Mechanism for Prevention of Alcohol-Induced Liver Injury by Dietary Methyl Donors

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Alcohol-induced liver injury (ALI) has been associated with, among other molecular changes, abnormal hepatic methionine metabolism, resulting in decreased levels of S-adenosylmethionine (SAM). Dietary methyl donor supplements such as SAM and betaine mitigate ALI in animal models; however, the mechanisms of protection remain elusive. It has been suggested that methyl donors may act via attenuation of alcohol-induced oxidative stress. We hypothesized that the protective action of methyl donors is mediated by an effect on the oxidative metabolism of alcohol in the liver. Male C57BL/6J mice were administered a control high-fat diet or diet enriched in methyl donors with or without alcohol for 4 weeks using the enteral alcohol feeding model. As expected, attenuation of ALI and an increase in reduced glutathione:oxidized glutathione ratio were achieved with methyl donor supplementation. Interestingly, methyl donors led to a 35% increase in blood alcohol elimination rate, and while there was no effect on alcohol metabolism in the stomach, a profound effect on liver alcohol metabolism was observed. The catalase-dependent pathway of alcohol metabolism was induced, yet the increase in CYP2E1 activity by alcohol was blunted, which may be mitigating production of oxidants. Additional factors contributing to the protective effects of methyl donors in ALI were increased activity of low- and high-Km aldehyde dehydrogenases leading to lower hepatic acetaldehyde, maintenance of the efficient mitochondrial energy metabolism, and promotion of peroxisomal β-oxidation. Profound changes in alcohol metabolism represent additional important mechanism of the protective effect of methyl donors in ALI.

Key Words: acetaldehyde; catalase; cytochrome P4502E1; S-adenosylmethionine; peroxisome proliferator–activated receptor alpha.

Hepatic methionine metabolism, also referred to as one-carbon metabolism, is critical for a number of vital liver functions. One of the most important roles is the production of S-adenosylmethionine (SAM), the principal methyl donor required for the methylation of DNA, RNA, proteins, and phospholipids and as a precursor for polyamine and reduced glutathione (GSH) synthesis. Increasing evidence has shown that deficiencies or impairment of this pathway can contribute to the pathogenesis and progression of liver disease (Mato and Lu, 2007). Experimental animal models using diets deficient in methyl donors, such as methionine or choline, result in the spontaneous development of nonalcoholic steatohepatitis (Oz et al., 2008) and hepatocellular carcinoma (HCC) (Shivapurkar and Poirier, 1983). Interestingly, the methionine adenosyltransferase (MatIα) knockout mouse model that results in the chronic depletion of SAM and the glycine N-methyltransferase (Gnmt) knockout mouse model that results in the overproduction of SAM (Luka et al., 2006) both promote steatohepatitis (Lu et al., 2001; Mato and Lu, 2007) and HCC (Martinez-Chantar et al., 2002; Tseng et al., 2003). These observations suggest that SAM is tightly regulated and should be well balanced in order to maintain liver physiological function.

Aberrant hepatic methionine metabolism is commonly observed in patients with alcoholic liver disease. Both decreases in SAM and folate (Cravo et al., 1996) and elevations in homocysteine (de la Vega et al., 2001) in liver or serum samples have been reported. This condition is multifactorial in origin and results not only from poor nutrition but also due to the deleterious effects of alcohol on intestinal absorption and hepatic uptake of essential amino acids and B vitamins necessary to support one-carbon metabolism (Halsted et al., 1973). Moreover, the oxidative metabolism of alcohol resulting in changes in the hepatic redox state shifts the cycle away from recycling methionine for SAM production to synthesizing the antioxidant glutathione.
The use of dietary methyl donor supplementation in patients with alcoholic cirrhosis has produced mixed results (Mato et al., 1999; Vendemiale et al., 1989). Only one clinical study reported improved survival with long-term dietary supplementation with SAM alone but only among those with less advanced liver disease (Mato et al., 1999). Thus, better understanding of the mechanisms underlying the linkages between one-carbon metabolism and alcohol-induced liver injury (ALI), especially the protective effects of dietary supplementation with methyl donors, is needed. In this study, unlike previous works to date that have employed single nutritional supplements, we have used a combination of methionine and its metabolites (choline, betaine, and folate), as well as cofactors (vitamins B6, B12, and zinc), to fortify one-carbon metabolism in the liver. We report that the protective effects are mediated via profound changes in alcohol metabolism and describe the mechanistic underpinnings of this effect.

