand breaks [8]. The expressions of several histone MTs in isolated rat hepatocytes were affected by ethanol exposure, resulting in activations of histone residues at H3K4 and suppression at H3K9 that altered the expressions of genes relevant to alcoholic liver injury [60]. Our recent study of ethanol-fed CβS deficient mice demonstrated the association of activated ER stress gene pathways with reduced expression of the histone methyltransferase G9 and reduced levels of lysine residue H3K9 that affected promoter regions of relevant genes according to chromatin histone immunoprecipitation assays [61].

Efficacy of SAM in the treatment of ALD

Whereas numerous studies in ethanol-fed rodents, baboons, and micropigs have demonstrated positive preventive effects of methyl donors such as betaine [10, 53, 62–65] or SAM [17, 56, 57] in experimental ALD, there is no consensus on whether either compound is effective in reversing liver injury in the treatment of established clinical ALD. An Italian study of 17 ALD patients showed that 6 months of SAM at 1.2 g per day was effective in raising liver GSH levels, but did not report on its potential effects on histopathology [66]. A subsequent 2-year multicenter European study of 123 ALD patients randomized to receive placebo or SAM in the same dose showed reduction in mortality or liver transplant incidence in the SAM group, but this was only significant only after excluding the most severely ill patients from the analysis [67]. A later Cochrane meta-analysis of data from 434 patients in nine clinical trials found no differences in efficacy between SAM or placebo in treatment of clinical ALD [68]. Of note, none of these clinical trials reported on treatment effects on liver histopathology, which should be presumed to be the gold standard for demonstrated efficacy.

Recently, our group reported on a 6 month trial on the efficacy of SAM at 1.2 g/day in the treatment of ALD. Unlike the prior studies noted above, our additional goals were to determine potential mechanisms for SAM efficacy and its potential effects on biochemical parameters at 2–4 week intervals and on the histopathology of ALD according to liver biopsies that were obtained at baseline and at completion of the study [69]. An initial baseline study compared serum B-vitamin levels and methionine metabolites in 40 ALD patients, 26 chronic and actively drinking alcoholics without liver disease, and 28 healthy control subjects [24]. Whereas serum folate levels were relatively decreased in both alcoholic groups compared to controls, serum vitamin B6 levels were lower and vitamin B12 levels were elevated only in the ALD group compared to findings in the other two groups. Evaluation of methionine metabolites found elevated serum homocysteine and SAH levels in both alcoholic groups, whereas serum SAM levels were elevated in ALD patients compared to the other groups. Additionally, serum cystathionine was elevated and its ratio to α-aminobutyrate, the product of the vitamin B6-dependent cystathionase reaction, was decreased in the ALD group and was predictive of the presence of early cirrhosis in a subset of 24 ALD patient liver biopsies. Summarizing, this initial analysis demonstrated significant vitamin B6 deficiency in ALD patients that was associated with decreased cystathionase activity (Figure 1) which correlated with the severity of liver disease.

In the subsequent clinical study, 26 of 37 enrolled ALD patients completed a 24 week trial, receiving either SAM at a dose of 1.2 g per day or size, shape, and color matched placebo pills each in three daily doses [69]. All subjects were required to remain abstinent during the trial and both baseline and end-point liver biopsies were

obtained in six and eight subjects in each group. While levels of liver function tests improved with time in all patients, consistent with an abstinence effect, there were no group differences in this clinical response to either regimen. While there were no differences between the two groups in levels of serum SAH or homocysteine levels over time, SAM serum levels were increased during treatment compared to baseline, indicative of an adequate systemic response. Comparing liver histopathology scores in baseline and end-point biopsies, there were no changes of total scores within or between the two groups, although slight improvement in steatosis and inflammation was found in the placebo group but not in the SAM group. Summarizing these data, SAM was ineffective as a treatment for ALD in our study according to biochemical and histological parameters. Potential reasons for these negative results include relatively short 6 month period of treatment compared to the prior 2-year multicenter trial [67], and a 30% dropout rate due to recidivism of alcoholism during the course of the treatment protocol. The finding of elevated serum SAM levels in both groups at baseline suggests incomplete hepatic retention and/or subsequent abnormal intrahepatic metabolism of SAM that would be required for a potential clinical effect.

