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Creatine reduces hepatic TG accumulation in hepatocytes by stimulating fatty acid oxidation

Robin P. da Silva, Karen B. Kelly, Kelly-Ann Leonard, René L. Jacobs *

Metabolic and Cardiovascular Diseases Laboratory, Group on the Molecular and Cell Biology of Lipids, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada

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ABSTRACT

Non-alcoholic fatty liver disease encompasses a wide spectrum of liver damage including steatosis, non-alcoholic steatohepatitis, fibrosis and cirrhosis. We have previously reported that creatine supplementation prevents hepatic steatosis and lipid peroxidation in rats fed a high-fat diet. In this study, we employed oleate-treated McArdle RH-7777 rat hepatoma cells to investigate the role of creatine in regulating hepatic lipid metabolism. Creatine, but not structural analogs, reduced cellular TG accumulation in a dose-dependent manner. Incubating cells with the pan-lipase inhibitor diethyl p-nitrophenylphosphate (E600) did not diminish the effect of creatine, demonstrating that the TG reduction brought about by creatine does not depend on lipolysis. Radiolabeled tracer experiments indicate that creatine increases fatty acid oxidation and TG secretion. In line with increased fatty acid oxidation, mRNA analysis revealed that creatine-treated cells had increased expression of PPARα and several of its transcriptional targets. Taken together, this study provides direct evidence that creatine reduces lipid accumulation in hepatocytes by the stimulation of fatty acid oxidation and TG secretion.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) has been associated with obesity and decreased insulin sensitivity, and a fatty liver is considered the hepatic manifestation of the metabolic syndrome [1]. The hallmark of NAFLD is the accumulation of triglycerides (TG) in lipid droplets within hepatocytes. Current data suggests that 20–30% of North Americans have NAFLD, which could progress to more severe liver damage if left untreated [2]. Current clinical treatments for fatty liver are limited and so the search for safe and effective therapy is important.

In vivo, phosphatidylcholine (PC) synthesis is a major consumer of hepatic methyl groups accounting for approximately 40% of all transmethylation reactions, and is an important determinant of hepatic TG metabolism [3]. Hepatocytes have the highest activity of phosphatidylethanolamine *N*-methyltransferase (PEMT) and they synthesize a significant portion of PC via the sequential methylation of phosphatidylethanolamine (PE) [4]. This means that synthesizing a relatively small amount of PC via the PEMT enzyme could have a significant impact on methylation reactions. Fat accumulation and NASH progression have been associated with decreased availability of *S*-adenosylmethionine (AdoMet) as well as an increase in homocysteine levels and oxidative stress [5]. Dietary betaine, an important methyl donor for the remethylation of homocysteine to methionine, protects the liver from fat accumulation and lipid peroxidation in rodent models of both alcoholic and non-alcoholic fatty liver [6,7]. Betaine has been shown to elevate AdoMet, reduce homocysteine, and beneficially alter glutathione redox reactions in alcoholic liver injury [8]. It is possible that the provision of excess methyl groups leads to increased synthesis of PC thereby increasing lipid efflux from this tissue; PC plays a functional role in the packaging of lipoproteins and is the most abundant phospholipid found in these structures. In fact, mice that have a deletion of PEMT develop a fatty liver and have reduced hepatic VLDL secretion when fed a high-fat diet [9].

De novo creatine biosynthesis occurs in the liver via the AdoMet-dependent methylation of guanidinoacetate (GAA) and is a major consumer of hepatic methyl groups, estimated to account for 40% of total methylation reactions in the body [10]. Dietary creatine supplementation can reduce plasma GAA levels by 90% and thereby reduces demand on hepatic methylation [11]. Previously, we hypothesized that dietary creatine supplementation may spare AdoMet for PC synthesis, thus protecting the liver from TG accumulation. Dietary creatine supplementation prevented TG accumulation and the lowering of AdoMet in the liver of rats fed a high-fat diet (HFD) [12]. Interestingly, dietary creatine did not alter hepatic PC levels or PEMT activity; therefore, the

Abbreviations: ABCA1, ATP binding cassette protein; AMPK, adenosine monophosphate-activated protein kinase; CT, CTP:phosphocholine cytidylyltransferase; DGAT, acyl CoA:diacylglycerol acyltransferase; HDL, high density lipoproteins; HFD, high-fat diet; HCD, high-carbohydrate diet; HMGCS2, hydroxymethylglutaryl CoA synthase 2; GPAT, glycerol-3-phosphate acyltransferase; MHFD, moderate-high fat diet; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OA, oleate; PEMT, phosphatidylethanolamine *N*-methyltransferease; McA, McArdle RH-7777 cells

* Corresponding author at: 4-002E Li Ka Shing Building, 112th St and 87th Avenue NW, Edmonton, AB T6G 2E1, Canada. Tel.: +1 780 492 2343.
E-mail address: rjacobs@ualberta.ca (R.L. Jacobs).

