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ORIGINAL CONTRIBUTION

Plasma homocysteine level and hepatic sulfur amino acid metabolism in mice fed a high-fat diet

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Abstract
Purpose Obesity, a feature of metabolic syndrome, is a risk factor for cardiovascular disease, and elevated plasma homocysteine is associated with increased cardiovascular risk. However, little published information is available concerning the effect of obesity on homocysteine metabolism.
Methods Hepatic homocysteine metabolism was determined in male C57BL/6 mice fed a high-fat diet for 12 weeks.
Results High-fat diet increased plasma homocysteine but decreased hepatic homocysteine levels. Hepatic *S*-adenosylhomocysteine hydrolase levels were down-regulated in the obese mice, which was in part responsible for the decrease in hepatic *S*-adenosylmethionine/*S*-adenosylhomocysteine, which served as an index of transmethylation potential. Despite the decrease in hepatic cysteine, hepatic taurine synthesis was activated via up-regulation of cysteine dioxygenase. Hepatic levels of methionine adenosyltransferase I/III, methionine synthase, methylene tetrahydrofolate reductase, and gamma-glutamylcysteine ligase catalytic subunit were unchanged. Obese mice showed elevated betaine-homocysteine methyltransferase and decreased cystathionine beta-synthase activities, although the quantities of these enzymes were unchanged.
Conclusion This study suggests that plasma homocysteine level is increased in obesity-associated hepatic steatosis, possibly as a result of increased hepatic homocysteine efflux along with an altered sulfur amino acid metabolism.

Keywords Homocysteine · Sulfur amino acid metabolism · High-fat diet · Hepatic steatosis · Obesity

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Introduction

Although a recent study suggested that elevated plasma homocysteine is a marker, rather than a cause, of atherosclerotic disease [1], elevation of plasma homocysteine level is a recognized risk factor for cardiovascular diseases [2]. Mean plasma homocysteine was found to be significantly higher in both male and female patients with coronary artery disease compared with controls with angiographically normal coronary arteries [3]. An increased plasma homocysteine of only 12% above the upper limit of normal was associated with a 3.4-fold increase in the risk of myocardial infarction [4]. Many previous studies indicated that even a slight increase in plasma homocysteine is associated with a significantly increased risk of stroke and cognitive dysfunctions ranging in severity from mild cognitive impairment to Alzheimer's disease [5]. Hoogeveen et al. [6] and Okada et al. [7] showed that hyperhomocysteinemia is a stronger risk factor in patients with type 2 diabetes and with existing coronary disease.

Homocysteine is intermediate in the sulfur amino acid metabolic pathway. Methionine metabolism occurs primarily via the transsulfuration pathway, which results in

the transfer of methionine sulfur to serine to form cysteine. The first step in methionine metabolism is the formation of *S*-adenosylmethionine (SAM), catalyzed by methionine adenosyltransferase (MAT). SAM serves as a methyl donor for biological methylation reactions, and the co-product of transmethylation, *S*-adenosylhomocysteine (SAH), is hydrolyzed to yield homocysteine [8]. Decarboxylation of SAM provides an aminopropylic group for synthesis of polyamines such as spermidine and spermine. Homocysteine stands at the intersection of two competitive metabolic pathways: remethylation to methionine and transsulfuration to cystathionine [9]. Remethylation is independently mediated by two enzymes, methionine synthase (MS) and betaine-homocysteine methyltransferase (BHMT). MS is a cobalamin-dependent enzyme that requires methylene tetrahydrofolate reductase (MTHFR) as a folate cycle enzyme to generate the methyl donor, 5-methyltetrahydrofolate that is used for homocysteine remethylation by MS. Remethylation of homocysteine via BHMT requires betaine, a metabolite of choline. The transsulfuration of homocysteine to cysteine via cystathionine is mediated by the consecutive actions of cystathionine β-synthase (CβS) and cystathionine γ-lyase (CγL). Cysteine is metabolized in the liver to yield either taurine, inorganic sulfate, or glutathione (GSH). Cysteine dioxygenase (CDO) catalyzes the oxidation of this amino acid to cysteine sulfinate, which is mainly converted to taurine via hypotaurine by cysteine sulfinate decarboxylase (CDC). Synthesis of GSH is mediated consecutively by γ-glutamylcysteine ligase (GCL) and GSH synthetase [10].

