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IMPAIRED HOMOCYSTEINE TRANSSULFURATION IS AN INDICATOR OF ALCOHOLIC LIVER DISEASE

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Abstract

Background & Aims—Although abnormal hepatic methionine metabolism plays a central role in the pathogenesis of experimental alcoholic liver disease (ALD), its relationship to the risk and severity of clinical ALD is not known. The aim of this clinical study was to determine the relationship between serum levels of methionine metabolites in chronic alcoholics and the risk and pathological severity of ALD.

Methods—Serum levels of liver function biochemical markers, vitamin B6, vitamin B12, folate, homocysteine, methionine, S-adenosylmethionine, S-adenosylhomocysteine, cystathionine, cysteine, α-aminobutyrate, glycine, serine, and dimethylglycine were measured in 40 ALD patients, of whom 24 had liver biopsies, 26 were active drinkers without liver disease, and 28 were healthy subjects.

Results—Serum homocysteine was elevated in all alcoholics, whereas ALD patients had low vitamin B6 with elevated cystathionine and decreased α-aminobutyrate/cystathionine ratios, consistent with decreased activity of vitamin B6 dependent cystathionase. The α-aminobutyrate/cystathionine ratio predicted the presence of ALD, while cystathionine correlated with the stage of fibrosis in all ALD patients.

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Conclusions—The predictive role of the α- aminobutyrate/cystathionine ratio for the presence of ALD and the correlation between cystathionine serum levels with the severity of fibrosis point to the importance of the homocysteine transsulfuration pathway in ALD and may have important diagnostic and therapeutic implications.

Keywords

alcohol; methionine; cystathionine; vitamin B6

INTRODUCTION

Multiple interacting pathways that include aberrant methionine metabolism contribute to the development and progression of alcoholic liver disease (ALD) (Fig. 1). Chronic ethanol administration to experimental animals increases levels of plasma homocysteine (HCY), reduces liver S-adenosylmethionine (SAM) and increases S-adenosylhomocysteine (SAH) [1]. Acetaldehyde induced inhibition of methionine synthase (MS) activity is associated with increased activity of betaine homocysteine methyltransferase (BHMT), which uses betaine as a substrate to methylate HCY to methionine and dimethylglycine (DMG) [2]. Reactive oxygen species generated by ethanol metabolism impairs the expression of methionine adenosyl transferase (MAT), with consequent reduced levels of SAM [3], which is the universal substrate for methyltransferase reactions that generates SAH, an inhibitor of methylation. SAH is also a substrate for the bidirectional enzyme SAH hydrolase (SAHH) that can both regenerate HCY, or can enhance levels of SAH when HCY is elevated [1]. HCY is mainly reduced through the transsulfuration pathway that includes two vitamin B6 dependent enzymes, cystathionine-β-synthase (CβS) and γ-cystathionase, ultimately producing the antioxidant glutathione. α-Aminobutyrate (ABU) is produced during the conversion of cystathionine (CYSTAT) to cysteine as a byproduct of the transsulfuration pathway [1]. Since SAM facilitates the CβS reaction, reduced liver SAM levels are associated with decreased production of glutathione [4] [5]. Reduced SAM and elevated SAH predictably reduce the potential for methylation reactions, potentially contributing to the activation of many genes relevant to liver injury [5]. Ethanol-induced increases in hepatic SAH also potentiates pro-inflammatory cytokine TNFα and cell death in ethanol-fed mice [6]. The central role of aberrant methionine metabolism in the pathogenesis of alcoholic steatohepatitis was underscored by findings that its pathology and many of its mechanisms can be prevented by the addition of SAM to folate deficient and ethanol containing diets in a micropig model [7,8].

The goal of our study was to identify changes in serum methionine metabolites predictive of clinical ALD. We hypothesized that profiles of serum methionine metabolites are influenced by alcohol consumption and may predict and correlate with the severity of hepatic histopathology in ALD. We compared serum parameters of hepatic methionine metabolism in ALD patients, in active drinkers without evidence of liver disease, and in healthy subjects.

