Amino Acids (2015) 47:839–846
DOI 10.1007/s00726-014-1913-x

ORIGINAL ARTICLE

Betaine supplementation prevents fatty liver induced by a high-fat diet: effects on one-carbon metabolism

Rafael Deminice · Robin P. da Silva · Simon G. Lamarre · Karen B. Kelly · René L. Jacobs · Margaret E. Brosnan · John T. Brosnan

Received: 9 October 2014 / Accepted: 30 December 2014 / Published online: 11 January 2015

Abstract The purpose of this study was to examine the effects of betaine supplementation on the regulation of one-carbon metabolism and liver lipid accumulation induced by a high-fat diet in rats. Rats were fed one of three different liquid diets: control diet, high-fat diet and high-fat diet supplemented with betaine. The control and high-fat liquid diets contained, respectively, 35 and 71 % of energy derived from fat. Betaine supplementation involved the addition of 1 % (g/L) to the diet. After three weeks on the high-fat diet the rats had increased total liver fat concentration, liver triglycerides, liver TBARS and plasma TNF-α. The high-fat diet decreased the hepatic *S*-adenosylmethionine concentration and the *S*-adenosylmethionine/*S*-adenosylhomocysteine ratio compared to the control as well as altering the expression of genes involved in one-carbon metabolism. Betaine supplementation substantially increased the hepatic *S*-adenosylmethionine concentration (~fourfold) and prevented fatty liver and hepatic injury induced by the high-fat diet. It was accompanied by the normalization of the gene expression of BHMT, GNMT and MGAT, which code for key enzymes of one-carbon metabolism related to liver fat accumulation. In conclusion, the regulation of the expression of MGAT by betaine supplementation provides an additional and novel mechanism by which betaine supplementation regulates lipid metabolism and prevents accumulation of fat in the liver.

Keywords Betaine · Liver · High-fat diet · *S*-adenosylmethionine

Abbreviations

BHMT	Betaine-homocysteine *S*-methyltransferase
CβS	Cystathionine-β-synthase
CDD	Choline deficient diet
ChDh	Choline dehydrogenase
CDO	Cysteine dioxygenase
Chka	Choline kinase alpha
Chkb	Choline kinase beta
Cta	Choline-phosphate cytidylyltransferase A
Cth	Cystathionase
CTP	Phosphorylcholine cytidylyltransferase
ET	Ethanolamine phosphate cytidylyltransferase 2
Gclc	Glutamyl-cysteine synthetase
Gnmt	Glycine *N*-methyltransferase
Hcy	Homocysteine
HHcy	Hyperhomocysteinemia
Mat1	Methionine adenosyltransferase
Mgat1	Monoacylglycerol *O*-acyltransferase 1
MS	Methionine synthase
Mthfr	5,10-Methylenetetrahydrofolate reductase
PE	Phosphatidylethanolamine
PEMT	Phosphatidylehanolamine N-methyltranferase
PC	Phosphatidylcholine
SAH	*S*-adenosylhomocysteine
SAM	*S*-adenosylmethionine

R. Deminice · R. P. da Silva · S. G. Lamarre · M. E. Brosnan · J. T. Brosnan
Department of Biochemistry, Memorial University of Newfoundland, St. John's, Canada

R. Deminice (✉)
Department of Physical Education, Faculty of Physical Education and Sport, State University of Londrina, Rodovia Celso Garcia Cid, Pr 445 km 380, Campus Universitário, Londrina, Paraná, Brazil
e-mail: deminice@ig.com.br

R. P. da Silva · K. B. Kelly · R. L. Jacobs
Department of Agricultural, Food and Nutritional Science and the Group on the Molecular and Cell Biology of Lipids, University of Alberta, Edmonton, Canada

Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases throughout the world (Brunt 2010). NAFLD is a pathological state that develops in the absence of alcohol abuse and includes a wide spectrum of liver abnormalities ranging from simple accumulation of triglyceride in hepatocytes to nonalcoholic steatohepatitis (NASH) and cirrhosis (Duvnjak et al. 2007). However, the mechanisms involved in nonalcoholic fatty liver disease are not totally understood (Duvnjak et al. 2007; Day and James 1998). The two-hit model proposes that the first hit involves a simple accumulation of fat in the liver, increasing the susceptibility of liver to more severe damage resulting from the second hit which involves mitochondrial dysfunction, lipid peroxidation and inflammation (Day and James 1998). Over the last few years, fat accumulation and NASH progression have been associated with impairment of hepatic one-carbon metabolism (Dahlhoff et al. 2013), resulting in decreased availability of *S*-adenosylmethionine (SAM) as well as an increase in homocysteine (Hcy) levels (Kim and Kim 2005; Kwon do et al. 2009). A recent review on the role of one-carbon metabolism-mediated regulation of lipid metabolism by da Silva et al. (2014) provides relevant background information.