Hepatic homocysteine metabolism can be altered in response to pathophysiological conditions, such as diabetes, hepatic disease, and alcohol consumption. Plasma homocysteine levels were approximately 40% lower in streptozotocin-induced type 1 diabetic rats than in control rats, and insulin treatment maintained a plasma homocysteine level comparable with that of controls [11]. Zucker diabetic fatty (ZDF) rats, a type 2 diabetic model, also showed decreased plasma homocysteine levels [12]. This reduction appeared to be a result of increased CβS, CγL, and BHMT activities. In HepG2 cells, a human cell culture model, insulin treatment decreased the activity of CβS at the transcriptional level [13], indicating that insulin can regulate plasma homocysteine levels through inhibition of CβS expression. On the other hand, experimental and clinical studies of chronic alcohol consumption have consistently documented elevated total plasma homocysteine levels [14]. However, little published information is available concerning the effect of obesity-associated hepatic steatosis on sulfur amino acid metabolism. In this study, hepatic sulfur amino acid metabolism was determined in C57BL/6 mice fed the high-fat diet originally introduced by Surwit et al. [15].

Materials and methods

Animals

Male 8-week-old C57BL/6 mice were used in these studies. All animals were maintained at the Korea Research Institute of Bioscience and Biotechnology (Daejeon, Korea). The mice were housed in plastic cages in a room with controlled temperature (22 ± 2 °C) and maintained on a reverse 12-h light/dark cycle. The mice were randomly divided into two groups: standard chow diet ($n = 10$; Teklad #2018; Harlan Laboratories, Indianapolis, IN, USA) and high-fat diet ($n = 10$; #100244; Dyets Inc., Bethlehem PA, USA). The high-fat diet consisted of 21% milk fat, 0.15% cholesterol, 19.5% casein, 15% cornstarch, 34.1% sucrose, 5% cellulose, 3.5% salt mixture, 1% vitamin mixture, 0.4% calcium carbonate, 0.004% ethoxyquin, and 0.3% methionine. The standard chow diet contained 18.6% crude protein, 6.2% fat, 44.2% carbohydrate, 3.5% crude fiber, 14.7% neutral detergent fiber, 5.3% ash, and 0.4% methionine. After 12 weeks on the diets, mice were killed by decapitation. All animal experiments were approved by the Institutional Animal Care and Use Committee and performed in accordance with the institutional guidelines.

Chemicals and antibodies

DL-Homocysteine, L-methionine L-cysteine, L-glutathione, taurine, hypotaurine, *N*-(2-mercaptopropionyl) glycine, tris-(2-carboxyethyl)-phosphine hydrochloride (TCEP), trichloroacetic acid (TCA), ethylenediaminetetraacetic acid (EDTA), ammonium-7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonic acid (SBD-F), and sodium acetate were purchased from Sigma Chemical (St. Louis, MO, USA). Antibodies against MATI/III, MTHFR, and actin were purchased from Santa Cruz Biotech (Santa Cruz, CA, USA), and anti-SAHH, anti-MS, and anti-CDO antibodies were purchased from Abcam (Cambridge, UK). Anti-BHMT and anti-GCL catalytic subunit (GCLC) antibody were purchased from Everest Biotech (Oxford, UK) and NeoMarkers Inc. (Fremont, CA, USA), respectively. Anti-CβS antibody was kindly provided by Dr. Matherly (School of Medicine, Wayne State University, Detroit, MI, USA). All other chemicals and solvents were reagent grade or better.

Plasma analysis and determination of lipid peroxidation and triglycerides in the liver

At the end of the experimental period, blood samples were taken from the orbital venous congestion to determine the concentrations of plasma biomarkers. Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST),

and glucose levels were measured using an automatic chemistry analyzer (Model 1750; Hitachi, Tokyo, Japan). Plasma leptin levels were determined using a radioimmunoassay kit (DY498 Mouse Leptin; R&D Systems, Inc., USA). Plasma insulin was measured using an assay from Alpco Diagnostics (Cat# 80-INSMS-E01; Salem, NH) in 96-well plates.