PATIENTS AND METHODS

Patients

Forty patients with clinical and biochemical features of ALD provided data for the present study, including 24 who had liver biopsies within one week of enrollment. The reasons for inability to obtain the liver biopsy in 16 patients included ascites or coagulopathy (8 cases) and patient refusal (8 cases). The enrolled patients were gender matched with 26 active drinkers (AD) without clinical evidence of liver disease and were age- and gender-matched with 28 healthy subjects (HS). The diagnosis of chronic alcoholism was based on criteria from the Diagnostic and Statistical Manual of Mental Disorders (fourth edition) and the

World Health Organization [9]. Patients were categorized according to the Child score (based on levels of bilirubin, albumin, international normalized ratio (INR), severity of ascites and hepatic encephalopathy) [10] and to the model for end-stage liver disease (MELD) score (based on levels of INR, bilirubin, and creatinine) [11]. Exclusion criteria included Child C cirrhosis, insufficient history for alcohol abuse, and laboratory testing consistent with viral or autoimmune hepatitis, Wilson disease, or hemochromatosis. The entry criteria for AD subjects included a history of chronic alcoholism without clinical or biochemical stigmata of chronic liver disease or other significant illness. Inclusion in the HS group required drinking on average less than two drinks per day (men) or one drink per day (women), normal liver biochemistry, and absence of significant illness. All subjects were enrolled from the University of California Davis Health Systems Hepatology clinic, Emergency Department, Primary Care Network clinics, or by flyers posted in the community. The study was performed at the University of California Davis Clinical and Translational Science Center (CTSC) Clinical Research Center (CCRC). The Institutional Review Board approved the protocol (# 200311168-7), and all patients provided written informed consent. The study was conducted according to the guidelines of the Declaration of Helsinki under provisions of “Good Clinical Practices” as defined in the U.S. Code of Federal Regulations on the Protection of Human Subjects for the United States.

Laboratory evaluations

Fasting venous blood was used for determinations of complete blood count, serum AST, ALT, bilirubin, alkaline phosphatase, albumin, INR, creatinine, folate and vitamin B12. Vitamin B6 was measured as pyridoxal-5’-phosphate by co-author J.F.G. as the semicarbazone-derivative by reverse-phase HPLC with fluorescence detection [12]. Cytokines TNFα, Interleukin 6 (IL-6), and IL-10 were measured using high sensitivity cytokine ELISA reactions (R&D Systems, Minneapolis, Minnesota). Patients were screened for alcohol use by the modified measurement of serum carbohydrate deficient transferrin (%CDT) turbidimetric immunoassay (Bio-Rad Laboratories, Inc. Hercules, California) [13]. For measurements of serum methionine metabolites, about 4 cc of blood were clotted in a serum separator tube for 15 minutes at room temperature, then placed in ice until centrifugation at 3,000 rpm for 15 minutes within 30 minutes of venipuncture. Serum was removed and stored at –80°C, until sent to Metabolite Laboratories, University of Colorado Health Sciences Center for measurements by co-author S.P.S. of serum SAM, SAH, HCY, cystathionine, cysteine, methionine, ABU, DMG, serine, and glycine using stable isotope dilution gas or liquid chromatography/mass spectrometry [14].

Liver histopathology

Liver biopsies were obtained percutaneously from ALD patients using a 16G Jamshidi needle and ultrasound guidance. Safety criteria required INR <1.5 and a platelet count >50,000/mm^3. Biopsies were considered valid for the analysis if they were >15 mm in length, 1.4 mm wide, and contained more than five portal tracts. Each biopsy specimen was fixed in formalin and shipped to co-author S.W.F. for scoring. The liver biopsy grading was blinded using a Nikon E400 microscope equipped with a digital camera and Nikon Metomorph computer software (Molecular Devices Corporation, Downingtown, Pennsylvania) to make quantitative morphometric measurements of abnormalities per square millimeter of biopsy surface. Using published criteria [15] a quantitative score was applied to each of the following parameters: steatosis (four-point scale: grade 0: steatosis involving <10% of hepatocytes; grade 1: steatosis up to 30%; grade 2: steatosis between 30 and 60%; and grade 3: steatosis >60%) , fibrosis by Sirius red stain (five-point scale, stage 0: no fibrosis; stage 1: zone 3 perisinusoidal fibrosis; stage 2: perisinusoidal fibrosis and periportal fibrosis; stage 3: focal or extensive bridging fibrosis; stage 4: cirrhosis), mononuclear and polymorphonuclear inflammation (four-point scale based on inflammatory foci per 20x:

grade 0; 1 with 1-2 foci; 2 with 3-4 foci; 3 with > 4 foci). Mallory-Denk hyaline bodies were counted in 10 fields (× 20 magnification) to determine the average number per field.