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mechanism(s) through which creatine reduces fatty liver does not appear to be related to AdoMet availability. In the current study, we utilized the McArdle RH-7777 (McA) immortalized hepatoma cell line, an established model for the study of hepatic lipid metabolism that does not express PEMT [13–16], to assess whether creatine might have a direct action on TG synthesis in liver cells. We have now demonstrated that creatine prevents TG accumulation in McA cells incubated with exogenous oleic acid. Our data suggest that creatine, potentially through increased PPARα activity, stimulates fatty acid oxidation and TG secretion.

2. Materials and methods

2.1. Cell culture conditions for McA cells

McArdle RH-7777 cells were obtained from American Type Culture Collection (ATCC) and cultured in high glucose Dulbecco's Modified Eagles Medium (DMEM) (Life Technologies) containing 10% fetal bovine serum and 10% horse serum within a humidified incubator maintained at 37 °C and 5% CO_2. Concentrated stock solutions of compounds were made in PBS and a volume of 200 μL or less was added to 10 mL of DMEM containing the 20% serum noted above. Where appropriate, oleic acid (Sigma) was delivered to cells in DMEM complexed with BSA (BSA-OA).

500,000 McA cells were plated in 10 cm culture plates and made to a final volume of 10 mL with DMEM containing serum. Concentrated stock solutions of creatinine, guanidinoacetate (GAA), guanidinopropionic acid (GPA) or methylguanidine (MGU) were added to medium and cells were grown for 48 h. After 48 h cells were washed three times in PBS and fresh DMEM (with no serum) containing the respective compounds, with or without oleic acid, and were incubated for a further 4 h at 37 °C. After 4 h the medium was removed and the cells were washed 3 times with PBS and collected in 1 mL of ice-cold lysis buffer containing 20 mM Tris–HCl, 50 mM NaCl, 50 mM NaF, 5 mM NaPyrophosphate and 250 mM sucrose. For RNA isolation, cells were treated in a similar fashion but instead of lysis buffer, 2 mL of ice-cold Trizol® (Invitrogen) was added directly to the petri dish. Cell viability was greater than 95% for all treatments as assessed by trypan blue exclusion.

2.2. Lipid analysis

Total lipids were extracted from cells using a Folch-type extraction using 2:1 chloroform:methanol [17]. The lipid was re-suspended in 2-propanol and assayed for total triglycerides using a commercially available kit from Sekisui Diagnostics (Charlottetown, PEI, Canada, catalogue # 236-60). Hepatic phosphatidylcholine and phosphatidylethanolamine were measured by a phosphorous assay after separation of lipid species by thin-layer chromatography, as described by Jacobs et al. [18]. TG mass in the medium was determined using the GC-FID method of Kuksis [19] using tridecanoylglycerol as an internal standard.

2.3. Lipid synthesis

For experiments measuring the incorporation of oleic acid into lipid species, 9,10-^{3}H-oleic acid (Perkin Elmer) was added to unlabeled oleic acid and complexed to BSA. Cells were incubated with the BSA–^{3}H-oleic acid and lipids were extracted from the cell lysates and separated on TLC plates, as described previously [20]. Bands were detected using iodine vapor, scraped into scintillation vials containing 10 mL of Ultima Gold scintillation fluid (Perkin Elmer). For de novo fatty acid synthesis, ^{14}C-acetate (Perkin Elmer) was added directly into DMEM and incubated for 4 h at 37 °C. Cells were rinsed 3 times with PBS and collected in 1 mL of ice cold lysis buffer (above).

2.4. Fatty acid oxidation

Total fatty acid oxidation was measured by incubating McA cells with BSA–oleic acid labeled with 9,10-^{3}H-oleic acid for 4 h after which the cell culture medium was collected, acidified with 1 M ascorbic acid and extracted using 2:1-chloroform:methanol. The aqueous phase (acid soluble oxidation products) was collected and radioactivity was determined by scintillation counting using Ultima Gold (Perkin Elmer) liquid scintillation fluid. This measurement represents the total catabolism of oleate to acid soluble aqueous metabolites. In a separate method, we determined complete oxidation of fatty acids (oxidation to CO_2) by incubating McA cells in air-tight flasks with 1-^{14}C-oleic acid (Perkin Elmer) labeled BSA–oleic acid complex for 4 h at 37 °C. After this time, 400 μL of concentrated perchloric acid was injected into the culture medium through a septum. CO_2 was collected for 2 h after injecting 300 μL of 1 M KOH into the center-well containing a piece of filter paper. The center-well and contents were added to Ultima Gold (Perkin Elmer) liquid scintillation fluid and radioactivity was determined.

2.5. Western blots

Cell lysates were added to Laemmli sample buffer and resolved using SDS-PAGE. Proteins were subsequently transferred to PVDF membranes, incubated with antibodies using an HRP-conjugated secondary antibody and detected using a Chemidoc (Bio-rad). All antibodies were obtained from Cell Signaling Technology; we used rabbit anti-AMPKα (#2532), rabbit anti-(phospho-Thr172) AMPKα (#2531), rabbit anti-ACC (#3662) and rabbit anti-(phospho-Ser79) ACC (#3661).

