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Creatine Supplementation Prevents the Accumulation of Fat in the Livers of Rats Fed a High-Fat Diet[1,2]

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Abstract

The aim of the present study was to examine the effects of creatine supplementation on liver fat accumulation induced by a high-fat diet in rats. Rats were fed 1 of 3 different diets for 3 wk: a control liquid diet (C), a high-fat liquid diet (HF), or a high-fat liquid diet supplemented with creatine (HFC). The C and HF diets contained, respectively, 35 and 71% of energy derived from fat. Creatine supplementation involved the addition of 1% (wt:v) of creatine monohydrate to the liquid diet. The HF diet increased total liver fat concentration, liver TG, and liver TBARS and decreased the hepatic *S*-adenosylmethionine (SAM) concentration. Creatine supplementation normalized all of these perturbations. Creatine supplementation significantly decreased the renal activity of L-arginine:glycine amidinotransferase and plasma guanidinoacetate and prevented the decrease in hepatic SAM concentration in rats fed the HF diet. However, there was no change in either the phosphatidylcholine:phosphatidylethanolamine (PE) ratio or PE *N*-methyltransferase activity. The HF diet decreased mRNA for PPARα as well as 2 of its targets, carnitine palmitoyltransferase and long-chain acylCoA dehydrogenase. Creatine supplementation normalized these mRNA levels. In conclusion, creatine supplementation prevented the fatty liver induced by feeding rats a HF diet, probably by normalization of the expression of key genes of β-oxidation. J. Nutr. 141: 1799–1804, 2011.

Introduction

Nonalcoholic fatty liver disease is one of the most common chronic liver diseases throughout the world (1). It is a clinical pathological state that develops in the absence of alcohol abuse and is characterized by the accumulation of TG in hepatocytes. It can progress to NASH[6] and in some cases to fibrosis and cirrhosis (2). However, the mechanisms involved in nonalcoholic fatty liver disease are uncertain (2,3). The 2-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 by the second hit that involves mitochondrial dysfunction, lipid peroxidation, and inflammation (4).

Fat accumulation and NASH progression have been associated with impairment of methionine metabolism in liver (5), resulting in decreased availability of SAM as well as an increase in homocysteine levels and oxidative stress (5,6). In support of this mechanism, supplementation with betaine, an important methyl donor for the remethylation of homocysteine to methionine, may protect the liver from fat accumulation and lipid peroxidation. This has been observed in both rodent models of alcoholic (7) and nonalcoholic (3) fatty liver. Kwon et al. (3) suggested that the elevation of SAM may play a critical role in the protective effect of betaine.

Creatine occurs naturally in food, especially in meat and fish. Creatine, in the form of creatine monohydrate, is taken by many athletes for its ergogenic properties (8). In humans, ~1–2 g of creatine is required to replace that lost by irreversible conversion to creatinine. In human omnivores, one-half of this is provided in the diet and the remainder is endogenously synthesized (8). The first step in creatine synthesis involves the reversible transfer of the amidino group of arginine to glycine to form GAA and ornithine in a reaction catalyzed by the enzyme AGAT; this

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[6] Abbreviations used: AGAT, arginine:glycine amidinotransferase; BHMT, betaine-homocysteine *S*-methyltransferase; GAA, guanidinoacetic acid; GAMT, *S*-adenosylmethionine:guanidinoacetate *N*-methyltransferase; GNMT, glycine N-methyltransferase; NASH, nonalcoholic steatohepatitis; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine *N*-methyltransferase; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine.
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enzyme is very active in kidneys. Next, the irreversible transfer of a methyl group from SAM to GAA is catalyzed by the enzyme GAMT, which is most active in the liver (8). The products of this reaction are creatine and SAH. Creatine synthesis is responsible for a considerable consumption of SAM in the liver (9), as much as 40% of the total body SAM requirement. Previous studies have shown that creatine supplementation downregulates renal AGAT activity (10) and therefore the endogenous formation of creatine. It also reduces homocysteine production (11,12). Because both PEMT and GAMT use the same hepatic SAM pool, creatine supplementation may increase SAM availability to PC formation via PEMT, thus increasing VLDL secretion and diminishing fat accumulation in liver.