Betaine (trimethylglycine) is an amino compound obtained from dietary sources (especially sea-food, spinach and beets) or synthesized endogenously from choline (Kharbanda 2013). Betaine is an important methyl donor used for the remethylation of Hcy to methionine (Fig. 1). Studies have shown that supplementation with betaine may protect the liver from fat accumulation, lipid peroxidation and inflammation in rodent models of alcoholic (Erman et al. 2004; Kim et al. 2008; Kharbanda et al. 2007) and nonalcoholic (Kwon do et al. 2009; Wang et al. 2010; Kawakami et al. 2012) fatty liver disease. Thus, fatty liver disease is linked to one-carbon metabolism through the reduction in methylation capacity (SAM/SAH ratio), which may be attenuated by betaine supplementation (Kharbanda 2013). A molecular approach may allow a deeper understanding of the alterations in one-carbon metabolism induced by high-fat diet and the modulations caused by betaine supplementation. The aim of the present study was to examine the effects of betaine supplementation on liver

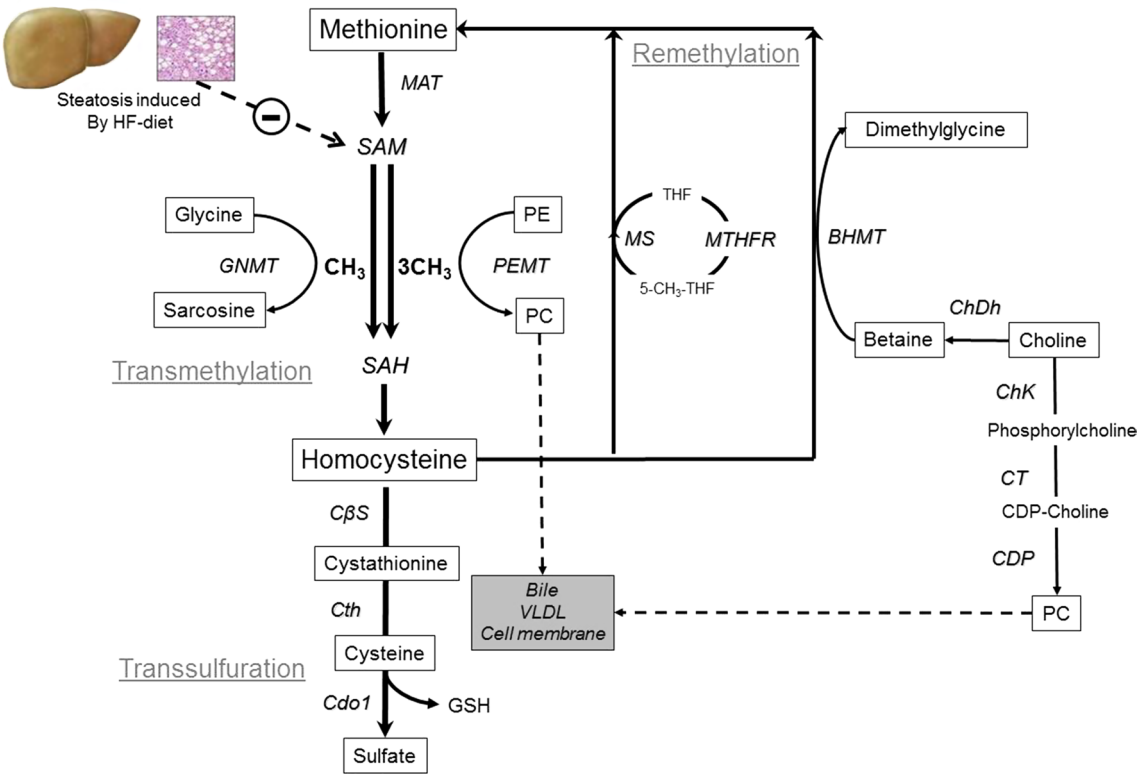

Fig. 1 Methionine metabolism related to fatty liver and betaine supplementation. HF diet decreases SAM availability for PC formation and VLDL secretion via PEMT. Betaine supplementation causes a threefold increase in hepatic SAM and alleviates fat liver accumulation induced by the HF diet. *MAT* Methionine adenosyltransferase, *SAM* *S*-adenosylmethionine, *SAH* *S*-adenosylhomocysteine, *CHDH* choline oxidase, *BHMT* betaine-homocysteine methyltransferase, *GNMT* glycine methyltransferase, *MS* methionine synthase, *MTHFR* 5,10-methylenetetrahydrofolate reductase, *CBS* cystathionine β-synthase, *CTH* cystathionase, *CDO1* cysteine dioxygenase

Table 1 Diet composition of control and high-fat diets

Ingredient	Control* (g/L)	HF diet* (g/L)
Casein (100 mesh)	41.4	41.4
L-Cysteine	0.5	0.5
DL-Methionine	0.3	0.3
Corn oil	8.5	48.5
Olive oil	28.4	28.4
Safflower oil	2.7	2.7
Maltose dextrin	115.2	25.6
Cellulose	10.0	10.0
Salt mix	8.75	8.75
Vitamin mix	2.5	2.5
Choline bitartrate	0.53	0.53
Xanthan gum	3.0	3.0

* Control diet provided 1.0 kcal/ml of which 35 % are fat derived (Lieber-DeCarli control rat diet#710027) and HF diet provided 1.0 kcal/ml of which 71 % are fat derived (Lieber-DeCarli fat-derived calorie rat diet #712031) according to Dyets Inc (Bethlehem, PA, USA)

fat accumulation and dysfunction of one-carbon metabolism induced by feeding a HF diet to rats.