Liver homogenate samples were comprised of livers from two mice. Pooled livers were homogenized in a threefold volume of cold 0.154 M KCl/50 mM Tris–HCl containing 1 mM EDTA (pH 7.4). Malondialdehyde (MDA) levels were measured by high-performance liquid chromatography (HPLC) [16].

Hepatic lipids were extracted using the procedures developed by Bligh and Dyer [17]. Triglyceride contents in liver were analyzed using an enzymatic kit (Sigma Chemical) at 540 nm.

Determination of sulfur amino acid metabolites in tissues and plasma

Liver homogenates were diluted in a threefold volume of ice-cold methanol for analysis of methionine and taurine levels. Methionine and taurine were derivatized with *O*-phthalaldehyde/2-mercaptoethanol and quantified by an HPLC system with a fluorescence detector (RF-10AXL; Shimadzu Co., Tokyo, Japan; Ex 385 nm and Em 515 nm). An Agilent Eclipse XDB-C18 column (4.6 × 150 mm, 3.5 μm; Wilmington, Delaware) and a Phenomenex Luna C18 column (4.6 × 150 mm, 5 μm; Torrance, CA, USA) were used for analysis of methionine and taurine, respectively [18, 19].

Liver homogenates were diluted in the same volume of ice-cold 12% perchloric acid. Denatured protein was removed by centrifugation at 10,000×*g* for 10 min, and the supernatant was used as a sample for measuring hepatic SAM, SAH, homocysteine, cysteine, GSH, oxidized glutathione (GSSG), and polyamines. Supernatants were stored at −70 °C. HPLC was used for determination of SAM and SAH [20]. The supernatant was directly applied to an HPLC system (SCL-10A; Shimadzu Co.) equipped with an ultraviolet detector (SPD-10Avp; Shimadzu Co.; 254 nm) and a TSK-GEL ODS-80TM column (4.6 × 250 mm; Tosoh Co., Tokyo, Japan). SAM was determined within 2 min of sample thawing because it is an unstable episulfonium ion. In our analytical method, the correlation coefficient of the standard curve was ≥0.99 and the intra-day coefficient of variation was <7%. Hepatic total GSH and GSSG concentrations were determined using the enzymatic recycling method of Griffith [21]. Polyamines were determined by the method of Fu et al. [22].

Hepatic homocysteine and cysteine and plasma homocysteine, cysteine, and GSH were quantified using the SBD-F method [23]. For sample preparation, *N*-(2-mercaptopropionyl)-glycine was added as an internal standard (50 μL) to samples (50 μL) and briefly vortex mixed. Following the addition of TCEP (100 g/L; 10 μL), tubes were capped, briefly vortex mixed, and incubated at room temperature for 30 min. TCA with 1 mM EDTA (100 g/L; 90 μL) was then added to each sample, briefly vortex mixed, and centrifuged at 13,000×*g* for 10 min. Supernatant (50 μL) was added to another tube containing 10 μL 1.55 M NaOH, 125 μL 0.125 M borate buffer (pH 9.5) with 4 mM EDTA, and 50 μL 1 g/L SBD-F in borate buffer. Samples were capped, briefly vortex mixed, incubated at 60 °C for 1 h, and then, a 10-μL aliquot was injected into the HPLC system for analysis.

Enzyme assays

The liver homogenate was centrifuged at 10,000×*g* for 20 min. The 10,000×*g* supernatant fraction was used for determination of enzyme activities. Protein was quantified using a bicinchoninic acid (BCA) protein assay kit (Thermo Scientific, IL, USA) with bovine serum albumin as a standard.

MAT activity was estimated by quantifying SAM production, as described above [24]. Both BHMT and MS activities were determined by measuring methionine formation [25, 26]. CβS activity was determined by the method of Costa et al. [27].