Summarizing these clinical studies, there is no consensus on the efficacy of SAM in the treatment of clinical ALD, contrary to expectations from effective preventive trials in experimental ethanol-fed animal models. Prior extensive clinical trials did not assess changes in histopathology. Our recent clinical studies demonstrated a potentially significant role of vitamin B6 in the pathogenesis of ALD, suggesting the importance of assuring adequate levels of this vitamin in future treatment trials. Lack of efficacy of SAM in treatment of ALD may reflect inability of damaged hepatocytes to retain and metabolize this compound in such a way as to improve parameters of alcoholic liver injury.

Conflict of interest statement

Author's conflict of interest disclosure: The author stated that there are no conflicts of interest regarding the publication of this article.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.

Received May 16, 2012; accepted July 23, 2012; previously published online August 24, 2012

References

1. Gao B, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. Gastroenterology 2011;141:1572–85.
2. Lieber CS, Jones DP, Decarli LM. Effects of prolonged ethanol intake: production of fatty liver despite adequate diets. J Clin Invest 1965;44:1009–21.
3. Lucey MR, Mathurin P, Morgan TR. Alcoholic hepatitis. N Engl J Med 2009;360:2758–69.
4. Halsted CH. Nutrition and alcoholic liver disease. Semin Liver Dis 2004;24:289–304.
5. Kharbanda KK. Alcoholic liver disease and methionine metabolism. Semin Liver Dis 2009;29:155–65.
6. Halsted CH, Villanueva J, Chandler CJ, Stabler SP, Allen RH, Muskhelishvili L, et al. Ethanol feeding of micropigs alters methionine metabolism and increases hepatocellular apoptosis and proliferation. Hepatology 1996;23:497–505.
7. Halsted CH, Villanueva JA, Devlin AM, Niemela O, Parkkila S, Garrow TA, et al. Folate deficiency disturbs hepatic methionine metabolism and promotes liver injury in the ethanol-fed micropig. Proc Natl Acad Sci USA 2002;99:10072–7.
8. Lu SC, Huang ZZ, Yang H, Mato JM, Avila MA, Tsukamoto H. Changes in methionine adenosyltransferase and S-adenosylmethionine homeostasis in alcoholic rat liver. Am J Physiol Gastrointest Liver Physiol 2000;279:G178–85.
9. Trimble KC, Molloy AM, Scott JM, Weir DG. The effect of ethanol on one-carbon metabolism: increased methionine catabolism and lipotrope methyl-group wastage. Hepatology 1993;18:984–9.
10. Kharbanda KK, Todero SL, King AL, Osna NA, McVicker BL, Tuma DJ, et al. Betaine treatment attenuates chronic ethanol-induced hepatic steatosis and alterations to the mitochondrial respiratory chain proteome. Int J Hepatol 2012;2012:962183.
11. Lu SC, Alvarez L, Huang ZZ, Chen L, An W, Corrales FJ, et al. Methionine adenosyltransferase 1A knockout mice are predisposed to liver injury and exhibit increased expression of genes involved in proliferation. Proc Natl Acad Sci USA 2001;98:5560–5.
12. Ruiz F, Corrales FJ, Miqueo C, Mato JM. Nitric oxide inactivates rat hepatic methionine adenosyltransferase in vivo by S-nitrosylation. Hepatology 1998;28:1051–7.
13. Clarke S, Banfield K. S-adenosylmethionine-dependent methyltransferases. In: Carmel R, Jacobsen D, editors. Homocysteine in health and disease Cambridge: Cambridge University Press, 2001:63–78.
14. Prudova A, Bauman Z, Braun A, Vitvitsky V, Lu SC, Banerjee R. S-adenosylmethionine stabilizes cystathionine beta-synthase and modulates redox capacity. Proc Natl Acad Sci USA 2006;103:6489–94.
15. Lee TD, Sadda MR, Mendler MH, Bottiglieri T, Kanel G, Mato JM, et al. Abnormal hepatic methionine and glutathione metabolism in patients with alcoholic hepatitis. Alcohol Clin Exp Res 2004;28:173–81.
16. Villanueva JA, Halsted CH. Hepatic transmethylation reactions in micropigs with alcoholic liver disease. Hepatology 2004;39:1303–10.