Materials and methods

Eighteen male Sprague–Dawley rats (initial weight ~120 g) were obtained from the Memorial University of Newfoundland Animal Care Unit after approval by the institution's Committee on Animal Care. All procedures were in accordance with the Guidelines of the Canadian Council on Animal Care. The animals were kept in individual cages on a 12/12 h light/dark cycle at a mean temperature of 22 °C and were randomly divided into 3 groups of six rats each: control (C); high-fat (HF) and high-fat with betaine (HFB). The animals had free access to food throughout the 3 weeks and food intake was measured daily to assess total energy, total fat and betaine consumption. Composition of the diets is presented in Table 1.

The control standard liquid diet provided 35 % of energy from fat, 18 % from protein and 47 % from carbohydrates. Both high-fat groups received a high-fat liquid diet with 71 % of energy derived from fat, 18 % from protein and 11 % from carbohydrates. The diets had the same amount of vitamins, minerals, S-containing amino acids and fiber. The diets were purchased from Dyets Inc (Bethlehem, PA, USA). Betaine supplementation was performed by adding 1 % (g/L) betaine to the high-fat liquid diet.

Tissue preparation, histopathological evaluation and liver fat were performed as described by Deminice et al. (2011). A portion of fresh liver tissue was stained with Oil Red O. Liver total fat and total triglycerides were determined after chemical extraction using chloroform–methanol (2:1) solution.

Assay of metabolites of one-carbon metabolism

Liver SAM and *S*-adenosylhomocysteine (SAH) levels were determined by HPLC using a method described by Jacobs et al. (2005). Total plasma Hcy and cysteine concentrations were determined by reverse-phase HPLC and fluorescence detection of ammonium 7-fluoro-2-oxa-1,3-diazole-4-sulphonate (SBDF) thiol adducts as per Vester and Rasmussen (1991). Hepatic phosphatidylcholine and phosphatidylethanolamine were measured by a phosphorous assay after separation by thin-layer chromatography, as described by Jacobs et al. (2008).

Indices of hepatic injury

Plasma TNF-α and hepatic thiobarbituric acid reactive species (TBARS) were determined using commercially available kits from Invitrogen Corporation (catalog # KRC3014) and ZeptoMetrix Corporation (catalog # 081192), respectively.

Gene expression

RNA was isolated from 50 mg of frozen liver using Trizol® (Invitrogen). Total RNA was quantified by spectrophotometry at OD 260/280 (NanoDrop2000c, ThermoScientific, USA). RNA quality was assessed with an Agilent 2100 bioanalyser, using an RNA 6000 Nano kit. Samples were treated with DNAse I (Invitrogen) to digest genomic DNA; RNA was then reverse transcribed using Superscript II (Invitrogen). Primer sets and a corresponding probe for each of the following genes were designed using the Universal Probe Library (Roche) based on the NCBI reference nucleotide sequences for *rattus norvegicus:* Mat1a, Gnmt, Mthfr, Mtrr, ChDh, Bhmt1, Cdo1, Gclc, Gss, Cth, Mogat1, Lipin1, Chka, Chkb, Pcyt1a, Pcyt2a and Pemt. Each primer pair and probe combination was tested by qPCR (StepOnePlus, Applied Biosystems). Primer mixes for each gene were combined in a single assay, which was used to pre-amplify the cDNA of the genes of interest in each sample. Pre-amplification was tested by qPCR using a probe for cyclophilin. Forty-eight gene assays and cDNA samples were loaded into separate wells on a 48-by-48 gene expression chip (Fluidigm). qPCR was run on the Biomark™ system (Fluidigm) for 40 cycles. All samples were performed in triplicate and cyclophilin A was used as reference gene to normalize the reactions. The relative quantitation was determined by the $2^{-\Delta\Delta CT}$ method.

Data reporting and analysis

Data were reported as mean ± standard error of the mean (SEM). Groups were compared by analysis of variance (ANOVA) and Tukey post-test was used to identify specific differences between pairs of treatments using the SPSS 18.0 statistical package. The level of significance was $P < 0.05$ in all cases. Some of the data for the control and high-fat fed rats have already been published Deminice et al. (2011) and are given here for ease of comparison.

Results

Three weeks of HF or HFB diet resulted in higher ($P < 0.05$) fat intake, but with no significant differences in body weight or food intake between the three diet groups (Table 2). The HF diet group had increased liver and epididymal fat pad mass compared to the control animals. Betaine supplementation prevented the increase in epididymal fat pad weight, but did not prevent the increased liver weight that resulted from the high-fat diet (Table 2).

Supplementation of the HF diet with betaine prevented the hepatic accumulation of total lipid and triglycerides (Fig. 2b, c). The effects of betaine supplementation on liver lipid content were clearly evident in the Oil Red O-stained histological sections (Fig. 2a). In addition, the HF diet group had increased hepatic TBARS and plasma TNF-α compared to the control and HFB diets (Fig. 2d, e).