Statistical analysis

Descriptive characteristics were calculated for all variables for ALD patients, AD, and HS groups. Variables that were not normally distributed were transformed as appropriate prior to analysis. If no suitable transformation was found, non-parametric analysis was used. The data from all groups were analyzed for differences using analysis of variance (ANOVA). Correlations were made among biochemical parameters, clinical Child and MELD scores, and histology scores using multiple variable analysis. Logistic regression was used to identify predictors of liver disease among all alcoholics with and without liver disease. All analyses were conducted with SAS v. 8.2 or higher (Cary, North Carolina). With 40 ALD, more than 20 AD, and more than 20 HS evaluable subjects, the study would have at least an 80% power at an α-value of 0.05 (two-tailed) to detect a significant difference in methionine metabolites varying between groups.

RESULTS

Clinical features

As shown in Table 1, AD subjects were younger than ALD and HS. The prevalent ethnicity was Caucasian, followed by African American, Hispanic, Asian, and Native American. The mean body mass index (BMI) (weight in kg per height in m^2) of all subjects was similar in the three groups. Twenty-one ALD patients were actively drinking at the time of the enrollment with the last drink 1 to 7 days prior to enrollment, and the remainder had been sober for more than 8 days. The weekly historical average of drinks for patients with ALD was 59.6 ± 44.1 (mean ± SD) and for AD was 40.2 ± 12.9, corresponding to 99.6 ± 75 and 69 ± 22 grams of daily alcohol, respectively (n.s.). Seventeen (42.5%) ALD patients used over the counter multivitamins, 10 (25%) used folic acid, and 5 (12.5%) thiamine supplements.

Clinical parameters

There were no differences in biochemical parameters, vitamin levels, or methionine metabolite levels between ALD patients who did or did not undergo liver biopsies. Serum AST, ALT, bilirubin, alkaline phosphatase, and INR were higher in the ALD group than the other groups (Table 2), and the median AST/ALT ratio in the ALD patients was 1.72. ALD patients had a mean MELD score of 11 ± 5.2 and Child score of 6.7 ± 1.6, distributed as 19 patients in Child class A and 21 in Child class B. The serum % CDT, an index of recent alcohol consumption, was similar and elevated in all ALD patients and in AD compared to HS, and was similar in ALD subjects who were actively drinking and who were abstinent at the time of the enrollment, confirming its limited value in the presence of ALD [16]. Serum folate levels were within normal range, but lower in ALD and AD compared to HS. Vitamin B12 levels were higher in ALD than in AD and HS. Vitamin B6 levels were lower in ALD than in AD and HS.

Serum methionine metabolites

Serum HCY levels were similar in ALD and AD and higher than in HS, while SAH levels were higher in ALD patients than in HS (Table 3). Values for HCY and SAH levels were positively correlated among all subjects ($r = 0.29$, $p = 0.005$). Serum SAM levels were higher in ALD patients compared to AD and HS, and there were no differences in the SAM/SAH ratio among the groups. Serum methionine and cysteine were marginally increased in ALD and serine and glycine were similar in all groups. Serum CYSTAT, the substrate for

cystathionase (Fig. 1), was increased by more than 2-fold in ALD, while ABU was moderately decreased and the ABU/CYSTAT ratio was decreased by more than 3-fold in ALD, consistent with reduction in cystathionase activity. DMG, a byproduct of BHMT (Fig. 1), was increased in ALD patients, consistent with activation of the BHMT alternate pathway of HCY transmethylation.

Multivariate analysis indicated these metabolite levels were not influenced by use of supplemental multivitamins containing vitamin B6 or folic acid.

HCY and SAH levels were positively correlated with AST ($r = 0.44$, $p < 0.0001$ and $r = 0.27$, $p = 0.01$, respectively) and alkaline phosphatase levels ($r = 0.37$, $p = 0.0002$ and $r = 0.4$, $p = 0.0003$, respectively). CYSTAT levels were positively correlated with AST ($r = 0.4$, $p < 0.0001$), bilirubin ($r = 0.57$, $p < 0.0001$), alkaline phosphatase ($r = 0.57$, $p < 0.0001$), and INR ($r = 0.53$, $p < 0.0001$), as well as MELD and Child scores ($r = 0.66$, $p < 0.0001$, and $r = 0.6$, $p < 0.0001$). Levels of vitamin B6 correlated negatively with levels of serum AST ($r = -0.37$, $p < 0.006$) and correlated negatively with CYSTAT levels ($r = -0.24$, $p = 0.02$) and positively with the ABU/CYSTAT ratio ($r = 0.37$, $p = 0.0006$).

Cytokine levels

There were no differences among the three groups in values for IL-6 and TNFα, while IL-10 levels were lower in ALD (Table 4).