2.6. Gene expression analysis

Total RNA was isolated from McA cells using the Trizol® (Invitrogen) method. RNA quality was assessed with an Agilent 2100 bioanalyser, using an RNA 6000 Nano kit (Agilent Technologies). 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 Diagnostics) based on the NCBI reference nucleotide sequences for *Rattus norvegicus*. Each primer pair and probe combination was tested by qPCR (StepOnePlus, Applied Biosystems) to confirm that amplification conditions were suitable for use in the Biomark™ gene chip (Fluidigm). A mix containing primers for all genes combined in a single assay was used to pre-amplify the cDNA in each sample. Pre-amplification was performed in order to enrich the template cDNA for use in the Biomark™ gene chip. All pre-amplified samples were tested on the StepOnePlus qPCR machine using the probe for cyclophilin (Ppia) before loading of the Biomark™ gene chip. Ninety-six primer pairs and pre-amplified samples were loaded into separate wells on a 96-by-96 gene chip (Fluidigm). qPCR was run on the Biomark™ system (Fluidigm) for 40 cycles. Relative RNA expression for each gene in a sample was calculated using the comparative threshold (ΔΔCT) method. Values were normalized to the endogenous housekeeping gene cyclophilin. All samples were assayed in triplicate for each primer pair.

2.7. Statistical analysis

Data are expressed as the means ± standard deviation ($N = 4$–6 for each measurement). Data were analyzed using one-way ANOVA or Student's *t*-test where appropriate. The time course experiments were fitted using non-linear regression. All analyses were done using GraphPad Prism software. A p-value <0.05 was taken as statistically significant.

3. Results

3.1. Creatine prevents TG accumulation

To determine whether creatine directly affects hepatic lipid metabolism in McA cells we performed incubations with increasing concentrations of oleate. Creatine reduced TG levels by 40–60% in cells incubated with oleate concentrations ranging from 0.1 to 0.4 mM (Fig. 1A). Furthermore, there was a dose-dependent reduction in cellular TG in creatine-treated cells (Fig. 1B). Reduction in cellular TG was observed when the concentration of creatine in the medium was 0.3 mM or higher. To determine whether structural analogs of creatine might have a similar effect on lipid metabolism, we incubated cells with creatinine, GAA, GPA or MGU (Fig. 2A). None of the creatine analogs affected cellular TG levels in McA cells (Fig. 2B). These data support the notion that the observed effect on lipid metabolism is specific to creatine.

3.2. Fatty acid transport and lipolysis are not affected by creatine treatment

Two possible explanations for the reduction of lipid accumulation in McA cells could be: 1) a reduction in fatty acid uptake or 2) an increased mobilization of TG through the action of intracellular lipases. We did not observe any difference in the rate of oleate uptake by McA cells treated with creatine (Fig. 3A). To determine whether the TG lowering effect of creatine was dependent on lipase activity, cells were incubated with the pan-lipase inhibitor diethyl nitrophenylphosphate (E600) (Fig. 3B). The presence of E600 caused a significant increase in lipid accumulation in all conditions tested; however, the effect of creatine-treatment was not diminished by the presence of the lipase inhibitor. This finding suggests that the mechanism by which creatine prevents the TG accumulation does not involve modulation of lipase activity.