The HF diet decreased liver [SAM] by about 20 % when compared with the control (Table 3). Betaine supplementation increased liver SAM concentration almost fourfold, increased liver SAH by more than twofold and doubled the ratio of SAM to SAH compared to rats fed the HF diet. Betaine supplementation also increased liver PC and the ratio of PC to PE compared to rats fed either control or HF diets. Although no changes were found in plasma Hcy concentration between the groups, plasma cysteine was elevated in both the HF and HFB groups.

We examined the transcription levels for genes involved in one-carbon and lipid metabolism. Expression of BHMT1, GNMT, and MGAT1 was greater in the HF group than in the C group. Betaine supplementation normalized the abundance of these mRNA to levels measured in control animals (Table 4). Neither the HF diet, nor HFB diet had any effect on the mRNA abundance of the PEMT gene, nor were the genes involved in the transsulfuration pathway affected by HF but HFB did affect cystathionine β-synthase (CBS) expression which was increased and cystathionase (Cth) expression which was decreased by betaine supplementation.

Table 2 Weight gain, liver and fat pad weights, total energy, fat and betaine intake for rats fed the C, HF and HFB diets for 3 weeks

	C	HF	HFB
	Mean ± SEM	Mean ± SEM	Mean ± SEM
Weight gain (g)	183.5 ± 20.5	193.3 ± 15.6	178.7 ± 10.9
Liver weight (% body weight)	4.24 ± 0.26[a]	4.82 ± 0.40[b]	4.75 ± 0.19[b]
Epididymal fat pad (% body weight)	1.61 ± 0.10[a]	1.84 ± 0.12[b]	1.42 ± 0.11[a]
Food intake (mL/d)	120.0 ± 11.4	121.7 ± 5.6	117.4 ± 8.5
Energy intake (kJ/d)	502.1 ± 45.9	508.6 ± 24.3	490.8 ± 35.8
Fat intake (kJ/d)	175.7 ± 16.1[a]	361.2 ± 16.1[b]	334.0 ± 25.1[b]
Betaine INTAKE (g/kg.d)			3.1 ± 0.4

Values are given as mean ± SEM

[ab] Mean values with unlike letters were significantly different ($P < 0.05$ by ANOVA with Tukey post-test)

Discussion

The positive effects of betaine on fatty liver disease have been attributed to the fact that it is an important methyl donor, resulting in a considerable increase in hepatic SAM concentrations (Kwon do et al. 2009; Kharbanda 2013; Wang et al. 2010; Kawakami et al. 2012; Jung et al. 2013). The increased SAM availability is thought to regulate phosphatidylcholine (PC) synthesis by PEMT and this normalizes VLDL production rates (Kharbanda et al. 2009), preventing hepatic fat accumulation induced either by a high-fat diet (Kwon do et al. 2009; Wang et al. 2010; Kawakami et al. 2012) or by ethanol ingestion (Kharbanda et al. 2007; Jung et al. 2013). Our results confirmed that betaine supplementation substantially increased SAM concentration in the liver (Table 1) and prevented fatty liver; it also prevented the elevation of the levels of two markers of liver damage, TBARS and TNF-α (Fig. 2). Betaine supplementation increased hepatic PC levels and the hepatic PC/PE ratio (Table 2), although there were no changes in PEMT gene expression. A decreased hepatic PC/PE ratio has been previously associated with hepatic dysfunction (Li et al. 2006; Li and Vance 2008). Increased PC levels and hepatic PC/PE ratio may result in increased export of lipids from the liver (Kharbanda et al. 2009; Li et al. 2006), preventing fatty liver induced by a high-fat diet. Wang et al. (2014) recently demonstrated that betaine supplementation attenuated hepatic steatosis and restored reduced levels of microsomal triglyceride transfer protein (MTTP) gene expression in mice fed with high-fat diet, which catalyzes the transfer of PC between membranes, required for the secretion of VLDL in the liver. These

