L-Serine Supplementation Attenuates Alcoholic Fatty Liver by Enhancing Homocysteine Metabolism in Mice and Rats

Woo-Cheol Sim, Hu-Quan Yin, Ho-Sung Choi, You-Jin Choi, Hui Chan Kwak, Sang-Kyum Kim, and Byung-Hoon Lee

Abstract

Background: Hyperhomocysteinemia plays an important role in the development of steatosis, and studies indicate that homocysteine-lowering treatment inhibits the development of fatty liver.

Objective: We evaluated the effects of L-serine on alcoholic fatty liver and homocysteine metabolism.

Methods: In a binge ethanol study, male C57BL/6 mice were divided into 4 groups: control, ethanol + vehicle, and ethanol + 20 or 200 mg/kg L-serine. Mice were gavaged with ethanol (5 g/kg body weight) 3 times every 12 h with or without L-serine which was given twice 30 min before the last 2 ethanol doses. Control mice were fed isocaloric dextran-maltose. In a chronic ethanol study, male Wistar rats were divided into 3 groups: control, ethanol, and ethanol + L-serine. Rats were fed a standard Lieber-DeCarli ethanol diet (36% ethanol-derived calories) for 4 wk with or without dietary L-serine supplementation (1%; wt:vol) for the last 2 wk. In control rats, the ethanol-derived calories were replaced with dextran-maltose. The effects of L-serine were also tested in AML12 cells manipulated to have high homocysteine concentrations by silencing the genes involved in homocysteine metabolism.

Results: Binge ethanol treatment increased serum homocysteine and hepatic triglyceride (TG) concentrations by >5-fold vs. controls, which were attenuated in the 200-mg/kg L-serine treatment group by 60.0% and 47.5%, respectively, compared with the ethanol group. In the chronic ethanol study, L-serine also decreased hepatic neutral lipid accumulation by 63.3% compared with the ethanol group. L-Serine increased glutathione (GSH) and S-adenosylmethionine (SAM) by 94.0% and 30.6%, respectively, compared with the ethanol group. Silencing betaine homocysteine methyltransferase, cystathionine β-synthase, or methionine increased intracellular homocysteine and TG concentrations by >2-fold, which was reversed by L-serine when L-serine–independent betaine homocysteine methyltransferase was knocked down.

Conclusion: These results demonstrate that L-serine ameliorates alcoholic fatty liver by accelerating L-serine–dependent homocysteine metabolism.

Keywords: alcoholic fatty liver, homocysteine, nutrition and disease, SREBP1, sulfur amino acid metabolism

Introduction

Alcoholic fatty liver is a mild, early-stage liver disease induced by excess consumption of ethanol. There is no standard treatment because it is generally considered to be a benign and reversible condition. However, it is now recognized that without proper treatment fatty liver can lead to steatohepatitis, fibrosis, and ultimately end-stage liver disease. The molecular mechanisms that contribute to alcoholic fatty liver include increased FA synthesis by sterol regulatory element binding protein (SREBP)1, decreased FA

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3 Supplemental Table 1, Supplemental Method, and Supplemental Figures 1 and 2 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

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7 Abbreviations used: Akt, protein kinase B; ALD, alcoholic liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BHMT, betaine homocysteine methyltransferase; C, control; CBS, cystathionine β-synthase; E, ethanol diet; ER, endoplasmic reticulum; ES20, binge ethanol + 20 mg/kg L-serine; ES200, binge ethanol + 200 mg/kg L-serine; EV, ethanol diet + vehicle; FAS, FA synthase; GSH, reduced glutathione; INSIG1, insulin-induced gene 1; MS, methionine synthase; mTOR, mammalian target of rapamycin; nSREBP, nuclear form of sterol regulatory element binding protein; PI3K, phosphoinositide-3-kinase; pSREBP, premature form of sterol regulatory element binding protein; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; siRNA, small interfering RNA; SREBP, sterol regulatory element binding protein; 5-methylTHF, 5-methyltetrahydrofolate; 4EBP1, eukaryotic translation initiation factor 4E binding protein 1.
oxidation by peroxisome proliferator-activated receptor-α, and impaired VLDL lipoprotein secretion by phosphatidylethanolamine methyltransferase (1).