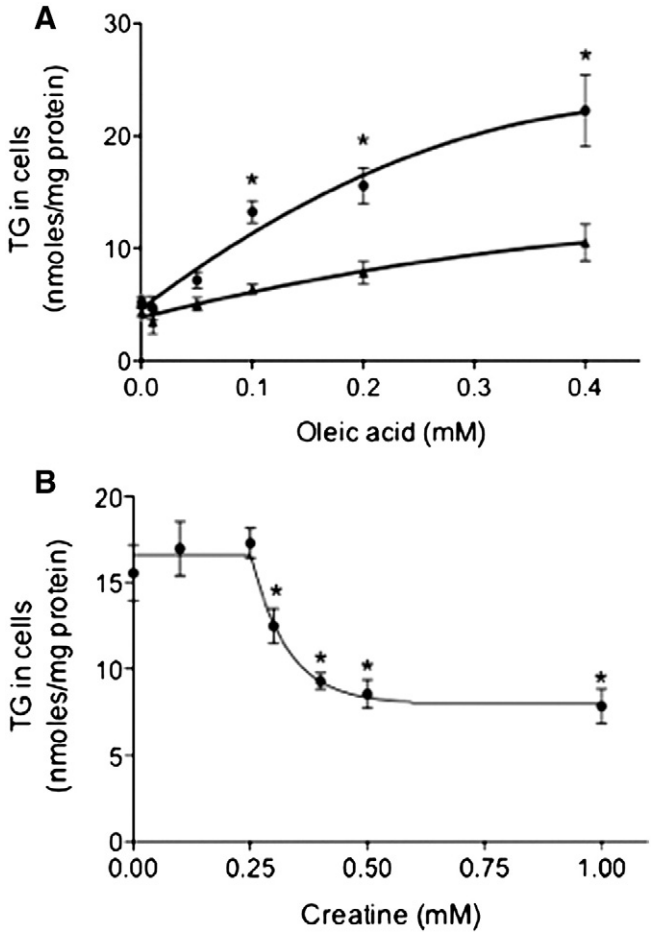

Fig. 1. Creatine reduces TG accumulation in McA cells. Cellular TG measured in the presence of increasing concentrations of oleic acid (A) and creatine (B). An asterisk denotes a significant difference and $P < 0.05$ was taken as significant.

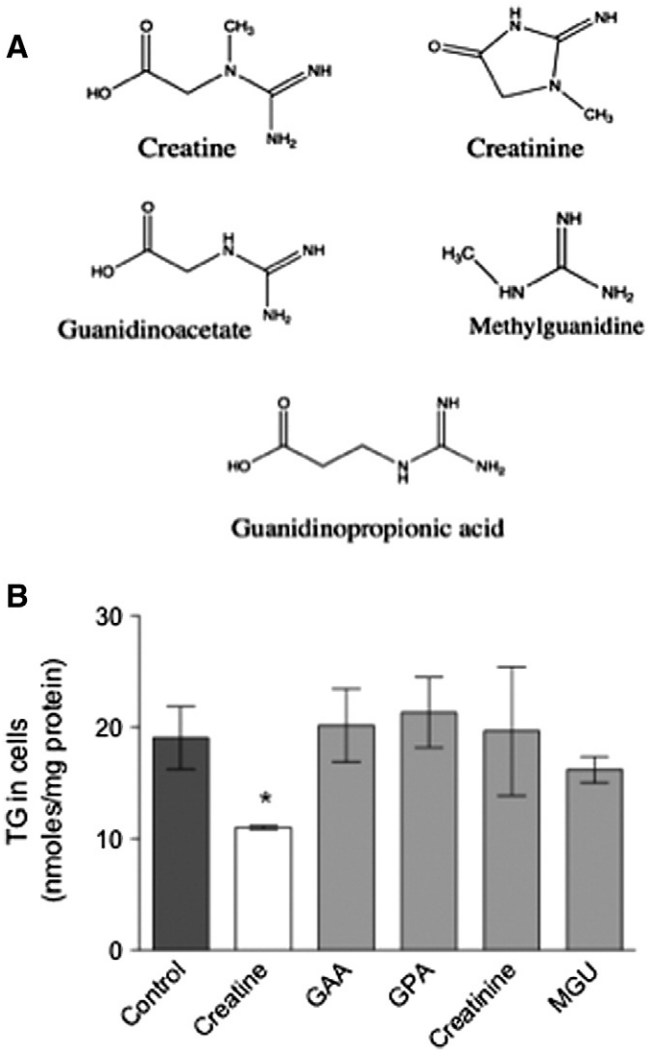

Fig. 2. Creatine specifically reduces TG accumulation in McA cells. Structures of creatine and physiologically relevant analogous molecules (A). Cellular TG measured in McA cells incubated with creatine and structural analogs (B). An asterisk denotes a significant difference and $P < 0.05$ was taken as significant.

3.3. Lipid synthesis and efflux are altered by creatine

To further study the dynamics of lipid metabolism, cells were incubated with ^{3}H-oleate. Incorporation of ^{3}H-oleate into TG was significantly reduced in the McA cells incubated with creatine compared to vehicle controls (Fig. 4A). The percentage of labeled TG secreted by McA cells was proportionally higher in the creatine-treated group when compared to control (Fig. 4B). Furthermore, TG mass was increased in the medium of creatine-treated cells (Fig. 4C). These data demonstrate that TG secretion by creatine-treated McA cells was significantly higher.

The incorporation of ^{3}H-oleate into PC and PE was not different in the creatine-treated cells when compared to control cells (Fig. 5A). Total PC and PE mass was modestly but significantly reduced in the cells treated with creatine; however, the PC/PE ratio was unaltered (Fig. 5B). In addition, the proportion of labeled PC was significantly greater in the cell culture medium from the creatine treated McA cells