The aim of the present study was to examine the effects of creatine supplementation of rats fed a high-fat diet on liver fat accumulation, methionine metabolism, and lipid peroxidation. Creatine supplementation does indeed prevent development of fatty liver in response to a high-fat diet but by a mechanism other than increased SAM availability.

Materials and Methods

Rats and treatment. Male Sprague-Dawley rats (initial weight ~120 g) were obtained from the Memorial University of Newfoundland Animal Care Unit. All procedures were approved by the Animal Care Committee of the same institution and were in accordance with the Guidelines of the Canadian Council on Animal Care. The rats were kept in individual cages on a 12-h-light/-dark cycle at a mean temperature of 22°C and were randomly assigned to 3 groups of 6 rats each: control (C); high-fat (HF); and high-fat with creatine (HFC). Group C was fed with a standard liquid diet with 35% of energy from fat, 18% from protein and 47% from carbohydrates (Dyets catalogue no. 710027). The high-fat groups received a high-fat liquid diet with 71% of energy derived from fat, 18% from protein, and 11% from carbohydrates (Dyets catalogue no. 712031). The diets were purchased from Dyets. The overall compositions of both the C and HF diets were identical to those described by Lieber et al. (13) Creatine supplementation was performed by adding 1% (wt:v) creatine monohydrate to the HF diet. The rats had free access to food throughout the 3 wk. Food intake was measured daily to assess total energy, total fat, and creatine consumption. Body weight was measured twice each week to determine the weight gain.

Tissue preparation. After the experimental period, the rats were anesthetized with an i.p. injection of sodium pentobarbital (65 mg/kg i.p.). The fed rats were killed between 0800 and 1000 h. Blood was collected into heparinized tubes, centrifuged, and the plasma stored at −80°C. A portion of the liver was freeze-clamped with aluminum tongs precooled in liquid nitrogen, weighed, and stored at −80°C. A portion of fresh liver tissue was weighed and cut in small cubes of ~5 × 5 × 5 mm and embedded using the Optimal Cutting Temperature Compound (Leica) for Oil-Red-O histopathologic evaluation. One kidney was removed and immediately homogenized in 50 mmol/L ice-cold potassium phosphate buffer (pH 7.4) with a Polytron (Brinkmann Instruments) for 25 s at 50% output. This homogenate was used for the analysis of AGAT activity.

Hepatic histology and lipid analysis. For histopathologic evaluation, 7-μm sections of OCT-blocked liver tissue were immersed in propylene glycol for 7 min and stained with Oil-Red-O for 10 min. The sections were washed 3 times with distilled water, counterstained in hematoxylin for 20 min, and washed again in distilled water before microscopic analysis. Adobe Photoshop CS3 software (Adobe) was used to estimate the percentage of red pixels in each image.

Liver total fat was determined by homogenizing 1 g of liver in 1.5 mL of distilled water; 5 mL of chloroform-methanol (2:1) was added and the tubes were thoroughly mixed. After centrifugation, the chloroform phase was transferred to a preweighed tube, the extraction repeated twice, and the chloroform phases combined, evaporated to dryness, and reweighed. The fat was resuspended in 1 mL 1-propanol for the measurement of total liver TG and cholesterol by using commercially available kits from Diagnostic Chemicals (catalogue nos. 236–17 and 234–60, respectively, for TG and cholesterol). Hepatic PC and PE were measured by a phosphorous assay after separation by TLC, as described by Jacobs et al. (14).

Creatine, GAA, and sulfur-containing metabolites. Both GAA and creatine were assayed by the HPLC method of Buchberger and Ferdig (15). For SAM and SAH determinations, freeze-clamped liver samples were homogenized in ice-cold 8% (wt/v) trichloroacetic acid and the homogenates centrifuged at 13,000 × *g* for 5 min at 4°C. The supernatants were analyzed by HPLC using a Vydac C_{18} column (model 2187P54) equilibrated with 96% of buffer A (50 mmol/L NaH_2PO_4 containing 10 mmol/L heptanesulfonic acid at pH 3.2) and 4% acetonitrile. SAM and SAH were separated by means of a gradient of 96–80% of buffer A and 4–20% of acetonitrile for 15 min. SAM and SAH peaks were detected at 258 nmol/L and quantified using Millennium^{32} (version 2) software (Waters, Milford, MA). Total plasma homocysteine and cysteine concentrations were determined by reverse-phase HPLC and fluorescence detection of ammonium 7-fluoro-2-oxa-1,3-diazole-4-sulphonate thiol adducts by the method of Vester and Rasmussen (16).