Mice fed homocysteine or a high-methionine, low-folate diet have increased homocysteine concentrations and an increased incidence of liver disease, characterized by hepatic steatosis (2). Chronic ethanol consumption increases homocysteine accumulation in the liver, which is closely linked to the development of various liver diseases. Moreover, patients with chronic liver disease, as well as alcoholics, develop substantially increased serum homocysteine concentrations regardless of the stage of steatosis, from mild fibrosis to severe cirrhosis (3, 4). Homocysteine is removed metabolically in the liver by several biochemical reactions. These include remethylation into methionine by methionine synthase (MS) or betaine homocysteine S-methyltransferase (BHMT) and trans-sulfuration by cystathionine β-synthase (CBS). L-Serine is required either as a methyl group donor for MS or as a substrate for CBS. All of these metabolic pathways maintain homeostasis of homocysteine, methionine, S-adenosylhomocysteine (SAM), and S-adenosylhomocysteine (SAH).

Prolonged ethanol feeding led to depletion of SAM and an increase in SAH, along with early decreased MS and late increased BHMT (5, 6). An attempt was made to correct defective cellular methylation and to decrease homocysteine concentrations by using folate, vitamin B-6, or vitamin B-12. SAM prevents alcoholic liver disease (ALD), not only as a methyl donor or reduced glutathione (GSH) precursor but also by activating adenosine monophosphate–activated protein kinase (7). Several disadvantages, including unstable property and high cost, prohibit the development of SAM as an antisteatotic drug. Betaine is thus far the most promising treatment modality for ALD that prevents alcohol-induced steatosis, apoptosis, protein damage, and altered signaling events by decreasing homocysteine concentrations and generating SAM in vivo (8). However, to date, no clinical trials of betaine have been conducted.

L-Serine reduces homocysteine concentrations in hepatocytes treated with methionine in rats fed a high-protein diet (9) and in humans who ingested meals fortified with methionine (10). However, it remains to be established whether the homocysteine-lowering effects of l-serine can ameliorate fatty liver. A recent study indicated that lowering homocysteine by l-serine was not sufficient to inhibit fatty liver induced by choline deprivation in rats (11). These data prompted us to investigate whether reversal of alcoholic fatty liver by l-serine supplementation is partially attributable to homocysteine-mediated SREBP activation. Data supporting this hypothesis could contribute to recommendations for the possible use of l-serine alone or as a supplement for the treatment of ALD.

Methods

Materials. L-Serine, D-serine, methionine, homocysteine, and Nile Red were purchased from Sigma Chemical. AML12 was obtained from the American Type Culture Collection (ATCC) and cultured following ATCC guidelines.

Animal experiments. Animals used in the study were purchased from Japan SLC, Inc., housed in an air-conditioned room (24°C) with a 12-h light/dark cycle, and acclimatized over 1 wk to a nonpurified diet (Supplemental Table 1). The experiments using animals were carried out in accordance with animal experiment guidelines with the approval of the Institutional Animal Care and Use Committee of Seoul National University.

Binge ethanol study. Male C57BL/6 mice (20 g) were randomly divided into 4 groups: control (G), binge ethanol + vehicle (EV), binge ethanol + 20 mg/kg l-serine (ES20), and binge ethanol + 200 mg/kg l-serine (ES200). They were fed the nonpurified diet throughout the experiments. Three mice in each group were gavaged with 5 g/kg of ethanol or isocaloric dextran-maltose 3 times every 12 h. L-Serine dissolved in tap water was administered twice by oral gavage 30 min before the last 2 ethanol doses, and the mice were killed by cardiac puncture after Zoletil (10 mg/kg tiletamine, 10 mg/kg zolazepam, ip; Virbac) anesthesia 24 h after the last dose. The binge ethanol study was performed twice independently (Supplemental Figure 1).