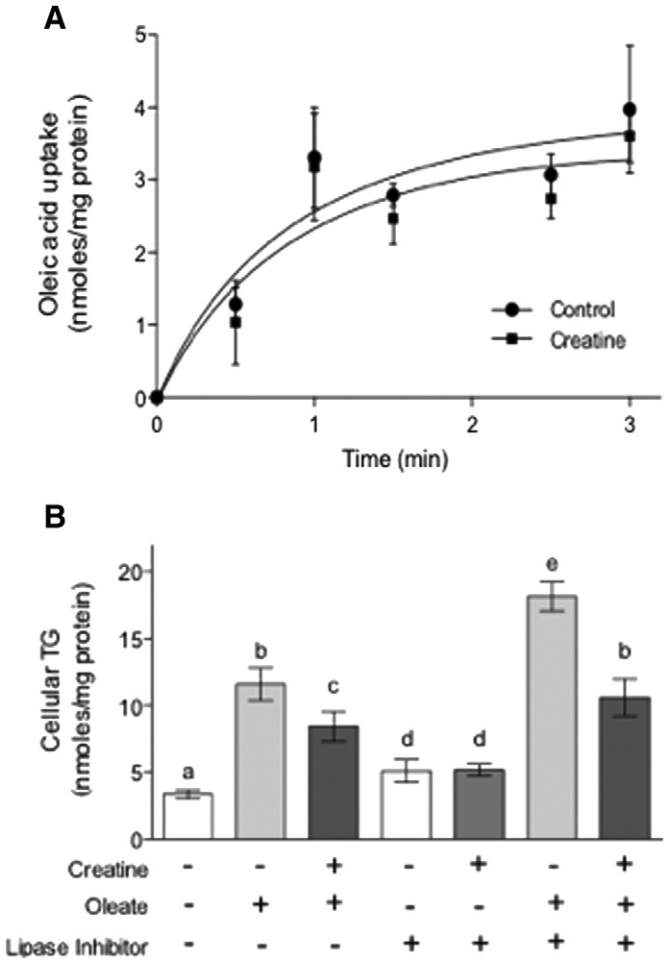

Fig. 3. Creatine has no effect the uptake of fatty acids and does not require the activity of lipases to lower cellular TG in McA cells. Uptake of ^{3}H-oleic acid in cells treated with creatine (A); solid circles (●) represent control cells and solid squares (■) represent creatine treated cells. McA cells were treated with creatine, oleic acid and/or a lipase inhibitor (E600) (B). Different letters denote a significant difference and $P < 0.05$ was taken as significant.

(Fig. 5A and C) supporting the idea that lipoprotein secretion from these cells is increased.

3.4. Creatine increases fatty acid catabolism and decreases fatty acid synthesis

Two radiolabelled methods were employed to determine the rate of fatty acid oxidation and catabolism by the McA cells. The production of $^{14}CO_2$ from ^{14}C-oleic acid was doubled in cells treated with creatine compared with untreated cells (Fig. 6A). Similarly, the acid-soluble products of ^{3}H-oleic acid increased by 30% in creatine-treated cells (Fig. 6A).We assessed fatty acid synthesis in the McA cells following a ^{14}C-acetate incubation; incorporation of de novo-synthesized fatty acids into TG and phospholipids was significantly lower in cells treated with creatine (Fig. 6B).

3.5. AMP-activated protein kinase (AMPK) is not regulated by creatine

Increased AMPK activity is associated with a reduction in fatty acid synthesis and an elevation in fatty acid oxidation. Phosphorylation of the AMPK catalytic subunit (AMPK-α) at Thr-172 is essential for AMPK activation [21]; we postulated that AMPK phosphorylation would be increased by creatine treatment. However, no difference was found in the relative amount of active phospho-(Thr-172)-AMPK

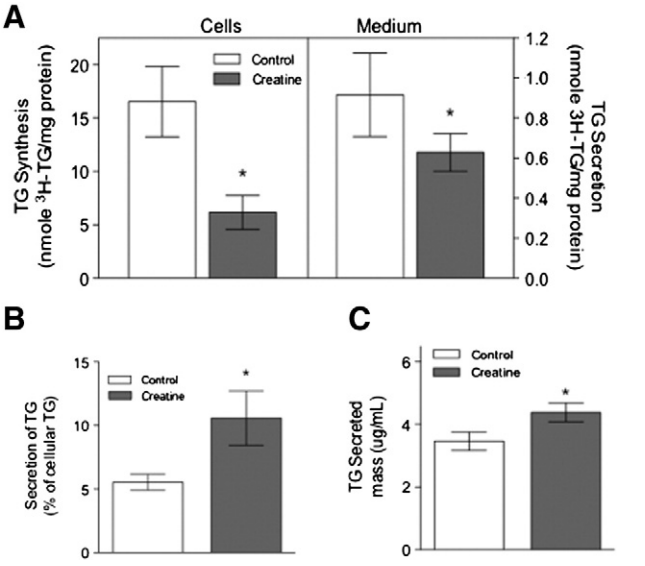

Fig. 4. The presence of creatine alters synthesis and secretion of TG by McA cells. ^{3}H-oleic acid incorporation in TG and the secretion of ^{3}H-labeled TG into the culture medium (A). The percentage of ^{3}H-labeled TG in the medium relative to ^{3}H-labeled TG in cells (B). The mass of TG secreted into the medium (C). An asterisk indicates a significant difference from control. $P < 0.05$ was taken as significant.

between the control and creatine treated McA cells (Fig. 7A and C). AMPK regulates the activity of acetyl-CoA carboxylase by phosphorylation at Ser-79 [22]. No difference was observed in the phospho-(Ser-79)-ACC between the control and creatine-treated McA cells (Fig. 7A and B).