AGAT activity assay and immunoblot. Protein was assayed using the Biuret method. Kidney AGAT activity was determined as described by Van Pilsum et al. (17). For the Western blotting of kidney AGAT, proteins were separated by SDS-PAGE (12% polyacrylamide gel) and transferred by electroblotting to nitrocellulose membranes. AGAT-protein was detected using an affinity-purified anti-AGAT rabbit polyclonal antibody raised against the sequence RPDPIDWSLKYKTPDFE, amino acids 142–159 of rat AGAT (accession no. P50442–1) (Open Biosystems). The blots were incubated at 4°C overnight with the primary antibodies diluted in 5% nonfat dried milk, followed by a 45-min incubation with HRP-conjugated anti-rabbit IgG (Bio-Rad) and visualized by chemiluminescence. Band detection was performed using an enhanced luminol system (Immunn-Star, Bio-Rad) and analyzed with an Alpha Innotech Chemilmager 4400 (Alpha Innotech).

Gene expression. Real-time reactions were carried out to analyze the gene expressions of *PEMT*, *BHMT1*, *GNMT*, *MGAT*, *PPARα*, *CD36*, *CPT1a*, *LCAD*, *VLCAD*, and *UCP2*. Total RNA was isolated from frozen liver using Trizol (Invitrogen). RNA quality was assessed with an Agilent 2100 bioanalyzer by 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 gene of interest were designed using the Universal Probe Library (Roche) based on the NCBI reference nucleotide sequences for *Rattus norvegicus*. Each primer pair and probe combination was tested by qPCR (StepOnePlus, Applied Biosystems). Primer mixes for each gene were combined in a single assay that was used to preamplify the cDNA of the genes of interest in each sample. Preamplification was tested using a probe for cyclophilin by qPCR. 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. Relative RNA expression for each gene in a sample was standardized to the endogenous housekeeping gene cyclophilin (*Ppia*) and calculated using the $\Delta\Delta C_T$ method. All assays were performed in triplicate.

Insulin, glucose, and hepatic TBARS. Plasma glucose was measured using hexokinase and glucose-6-phosphate dehydrogenase as described by Bergmeyer et al. (18). Plasma insulin was measured using a commercially available kit from Crystal Chem (catalogue no. INSKR020) using a rat insulin standard. Liver TBARS was determined using commercially available kits from ZeptoMetrix (catalogue no. 081192).

Statistical analysis. Data were reported as mean ± SD. Groups were compared by ANOVA and orthogonal contrasts were used to identify specific differences between pairs of treatments using the SAS statistical package (version 8.2). In all analyses, the level of significance was set at $P < 0.05$.

TABLE 1 Weight gain, liver weight, and intake of energy, fat, and creatine in rats fed C, HF, or HFC for 3 wk[1]

	C	HF	HFC
Weight gain, *g*	184 ± 21	193 ± 16	174 ± 13
Liver weight, *% body weight*	4.2 ± 0.3[a]	4.8 ± 0.4[b]	4.1 ± 0.2[a]
Energy intake, *kJ/d*	502 ± 46	509 ± 24	480 ± 24
Fat energy Intake, *kJ/d*	176 ± 16[a]	361 ± 16[b]	341 ± 17[b]
Creatine intake, *g/(kg × d)*	—	—	3.1 ± 0.1

[1] Values are means ± SD, $n = 6$. Means in a row with superscripts without a common letter differ, $P < 0.05$. C, control; HF, high-fat; HFC, high-fat liquid diet with creatine.