Chronic ethanol feeding study. Male Wistar rats (250 g) were divided into 3 groups: C, ethanol diet (E), and ethanol diet + 1% l-serine (ES). The rats were fed a standard Lieber-DeCarli ethanol diet (36% ethanol-derived calories; Dyets) for 4 wk (12); pair-fed control rats were administered dextran-maltose to match the alcohol-derived calories in the ethanol diet. For pair-feeding, 2 rats were housed in a single cage. The food intake of each cage in the E group was determined daily between 0900 and 1000 h, and the same amount of the food was then given on the following day to the C and ES groups. Pair-feeding was conducted throughout the study. The ethanol diet was supplemented either with or without 1% (wt/vol) l-serine for the last 2 wk.

Histopathologic evaluation. For Oil Red O staining, frozen liver tissues were cut into 7-μm sections and affixed to microscope slides. Sections were stained with Oil Red O solution buffer and counterstained with Harris hematoxylin.

TG analysis. Liver and cellular TGs were determined by a modified Folch method (13) by using a Serum Triglyceride Determination kit (Sigma) following the manufacturer’s protocol.

Serum biochemistry. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), TGs, and cholesterol were monitored by standard clinical chemistry assays on a Tokyo Boeki Protege 24 Chemistry Analyzer (Tokyo Boeki Machinery Limited). Serum and cellular total homocysteine concentrations were quantified by using an Axis Homocysteine EIA Reagent kit (Axis-Shield) following the manufacturer’s protocol with SpectraMax 340 (Molecular Devices).

Determination of sulfur amino acids and metabolites. Liver homogenates were diluted, and denatured protein was removed by centrifugation at 10,000 × g for 10 min; the supernatant was used to measure hepatic homocysteine, SAM, SAH, cysteine and GSH. An HPLC method was used to determine SAM and SAH (14), and total homocysteine, total cysteine, and total GSH were quantified by using a 7-benzox-2-oxa-1,3-diazole-4-sulfonic acid (SBD-F) method (15).

For hepatic methionine and cystathionine analysis, liver homogenates were diluted in ice-cold methanol. They were then derivatized with O-phthalaldehyde/2-mercaptoethanol and quantified by using an HPLC (SCL-10A; Shimadzu) system with a fluorescence detector (RF-10AXL, Ex 385 nm and Em 515 nm; Shimadzu) (16).

Western blotting. Proteins were extracted from cell and liver homogenate by using a PRO-PREP protein extraction kit (Intron Biotechnology). Extracted proteins were separated by SDS-PAGE and then transferred to a polyvinylidene difluoride membrane (Millipore). Western blotting was probed with the specific antibodies against insulin-induced gene 1 (INSIG1), FA synthase (FAS), premature form of SREBP (pSREBP), nuclear form of SREBP (nSREBP), CBS (Santa Cruz Biotechnology), MS (Abcam), and GAPDH (Cell Signaling Technology).

Homocysteine and methionine treatment. AML12 cells were seeded with 1.2 × 10⁵ cells per well in 100-mm dishes in medium containing 10% FBS for 24 h. The cells were treated with 5 mM homocysteine or 5 mM methionine in the absence or presence of 10 mM l-serine. After 24 h, the cells were collected for homocysteine and TG measurement.

RNA interference. AML12 cells were seeded with 2 × 10⁵ cells per well in 6-well plates in medium containing 10% FBS for 24 h. The cells were transiently transfected with Ms, Ost, and Bhom small interfering RNA
(siRNA; Santa Cruz Biotechnology) by using the Fugene HD Transfection Reagent (Promega) as recommended by the manufacturer’s protocol. After 48 h, the cells were collected for homocysteine and TG measurement.

**Statistical analysis.** All results are presented as means ± SDs. In vivo data were evaluated by 1-factor ANOVA followed by Tukey’s multiple-comparison procedure. In vitro data were analyzed by 2-factor ANOVA followed by Bonferroni post-test. For unequal variances, data were evaluated by Kruskal-Wallis test. P < 0.05 was considered significant. All data were analyzed by GraphPad Prism 5 (GraphPad Software).

**Results**

**L-Serine ameliorates ethanol-induced steatosis and improves homocysteine metabolism in the binge and the chronic ethanol study.** There tended to be more Nile Red-positive cells among ethanol-treated samples than in controls (P = 0.06) ([Supplemental Method](#)). However, cotreatment with 10 mM L-serine reduced lipid accumulation and tended to return lipid concentrations to control levels (P = 0.06) ([Supplemental Figure 2](#)).