Liver histopathology

Liver biopsies were performed in 24 ALD patients, including 15 ALD actively drinking and 9 ALD non-drinking at the time of enrollment. The mean time elapsed between the enrollment and the liver biopsy was 7.9 ± 4.4 days (range 2-22 days). The ALD actively drinking group continued to drink during this interval, except for two who had an interval of 7.5 ± 0.7 days between the enrollment and the liver biopsy. Actively drinking ALD subjects had a higher degree of steatosis and lower stage of fibrosis compared to non active ALD (Table 5). In the entire group of ALD patients, the stages of fibrosis and steatosis were inversely correlated ($r = -0.46$, $p = 0.021$), while the grades of inflammation and steatosis were positively correlated ($r = 0.4$, $p = 0.046$). The duration of abstinence from alcohol was negatively associated with the grade of steatosis ($r = -0.47$, $p = 0.021$) and was positively associated with the stage of fibrosis ($r = 0.41$, $p = 0.047$). AST was positively correlated with the grade of steatosis, inflammation, and with the frequency of Mallory-Denk bodies (Table 6). Among the parameters of liver function, INR, MELD, and Child scores were all positively correlated with the stage of fibrosis. The average number of Mallory-Denk bodies was positively correlated with the grade of steatosis ($r = 0.53$, $p = 0.008$) and inflammation ($r = 0.52$, $p = 0.01$).

Regressions

According to logistic regression analysis of data from all three alcoholic groups, the ABU/CYSTAT ratio was a predictor of the likelihood of ALD ($r = -0.38$, $p < 0.0001$). However, although CYSTAT and ABU/CYSTAT correlated with the stage of fibrosis (Table 6), the small number of liver biopsies did not allow for the determination of predictors of the severity of fibrosis.

DISCUSSION

Although others related changes in serum methionine metabolite profiles to abnormal biochemistry in ALD patients [17], our study is the first to correlate serum methionine metabolite levels with hepatic histopathology. The population of patients was characterized

by a long history of heavy drinking with typical clinical features of ALD including elevated AST, MELD, and Child scores. Heavy drinking was demonstrated by elevated %CDT levels in all alcoholics, regardless of the duration of sobriety in the ALD group (Table 2). Ours is the first report on serum folate levels in an alcoholic population after the introduction of the 1998 US governmental mandate for supplemental dietary folic acid. Folate deficiency was established as a feature of chronic alcoholism over four decades ago [18] and is attributable to combinations of poor diet, impaired folate absorption, and increased urinary folate excretion [19]. Although folate levels were in the normal range in all groups, the lower levels in ALD and AD subjects confirm the risk of chronic alcoholism for lower folate levels [18]. The finding of increased serum vitamin B12 levels in ALD patients can be attributed to impaired hepatic uptake or retention, or increased release of this vitamin by injured hepatocytes [20]. Relatively lower circulating levels of vitamin B6 were previously described in alcoholics and can be attributed to dietary deficiency, malabsorption [21], or to displacement of the vitamin from its protein carrier by acetaldehyde with subsequent degradation by phosphatases [22]. The variability of IL-6 and TNFα levels could be ascribed to differences in the duration of abstinence [23], the severity of liver disease [24], and/or to genetic polymorphisms [25]. IL-10 was significantly lower in the ALD patients compared to the other 2 groups, contrasting with results of others [26,27], including a study showing a higher prevalence of polymorphisms in the IL-10 promoter in association with increased risk of advanced liver disease [28].

The observed elevations in plasma HCY levels among ALD and AD subjects (Table 3) is consistent with prior data [17,29,30], and can be attributed to inhibitory effects of acetaldehyde on MS activity [2], although a block in transsulfuration could play an additional role (Fig. 1). Elevated HCY can contribute to the development of histopathological damage in the liver through induction of oxidative liver injury [7] and by ER stress pathways of steatosis and apoptosis [8]. Whereas serum HCY correlated with AST, it did not correlate with the histopathologic score or to the likelihood of liver disease among all chronic alcoholics. Although MS is reduced with chronic alcohol exposure, the observed maintenance of serum methionine levels and increase in serum DMG levels are consistent with a compensatory increase in BHMT activity in experimental models of ALD (Table 3) [1]. HCY can also be converted to SAH by the reversible enzyme SAHH [1], as indicated by the correlation between HCY and SAH levels.