**MATERIALS AND METHODS**

**Animals, diets, and treatment.** Male C57BL/6J mice (8 weeks old; Jackson Laboratory, Bar Harbor, ME) were randomized into two groups and provided either a standard (Isopro RMH 3000; Lab Diet, Richmond, IN) or methyl-enriched (TD.01310; Harlan-Teklad, Madison, WI) rodent chow for 1 week prior to and immediately following surgical intragastric intubation until the intragastric feeding was initiated (Kono et al., 2000). Following surgery, mice were housed in individual metabolic cages and allowed a week to recover with ad libitum access to food and water. Following the recovery period, mice were administered via gastric cannula high-fat diets (HFDs) prepared as detailed in Thompson and Reitz (1978), which contained either maltose-dextrin (isoscaloric control, referred to as “control”) or ethyl alcohol (referred to as “alcohol”) with or without methyl donor enrichment (added per kilogram diet: 15 g betaine, 13.2 g choline chloride, 13.6 mg folic acid, 7.5 g L-methionine, 0.13 mg vitamin B12, and 648 mg zinc sulfate). The high-fat (corn oil–based) diet was then incrementally adjusted 1 g/kg every 4 days until the dose reached 0.33 mM dithiothreitol. For high-

**Quantitative analysis was performed by averaging percent positively stained nuclei to total nuclei within 10 random fields at ×200.**

**Immunohistochemical detection of BrdU.** Formalin-fixed paraffin-embedded liver sections (6 μm) were mounted on glass slides. Sections were deparaffinized and rehydrated, and immunodetection was performed using DAKO EnVision System HRP (DakoCytomation, Carpinteria, CA) with primary anti-BrdU monoclonal antibody (clone Bu20a; DAKO) diluted in PBS containing 1% bovine serum albumin and incubated overnight at 4°C. Quantitative analysis was performed by averaging percent positively stained nuclei to total nuclei within 10 random fields at ×200.

**Clinical chemistry.** Alcohol and acetaldehyde concentrations in serum, urine, or liver tissue homogenates were determined as described elsewhere (Bergmeyer, 1988). Serum alanine aminotransferase (ALT) levels were determined spectrophotometrically with the Thermo Scientific Infinity ALT Liquid stable reagent (Thermo Electron, Melbourne, Australia).

**Determination of aminothioles.** The content of one-carbon metabolites in liver tissue extracts was determined using high-performance liquid chromatography with coulometric electrochemical detection as previously described (Trasler et al., 2003).

**Protein level and activity measurements.** Cytosol, membrane, mitochondrial, and nuclear proteins were obtained by subcellular fractionation of liver tissue using the Qproteome Cell Compartment Kit (Qiagen, Valencia, CA). Proteins were resolved using 4–12% Bis-Tris gradient gel electrophoresis (Invitrogen, Carlsbad, CA) and transferred to 0.45-

**Hepatic acetaldehyde content.** Levels of acetaldehyde were determined in liver supernatants as described in Bergmeyer (1988) and modified for use in a 96-

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**Histopathology.** Formalin-fixed paraffin-embedded liver or kidney sections (6 μm) were stained with hematoxylin/eosin (H&E) or periodic acid Schiff (PAS) stain, respectively, for light microscopy evaluation of liver or kidney pathology. H&E-stained slides were scored as described by Nanji et al. (1989) as follows: steatosis (the percentage of hepatocytes containing fat): < 25% = 1+, < 50% = 2+, < 75% = 3+, and > 75% = 4++; inflammation and necrosis: one focus per low-power field = 1+ and two or more foci = 2+. PAS-stained slides were scored as follows: +, slight and focal lesions (less than 30% of area affected); 2+, moderate and diffuse lesions (30–70% affected); and 3+, severe and massive lesions (more than 70% affected).
null
was observed in mice given MDHFD + alcohol over those receiving HFD + alcohol (Table 1).