In the binge ethanol study, the EV group had a higher percentage of TGs than did the C group. Hepatic neutral lipid accumulation was significantly attenuated in the ES200 group compared with the E group as seen in the Oil Red O staining and biochemical analysis of hepatic TGs (Figure 1A, B). Serum ALT and homocysteine concentrations were increased in the EV group by 2.0- and 5.6-fold compared with the C group (Figure 1C, D). The ES200 group showed significantly reduced homocysteine concentrations compared with the E group (Figure 1D). Oil Red O staining of the liver performed in the chronic ethanol study demonstrated that ethanol-induced fatty infiltration was substantially attenuated in the ES group compared with the E group (Figure 2A). Moreover, serum ALT, AST, hepatic TG, and serum total cholesterol concentrations were lower in the ES group than in the E group (Figure 2B, C, E, G). These results clearly demonstrate that L-serine ameliorates ethanol-induced fatty liver.

The hepatic homocysteine concentration in the EV group increased by 1.5-fold over the C group and decreased in the ES200 group to the concentration of the C group (Table 1). In the chronic ethanol study, feeding ethanol with or without L-serine did not change the hepatic homocysteine concentration. However, the decreased concentrations of SAM and GSH observed in the E group were completely recovered in the ES group. As a result, the SAM:SAH ratio was also restored in the ES group (Table 1).

**L-Serine reduces the expression of the proteins associated with lipogenesis and homocysteine metabolism in the binge ethanol study.** Increased expression of SREBP1 and FAS in the EV group was inhibited by L-serine (Figure 3A–C). The expression of INSIG1, an endoplasmic reticulum (ER)-associated protein that regulates proteolytic cleavage of membrane-bound SREBPs, was higher in the ES200 group than in the EV group (Figure 3A, D). The expression of MS tended to be lower in the EV group than in the C group (P = 0.07), although the expression was greater in the ES200 group than in the EV group (Figure 4A, B).

**The lipid-lowering effect of L-serine is associated with L-serine-dependent homocysteine metabolism.** The incubation of AML12 cells with homocysteine increased intracellular homocysteine by 4.7-fold and TG concentrations by 2.3-fold over controls. When cells were coincubated with l-serine, however, homocysteine and TG concentrations decreased by 64% and 41%, respectively. The homocysteine- and TG-lowering effects

**FIGURE 1** Oil Red O staining of the liver (A) and concentrations of hepatic TGs (B), serum ALT (C), and serum tHcy (D) of mice in the binge ethanol study. Values in panels B–D are means ± SDs, n = 3 mice per group. Within each graph, labeled means without a common letter differ (P < 0.05). Statistical analysis was performed by using 1-factor ANOVA with Tukey’s multiple-comparison procedure. A second independent experiment gave similar results. ALT, alanine aminotransferase; C, control; ES20, binge ethanol + 20 mg/kg L-serine; ES200, binge ethanol + 200 mg/kg L-serine; EV, binge ethanol + vehicle; tHcy, total homocysteine.
of l-serine were also seen in a methionine-induced intracellular hyperhomocysteine model (Figure 5A, B). siRNA-mediated silencing of Bhmt, Cbs, or Ms significantly increased cellular homocysteine and TG concentrations by 2- to 3-fold. l-Serine treatment reduced homocysteine and TG concentrations in Bhmt or Cbs knockdown cells. However, in Ms-silenced cells, l-serine had little effect on homocysteine and TG concentrations (Figure 6A, C). The effects of l-serine were greater in cells knocked down with siRNA for Bhmt than Cbs. Cbs/Ms double knockdown amplified the accumulation of homocysteine and TGs, which was not reversed by l-serine (Figure 6B, D). These data indicate that l-serine plays a role in efficiently reducing homocysteine and TG concentrations only when both of the l-serine–dependent homocysteine-metabolizing enzyme systems are intact.