Surprisingly, we observed higher serum SAM levels in ALD patients than in AD and HS subjects (Table 3). This novel finding is inconsistent with findings of reduced hepatic levels of SAM in various experimental animal models of ALD [1,5,8] and in liver biopsies of patients with alcoholic hepatitis [29]. Due to the small size of clinical liver biopsies, we could not make comparisons of serum and liver levels of SAM and SAH. We propose that levels of SAM in the serum of patients with ALD do not reflect their concentrations in the liver, but probably reflect reduced retention of SAM by injured hepatocytes. An animal model with simultaneous severe depletion of liver SAM with unchanged serum SAM levels has been reported, suggesting that changes in serum do not parallel those in the liver [31].

HCY levels are regulated by two intersecting pathways of transmethylation and transsulfuration (Fig. 1). While transmethylation of HCY is controlled by both MS and BHMT, transsulfuration is regulated by two vitamin B6 dependent enzymes, CβS and cystathionase [1]. A previous study of liver biopsies from ALD patients and control subjects found reduced expressions of genes relevant to methionine metabolism including CβS [32]. Previous studies of diet-induced vitamin B6 deficiency found that HCY levels were unaffected while CYSTAT levels were increased [33] suggesting a greater effect of deficiency of vitamin B6 on cystathionase than on CβS. Clinical Vitamin B6 deficiency was associated with increased CYSTAT and reduced ABU [34]. This finding was confirmed in

experimental vitamin B6- deficient rats [35]. The observed two-fold increase in CYSTAT and the 3-fold reduction in ABU/CYSTAT ratio in ALD patients (Table 3) are consistent with impairment of the vitamin B6 dependent cystathionase reaction. An alternate attribution of more than two-fold increased serum CYSTAT levels in ALD to export from injured hepatocytes is less likely in view of minimal to no increase in serum levels of several other methionine metabolites, serine and glycine. Furthermore, the consistency of elevated CYSTAT with the reduced ABU/CYSTAT substrate to product ratio is indicative of altered metabolism of CYSTAT in the ALD patients. The moderately increased cysteine levels observed in this study could be attributed to reduction of the downstream activity of cysteine dioxygenase which catabolizes cysteine to cysteine sulfinic acid [36].

The findings of positive correlations between serum CYSTAT with parameters of liver function, including albumin, INR, and the MELD score, confirms results from a previous study in ALD patients [17]. Since vitamin B6 is a co-factor for cystathionase, the findings of lower vitamin B6 levels in the ALD group (Table 2) and the correlation of vitamin B6 levels with the observed increase in CYSTAT and reduced ABU and ABU/CYSTAT ratio and AST levels in all ALD patients (Table 3) suggest a primary role for vitamin B6 deficiency in disturbing the transsulfuration pathway in this group. The significant effect of vitamin B6 deficiency in the present study is supported by the prior observation that vitamin B6 deficiency promotes ALD in ethanol fed rats [37]. Furthermore, our study linked abnormal transsulfuration to the presence and severity of ALD by finding a relationship between elevated CYSTAT with AST levels and the stage of fibrosis in the ALD group, and that the ABU/CYSTAT ratio was a valid predictor of ALD among all chronic alcoholics. The reduced ABU/CYSTAT ratio in ALD and its predictive value of the presence of liver disease among alcoholics is a new finding that points to the potential importance of vitamin B6 deficiency in the pathogenesis of ALD, as underscored by the positive correlation between B6 levels and both the MELD and Child scores. The pathophysiological significance of the ABU/CYSTAT ratio relies on its connection with glutathione synthesis and consequently impaired antioxidant mechanisms leading to liver injury. Moreover, the ratio could potentially be used to guide the decision to perform the liver biopsy in subjects at risk of ALD. The cross-sectional design of our study does not allow us to achieve conclusive results on the cause-effect relation between alcohol, methionine metabolism, and ALD. However, the correlation between aberrant transsulfuration pathway metabolites and parameters of liver function as well as with histological parameters points to a central role of this pathway in the development of chronic liver injury related to alcohol use.

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Abbreviations

ALD	alcoholic liver disease
HCY	homocysteine
SAM	S-adenosylmethionine

SAH	S-adenosylhomocysteine
MS	methionine synthase
BHMT	betaine homocysteine methyltransferase
DMG	dimethylglycine
MAT	methionine adenosyl transferase
SAHH	S-adenosylhomocysteine hydrolase
CβS	cystathionine-β-synthase
ABU	α-aminobutyrate
CYSTAT	cystathionine
AD	active drinkers
HS	healthy subjects
INR	international normalized ratio
MELD	model for end-stage liver disease
IL	Interleukin
%CDT	% carbohydrate deficient transferrin