Immunoblot analysis

To determine the protein levels of MATI/III, SAHH, BHMT, MS, MTHFR, CβS, GCLC, CDO, and actin, the S-9 fraction was diluted to 2 mg/mL in loading buffer that contained a reducing agent. Lysate (10 μL) was resolved by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA, USA), blocked in 5% milk powder in 0.1% Tween 20 in phosphate-buffered saline solution (PBS-T), probed with appropriate antibodies, and then visualized by enhanced chemiluminescence using a Chemidoc XRS digital imaging system (Bio-Rad Laboratories) or a LAS 4000 mini-system (Fuji Photo Film Co., Tokyo, Japan). Immunoquantitation was accomplished by the Quantity One analysis program (Bio-Rad Laboratories) or Multi Gauge 3.0 software (Fuji Photo Film Co.).

Statistical analysis

Data are expressed as the mean ± standard deviation (SD) and were analyzed using a two-tailed Student's *t*-test. The acceptable level of significance was established at $P < 0.05$, except when otherwise indicated.

Results

Changes in sulfur amino acid metabolites and liver physiology in control and obese mice fed a high-fat diet

The high-fat diet caused a marked increase in total body weight of 4.7 ± 0.8–13.3 ± 3.7 g over 12 weeks (24.7 ± 1.2–29.4 ± 1.8 g in control mice; and 24.6 ± 1.2–37.9 ± 3.7 g in mice fed a high-fat diet). Hepatic triglyceride contents were also elevated ~4.1-fold compared with control mice (9.0 ± 1.2 mg/g liver in control mice; and 37.1 ± 10.3 mg/g liver in mice fed a high-fat diet). Liver weights per 100 g body weight measured at terminal kill were not significantly changed (4.14 ± 0.27 in control mice; and 3.91 ± 0.47 in mice fed a high-fat diet). Neither plasma ALT nor AST activities were altered in mice fed a high-fat diet (39.2 ± 14.9 and 86.0 ± 35.4 IU/L in control mice; and 35.9 ± 10.3 and 96.9 ± 45.6 IU/L in obese mice). No significant difference in MDA formation was observed between the control and high-fat diet groups (control, 24.3 ± 2.3 nmol/g liver; and high-fat diet, 21.2 ± 2.3 nmol/g liver). Also, neither plasma insulin nor glucose levels were affected by a high-fat diet (1.17 ± 0.76 ng/mL and 186 ± 45 mg/dL in controls; and 0.71 ± 0.56 ng/mL and 195 ± 25 mg/dL in mice fed a high-fat diet). On the other hand, plasma leptin level was significantly increased in mice fed a high-fat diet (0.91 ± 0.26 μg/L in control mice; and 13.41 ± 8.24 μg/L in mice fed a high-fat diet).

Plasma total homocysteine, cysteine, and GSH concentrations were determined in mice fed a high-fat diet for 12 weeks (Fig. 1). Plasma total homocysteine level in mice fed a high-fat diet was increased to ~157% of the level in control mice. On the other hand, the high-fat diet resulted in a 16 or 28% decrease in plasma total cysteine and GSH levels, respectively, relative to control mice.

The hepatic concentrations of major metabolites and products in the transsulfuration pathway were monitored in mice fed a high-fat diet for 12 weeks (Table 1). SAH and taurine levels in the liver were elevated significantly, while that of SAM was reduced, in mice fed a high-fat diet. The ratio of SAM/SAH, which serves as an index of transmethylation potential, was decreased by 47.7%. Hepatic total cysteine and homocysteine levels were decreased to ~76 and ~73%, respectively, of those in control mice. Thus, the ratio of plasma homocysteine/hepatic homocysteine increased from 0.33 ± 0.06 in control mice to 0.71 ± 0.19 in mice fed a high-fat diet, but the ratio of plasma cysteine/hepatic cysteine was not significantly different between control (1.02 ± 0.24) and obese (1.10 ± 0.21) mice. On the other hand, neither methionine nor total GSH levels in the liver were altered.