**Discussion**

Hyperhomocysteinemia can induce ER stress, which activates lipogenesis by increasing SREBP1 expression (17). One of the mechanisms of alcoholic fatty liver is a disturbance in hepatic sulfur-amino acid metabolism, leading to high homocysteine concentrations and a low SAM:SAH ratio (18). However, a striking species difference between rats and mice exists in the induction of hyperhomocysteinemia in response to ethanol. Shinohara et al. (19) demonstrated that rats are more resistant to ethanol-induced hyperhomocysteinemia and steatosis due to increased expression of BHMT, which resulted in effective homocysteine metabolism. Our results are in accordance with previous reports that demonstrated ethanol-induced

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**FIGURE 2** Oil Red O staining of the liver (A) and concentrations of serum ALT (B), serum AST (C), serum tHcy (D), hepatic TGs (E), serum TGs (F), and serum tCH (G) of rats in the chronic ethanol feeding study. Values in panels B–G are means ± SDs, n = 8–10 rats per group. Within each graph, labeled means without a common letter differ (P < 0.05). Statistical analysis was performed by using 1-factor ANOVA with Tukey’s multiple-comparison procedure. ALT, alanine aminotransferase; AST, aspartate aminotransferase; C, control; E, ethanol diet; ES, ethanol diet + 1% l-serine; tCH, total cholesterol; tHcy, total homocysteine.

**TABLE 1** Effects of l-serine on concentrations of sulfur amino acids and metabolites in the liver obtained from mice in the binge ethanol study and rats in the chronic ethanol feeding study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Binge ethanol study (mice)</th>
<th>Chronic ethanol feeding study (rats)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>EV ES20 ES200</td>
</tr>
<tr>
<td>Homocysteine, nmol/g</td>
<td>48.5 ± 6.9</td>
<td>71.3 ± 3.0*</td>
</tr>
<tr>
<td>Cysteine, nmol/g</td>
<td>287 ± 52.8</td>
<td>277 ± 157</td>
</tr>
<tr>
<td>SAM, nmol/g</td>
<td>59.3 ± 29.5</td>
<td>25.2 ± 23.6</td>
</tr>
<tr>
<td>SAH, nmol/g</td>
<td>26.2 ± 0.5</td>
<td>31.1 ± 12.9</td>
</tr>
<tr>
<td>SAM:SAH</td>
<td>2.3 ± 1.1</td>
<td>1.0 ± 1.1</td>
</tr>
<tr>
<td>Methionine, nmol/g</td>
<td>670 ± 175</td>
<td>733 ± 291</td>
</tr>
<tr>
<td>Cystathionine, nmol/g</td>
<td>39.6 ± 4.7</td>
<td>24.4 ± 1.5</td>
</tr>
<tr>
<td>GSH, mmol/g</td>
<td>0.6 ± 1.3</td>
<td>6.0 ± 3.4</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs, n = 3 mice and rats per group. Data were analyzed by 1-factor ANOVA, followed by Tukey’s multiple-comparison procedure. Labeled means in a row for a study without a common letter differ, P < 0.05; C, control; E, ethanol diet; ES, ethanol diet + 1% l-serine; ES20, binge ethanol + 20 mg/kg l-serine; ES200, binge ethanol + 200 mg/kg l-serine; EV, binge ethanol + vehicle; GSH, reduced glutathione; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine.

2 Homocysteine in rat liver and cystathionine in mouse liver were analyzed by Kruskal-Wallis test because of unequal variances.
hyperhomocysteinemia in mice but not rats. Moreover, a significant homocysteine-lowering effect of L-serine was observed in mice but not rats. To explain the antisteatotic effects of L-serine in ethanol-fed rats, we determined the concentrations of sulfur amino acids and metabolites in the liver. SAM is an important methyl group donor in many biological reactions. Various methyltransferases convert SAM to SAH, which is further metabolized to homocysteine by SAH hydrolase. Although the hydrolysis of SAH is very efficient, the production and accumulation of SAH are favored in hyperhomocysteinemia. SAH inhibits phosphatidylethanolamine methyltransferase and VLDL secretion, leading to hepatosteatosis (20). Therefore, the SAM:SAH ratio is associated with liver injury (21). Our data in the chronic ethanol study clearly show that L-serine increased hepatic SAM without effects on the SAH concentration. L-Serine also increased the hepatic GSH concentration in ethanol-fed rats. On the basis of previous reports that described that disturbance of SAM/SAH homeostasis as well as oxidative stress contribute to the development of alcoholic fatty liver (22), normalizing sulfur amino acid metabolism by L-serine may play an important role in the antisteatotic effects in rats.