17. Lieber CS, Casini A, DeCarli LM, Kim CI, Lowe N, Sasaki R, et al. S-adenosyl-L-methionine attenuates alcohol-induced liver injury in the baboon. Hepatology 1990;11:165–72.
18. Zhuge J, Cederbaum AI. Depletion of S-adenosyl-l-methionine with cycloleucine potentiates cytochrome P450 2E1 toxicity in primary rat hepatocytes. Arch Biochem Biophys 2007;466:177–85.
19. Leevy CM, Cardi L, Frank O, Gellene R, Baker H. Incidence and significance of hypovitaminemia in a randomly selected municipal hospital population. Am J Clin Nutr 1965;17:259–71.
20. Herbert V, Zalusky R, Davidson CS. Correlation of folate deficiency with alcoholism and associated macrocytosis, anemia, and liver disease. Ann Intern Med 1963;58:977–88.
21. Cravo ML, Camilo ME. Hyperhomocysteinemia in chronic alcoholism: relations to folic acid and vitamins B(6) and B(12) status. Nutrition 2000;16:296–302.
22. Pfeiffer CM, Hughes JP, Lacher DA, Bailey RL, Berry RJ, Zhang M, et al. Estimation of trends in serum and RBC folate in the U.S. population from pre- to postfortification using assay-adjusted data from the NHANES 1988–2010. J Nutr 2012;142:886–93.
23. Li SF, Jacob J, Feng J, Kulkarni M. Vitamin deficiencies in acutely intoxicated patients in the ED. Am J Emerg Med 2008;26: 792–5.
24. Medici V, Peerson JM, Stabler SP, French SW, Gregory JF 3rd, Virata MC, et al. Impaired homocysteine transsulfuration is an indicator of alcoholic liver disease. J Hepatol 2010;53:551–7.
25. Shane B. Folate metabolism. In: Picciano M, Stokstad ELR, Gregory JF, editors. Folic acid metabolism in health and disease. New York, NY: Wiley, Liss, 1990:65–8.
26. Halsted CH, Ling EH, Luthi-Carter R, Villanueva JA, Gardner JM, Coyle JT. Folylpoly-gamma-glutamate carboxypeptidase from pig jejunum. Molecular characterization and relation to glutamate carboxypeptidase II. J Biol Chem 1998;273:20417–24.
27. Qiu A, Jansen M, Sakaris A, Min SH, Chattopadhyay S, Tsai E, et al. Identification of an intestinal folate transporter and the molecular basis for hereditary folate malabsorption. Cell 2006;127:917–28.
28. Said HM. Intestinal absorption of water-soluble vitamins in health and disease. Biochem J 2011;437:357–72.
29. Horne DW, Reed KA, Hoefs J, Said HM. 5-Methyltetrahydrofolate transport in basolateral membrane vesicles from human liver. Am J Clin Nutr 1993;58:80–4.
30. Van Hoozen CM, Ling EH, Halsted CH. Folate binding protein: molecular characterization and transcript distribution in pig liver, kidney and jejunum. Biochem J 1996;319:725–9.
31. Steinberg SE, Campbell CL, Hillman RS. The role of the entero-hepatic cycle in folate supply to tumour in rats. Br J Haematol 1982;50:309–16.
32. Sikka PK, McMartin KE. Determination of folate transport pathways in cultured rat proximal tubule cells. Chem Biol Interact 1998;114:15–31.
33. Subramanian VS, Marchant JS, Said HM. Apical membrane targeting and trafficking of the human proton-coupled transporter in polarized epithelia. Am J Physiol Cell Physiol 2008;294:C233–40.
34. Halsted CH, Griggs RC, Harris JW. The effect of alcoholism on the absorption of folic acid (H3-PGA) evaluated by plasma levels and urine excretion. J Lab Clin Med 1967;69:116–31.