In polyamine synthesis, SAM is also the source of a propylamine group for spermidine and spermine following its decarboxylation by SAM decarboxylase [28]. To determine whether the decrease in hepatic SAM was associated with an increased utilization of SAM for polyamine synthesis, hepatic levels of polyamines, including putrescine, spermidine, and spermine, were determined (Table 1). Although the hepatic putrescine produced from ornithine by ornithine decarboxylase was significantly

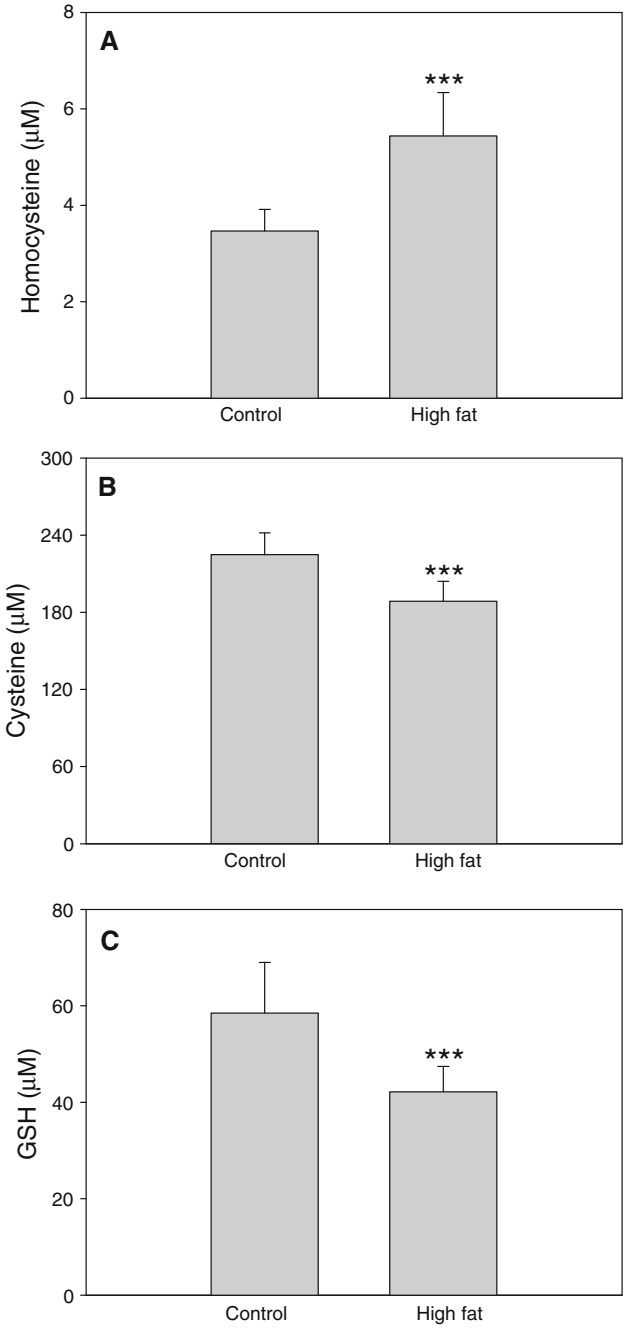

Fig. 1 Plasma concentrations of homocysteine (**a**), cysteine (**b**), and GSH (**c**) in control and obese mice fed a high-fat diet. Each value represents the mean ± SD of 10 mice. ***Significantly different from the control at $P < 0.001$

Table 1 Hepatic sulfur amino acid metabolite concentrations in control and obese mice fed a high-fat diet

	Control	High fat
Methionine (nmol/g liver)	26.0 ± 6.1	26.9 ± 3.5
SAM (nmol/g liver)	192.9 ± 40.6	140.3 ± 21.8*
SAH (nmol/g liver)	93.3 ± 12.1	130.7 ± 15.9*
Homocysteine (nmol/g liver)	10.8 ± 2.1	7.8 ± 1.1*
Cysteine (nmol/g liver)	229.3 ± 44.6	173.5 ± 17.4*
Taurine (μmol/g liver)	12.4 ± 3.0	17.8 ± 1.2**
GSH (μmol/g liver)	14.7 ± 2.0	12.5 ± 1.1
Putrescine (nmol/g liver)	36.1 ± 7.4	62.3 ± 21*
Spermidine (nmol/g liver)	868 ± 133	885 ± 134
Spermine (nmol/g liver)	754 ± 102	819 ± 123

Each value represents the mean ± SD of five pooled samples, each comprising the livers of two mice

*, ** Significantly different from the control at $P < 0.05$ or $P < 0.01$, respectively

increased by ~70% in mice fed a high-fat diet compared with controls, hepatic spermidine and spermine were not significantly affected. These results suggest that decreased hepatic SAM levels could not be attributed to elevated utilization of SAM for polyamine synthesis.