**Methyl Donor–Enriched Diet Has No Effect on First-Pass Metabolism of Alcohol**

Although many tissues can metabolize alcohol, considerable metabolism occurs in the liver and, to a lesser extent, in the stomach. The lack of liver injury along with reduced UAC and increase in BAE rate in mice given MDHFD + alcohol could lead one to speculate that increased metabolism of alcohol in the stomach prevented significant quantities of alcohol reaching the liver. Stomach catalase/D-amino acid oxidase pathway has been shown to be partially responsible for the protective action of glycine on ALI (Iimuro et al., 1996). To address the role of first-pass metabolism in the protection afforded by the methyl donor–enriched diet, we assessed the activity of stomach ADH and catalase in mice fed the diets for 28 days and observed no differences between control and methyl donor–enriched diet groups (Supplementary table 1). In addition, disposition of alcohol after a single acute dose was unaffected by methyl donors when mice were fed *ad libitum* with a pelleted control or methyl donor–enriched diet for 2 weeks followed by an oral bolus (1.5 g/kg) of alcohol (data not shown).

**Methyl Donor–Enriched Diet Leads to Stimulation of the Catalase-Dependent Alcohol Metabolism in the Liver**

During moderate alcohol consumption, the majority of alcohol is metabolized to acetaldehyde by ADH in the liver, but under conditions of chronic high-dose exposures, a significant portion is metabolized by the catalase-peroxide complex and, to a lesser degree, Cyp2e1. Thus, hepatic activity of all three enzymes was measured in this study. ADH activity was found to be marginally, but not significantly, increased in both alcohol-treated groups, and no effect of the methyl donors was observed (Fig. 3A). While Cyp2e1 activity (Fig. 3B) and protein levels (Fig. 3D) were significantly elevated in both alcohol-fed groups, the induction in MDHFD + alcohol group was significantly lower than that in HFD + alcohol group.

**FIG. 2.** Representative plots of daily UAC. Typical urine alcohol levels in mice administered different diets as indicated. The dose of alcohol administered over the 28 days of treatment is plotted on the right y-axis.

**FIG. 3.** Liver alcohol metabolism. Liver activity of (A) ADH, (B) Cyp2e1, and (C) catalase was determined in mice administered a control HFD or MDHFD with or without alcohol for 4 weeks. Asterisks indicate statistically significant (**p < 0.01 and ***p < 0.001) differences using one-way ANOVA followed by Tukey’s post hoc test. N = three to five animals per group. (D) Representative Western blots for ADH, catalase, and Cyp2e1. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and cadherin were used for cytosol and membrane protein loading controls, respectively.
Interestingly, activity of catalase was increased significantly in mice given a MDHFD + alcohol compared to all other groups (Fig. 3C) even though protein levels did not change significantly (Fig. 3D). The activity of acyl-CoA oxidase, enzyme that provides hydrogen peroxide needed for catalase-dependent alcohol metabolism, was also significantly increased only in the MDHFD + alcohol group (Fig. 4C). Since acyl-CoA oxidase is controlled by PPARα, we also assessed liver (nuclear) protein levels of PPARα (Fig. 4A) and expression of CYP4A10 (Fig. 4B), a PPARα-controlled gene. All were induced significantly in the MDHFD + alcohol group.

**Methyl Donor–Enriched Diet Reduces Hepatic Acetaldehyde Content**

Alcohol metabolism results in the production of acetaldehyde, a reactive metabolite, which in turn is rapidly metabolized to acetate by ALDH. The activity of liver ALDH1 and 2 in the MDHFD + alcohol group was statistically different from all other groups (Fig. 5A), and protein levels for ALDH1 and ALDH2 were increased (Fig. 5B). Although liver ALDH2 protein content was also increased in mice given HFD + alcohol (Fig. 5B), it did not result in an increase in enzyme activity (Fig. 5A). Kidney activity of ALDH1 and 2 was much lower than that in the liver, and little effect of the methyl donor supplementation was observed (Supplementary fig. 2). Striking differences in hepatic acetaldehyde content were also observed between two alcohol-fed groups. Levels in mice given HFD + alcohol were eightfold higher than those receiving MDHFD + alcohol (Fig. 5C). Hepatic acetaldehyde content was determined to also strongly correlate with liver injury (Fig. 5D).