Many investigators have attempted to control alcoholic steatosis by maintaining the folate and methionine cycle; however, unexpected pitfalls were encountered. Although choline supplementation in ethanol-fed rodents may produce sufficient betaine with satisfactory effects, it may not be effective in primates because of the low activity in the choline oxidase pathway (23). Betaine exerts homocysteine-lowering and thus antisteatotic effects; however, its supplementation is associated with increased plasma cholesterol (24), which may increase...

FIGURE 3 Western blot images (A) and expressions of nSREBP1 (B), FAS (C), and INSIG1 (D) in the liver of mice in the binge ethanol study. Representative data from 3 independent experiments are shown. Protein amounts were normalized to GAPDH. Values in panels B–D are means ± SDs, n = 3 mice per group. Within each graph, labeled means without a common letter differ (P < 0.05). Statistical analysis was performed by using 1-factor ANOVA with Tukey’s multiple-comparison procedure. C, control; ES20, binge ethanol + 20 mg/kg L-serine; ES200, binge ethanol + 200 mg/kg L-serine; EV, binge ethanol + vehicle; nSREBP1, nuclear form of sterol regulatory element binding protein 1.

FIGURE 4 Western blot images (A) and expressions of MS (B) and CBS (C) in the liver of mice in the binge ethanol study. Representative data from 3 independent experiments are shown. Protein amounts were normalized to GAPDH. Values in panels B and C are means ± SDs, n = 3 mice per group. Within each graph, labeled means without a common letter differ (P < 0.05). Statistical analysis was performed by using 1-factor ANOVA with Tukey’s multiple-comparison procedure. C, control; CBS, cystathionine β-synthase; ES20, binge ethanol + 20 mg/kg L-serine; ES200, binge ethanol + 200 mg/kg L-serine; EV, binge ethanol + vehicle; MS, methionine synthase.
cardiovascular risk by 10–20% (25). Moreover, chronic hypercholesterolemia induces hepatic steatosis and fibrosis (26). Because homocysteine and cholesterol are independent risk factors for fatty liver and cardiovascular disease, a treatment option targeting both would be advantageous. In the present study, L-serine treatment in rats significantly reduced serum cholesterol concentrations without a significant change in homocysteine concentrations. At present, we do not know how L-serine lowered serum cholesterol concentrations, and further study is needed to answer this question.

Chronic ethanol ingestion induces a deficiency in many nutrients, including folate, choline, B vitamins, and betaine and methionine (8). Several studies of experimental ALD, as well as in humans supplemented with deficient nutrients, revealed a significant decrease in homocysteine concentrations and/or attenuation of ethanol-induced liver injuries (10, 18, 27). Folate, betaine, and B-vitamin supplementation have been used to correct perturbed methionine metabolism, but little attention has been paid to L-serine. Our data support the use of nutrient replenishment in disease reversal. For example, ethanol consumption in Lieber-DeCarli diet–fed rats for 4 wk significantly reduced serum concentrations of several amino acids, including L-serine, by >60%. At this time, the mechanism of this amino acid deficiency remains unknown. However, given the role of L-serine in homocysteine metabolism, such as in the production of 5-methyltetrahydrofolate (5-methylTHF) in the MS-catalyzed pathway, as well as in the conjugation reaction with homocysteine catalyzed by CBS, it was important to examine the effects of L-serine on the homocysteine metabolism and the lipogenesis pathway.