Hepatic sulfur amino acid metabolizing enzymes in control and obese mice fed a high-fat diet

Levels of the hepatic enzymes involved in sulfur amino acid metabolism were determined by immunoblot analysis using specific antibodies (Fig. 2). Hepatic levels of MATI/III, BHMT, MS, MTHFR, CβS, and GCLC were not significantly changed in obese mice. However, SAHH, which converts SAH to adenosine and homocysteine, was down-regulated to 77% of the level in control mice. Similarly, CDO, which has a critical role in the synthesis of taurine from cysteine, was up-regulated in the obese mice.

We determined the activities of MAT, BHMT, MS, and CβS, each of which can be regulated by post-translational modifications and allosteric mechanisms [29] (Table 2). Although the levels of CβS and BHMT in the liver were unchanged, CβS activity was significantly decreased ($P < 0.001$) to ~78% of that of controls, and BHMT activity was increased to ~144% in mice fed a high-fat diet. However, MAT and MS activities were unaffected by the high-fat diet.

Discussion

This study demonstrated altered sulfur amino acid metabolism in C57BL/6 mice fed a high-fat diet for 12 weeks. The high-fat diet resulted in elevated plasma homocysteine but decreased plasma cysteine and GSH levels. These results differ from those in diabetic animals treated with streptozotocin and ZDF rats, each of which showed a decrease in plasma homocysteine levels. These results raise the possibility that plasma homocysteine levels are differently regulated in diabetes and obesity/hepatic steatosis.

Elevation of plasma homocysteine level is an independent risk factor for cardiovascular disease, stroke, Alzheimer's disease [5], and osteoporotic fractures [30]. Pathophysiologically, elevated homocysteine is associated with increased thrombogenicity, increased oxidative stress status, over-activation of redox-sensitive inflammatory pathways, impaired endothelial function, and atherogenesis [31].

In mammals, the liver plays a central role in the metabolism of sulfur amino acids, because nearly one-half of the daily methionine intake is metabolized there [32]. In this study, hepatic levels of homocysteine-clearing enzymes such as BHMT, MS/MTHFR, and CβS were not significantly different between control and obese mice. However, the hepatic activity of CβS, an enzyme that mediates irreversible transsulfuration of homocysteine to cystathionine, was decreased and that of BHMT, which converts homocysteine to methionine using betaine as a methyl donor, was increased. The activity of BHMT and CβS is allosterically regulated with no change in their protein levels. SAM has been suggested to serve as a regulator of homocysteine metabolism [29]. SAM is a potent inhibitor of BHMT activity and an activator and stabilizer of CβS [33]. In this study, decreased SAM levels possibly explain not only the increased BHMT activity, but also the decreased CβS activity. These results indicate that functional methods, such as enzyme assays, are more valuable than mRNA-based or immunoblot assays for evaluating sulfur amino acid metabolism.

Plasma homocysteine level was increased and hepatic homocysteine level decreased in mice fed a high-fat diet, which resulted in a greater than twofold increase in the ratio of plasma homocysteine/hepatic homocysteine, relative to control mice. Considering that the liver is the major source of plasma homocysteine [34], these results raise the possibility that efflux of hepatic homocysteine to the blood may be involved in the elevation of plasma homocysteine levels. Further studies to determine the effects of saturated free fatty acids and leptin on cellular homocysteine efflux are currently underway in our laboratory.

Several experimental approaches have been developed to induce hyperhomocysteinemia in animals, including oral or parenteral administration of methionine or homocysteine, dietary deficiency of folate, choline, cobalamin (vitamin B12), and/or pyridoxine (vitamin B6), and genetic alterations of enzymes such as CβS, MS, and MTHFR [35].