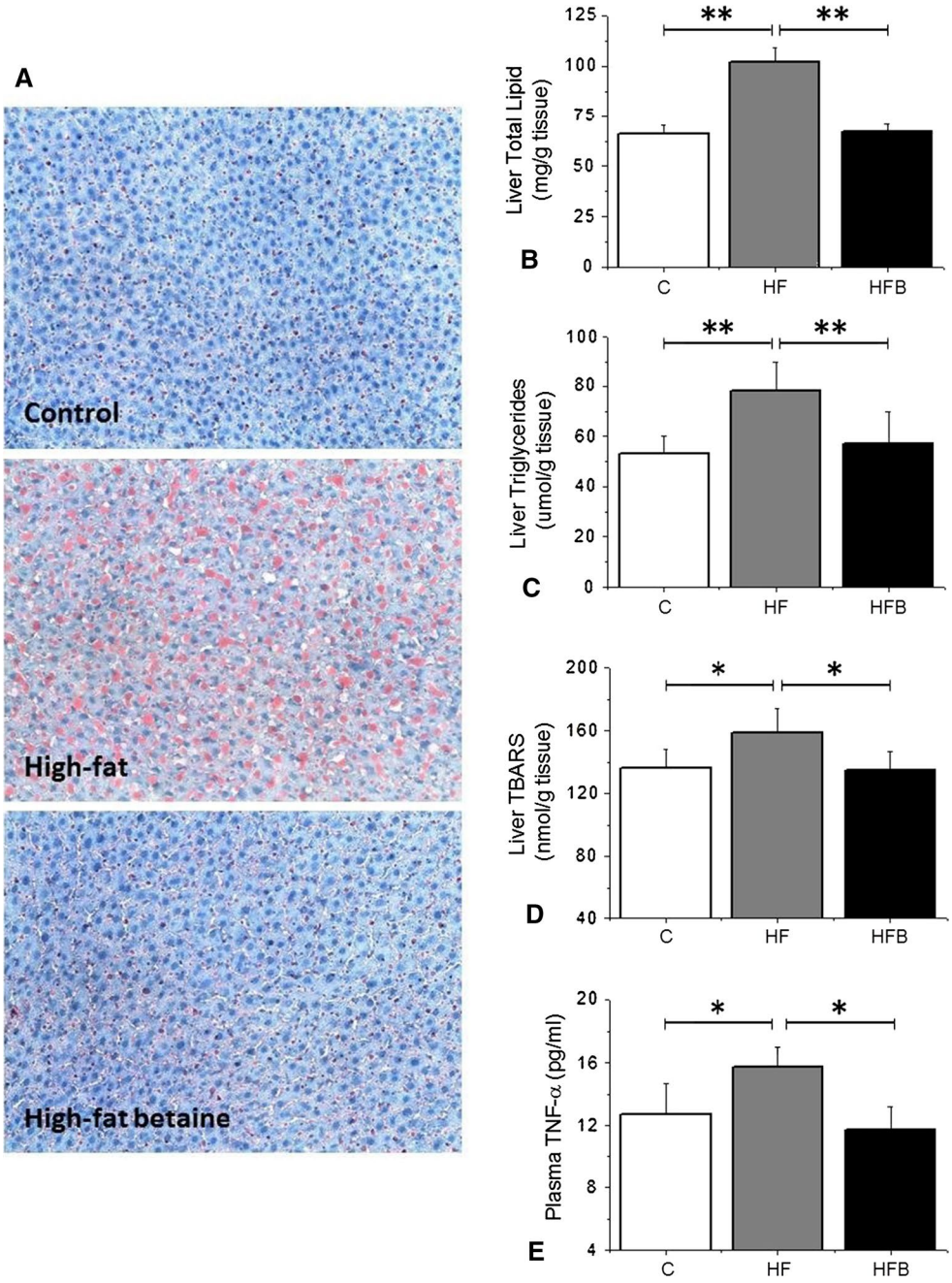

Fig. 2 Betaine supplementation prevented fatty liver and liver injury induced by HF diet. **a** Liver samples stained with Oil red O. **b** Liver total lipid. **c** Liver triglycerides. **d** Liver thiobarbituric acid reactive species (TBARS). **e** Plasma tumor necrosis factor alpha (TNF-a). Values are given as mean ± SEM ($n = 6$). *Indicates $P < 0.05$ and **$P < 0.01$ by ANOVA with Tukey post-test

data reinforce the effect of betaine supplementation on phospholipid metabolism and its importance in fatty liver disease.

We also found that the HF diet induced the expression of key genes involved in one-carbon metabolism (BHMT, GNMT) and lipid metabolism (MGAT1), and that betaine supplementation can prevent such alterations. Alterations in BHMT and GNMT caused by changes in SAM concentration were previously reported (Obeid 2013); however, we consider the alterations in the expression of these genes both by feeding a high-fat diet and by supplementation of HF-fed rats with betaine as novel mechanisms by which betaine supplementation regulates one-carbon metabolism and prevents hepatic steatosis.

BHMT catalyzes the transfer of a methyl group from betaine to homocysteine, yielding methionine and dimethylglycine. The mitochondrial metabolism of dimethylglycine provides two additional one-carbon groups. Our data revealed that 3 weeks on the high-fat diet increased gene expression of BHMT and decreased the hepatic concentration of SAM; these changes were prevented by betaine supplementation. Increased BHMT gene expression in the HF-fed group would tend to increase the availability of SAM and, indeed, may be a reflection of enhanced SAM

Table 3 One-carbon metabolism-related metabolites for rats fed the control (C), high fat (HF) and high-fat supplemented with betaine (HFB) diets for 3 weeks

	C	HF	HFB
	Mean ± SEM	Mean ± SEM	Mean ± SEM
Liver			
SAM (nmol/g tissue)	67.2 ± 6.3[a]	55.2 ± 5.4[b]	262.4 ± 16.7[c]
SAH (nmol/g tissue)	7.1 ± 1.1[a]	7.9 ± 0.8[a]	18.8 ± 2.6[b]
SAM/SAH	9.6 ± 0.8[a]	7.1 ± 0.8[a]	14.2 ± 2.4[b]
PC (nmol/mg protein)	131 ± 4.4[a]	145 ± 4.8[a]	155 ± 5.3[b]
PE (nmol/mg protein)	54.3 ± 2.2	56.7 ± 3.7	49.4 ± 1.5
PC/PE	2.4 ± 0.1[a]	2.6 ± 0.1[a]	3.1 ± 0.1[b]
Plasma			
Total homocysteine (μmol/L)	6.4 ± 1.0	6.2 ± 1.0	7.5 ± 1.1
Total cysteine (μmol/L)[$]	196.0 ± 14.7[a]	219.2 ± 11.8[ab]	241.3 ± 15.4[b]