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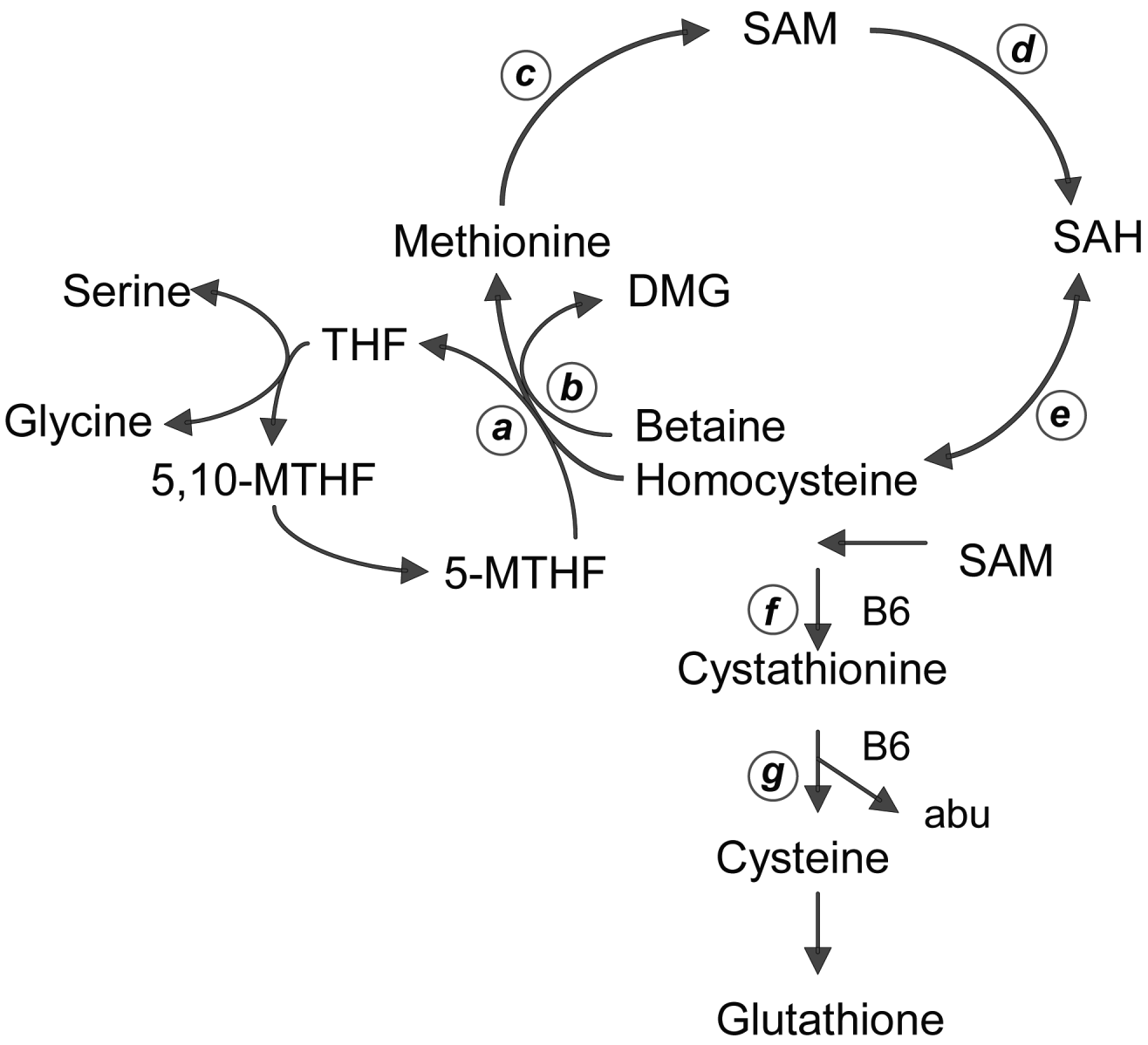

Fig. 1. Methionine metabolism

Homocysteine in the liver is converted to methionine by two reactions: methionine synthase (MS) *(a)* and betaine homocysteine methionine transferase (BHMT) *(b)*. 5-methyltetrahydrofolate (MTHF) is a substrate for MS which produces tetrahydrofolate (THF). BHMT uses betaine as a substrate with production of dimethylglycine (DMG). Methionine is converted by methionine adenosyltransferase (MAT) *(c)* to S-adenosylmethionine (SAM), which is irreversibly converted to S-adenosylhomocysteine (SAH) by donating its methyl moiety to DNA methyltransferases (DNMTs) *(d).* SAH hydrolase *(e)* regulates the bi-directional reaction that leads to the synthesis of homocysteine from SAH or vice versa. The transsulfuration pathway is regulated by the vitamin B6-dependent enzymes cystathionine β synthase *(f)* and cystathionase *(g)* which both catalyze irreversible reactions. Alpha-aminobutyric acid (abu) is the collateral product of the reaction catalyzed by cystathionase.