INSIG1 blocks proteolytic activation of SREBP, and activation of the ER stress by thapsigargin suppresses protein synthesis and activates proteolysis of SREBP followed by the disappearance of INSIG1 (28). In a mouse model, Insig1 mRNA was downregulated by ethanol feeding (29). In the present study, INSIG1 expression was inhibited by ethanol feeding; L-serine treatment attenuated these effects. We can conclude that L-serine ameliorated SREBP activation and TG accumulation by inhibiting INSIG1 suppression. When the homocysteine concentration was increased by incubation with methionine or homocysteine, cotreatment with L-serine significantly decreased intracellular homocysteine and TG concentrations.

**FIGURE 5** Intracellular tHcy (A) and TG (B) concentrations in homocysteine (5 mM) or methionine (5 mM)-treated AML12 cells in the absence or presence of L-serine (10 mM). Homocysteine and TG concentrations were normalized by intracellular proteins. Values are means ± SDs, n = 3 (means of triplicates). Within each graph, labeled means without a common letter differ (P < 0.05). Statistical analysis was performed by using 2-factor ANOVA with Bonferroni post-test. tHcy, total homocysteine.

**FIGURE 6** Intracellular tHcy and TGs in Bhmt-, Cbs-, or Ms-siRNA transfected AML12 cells (A, C) and Ms/Cbs-siRNA cotransfected AML12 cells (B, D) in the absence or presence of L-serine (10 mM). Homocysteine and TG concentrations were normalized by intracellular proteins. Values are means ± SDs, n = 3 (means of triplicates). Within each graph, labeled means (within L-serine treatment in panels B and D) without a common letter differ (P < 0.05). Statistical analysis was performed by using 2-factor ANOVA with Bonferroni post-test. Bhmt, betaine homocysteine S-methyltransferase; Cbs, cystathionine β-synthase; mock, control siRNA; Ms, methionine synthase; siRNA, small interfering RNA; total homocysteine.
effects on homocysteine and TG concentrations were similar when hyperhomocysteinemia was established in an in vitro model by knockdown of homocysteine-metabolizing enzymes. It is important to note, however, that the homocysteine and TG-lowering effects of l-serine were more pronounced in BhmtriRNA–transfected cells than in Cbsr-siRNA–transfected cells. These results indicate that BHMT-dependent homocysteine metabolism was not involved in l-serine–induced homocysteine metabolism. Moreover, the effects of l-serine were almost abolished when Ms was knocked down by siRNA, indicating that the MS-dependent remethylation pathway is essential in this process; siRNA-mediated knockdown of both Ms and Cbs corroborated this result.

In addition to the basic proteogenic and metabolic action of amino acids, some exert unexpected biological effects by acting as signaling molecules in the regulation of diverse signaling pathways. For example, l-threonine regulated G1/S phase transition of mouse embryonic stem cells through lipid raft/caveola-dependent activation of the phosphoinositide-3-kinase (PI3K)/protein kinase B (Akt)-MAPK-mammalian target of rapamycin (mTOR) signaling pathway (30). Oral supplementation with l-aspartate and l-glutamate inhibited atherogenesis and fatty liver disease in cholesterol-fed rabbits (31). Oral leucine enhanced myocardial protein synthesis in rats through increased phosphorylation of mTOR and eukaryotic translation initiation factor 4E binding protein 1 (4EBP1) 32). There have been some reports describing homocysteine-lowering effects of l-serine in methionine-loaded hepatocytes, in rats fed a methionine diet, and in healthy humans who ingested a low-protein meal fortified with methionine (9, 10, 33). However, the effects of l-serine on fatty liver and its association with homocysteine concentrations have not been investigated extensively. A recent study shows that although the plasma homocysteine concentration was decreased by l-serine supplementation in choline-deficient rats, fatty liver was not prevented (11). To our knowledge, this study is the first to demonstrate that the natural amino acid l-serine ameliorated alcoholic fatty liver by lowering homocysteine concentrations. Considering the safety of a natural amino acid, our results imply that l-serine may be a good candidate for treatment of hyperhomocysteinemia and the treatment of homocysteine-related disease, including alcoholic fatty liver.

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B-HL designed the study and wrote the manuscript; W-CS, H-QY, Y-JC, HCK, and S-KK conducted the research; W-CS, H-QY, and H-SC analyzed the data; W-CS and B-HL are responsible for the final content of the manuscript. All authors read and approved the final version of the manuscript.

References