3.6. Creatine increases mRNA abundance of genes involved in fatty acid oxidation

Creatine-treatment of McA cells significantly altered the mRNA abundance of genes involved in lipid transport, lipogenesis and lipid oxidation (Table 1). There was a marked increase in the abundance of *pparα* mRNA along with significant increases mRNAs of the transcriptional targets of PPARα such as *cpt1α*, *lfabp*, *mcad*, *lcad* and *vlcad*. We observed no change in the mRNA abundance of *pgc-1α*or *dgat1*, but *gpam*, *agpat2*, *agpat3*, and *dgat2* mRNA were modestly increased in the creatine-treated McA cells. *Lipin1* mRNA abundance was increased 3-fold by creatine treatment. *Apob* and *mtp* expression was increased by creatine treatment, which is consistent with the increase in TG secretion from these cells (Fig. 4B). Finally, the expression of the lipid trafficking genes *cd36* and *fatp1* were increased despite no observed changes in oleic acid uptake (Fig. 3A).

4. Discussion

We previously reported that creatine-supplementation prevents hepatic lipid accumulation and improves oxidative in rats fed a high-fat diet [12]. In this study, dietary creatine (1% *wt/wt*) increased plasma creatine from 90 μM (no dietary creatine) to 1 mM [12]. The purpose of the current study was to investigate the potential mechanisms responsible for these observations. We provide novel evidence that creatine can directly regulate lipid metabolism in McA cells. We find that creatine-treatment reduced cellular TG levels in a dose-dependent manner. The reduction in cellular TG levels was observed when a physiologically feasible concentration of creatine (300 μM) was added to the medium. The effect of creatine on TG accumulation was specific since none of the structural analogs tested displayed reduced cellular TG levels. It is of note that dietary GAA-supplementation elevates plasma creatine concentration and thus GAA could regulate hepatic TG metabolism in vivo [23]; however, we were unable to detect

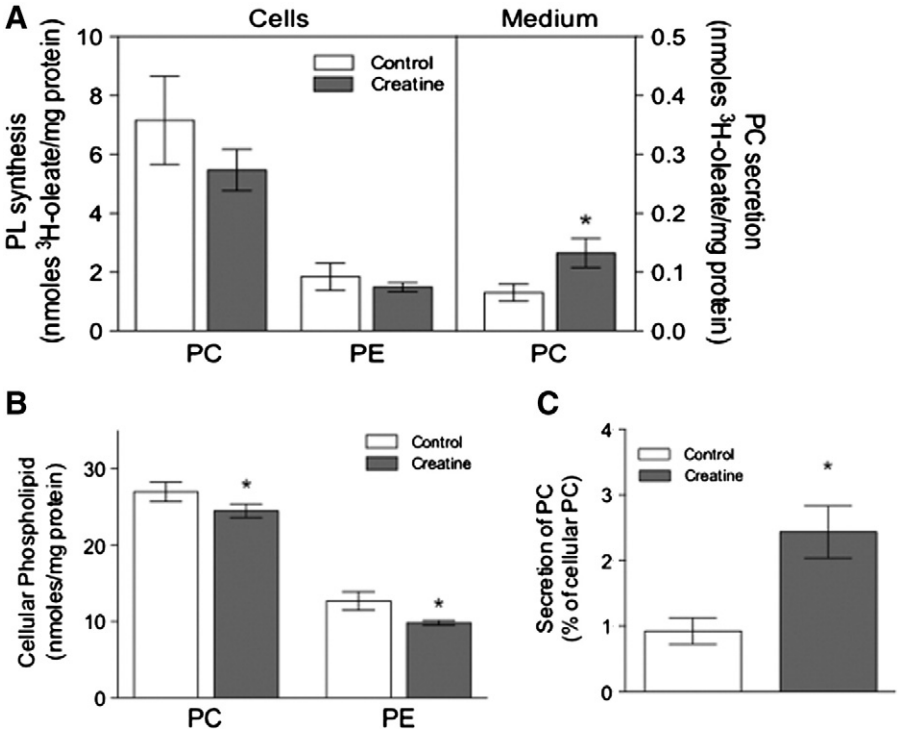

Fig. 5. The presence of creatine alters synthesis and secretion of phospholipid by McA cells. ^{3}H-oleic acid incorporation in cellular PC and PE and the radiolabel recovered in PC from the culture medium (A). Phospholipid content in cells (B). The percentage of 3H-labeled PC in the medium relative to 3H-labeled PC in cells (C). An asterisk indicates a significant difference from control. $P < 0.05$ was taken as significant.

guanidinoacetate N-methyltransferase mRNA in McA cells (data not shown). Furthermore, GAA-supplementation induces a fatty liver in mice fed a liquid high-fat diet [24].