TABLE 2 Liver total fat, TG, cholesterol, and TBARS and plasma glucose and insulin in rats fed C, HF, or HFC for 3 wk[1]

	C	HF	HFC
Liver			
Fat, *mg/g*	66.1 ± 14.1[a]	102 ± 23.3[b]	52.4 ± 12.0[a]
TG, *μmol/g*	22.5 ± 6.7[a]	42.2 ± 9.2[b]	16.6 ± 4.5[a]
Cholesterol, *μmol/g*	4.9 ± 0.7 [a]	6.1 ± 0.8 [b]	4.9 ± 0.6 [a]
TBARS, *nmol/g*	136 ± 2[a]	155 ± 16[b]	134 ± 10 [a]
Plasma			
Glucose, *mmol/L*	7.2 ± 1.4[a]	9.6 ± 1.7[b]	7.8 ± 1.2[ab]
Insulin, *pmol/L*	82.8 ± 24.1	97.4 ± 14.3	79.4 ± 20.7

[1] Values are means ± SD, $n = 6$. Means in a row with superscripts without a common letter differ, $P < 0.05$. C, control; HF, high-fat; HFC, high-fat liquid diet with creatine.

Results

There were no differences in body weight gain or food intake for the 3 groups during the 3-wk experimental period. As expected, fat intake was higher ($P < 0.05$) in the HF and HFC groups than in the C group. The liver weight was elevated in the HF group and this was prevented by creatine supplementation (**Table 1**).

Three weeks of ingesting the HF diet resulted in a significant increase in hepatic total fat (55%), TG (87%), and total cholesterol (25%) compared to rats fed the control diet. Supplementation of the HF diet with creatine prevented the hepatic accumulation of these lipids. Increased hepatic TBARS was evident after 3 wk of feeding the HF diet: this increase was prevented by creatine supplementation (**Table 2**). The effects of creatine supplementation on liver fat were clearly evident in the Oil-Red-O–stained histological sections (**Fig. 1**). The difference in fat accumulation between liver sections from the HF and HFC groups was confirmed by image analysis. Ingestion of the HF diet increased plasma glucose, which was partially reversed by creatine supplementation. The plasma insulin concentration did not differ among the groups.

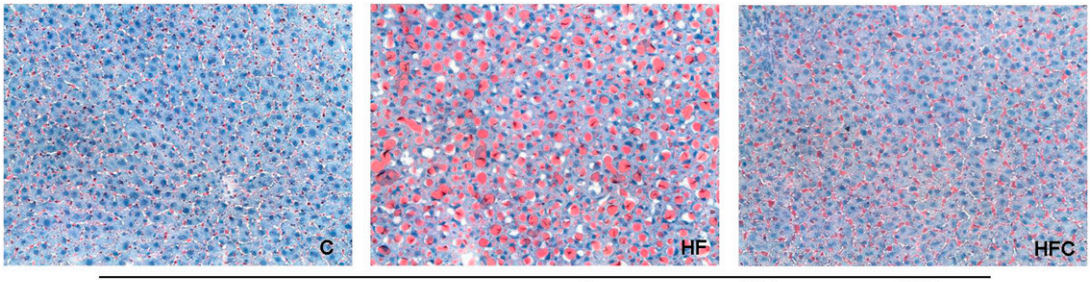
The plasma creatine concentration was greater in the HFC group than in the other 2 groups. The HF diet decreased the liver SAM concentration by 18% compared to the C group. Creatine supplementation prevented the decrease in liver SAM such that the concentration did not differ between the HFC and C groups. The plasma Hcy concentration did not differ among the groups. Creatine supplementation increased plasma cysteine; the reason for this is not known (**Table 3**).

As has been reported (10,19), creatine supplementation led to decreased renal AGAT activity and concentration (**Fig. 2**) and the plasma GAA concentration in the HFC group compared to the C and HF groups (Table 3). Creatine supplementation increased liver PE and decreased the PC:PE ratio compared to rats fed the other diets. *PEMT* mRNA expression did not differ among any of the groups (**Table 4**).