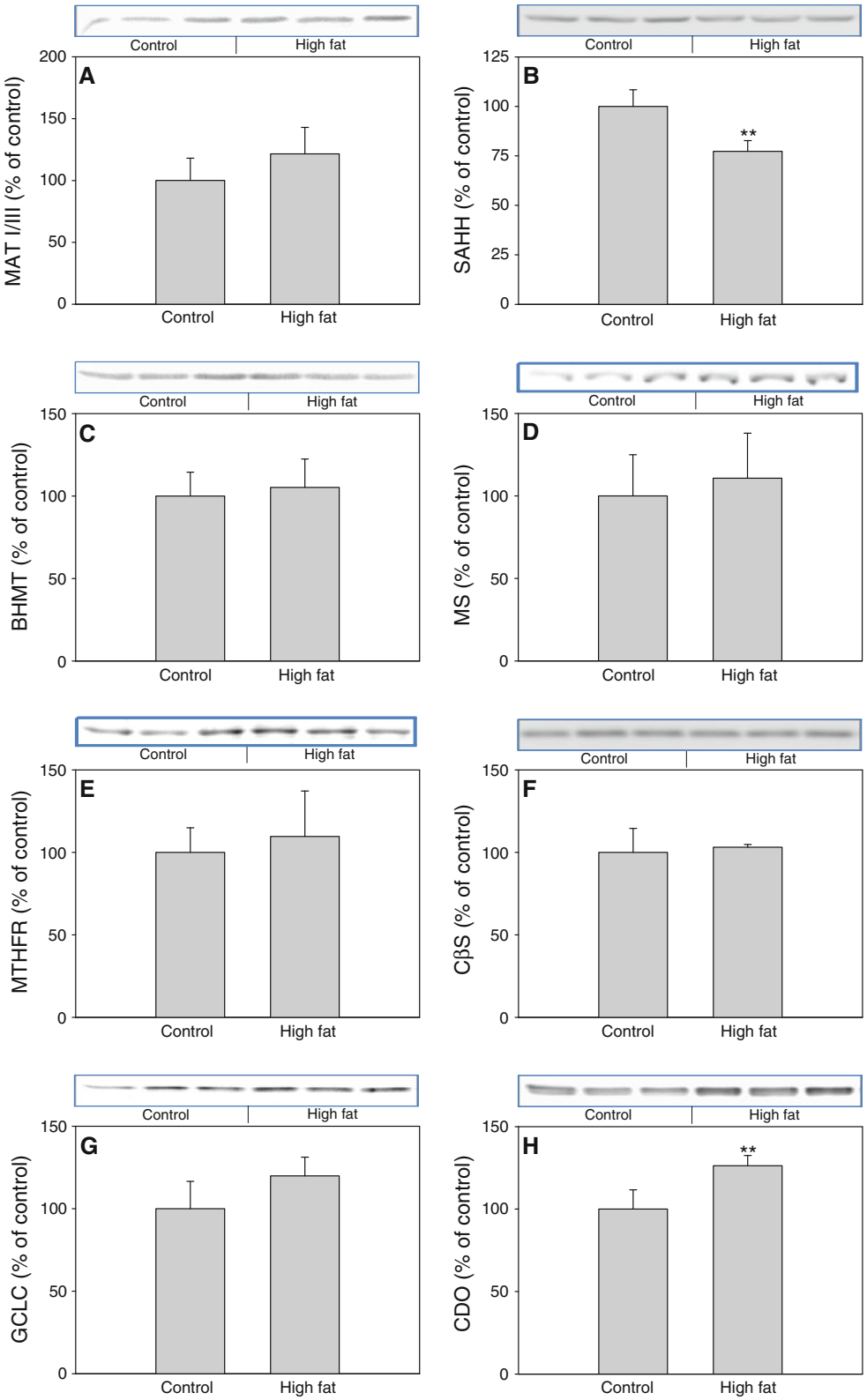

Fig. 2 Hepatic expression of the sulfur amino acid-metabolizing enzymes MATI/III (**a**), SAHH (**b**), BHMT (**c**), MS (**d**), MTHFR (**e**), CβS (**f**), GCLC (**g**), and CDO (**h**) in control and obese mice fed a high-fat diet. Each value represents the mean ± SD of five pooled samples, each comprising the livers of two mice. **Significantly different from the control at $P < 0.01$