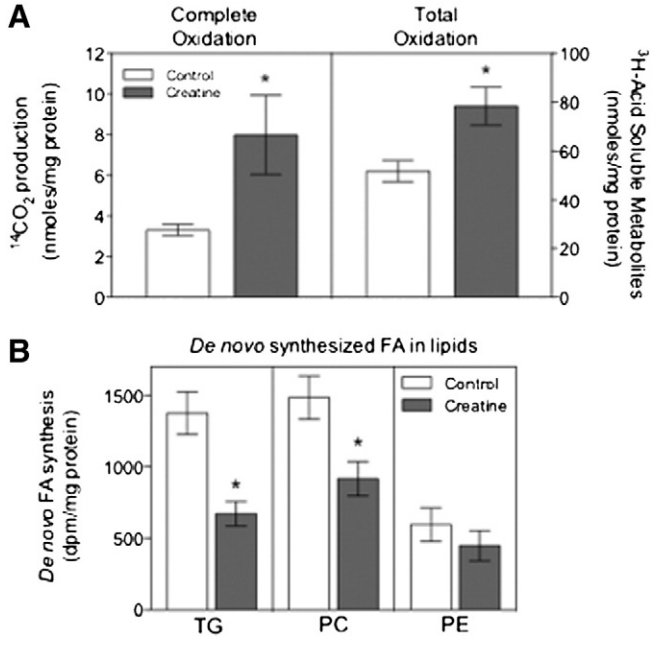

Fig. 6. Creatine treatment increased fatty acid oxidation and decreased fatty acid synthesis in McA cells. Complete oxidation of oleic acid (oleic acid to CO_2) was measured using a ^{14}C-oleic acid label. Total oxidation (incomplete and complete oxidation measured as acid soluble oxidation products) of oleic acid was measured using a ^{3}H-oleic acid label (A). De novo fatty acid synthesis was measured by the incorporation of ^{14}C-acetate into lipid species (B). Different letters denote a significant difference and $P < 0.05$ was taken as significant.

Our experiments clearly show that creatine stimulates fatty acid oxidation and lipid secretion, and reduces fatty acid synthesis in cultured hepatocytes. Increased *ppara* mRNA was observed in creatine-supplemented rats on a high fat diet [12] but hepatic lipid oxidation or VLDL secretion was not measured in these animals. Creatine also increased mRNA abundance of *ppara* as well as several of its downstream targets [25,26], such as, *cpt1a*, *acox1*, *lcad*, *lfabp* and *mtp* in the McA cells. Increased *cpt1a*, *acox1*, *lcad*, *mcad* and *vlcad* mRNA provides a potential mechanism for the stimulation of oleic acid oxidation by creatine. TG secretion into the medium was also increased in McA cells treated with creatine, which is consistent with an increase in *apob* and *mtp* mRNA abundance. However, we must note that *apob or mtp* mRNA abundance does not appear to be a regulatory mechanism of lipoprotein secretion, but rather the degradation of ApoB protein and the activity of MTTP [27]. Taken together, our data suggests that creatine alters expression of *ppara* and its downstream targets, which increases fatty acid oxidation and lipid secretion.

In addition to *ppara*, creatine supplementation resulted in a 3-fold increase in *lipin1* expression. Lipin1 is one of the phosphatidic acid phosphatase (PAP) enzymes responsible for converting phosphatidic acid (PA) into diacylglycerol (DAG) in one of the major pathways for TG synthesis. However, we observed an increase in *lipin1* along with a decrease in cellular TG synthesis. Recently, lipin1 has been recognized as a transcriptional co-activator of PPARα [28], and hepatic expression of lipin1 is known to be induced by glucocorticoids and fasting [29]. Interestingly, fatty liver dystrophic mice, which have a negative mutation in *lipin1*, have dramatically elevated hepatic TG content and aberrant lipoprotein metabolism. It is unclear how the creatine influences *lipin1* or *ppara* expression; however, both *lipin1* and *ppara* expressions are stimulated by PGC-1α and, these three proteins work together to elicit an increase in hepatic fatty acid oxidation [28]. We did not observe any change in *pgc-1α* mRNA abundance, which suggests that creatine does not affect the regulation of this transcriptional modulator at the pre-translational level. With our current data we cannot make the connection between lipin1 and the observed changes in lipid metabolism