We examined the abundance of genes involved in the metabolism of S-containing amino acids, fatty acids, and phospholipids. Expression of *BHMT1*, *GNMT*, and *MGAT1* was greater in the HF group than in the C group. Creatine supplementation normalized to control levels the abundance of these genes. With regard to fatty acid metabolism, rats fed the HF diet had reduced *PPARα* mRNA levels as well as those of its downstream targets *CPT1a* and *LCAD*, genes of mitochondrial *β*-oxidation, compared to rats fed the C diet (Table 4). Creatine supplementation normalized the expression of these genes. Previously, Koonen et al. (20) showed that the hepatic expression of *CD36*, a fatty acid transporter, alters the in vivo rate of fatty acid uptake and TG storage in mice. In our study, *CD36* was not affected in the HF group, but it was reduced in livers of the HFC-fed rats compared to the C group. The mRNA levels for *VLCAD* and *UCP2* were not affected by the diets (data not shown).

Discussion

The principal finding of the present study is that creatine supplementation prevented the hepatic fat accumulation that occurs upon feeding a high-fat diet to rats for 3 wk. The effects of betaine supplementation on SAM availability and the prevention of fatty liver are well known (3,21,22). Previous studies have shown that betaine supplementation regulates PC synthesis and normalizes VLDL production rates (22), thus preventing either high fat diet- or ethanol-induced fatty liver



	C	HF	HFC
Image quantification (% of red pixel)	23.8±7.3[a]	37.5 ±10.5[b]	21.1 ±5.6[a]

FIGURE 1 Oil-red-O–stained liver sections of rats fed a C, HF, or HFC for 3 wk. Values are means ± SD, $n = 3$ Means without a common letter differ, $P < 0.05$. C, Control; HF, high fat; HFC, HF liquid diet with creatine.

TABLE 3 Plasma creatine, GAA, homocysteine, and cysteine concentrations and liver SAM and SAH concentrations and their ratio in rats fed C, HF, or HFC for 3 wk[1]

	C	HF	HFC
Plasma			
Creatine, *mmol/L*	0.085 ± 0.019[a]	0.093 ± 0.015[a]	0.995 ± 0.152[b]
GAA, *μmol/L*	5.1 ± 0.6[b]	6.1 ± 0.4[b]	1.7 ± 0.1[a]
Homocysteine, *μmol/L*	6.4 ± 1.0	6.2 ± 1.0	5.0 ± 0.8
Cysteine, *mmol/L*	0.196 ± 0.015[a]	0.219 ± 0.012[a]	0.300 ± 0.026[b]
Liver			
SAM, *nmol/g*	67.2 ± 6.3[b]	55.2 ± 5.4[a]	66.4 ± 7.1[b]
SAH, *nmol/g*	7.1 ± 1.1	7.9 ± 0.8	7.5 ± 0.5
SAM:SAH	9.6 ± 0.8[b]	7.1 ± 0.8[a]	8.8 ± 0.7[b]

[1] Values are means ± SD, $n = 6$. Means in a row with superscripts without a common letter differ, $P < 0.05$. C, control; GAA, guanidinoacetic acid; HF, high-fat; HFC, high-fat liquid diet with creatine; SAH, *S*-adenosylhomocystein; SAM, *S*-adenosylmethionine.

(3,7,21–23). PC biosynthesis is required for the normal secretion of VLDL by hepatocytes. Both the PEMT (24) and liver-specific cytidylyltransferase α knockout mice (13,25) have impaired PC biosynthesis, which leads to fatty liver. Furthermore, it has been shown that a dramatic reduction in the PC:PE ratio results in steatohepatitis and liver failure (26). To our knowledge, this is the first study that shows that creatine can exert such a protective effect.