Values are given as mean ± SEM

[$] Total cysteine is cysteine plus 1/2 cystine

[ab] Mean values with unlike letters were significantly different ($P < 0.05$ by ANOVA with Tukey post-test)

Table 4 mRNA levels of hepatic genes involved in one-carbon and lipid metabolism for rats fed the control (C), high fat (HF) and high-fat supplemented with betaine (HFB) diets for 3 weeks

	C	HF	HFB
	Mean ± SEM	Mean ± SEM	Mean ± SEM
Transmethylation			
MAT1a	0.88 ± 0.18	1.01 ± 0.16	0.62 ± 0.06
GNMT	1.00 ± 0.02[a]	1.78 ± 0.09[b]	0.69 ± 0.10[a]
Remethylation			
MTHFR	1.08 ± 0.17	0.90 ± 0.03	1.28 ± 0.05
MSR	1.13 ± 0.24	1.08 ± 0.12	1.25 ± 0.15
CHDH	1.06 ± 0.16	0.83 ± 0.11	0.74 ± 0.12
BHMT1	0.61 ± 0.06[a]	1.2 ± 0.21[b]	0.28 ± 0.05[a]
Transsulfuration			
CBS	1.08 ± 0.09[a]	0.93 ± 0.05[a]	1.40 ± 0.15[b]
Cdo1	1.25 ± 0.40	1.27 ± 0.43	1.28 ± 0.48
Gclc	0.89 ± 0.11	0.86 ± 0.09	1.16 ± 0.22
Gss	1.04 ± 0.13	0.96 ± 0.03	0.93 ± 0.09
Cth	1.03 ± 0.10[a]	0.93 ± 0.07[a]	0.58 ± 0.03[b]
Phospholipid metabolism			
MGAT1	1.01 ± 0.12[a]	1.63 ± 0.13[b]	1.15 ± 0.16[a]
Lipin1	0.88 ± 0.13	1.09 ± 0.21	0.69 ± 0.09
Chka	1.01 ± 0.07	1.08 ± 0.13	1.60 ± 0.21
Chkb	1.01 ± 0.08	0.97 ± 0.04	0.88 ± 0.08
CTa	1.16 ± 0.12	0.94 ± 0.16	0.73 ± 0.09
ET	1.12 ± 0.11	0.85 ± 0.08	1.22 ± 0.19
PEMT	1.01 ± 0.06	1.00 ± 0.04	0.94 ± 0.10

Values are given as mean ± SEM of relative mRNA expression

[ab] Mean values with unlike letters were significantly different ($P < 0.05$ by ANOVA with Tukey post-test)

availability. Previous studies have shown that either a high-fat diet or ethanol intake decreased SAM availability and increased BHMT activity (Dahlhoff et al. 2013; Kharbanda et al. 2007). Decreased SAM can also result from reduced MAT1a gene expression (Kharbanda 2013) but we did not observe this in our study. The changes in mRNA abundance of genes affecting one-carbon metabolism caused by the HF diet were prevented by betaine supplementation. Increased SAM inhibits remethylation by BHMT thus reducing the excess of SAM; and activates transsulfuration via CBS to enhance the conversion of homocysteine into cysteine (Obeid 2013). These data are consistent with our finding that increased SAM caused by betaine supplementation led to decreased BHMT and increased CBS gene expression, which could lead to the increased cysteine plasma concentration observed in HFB compared to other groups. These data show the importance of BHMT for the regulation of one-carbon metabolism in fatty liver and the potential of betaine to regulate this pathway.

GNMT activity has the highest flux of all the methyltransferase enzymes when methionine is in excess in the diet and is important for the homeostasis of SAM. GNMT transfers the labile methyl group of SAM to a molecule of glycine and thereby prevents the accumulation of excessive [SAM] by forming sarcosine (Martínez-Chantar et al. 2008), which is metabolized within mitochondria. Studies have shown that GNMT knockout mice had steatosis, fibrosis and in some cases hepatocellular carcinoma (Martínez-Chantar et al. 2008; Varela-Rey et al. 2010; Liu et al. 2007). Rowling et al. (2002) showed the loss of GNMT in mice leads to the accumulation of hepatic SAM and to a marked increase in the hepatic SAM/SAH ratio. In contrast, activation of GNMT in rats by retinoic acid causes a reduction in SAM and global DNA methylation in the liver. It seems paradoxical that the HF diet should increase the gene expression of GNMT, given that SAM levels are reduced in the HF-fed rats. It is, however, possible that the decreased hepatic [SAM] was caused by the increased GNMT. Moreover, the decrease in GNMT expression in the betaine-supplemented rats may be partly responsible for the increased hepatic SAM levels.

MGAT codes for monoacylglycerol acyltransferase, which transfers a fatty acyl group, from fatty acyl-CoA, to monoacylglycerol to produce diacylglycerol. It is the first step of the monoacylglycerol pathway for the synthesis of triacylglycerol (Hall et al. 2012). The importance of MGAT action in both healthy and fatty livers was recently demonstrated (Hall et al. 2012; Lee et al. 2012). Lee et al.