Table 1

Demographic features of patients with alcoholic liver disease (ALD), active drinkers without liver disease (AD), and healthy subjects (HS)

In this and all subsequent tables, the data are presented as median (range) or percentage. Values without a common letter are significantly different (for example: a vs a or b vs b: not statistically different; a vs b: statistically different; a, b vs a or a, b vs b: not statistically different).

	Alcoholic Liver Disease (ALD) (n = 40)	Active drinkers w/o liver disease (AD) (n = 26)	Healthy Subjects (HS) (n = 28)	Overall *p* value
Age	46.5 (34-67)[a]	41 (23-66)[b]	45.5 (31-59)[a]	< .05
Gender (F/M)	12/28	4/22	10/18	
BMI (kg/m2)	25.8 (29-17.8)	26.4 (21-30)	26.6 (20-28)	0.8
Duration of abstinence (days)	8 (0-447)	1 (0-7)	n/a	0.09
Age at onset of heavy drinking	20 (10-62)	17 (13-30)[b]	n/a	< .05
Duration of drinking (years)	23.5 (2-48)	25 (2-40)	n/a	0.4
AUDIT score	15.5 (8-24)	13 (8-21)	n/a	0.2

Ethnicity				
Caucasian	27 (67.5%)	15 (57.7%)	16 (57.1%)	
African-American	4 (10%)	8 (30.7%)	2 (7.1%)	
Hispanic	6 (15%)	2 (7.6%)	5 (18%)	0.1
Asian	2 (5%)	0	3 (10.7%)	
Native American	1 (2.5%)	1 (4%)	2 (7.1%)	

Table 2

Clinical laboratory parameters

INR: International Normalized ratio; % CDT: percentage of carbohydrate deficient transferrin.

	Alcoholic Liver Disease (ALD) (n = 40)	Active drinkers w/o liver disease (AD) (n = 26)	Healthy Subjects (HS) (n = 28)	Overall *p* value
WBC K/mm^3 [4.5-11]	6.3 (3.6-15.4)	6.1 (4-9.5)	5.7 (4.5-10.4)	0.06
Hb mg/dL [12-16]	13 (9.2-17.2)[a]	14 (11-16.5)[b]	14.5 (11.8-17.3)[b]	<.02
Platelets K/mm^3 [130-400]	206 (55-403)[a]	254 (167-422)[b]	242 (167-449)[a, b]	<.02
AST U/L [15-37]	70.5 (47-350)[a]	28 (15-37)[b]	26 (14-37)[b]	<.0001
ALT U/L [5-37]	38 (14-211)[a]	24 (14-33)[b]	26 (12-36)[b]	<.0001
Total bilirubin mg/dL [0.3-1.3]	1.4 (0.5-7.3)[a]	0.7 (0.5-1.3)[b]	1 (0.6-1.3)[b]	<.0001
Alkaline Phosphatase U/L [35-115]	121 (52-425)[a]	62 (44-89)[b]	62 (34-99)[b]	<.0001
Albumin g/dL [3.2-4.6]	3.4 (2.4-4.3)[a]	4 (3.4-4.7)[b]	4 (3.1-4.5)[b]	<.0001
INR [0.75-1.19]	1.1 (0.9-1.8)[a]	0.9 (0.8-1.1)[b]	1 (0.9-1.1)[b]	<.0001
Creatinine mg/dL [0.44-1.4]	0.8 (0.5-1.39)	0.9 (0.62-1.2)	0.9 (0.6-1.2)	0.06
%CDT	3.9 (1.9-8.7)[a]	3.8 (1.6-6.9)[a]	2.6 (1.4-3.8)[b]	<.02
Folate ng/mL [3.5-16.1]	16.5 (0.4-20)[a]	14.8 (4.8-20)[a]	20 (9-20)[b]	<.05
Vitamin B12 pg/mL [200-600]	667 (288-1733)[a]	473 (220-2000)[b]	505 (322-1850)[b]	<.02
Vitamin B6 nmol/L [15-120]	44.5 (8.6-187.4)[a]	71.7 (22.4-195.8)[b]	83.6 (15-299)[b]	<.02

Table 3
Serum methionine metabolites and vitamin levels

HCY: homocysteine; SAH: S-adenosylhomocysteine; SAM: S-adenosylmethionine; CYSTAT: cystathionine; ABU: α-aminobutyrate; DMG: dimethylglycine.