Creatine supplementation is known to modulate methylation demand (10) and decrease the plasma homocysteine concentration (10,11), brought about by a remarkable (90%) downregulation of renal AGAT enzyme activity. In the present study, creatine supplementation also caused a significant reduction in the plasma GAA concentration (Table 3) and, consequently, in the endogenous formation of creatine. Creatine supplementation also prevented the decrease in liver SAM concentration seen in HF-fed rats. SAM is required to form several methylated compounds through transmethylation reactions (9), of which PC synthesis and creatine synthesis are quantitatively the most prominent. Phospholipid methylation is thought to be the major SAM-consuming pathway, because hepatic PC synthesis by PEMT is responsible for ~50% of hepatic Hcy formation (27). Creatine synthesis is also responsible for a very considerable consumption of hepatic SAM (9), perhaps as much as 40%. Because both PEMT and GAMT share the same hepatic SAM pool, we originally hypothesized that creatine supplementation could lead to a sparing effect on SAM use and result in increased PC formation. The increased hepatic SAM levels are consistent with such a sparing effect. However, PC levels and *PEMT* mRNA were unaltered by dietary treatment. Although creatine supplementation did increase PE and, consequently, resulted in a small decrease in the PC:PE ratio, it is unlikely that altered phospholipid metabolism plays an important role in either the development of steatosis in rats fed the HF diet or in its prevention by creatine. Nevertheless, we cannot rule out an increase in a key hepatic pool of PC that is not reflected in our measurements of total hepatic PC. We therefore examined the abundance of other key hepatic mRNA levels to see if they could shed light on how creatine supplementation might prevent fat accumulation.

The HF diet reduced mRNA levels for *PPARα* as well as those of its downstream targets *CPT1a* and *LCAD*. PPARα is essential in the modulation of lipid transport and metabolism, mainly through activating mitochondrial and peroxisomal fatty acid β-oxidation pathways (28,29). CPT1a is a regulatory enzyme in mitochondrial β-oxidation and a target of PPARα (29,30). LCAD, which is also a target of PPARα, catalyses a key reaction in β-oxidation (19). Creatine supplementation normalized these changes in mRNA levels. Creatine also reduced *CD36* mRNA levels (a fatty acid transporter). Together, these changes could provide a mechanism by which creatine reduces fat accumulation in the liver. Previous studies have shown impaired fatty acid metabolism induced in rats fed a high-fat diet (28,31,32) as well as in NASH patients (33). In this regard, the findings of Ayoma et al. (34), that PPARα-null mice have both hepatic steatosis and decreased mRNA levels for proteins involved in fatty acid oxidation, are of particular relevance. Abdelmegeed et al. (28) also found severe steatosis, inflammation, and increased parameters of oxidative stress in PPARα-null mice that were fed a high-fat diet for 3 wk. These authors concluded that PPARα plays a critical role in preventing steatosis and fat-related oxidative stress and inflammation via effects on fatty acid catabolism (32). To the best of our knowledge, our study is the first to demonstrate the regulation of PPARα expression and its downstream targets by creatine.

Feeding a high-fat diet increased the hepatic abundance of mRNA for both *BHMT* and *GNMT*. These RNA abundances