(2012) showed that MGAT1 expression was very low in normal liver but was highly expressed in the fatty liver. These authors also observed that suppression of MGAT1 expression protected liver from fat accumulation in three models: Ad-PPARγ-induced steatosis; 12-wk-HF diet-induced steatosis; and ob/ob mice with hepatic steatosis. The increased MGAT1 mRNA in the rats fed with HF diet observed in our study is consistent with increased hepatic triacylglycerol synthesis. Similarly, the decrease in the expression of this MGAT1 mRNA with betaine supplementation is consistent with the lack of hepatic steatosis in this model. Recent studies have proposed that modulation of fatty acid metabolism could represent a new mechanism for the hepatoprotective effect of betaine in nonalcoholic fatty liver (Wang et al. 2010; Dahlhoff et al. 2014; Song et al. 2007). Song et al. (2007) demonstrated that betaine supplementation increased fatty acid oxidation by the activation of the hepatic AMPK system in mice fed high-sucrose diet. Wang et al. (2010) verified raised circulating adiponectin levels in mice fed high-sucrose diet after betaine supplementation. Both studies suggest that betaine might have potential to improve adipose tissue function. To our knowledge, ours is the first study demonstrating that betaine supplementation suppresses MGAT1 expression induced by a HF diet. This result suggests that a decrease in the MGAT1 pathway of TG synthesis might be a novel and effective mechanism whereby betaine supplementation reduces the severity of HF-induced hepatic steatosis.

In conclusion, we have provided evidence that homeostasis of hepatic methionine metabolism is disturbed in rats on HF diet. Betaine supplementation prevented fatty liver in rats fed with HF diet, increasing SAM levels and preventing changes in the levels of BHMT, GNMT and MGAT mRNA. These changes in gene expression may represent additional, novel mechanisms by which betaine supplementation regulates one-carbon metabolism and prevents fatty liver induced by a HF diet.

Acknowledgments The authors acknowledge Ms. Kathy Clow for skilled technical assistance. Supported by Grants from the Canadian Institutes for Health Research (JTB/MEB), Natural Sciences and Engineering Research Council of Canada (RLJ grant number 386652) and Fundação de Amparo a Pesquisa do Estado de São Paulo, Brazil Protocol 07/08099-5 (RD). K.K. was supported by an Alexander Graham Bell Canada graduate scholarship from the Natural Sciences and Engineering Research Council of Canada. R.L.J. holds a Canadian Institutes of Health Research New Investigator Award.

Conflict of interest None of the authors has either financial or personal conflicts of interest to declare.

References

Brunt EM (2010) Pathology of nonalcoholic fatty liver disease. Nat Rev Gastroenterol Hepatol 7:195–203. doi:10.1038/nrgastro.2010.21

da Silva RP, Kelly KB, Al Rajabi A et al (2014) Novel insights on interactions between folate and lipid metabolism. BioFactors 40:277–283. doi:10.1002/biof.1154

Dahlhoff C, Desmarchelier C, Sailer M et al (2013) Hepatic methionine homeostasis is conserved in C57BL/6 N mice on high-fat diet despite major changes in hepatic one-carbon metabolism. PLoS One 8:e57387. doi:10.1371/journal.pone.0057387

Dahlhoff C, Worsch S, Sailer M et al (2014) Methyl-donor supplementation in obese mice prevents the progression of NAFLD, activates AMPK and decreases acyl-carnitine levels. Mol Metab 3:565–580. doi:10.1016/j.molmet.2014.04.010

Day CP, James OF (1998) Steatohepatitis: a tale of two "hits"? Gastroenterology 114:842–845

Deminice R, da Silva RP, Lamarre SG et al (2011) Creatine supplementation prevents the accumulation of fat in the livers of rats fed a high-fat diet. J Nutr 141:1799–1804. doi:10.3945/jn.111.144857

Duvnjak M, Lerotić I, Barsić N et al (2007) Pathogenesis and management issues for non-alcoholic fatty liver disease. World J Gastroenterol 13:4539–4550. doi:10.3748/wjg.v13.i34.4539

Erman F, Balkan J, Cevikbaş U et al (2004) Betaine or taurine administration prevents fibrosis and lipid peroxidation induced by rat liver by ethanol plus carbon tetrachloride intoxication. Amino Acids 27:199–205. doi:10.1007/s00726-004-0105-5

Hall AM, Kou K, Chen Z et al (2012) Evidence for regulated monoacylglycerol acyltransferase expression and activity in human liver. J Lipid Res 53:990–999. doi:10.1194/jlr.P025536

Jacobs RL, Stead LM, Devlin C (2005) Physiological regulation of phospholipid methylation alters plasma homocysteine in mice. J Biol Chem 280:28299–28305. doi:10.1074/jbc.M501971200

Jacobs RL, Lingrell S, Zhao Y et al (2008) Hepatic CTP:phosphocholine cytidylyltransferase-alpha is a critical predictor of plasma high density lipoprotein and very low density lipoprotein. J Biol Chem 283:2147–2155. doi:10.1074/jbc.M706628200