**Effect of MDHFD on Hepatic One-Carbon Metabolism**

Methyl donor–enriched diets were formulated to provide substantially increased amounts of cofactors (Cooney, 1993). Indeed, in both MDHFD and MDHFD + alcohol–treated mice, hepatic levels of SAM were significantly increased by 10- to 20-fold, respectively (Table 2). SAM:S-adenosylhomocysteine (SAH) ratios, an indicator of transmethylation potential, were also considerably improved fivefold to sevenfold. Homocysteine, a metabolic by-product of SAM methylation reactions, is typically methylated to regenerate methionine but during conditions of oxidative stress is diverted for GSH production. HFD + alcohol treatment reduced the levels of GSH and raised the levels of oxidized glutathione (GSSG) while reducing the GSH:GSSG ratio, an index of overall oxidant stress. MDHFD + alcohol treatment did not increase GSH but reduced the levels of GSSG.

**Methyl Donors Affect Cellular Energy Metabolism**

Analysis of the serum metabolome determined that feeding a methyl donor–enriched diet results in changes in carbohydrate and lipid metabolism following alcohol treatment (Fig. 6). Levels of acetate and citrate were reduced in mice given MDHFD + alcohol compared to those receiving HFD + alcohol, a condition that would preferentially stimulate carbohydrate metabolism over fatty acid oxidation. Lipid metabolism, however, appeared to be stimulated in mice given MDHFD + alcohol as serum lipids and, in particular very low-density lipoprotein, were found to be elevated.
DISCUSSION

The association between decreased hepatic SAM content and the severity of alcoholic hepatitis in humans, a well-established phenomenon (Herbert et al., 1963), has prompted studies of methyl donors in a variety of animal models of ALI. Administration of diets deficient in methionine, choline, or folate or combinations thereof to rats, mice, or mini-pigs have been shown to exacerbate ALI (Gyamfi et al., 2008). To the contrary, dietary supplementation with SAM or betaine to alcohol-fed rats (Garcia-Ruiz et al., 1995) or baboons (Lieber et al., 1990) attenuated liver injury, restored GSH, and improved mitochondrial function. Several hypotheses have been proposed to explain the mechanism of protection offered by methyl donors, including attenuation of oxidative stress by restoring levels of GSH (Garcia-Ruiz et al., 1995), an increase in SAM:SAH ratio (Barak et al., 1993), and an attenuated inflammatory response (Song et al., 2007). It is likely that these mechanisms are interdependent, yet the causality and temporality are not well understood.

In this study, the expected attenuation of ALI (e.g., restoration of the liver glutathione balance and attenuation of liver injury phenotypes) by dietary methyl donor supplementation was achieved in a subchronic mouse model and known hallmarks of methyl donor's protective effects (Purohit et al., 2007) were observed. While it is without a doubt that upregulation of the transsulfuration pathway, which leads to a decrease in oxidative stress via increased synthesis of glutathione, is a major contributor to protection, our data suggest a novel mechanism via the effect on alcohol metabolism in the liver.

Through observations of the daily changes in the urinary alcohol content, we detected an effect of the methyl donor–enriched diet on the oxidative metabolism of alcohol. Importantly, it has been reported that SAM accelerates the clearance of alcohol and acetaldehyde in humans (di Padova et al., 1984), but no mechanism for this effect was proposed. Thus, the phenotype studied here in the mouse model of ALI is relevant to clinical practice.

We found that a 35% increase in BAE rate in methyl donor–supplemented animals was due to changes in liver metabolism but not due to first-pass metabolism in the gut. Interestingly, the most pronounced effect of methyl donor supplementation was the induction of the catalase-dependent pathway since increased activity of both catalase and acyl-CoA oxidase, source of hydrogen peroxide necessary for catalase function, was observed.

Not only was alcohol metabolism enhanced through this route but also the activity of low- and high-\(K_m\) ALDH was also
TABLE 2
Effect of Methyl Donor–Enriched Diet on Hepatic One-Carbon Metabolism