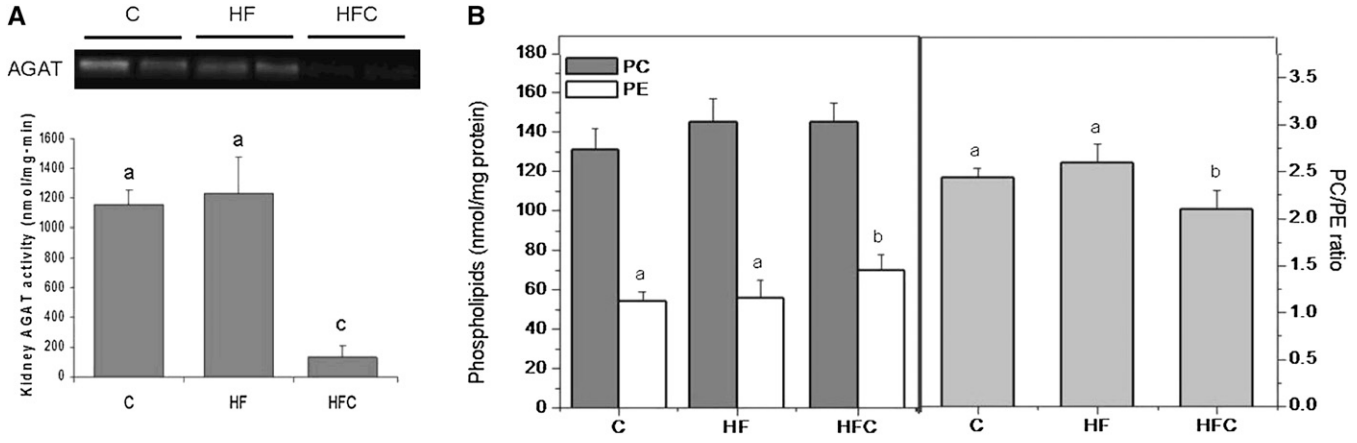


FIGURE 2 Renal AGAT protein and activity (*A*) and hepatic PC and PE levels and their ratio (*B*) in rats fed C, HF, or HFC diet for 3 wk. Values are means ± SD, $n = 6$. Labeled means without a common letter differ $P < 0.05$. AGAT, arginine:glycine amidinotransferase; C, control; HF, high fat; HFC, high-fat liquid with creatine; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

TABLE 4 *PEMT*, *PPARα*, *CD36*, *CPT1a*, *LCAD*, *BHMT*, *GNMT*, and *MGAT* mRNA levels in rats fed C, HF, or HFC for 3 wk[1]

mRNA	C	HF	HFC
PEMT	1.00 ± 0.14	1.00 ± 0.09	0.98 ± 0.17
PPARα	1.10 ± 029[b]	0.55 ± 022[a]	1.08 ± 0.29[b]
CD36	1.01 ± 0.016[b]	0.85 ± 0.27[b]	0.52 ± 0.19[a]
CPT1a	0.82 ± 0.25[b]	0.44 ± 0.22[a]	0.83 ± 0.20[b]
LCAD	1.04 ± 022[b]	0.55 ± 0.08[a]	0.88 ± 0.10[b]
BHMT	0.64 ± 0.12[a]	1.20 ± 0.52[b]	0.54 ± 0.22[a]
GNMT	1.00 ± 0.05[a]	1.73 ± 0.2[b]	0.88 ± 0.25[a]
MGAT	1.01 ± 0.23[a]	1.64 ± 0.29[b]	0.96 ± 0.36[a]

[1] Values are relative MRNA expression, standardized to cyclophilin, given as means ± SD, $n = 6$. Means in a row with superscripts without a common letter differ, $P < 0.05$. C, control; HF, high-fat; HFC, high-fat liquid diet with creatine.

were normalized in creatine-supplemented rats. These results suggest that feeding a high-fat diet may induce BHMT to promote remethylation of homocysteine, and GNMT to normalize the hepatic SAM:SAH ratio. BHMT transfers a methyl group from betaine to homocysteine to regenerate methionine. GNMT uses SAM to methylate glycine, producing sarcosine (N-methylglycine), which, via sarcosine dehydrogenase, is reconverted to glycine with the transfer of a 1-carbon group to the mitochondrial folate pool.

Creatine supplementation was at 3.1 g/(kg · d), which is appreciably higher than the 0.3 g/(kg·d) classically proposed for humans (35,36). However, the 1% creatine-supplemented diet was chosen, because it was previously shown to downregulate renal L-AGAT (10) and decrease the plasma Hcy concentration (11,12) in rats. It should be recognized that allometric scaling factors must be taken into account when comparing doses between rats of different sizes. In general, mass-specific metabolic rate scales as the 0.75 exponent of mass. Therefore, a 300-g rat has ~4 times the mass-specific metabolic rate of a 70-kg human. When expressed on this physiological basis, the rats received ~2.5-fold the creatine supplementation recommended for humans.

In conclusion, creatine supplementation prevented fatty liver induced by 3 wk of a high-fat diet in rats. This is the first study to our knowledge that shows this protective effect of creatine. This effect is not totally explained by modulation of methyl balance and consequent increased SAM availability, the explanation for the known effect of betaine on fatty liver. Our results do suggest that creatine supplementation also normalized some enzymes of methionine metabolism. In addition, and critically, creatine supplementation normalized the expression of *PPARα* as well as its downstream targets, *CPT1* and *LCAD*, genes that code for key mitochondrial enzymes of *β*-oxidation. Because the expression of these genes was decreased by the high-fat diet, their normalization suggests a possible mechanism by which creatine may prevent liver fat accumulation. Further studies are necessary to elucidate the exact mechanisms by which creatine supplementation modulates the expression of *PPARα*.

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