35. Halsted CH, Robles EA, Mezey E. Decreased jejunal uptake of labeled folic acid (3 H-PGA) in alcoholic patients: roles of alcohol and nutrition. N Engl J Med 1971;285:701–6.
36. Romero JJ, Tamura T, Halsted CH. Intestinal absorption of [3H] folic acid in the chronic alcoholic monkey. Gastroenterology 1981;80:99–102.
37. Naughton CA, Chandler CJ, Duplantier RB, Halsted CH. Folate absorption in alcoholic pigs: in vitro hydrolysis and transport at the intestinal brush border membrane. Am J Clin Nutr 1989;50:1436–41.
38. Villanueva JA, Devlin AM, Halsted CH. Reduced folate carrier: tissue distribution and effects of chronic ethanol intake in the micropig. Alcohol Clin Exp Res 2001;25:415–20.
39. Herbert V. Predicting nutrient deficiency by formula. N Engl J Med 1971;284:976–7.
40. Eichner ER, Hillman RS. The evolution of anemia in alcoholic patients. Am J Med 1971;50:218–32.
41. Herbert V. Experimental nutritional folate deficiency in man. Trans Assoc Am Physicians 1962;75:307–20.
42. Tamura T, Romero JJ, Watson JE, Gong EJ, Halsted CH. Hepatic folate metabolism in the chronic alcoholic monkey. J Lab Clin Med 1981;97:654–61.
43. Russell RM, Rosenberg IH, Wilson PD, Iber FL, Oaks EB, Giovetti AC, et al. Increased urinary excretion and prolonged turnover time of folic acid during ethanol ingestion. Am J Clin Nutr 1983;38:64–70.
44. McMartin KE, Collins TD, Eisenga BH, Fortney T, Bates WR, Bairnsfather L. Effects of chronic ethanol and diet treatment on urinary folate excretion and development of folate deficiency in the rat. J Nutr 1989;119:1490–7.
45. Tamura T, Halsted CH. Folate turnover in chronically alcoholic monkeys. J Lab Clin Med 1983;101:623–8.
46. Romanoff RL, Ross DM, McMartin KE. Acute ethanol exposure inhibits renal folate transport, but repeated exposure upregulates folate transport proteins in rats and human cells. J Nutr 2007;137:1260–5.
47. Leevy CM, Baker H, Tenhove W, Frank O, Cherrick GR. B-Complex vitamins in liver disease of the alcoholic. Am J Clin Nutr 1965;16:339–46.
48. Savage D, Lindenbaum J. Anemia in alcoholics. Medicine 1986;65:322–38.
49. Lumeng L. The role of acetaldehyde in mediating the deleterious effect of ethanol on pyridoxal 5′-phosphate metabolism. J Clin Invest 1978;62:286–93.
50. Labadarios D, Rossouw JE, McConnell JB, Davis M, Williams R. Vitamin B6 deficiency in chronic liver disease – evidence for increased degradation of pyridoxal-5′-phosphate. Gut 1977;18:23–7.
51. Lindenbaum J, Lieber CS. Alcohol-induced malabsorption of vitamin B12 in man. Nature 1969;224:806.
52. Kanazawa S, Herbert V. Total corrinoid, cobalamin (vitamin B12), and cobalamin analogue levels may be normal in serum despite cobalamin in liver depletion in patients with alcoholism. Lab Invest 1985;53:108–10.
53. Ji C, Kaplowitz N. Betaine decreases hyperhomocysteinemia, endoplasmic reticulum stress, and liver injury in alcohol-fed mice. Gastroenterology 2003;124:1488–99.
54. Song Z, Zhou Z, Uriarte S, Wang L, Kang YJ, Chen T, et al. S-adenosylhomocysteine sensitizes to TNF-alpha hepatotoxicity in mice and liver cells: a possible etiological factor in alcoholic liver disease. Hepatology 2004;40:989–97.
55. Esfandiari F, Villanueva JA, Wong DH, French SW, Halsted CH. Chronic ethanol feeding and folate deficiency activate hepatic