Table 2 Hepatic activity of sulfur amino acid-metabolizing enzymes in control and obese mice fed a high-fat diet

	Control nmol/min/mg protein	High fat
MAT	1.59 ± 0.6	1.53 ± 0.2
BHMT	1.29 ± 0.2	1.86 ± 0.1**
MS	0.29 ± 0.05	0.28 ± 0.04
CβS	10.3 ± 1.1	8.07 ± 0.5**

Each value represents the mean ± SD of five pooled samples, each comprising the livers of two mice

** Significantly different from the control at $P < 0.01$

Obesity induced by a high-fat diet is more clinically relevant than the above-mentioned models. Thus, our results enable investigators to induce hyperhomocysteinemia in animals for additional pathophysiological research into obesity-mediated diseases.

Of the major metabolites in the transsulfuration pathway, hepatic SAM was markedly decreased while SAH was increased in mice fed a high-fat diet. Consequently, SAM/SAH, which is used as a methylation index, was diminished by the high-fat diet. On the other hand, hepatic SAM levels were elevated in diabetic animals treated with streptozotocin [11] and ZDF [12]. In these studies, elevated hepatic SAM levels may have been a result of the increased MAT level, since it is up-regulated by glucocorticoids [36]. In this study, MAT and methionine were not significantly changed in obese mice, suggesting that SAM synthesis may be not altered and that MAT expression may not be altered in response to hepatic steatosis. SAM is the source of a methyl group for methylation and a propylamine group for polyamine synthesis. In this study, SAM/SAH was markedly decreased, suggesting a decreased methylation capacity. Thus, we determined whether the decrease in hepatic SAM was associated with an increased utilization of SAM for polyamine synthesis. Hepatic spermidine and spermine were not significantly affected by the high-fat diet, indicating that the decreased hepatic SAM levels could not be attributed to the elevation of SAM utilization for polyamine synthesis. Thus, the mechanism(s) underlying the decrease in hepatic SAM levels remain to be elucidated.

SAH, a demethylated product of SAM, is hydrolyzed to homocysteine by SAHH. Down-regulation of SAHH in the obese mice may be responsible for the accumulation of cellular SAH. The marked increase in SAH may be particularly important, because it is a potent inhibitor of many methyltransferases. DNA hypomethylation induced by SAH accumulation has been related to alterations in gene expression or other methylation-dependent functional biological reactions. In addition, some studies have shown that plasma SAH is a stronger risk factor of cardiovascular disease than is total plasma homocysteine [37].

Cysteine availability is a major factor determining the metabolic fate of cysteine to taurine, sulfate, or GSH in hepatocytes [34]. That is, low cysteine availability favors the synthesis of GSH, whereas greater cysteine availability enhances the partitioning of cysteine for catabolism to taurine mediated by CDO. Hepatic cysteine concentration is maintained substantially below the K_m of CDO for cysteine, so CDO expression is able to respond to changes in hepatic cysteine level. Regulation of CDO has recently been shown to occur at the level of CDO polyubiquitination, which is increased by low-protein diets; consequently, CDO is rapidly degraded [38]. However, hepatic CDO was up-regulated in the obese mice, resulting in the elevation of taurine synthesis, although hepatic cysteine level was decreased. Our results raise the possibility that CDO can be regulated not only by cysteine availability, but also by other factors involved in obesity and steatosis.

In this study, animals fed a high-fat diet exhibited the following alterations in hepatic sulfur amino acid metabolism: (1) increased plasma homocysteine and decreased hepatic homocysteine levels, (2) elevated hepatic taurine levels via up-regulation of CDO despite decreased hepatic cysteine levels, (3) decreased SAM/SAH, which may be associated with down-regulation of hepatic SAHH, and (4) increased CβS and decreased BHMT activity, both of which are allosterically regulated by SAM. These results warrant a further study to determine the factor(s) involved in regulation of homocysteine efflux and to investigate epigenetic regulation by DNA methylation.

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