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Alcohol</th>
<th>Control</th>
<th>Alcohol</th>
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<tbody>
<tr>
<td><strong>Methionine metabolism</strong></td>
<td></td>
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<tr>
<td>Methionine</td>
<td>1.0 ± 0.12</td>
<td>0.8 ± 0.05</td>
<td>5.0 ± 0.94</td>
<td>12.1 ± 2.95&lt;sup&gt;a,b&lt;/sup&gt;</td>
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<tr>
<td>SAM</td>
<td>0.3 ± 0.05</td>
<td>0.1 ± 0.02</td>
<td>2.2 ± 0.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.3 ± 0.27&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SAH</td>
<td>0.2 ± 0.03</td>
<td>0.2 ± 0.02</td>
<td>0.5 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.6 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SAM:SAH</td>
<td>1.4 ± 0.19</td>
<td>0.8 ± 0.14</td>
<td>5.3 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.1 ± 0.52&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adenosine</td>
<td>0.7 ± 0.04</td>
<td>0.6 ± 0.07</td>
<td>0.5 ± 0.06</td>
<td>0.5 ± 0.02</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>0.2 ± 0.02</td>
<td>0.2 ± 0.01</td>
<td>0.1 ± 0.01</td>
<td>0.2 ± 0.01</td>
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<tr>
<td><strong>Glutathione biosynthesis</strong></td>
<td></td>
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<tr>
<td>Cysteine</td>
<td>11 ± 1.1</td>
<td>9 ± 1.1</td>
<td>8 ± 0.5</td>
<td>9 ± 0.2</td>
</tr>
<tr>
<td>Glu-Cys</td>
<td>0.12 ± 0.02</td>
<td>0.23 ± 0.03</td>
<td>0.12 ± 0.02</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>GSH</td>
<td>84 ± 3</td>
<td>42 ± 4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>73 ± 8</td>
<td>60 ± 2</td>
</tr>
<tr>
<td>GSSG</td>
<td>0.49 ± 0.12</td>
<td>0.82 ± 0.10&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>0.28 ± 0.09</td>
<td>0.22 ± 0.02</td>
</tr>
<tr>
<td>GSH-GSSG</td>
<td>191 ± 43</td>
<td>55 ± 12&lt;sup&gt;d&lt;/sup&gt;</td>
<td>321 ± 92</td>
<td>277 ± 36</td>
</tr>
<tr>
<td>Cys-Gly</td>
<td>1.2 ± 0.09&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>1.2 ± 0.11&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>0.23 ± 0.03</td>
<td>0.41 ± 0.05</td>
</tr>
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</table>

Notes. Metabolite concentrations (nanomoles per milligram protein) were measured in liver tissue homogenates using coulometric electrochemical high-performance liquid chromatography detection as described in Materials and Methods section. Values given are the mean ± SEM from (n = 5–7 mice). Significance of the difference in means (p < 0.05, one-way ANOVA followed by Tukey’s post hoc test) is indicated by bold font and by an asterisk when compared to (a) HFD control, (b) HFD + alcohol, (c) MDHFD, or (d) MDHFD + alcohol. Cys-Gly, cysteinylglycine; Glu-Cys, glutaminecysteine.

Increased, which created favorable conditions for fast and efficient catabolism of alcohol and lower liver levels of acetaldehyde, a highly cytotoxic molecule and a key mediator of liver injury by alcohol (Seitz and Stickel, 2007). The effect of methyl donor supplementation on alleviating the net levels of acetaldehyde produced during feeding with alcohol is a potentially important part of the protection against ALI. Indeed, in humans, SAM was shown to favor the elimination of alcohol without increasing blood levels of acetaldehyde (di Padova et al., 1984).

It is not known, however, how SAM and/or other methyl donors can activate catalase and acyl-CoA oxidase, a potential means through which the methyl donor–supplemented diet appears to exhibit its protective action against ALI in the mouse. One possible mechanism could be through epigenetic regulation as it was suggested that the degree of methylation of the CpG-rich promoter of the catalase gene may be important for regulation of its expression (Reimer et al., 1994). In addition, it is plausible that a higher order regulatory mechanism that may affect both catalase and acyl-CoA oxidase is involved. A potential key pathway may be through PPARα, which controls expression of both proteins of interest, since induction of Cyp4a10 was also observed in the same group. Indeed, we observed that methyl donor supplementation led to an increase in nuclear protein levels for PPARα. It was suggested that SAM may act through a putative RNA methyltransferase PIMT (PPAR-interacting protein with methyltransferase domain) to enhance the transcriptional activity of both PPARs and retinoid-X-receptors (Zhu et al., 2001). Since in our study the marked elevations in liver methionine and SAM were detected in livers of methyl donor–supplemented mice, in both control and alcohol-treated groups, it is plausible that this mechanism may be responsible for an increase in nuclear levels of PPARα.