endoplasmic reticulum stress pathway in micropigs. Am J Physiol Gastrointest Liver Physiol 2005;289: G54–63.
56. Esfandiari F, You M, Villanueva JA, Wong DH, French SW, Halsted CH. S-adenosylmethionine attenuates hepatic lipid synthesis in micropigs fed ethanol with a folate-deficient diet. Alcohol Clin Exp Res 2007;31:1231–9.
57. Villanueva JA, Esfandiari F, White ME, Devaraj S, French SW, Halsted CH. S-Adenosylmethionine attenuates oxidative liver injury in micropigs fed ethanol with a folate-deficient diet. Alcohol Clin Exp Res 2007;31:1934–43.
58. Mandrekar P. Epigenetic regulation in alcoholic liver disease. World J Gastroenterol 2011;17:2456–64.
59. Shukla SD, Velazquez J, French SW, Lu SC, Ticku MK, Zakhari S. Emerging role of epigenetics in the actions of alcohol. Alcohol Clin Exp Res 2008;32:1525–34.
60. Pal-Bhadra M, Bhadra U, Jackson DE, Mamatha L, Park PH, Shukla SD. Distinct methylation patterns in histone H3 at Lys-4 and Lys-9 correlate with up- & down-regulation of genes by ethanol in hepatocytes. Life Sci 2007;81:979–87.
61. Esfandiari F, Medici V, Wong DH, Jose S, Dolatshahi M, Quinlivan E, et al. Epigenetic regulation of hepatic endoplasmic reticulum stress pathways in the ethanol-fed cystathionine beta synthase-deficient mouse. Hepatology 2010;51:932–41.
62. Barak AJ, Beckenhauer HC, Badakhsh S, Tuma DJ. The effect of betaine in reversing alcoholic steatosis. Alcohol Clin Exp Res 1997;21:1100–2.
63. Barak AJ, Beckenhauer HC, Tuma DJ. S-adenosylmethionine generation and prevention of alcoholic fatty liver by betaine. Alcohol 1994;11:501–3.
64. Kharbanda KK, Mailliard ME, Baldwin CR, Beckenhauer HC, Sorrell MF, Tuma DJ. Betaine attenuates alcoholic steatosis by restoring phosphatidylcholine generation via the phosphatidylethanolamine methyltransferase pathway. J Hepatol 2007;46:314–21.
65. Kharbanda KK, Rogers DD 2nd, Mailliard ME, Siford GL, Barak AJ, Beckenhauer HC, et al. A comparison of the effects of betaine and S-adenosylmethionine on ethanol-induced changes in methionine metabolism and steatosis in rat hepatocytes. J Nutr 2005;135:519–24.
66. Vendemiale G, Altomare E, Trizio T, Le Grazie C, Di Padova C, Salerno MT, et al. Effects of oral S-adenosyl-L-methionine on hepatic glutathione in patients with liver disease. Scand J Gastroenterol 1989;24:407–15.
67. Mato JM, Camara J, Fernandez de Paz J, Caballeria L, Coll S, Caballero A, et al. S-adenosylmethionine in alcoholic liver cirrhosis: a randomized, placebo-controlled, double-blind, multicenter clinical trial. J Hepatol 1999;30:1081–9.
68. Rambaldi A, Gluud C. S-adenosyl-L-methionine for alcoholic liver diseases. Cochrane Database Syst Rev 2006:CD002235.
69. Medici V, Virata MC, Peerson JM, Stabler SP, French SW, Gregory JF 3rd, et al. S-adenosyl-L-methionine treatment for alcoholic liver disease: a double-blinded, randomized, placebo-controlled trial. Alcohol Clin Exp Res 2011;35:1960–5.

Charles H. Halsted, MD is Professor Emeritus of Internal Medicine and Nutrition at the University of California Davis. He obtained his baccalaureate degree from Stanford University in 1958 and the Doctor of Medicine from the University of Rochester in 1962. After residency training in medicine at Cleveland Metropolitan General Hospital and subsequent fellowship training in gastroenterology at Johns Hopkins Hospital, he joined the faculty at the University of California Davis in 1974. He was Program Director of the National Institutes of Health Clinical Nutrition Research Unit at UC Davis, 1985–2004, and was Editor in Chief of the American Journal of Clinical Nutrition, 1997–2007. He is an elected Fellow of the American Gastroenterological Association, the American Society of Nutrition, and the American Association for the Advancement of Science, and member of the American Society for Clinical Investigation and the American Clinical and Climatological Association.