Jung YS, Kim SJ, Kwon do Y et al (2013) Alleviation of alcoholic liver injury by betaine involves an enhancement of antioxidant defense via regulation of sulfur amino acid metabolism. Food Chem Toxicol 62:292–298. doi:10.1016/j.fct.2013.08.049

Kawakami S, Han KH, Nakamura Y et al (2012) Effects of dietary supplementation with betaine on a nonalcoholic steatohepatitis (NASH) mouse model. J Nutr Sci Vitaminol (Tokyo) 58:371–375. doi:10.3177/jnsv.58.371

Kharbanda KK (2013) Methionine metabolic pathway in alcoholic liver injury. Curr Opin Clin Nutr Metab Care 16:89–95. doi:10.1097/MCO.0b013e32835a892a

Kharbanda KK, Mailliard ME, Baldwin CR et al (2007) Betaine attenuates alcoholic steatosis by restoring phosphatidylcholine generation via the phosphatidylethanolamine methyltransferase pathway. J Hepatol 46:314–321. doi:10.1016/j.jhep.2006.08.024

Kharbanda KK, Todero SL, Ward BW et al (2009) Betaine administration corrects ethanol-induced defective VLDL secretion. Mol Cell Biochem 327:75–78. doi:10.1007/s11010-009-0044-2

Kim SK, Kim YC (2005) Effects of betaine supplementation on hepatic metabolism of sulfur-containing amino acids in mice. J Hepatol 42:907–913. doi:10.1016/j.jhep.2005.01.017

Kim SJ, Jung YS, Kwon do Y et al (2008) Alleviation of acute ethanol-induced liver injury and impaired metabolomics of S-containing substances by betaine supplementation. Biochem Biophys Res Commun 368:893–898. doi:10.1016/j.bbrc.2008.02.003

Kwon do Y, Jung YS, Kim SJ et al (2009) Impaired sulfur-amino acid metabolism and oxidative stress in nonalcoholic fatty liver are alleviated by betaine supplementation in rats. J Nutr 139:63–68. doi:10.3945/jn.108.094771

Lee YJ, Ko EH, Kim JE et al (2012) Nuclear receptor PPARγ-regulated monoacylglycerol *O*-acyltransferase 1 (MGAT1)

expression is responsible for the lipid accumulation in diet-induced hepatic steatosis. Proc Natl Acad Sci USA 109:13656–13661. doi:10.1073/pnas.1203218109

Li Z, Vance DE (2008) Phosphatidylcholine and choline homeostasis. J Lipid Res 49:1187–1194. doi:10.1194/jlr.R700019-JLR200

Li Z, Agellon LB, Allen TM et al (2006) The ratio of phosphatidylcholine to phosphatidylethanolamine influences membrane integrity and steatohepatitis. Cell Metab 3:321–331. doi:10.1016/j.cmet.2006.03.007

Liu SP, Li YS, Chen YJ et al (2007) Glycine N-methyltransferase-/- mice develop chronic hepatitis and glycogen storage disease in the liver. Hepatology 46:1413–1425. doi:10.1002/hep.21863

Martínez-Chantar ML, Vázquez-Chantada M, Ariz U et al (2008) Loss of the glycine *N*-methyltransferase gene leads to steatosis and hepatocellular carcinoma in mice. Hepatology 47:1191–1199. doi:10.1002/hep.22159

Obeid R (2013) The metabolic burden of methyl donor deficiency with focus on the betaine homocysteine methyltransferase pathway. Nutrients 5:3481–3495. doi:10.3390/nu5093481

Rowling MJ, McMullen MH, Schalinske KL (2002) Vitamin A and its derivatives induce hepatic glycine *N*-methyltransferase and hypomethylation of DNA in rats. J Nutr 132:365–369

Song Z, Deaciuc I, Zhou Z et al (2007) Involvement of AMP-activated protein kinase in beneficial effects of betaine on high-sucrose diet-induced hepatic steatosis. Am J Physiol Gastrointest Liver Physiol 293:G894–G902. doi:10.1152/ajpgi.00133.2007

Varela-Rey M, Martínez-López N, Fernández-Ramos D et al (2010) Fatty liver and fibrosis in glycine N-methyltransferase knockout mice is prevented by nicotinamide. Hepatology 52:105–114. doi:10.1002/hep.23639

Vester B, Rasmussen K (1991) High performance liquid chromatography method for rapid and accurate determination of homocysteine in plasma and serum. Eur J Clin Chem Clin Biochem 29:549–554

Wang Z, Yao T, Pini M et al (2010) Betaine improved adipose tissue function in mice fed a HF-diet: a mechanism for hepatoprotective effect of betaine in nonalcoholic fatty liver disease. Am J Physiol Gastrointest Liver Physiol 298:G634–G642. doi:10.1152/ajpgi.00249.2009

Wang LJ, Zhang HW, Zhou JY et al (2014) Betaine attenuates hepatic steatosis by reducing methylation of the MTTP promoter and elevating genomic methylation in mice fed a high-fat diet. J Nutr Biochem 25:329–336. doi:10.1016/j.jnutbio.2013.11.007