Alcohol may impair PPARα-dependent signaling in the liver, which is an important factor in deregulation of lipid and overall metabolism, thus contributing to the development of ALI. Specifically, evidence exists that ALI is exacerbated in Pparα-null mice (Nakajima et al., 2004) and that PPARα agonists are protective against ALI (Fischer et al., 2003). Alcohol is also known to lead to a decrease in liver Pparα messenger RNA and protein as well as reduced expression of PPARα-controlled genes, such as acyl-CoA oxidase (Fischer et al., 2003; Lu et al., 2008). Our data suggest that methyl donors are able to block this deleterious effect of alcohol by activating PPARα and/or supporting peroxisomal and mitochondrial metabolism (see below), resulting in activation of catalase-dependent alcohol metabolism. It should be noted that the modest but significant activation of PPARα in methyl donor–enriched HFD group had no observable effect on alcohol metabolism, activity of acyl-CoA oxidase, or Cyp4a10 expression, which suggests that additional factors, such as increased stability of the enzymes (Horie and Suga, 1990), may be responsible for the overall protective effect.

We also observed that alcohol-induced activation of Cyp2e1, a protein that is known to contribute to liver oxidative stress (Lu and Cederbaum, 2008), was blunted by methyl donors.

![FIG. 6. Alterations in serum metabolites by methyl donors and alcohol. Metabolites in serum from mice (three per group) given a control HFD or MDHFD with or without alcohol for 4 weeks. Heatmap of metabolites significantly different (one-way ANOVA, p < 0.05) between two alcohol-treated groups is shown. Values are log<sub>2</sub> transformed and median centered and metabolites clustered using average linkage–centered correlation. A complete list of metabolite data is available in Supplementary table 2.](https://academic.oup.com/toxsci/article/115/1/131/1635653)
While SAM is known to be a weak inhibitor of Cyp2e1 (Caro and Cederbaum, 2005), the $K_i$ for inhibition is relatively high, and it is not likely that a direct effect of SAM on Cyp2e1 was responsible for the effect observed here. Interestingly, a similar protective effect on Cyp2e1 activation by alcohol was reported recently with betaine (1% in drinking water for 2 weeks) supplementation (Kim et al., 2008). The increase in Cyp2e1 protein levels and activity in the liver following treatment with alcohol is due to reduced protein turnover by the ubiquitin-dependent proteasomal pathway (Morishima et al., 2005) and not due to increased transcription. While blunted activation of Cyp2e1 in mice given MDHFD + alcohol was associated with remarkably higher proteasome activity as compared to HFD + alcohol group, we did not observe an inhibition of proteasome with alcohol in this mouse model (data not shown). Nonetheless, a blunted increase in Cyp2e1 activity in methyl donor diet + alcohol group may be an important factor for alleviation of liver injury by way of prevention of the formation of cytotoxic reactive oxygen species and lipid peroxides. Pathogenesis of alcohol-induced hepatic steatosis and steatohepatitis is thought to be dependent on oxidative stress, whereby lipid peroxidation products facilitate the inflammatory response (Day and James, 1998).

The metabolomic analysis suggested that additional factors contributing to the protective effects of methyl donors in ALI may be the improved maintenance of the efficient mitochondrial energy metabolism and promotion of peroxisomal $\beta$-oxidation of fatty acids. It is known that folate is responsible for stimulating methyl transfer pathways in the cytosol and mitochondria (Depeint et al., 2006). Indeed, serum acetate, a key transmembrane mitochondrial shuttle, was lower in the mice fed methyl donors and alcohol compared to alcohol group without supplementation, suggesting that mitochondrial function may be improved. In addition, methionine was found to be markedly elevated in serum of mice fed methyl donors and alcohol. Methionine and its metabolite carnitine play an important role in shuffling long-chain fatty acids into the mitochondrial matrix. Even though the elevation of serum carnitine in this group was not significant, it is evident that adequate levels were present in the liver to facilitate $\beta$-oxidation of fatty acids and dampen the untoward effects of alcohol on fatty acid metabolism. In view of our suggestion that a PPAR$\alpha$-related alcohol metabolism pathway may be induced by methyl donor supplementation, it is noteworthy that Makowski et al. (2009) demonstrated that PPAR$\alpha$ is a regulator of carnitine levels and that Par$\alpha$-null mice have lower levels of carnitine and fasted ketone levels suggestive of lowered rates of $\beta$-oxidation.

**SUPPLEMENTARY DATA**

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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