	Alcoholic Liver Disease (ALD) (n = 40)	Active drinkers w/o liver disease (AD) (n = 26)	Healthy Subjects (HS) (n = 28)	Overall *p* value
HCY μmol/L	10.2 (5.4-58.3)[a]	8.8 (5.8-23)[a]	6.4 (4.1-10)[b]	<.001
SAH nmol/L	33 (15-142)[a]	26 (13-74)[a, b]	20 (14-64)[b]	<.02
SAM nmol/L	120 (63-328)[a]	96 (49-341)[b]	88.5 (72-160)[b]	<.02
SAM/SAH	4.2 (0.4-9.2)	4 (1.2-10.3)	4.1 (1.2-7)	0.9
Methionine μmol/L	30 (11.4-77.2)[a]	24 (10.7-45.8)[b]	25.1 (17-35.4)[b]	<.02
CYSTAT nmol/L	300 (88-1786)[a]	111 (72-944)[b]	135 (86-538)[b]	<.001
Cysteine μmol/L	352 (231-528)[a]	318 (259-404)[a, b]	319 (255-383)[b]	<.05
ABU μmol/L	11.3 (3.9-38.2)[a]	20.4 (6.1-67.8)[b]	16.1 (7.4-31.8)[b]	<.05
ABU/CYSTAT	0.03 (0.005-0.22)[a]	0.13 (0.01-0.5)[b]	0.1 (0.02-0.2)[b]	<.001
DMG μmol/L	4.2 (2.3-89.4)[a]	3.5 (1.7-5.6)[b]	3.1 (1.7-7.6)[b]	<.001
Serine μmol/L	100 (51-168)	94 (59-119)	96.5 (64-134)	0.43
Glycine μmol/L	200 (120-341)	202 (115-305)	200 (158-314)	0.73

Table 4

Serum cytokine levels.

	Alcoholic Liver Disease (ALD) (n = 40)	Active drinkers w/o liver disease (AD) (n = 26)	Healthy Subjects (HS) (n = 28)	Overall *p* value
IL-6 pg/mL	2.9 (0.07-20.8)	1.3 (0.1-4.5)	0.7 (0.05-24.3)	0.08
TNFα pg/mL	5.8 (1.15-28.4)	4.7 (0.04-21.7)	3.44 (0.2-19.7)	0.4
IL-10 pg/mL	2.4 (0.01-27)[a]	3.5 (0.01-31.6)[b]	3.21 (0.01-27)[b]	< .02

Table 5

Hepatic histopathological parameters

Steatosis in actively drinking ALD vs non actively drinking ALD, $p = 0.006$; fibrosis in actively drinking ALD vs non actively drinking ALD, $p = 0.003$.

(mean ± SD)	Steatosis	Inflammation	Necrosis	Mallory Bodies	Fibrosis
Overall	2 (0-4)	1(0-3)	0 (0-3)	0.5 (0-4)	3 (0-4)
ALD-active	4 (1-4)[a]	1 (0-3)	0 (0-3)	1 (0-4)	2 (0-4)[a]
ALD-non active	0 (1-4)[b]	1 (1-4)	0 (1-4)	0 (1-4)	4 (1-4)[b]

Table 6
Correlations among liver biochemistry, methionine metabolites and histopathogy

Data are reported as correlation coefficient (r) and *p* value.

	Steatosis	Inflammation	Mallory-Denk Bodies	Fibrosis
AST	0.57 *p* = 0.003	0.48 *p* = 0.010	0.53 *p* = 0.008	0.17 *p* =0.40
ALT	-0.58 *p* = 0.002	0.28 *p* =0.17	0.16 *p* =0.45	-0.45 *p* = 0.020
INR	-0.56 *p* = 0.006	0.02 *p* =0.92	0.16 *p* =0.44	0.61 *p* = 0.002
MELD	0.38 *p* =0.08	0.06 *p* =0.77	-0.07 *p* =0.71	0.5 *p* = 0.020
Child	0.004 *p* =0.98	0.08 *p* =0.08	-0.33 *p* =0.11	0.42 *p* = 0.041
Cystathionine	0.39 *p* =0.05	-0.33 *p* =0.10	0.18 *p* =0.39	0.44 *p* = 0.028
ABU/Cystathionine	0.25 *p* =0.22	0.27 *p* =0.19	0.04 *p* =0.84	-0.46 *p* = 0.022