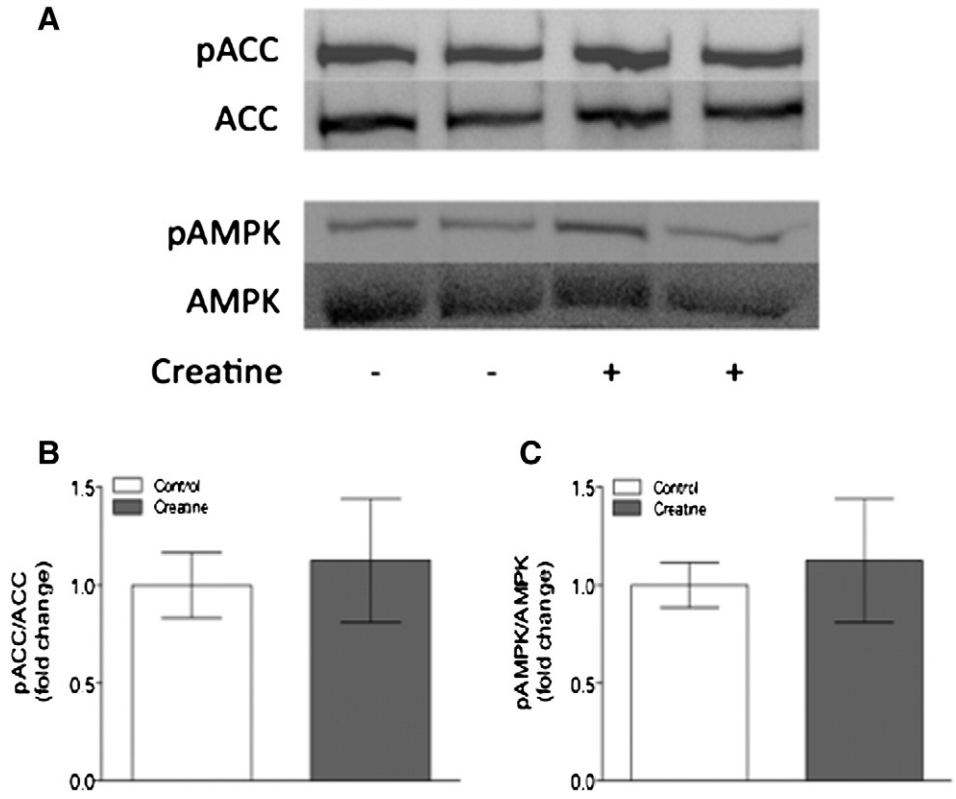

Fig. 7. AMPK and ACC phosphorylation are not changed when McA cells are incubated with creatine. Representative western blots for AMPK, ACC and respective phosphorylated forms (A). Ratio of integrated density values for pAMPK:AMPK (B). Ratio of integrated density values for pACC:ACC (C). Different letters denote a significant difference and $P < 0.05$ was taken as significant.

or activity of PPARα, but the role of lipin1 is more dynamic than as a simple DAG producing enzyme [30].

Recent evidence suggests that DGAT1 is primarily responsible for incorporation of exogenous oleic acid into the storage pool of TG, while both DGAT1 and DGAT2 are involved in the lipidation of the VLDL particles [31]. *Dgat2* mRNA was increased in McA cells treated with creatine, which agrees with the observed increase in lipid secretion. Although elevated *Gpam*, *agpat2*, *agpat3*, and *dgat2* mRNA abundance in the creatine-treated McA cells would suggest an increase in lipogenesis, we observed a reduction. It is possible that creatine reduces flux through lipogenesis by an alternate mechanism, or that a small increase in fatty acid synthesis is masked by a relatively larger increase in oxidation.

Our original hypothesis was that dietary creatine supplementation would reduce the methylation of guanidinoacetate and spare AdoMet for PC synthesis via PEMT [12]. The resulting increase in PC synthesis would stimulate VLDL secretion and reduce TG storage in the liver [12]. However, this mechanism was not supported by the hepatic phospholipid measurement in the *in vivo* study. Furthermore this mechanism cannot be the case in the McA cells; de novo creatine synthesis (GAA production) does not occur in McA cells. McA cells do not express the enzymes responsible for making GAA (data not shown), nor does the rat liver [10]. Secondly, McA cells display only trace PEMT expression [32] and therefore do not have the capacity to increase PC synthesis via this pathway. Therefore, our experiments on the McA cells show that creatine can affect lipid metabolism independent of methylation and PC synthesis via PEMT.

We also hypothesized that creatine could alter lipid metabolism by modulating AMPK activity. The phosphorylation state of AMPK affects the activity of ACC resulting in a decrease in fatty acid synthesis and an increase in fatty acid oxidation [33]. When McA cells were incubated with creatine for 48 h there was no effect on the phosphorylation state of Thr-172 of AMPK. While the phosphorylation state of ACC was not altered by creatine. The presence of a relatively large concentration of oleate (CoA ester) provided to the McA cells will allosterically inhibit ACC [34] and therefore the balance between fatty acid synthesis and oxidation is heavily shifted toward increased oxidation independent of the presence of creatine. Creatine itself does not appear to act in an acute manner, as a 4 h co-incubation with oleate did not prevent TG accumulation (data not shown); these results suggest that creatine is not working through an allosteric mechanism and that other signaling pathways are required. We must note here that creatine and ATP cannot be converted to creatine phosphate and ADP because McA cells, like primary rat hepatocytes, do not express the mRNA for any of the creatine kinase isoforms (data not shown). Thus creatine present within these cells cannot influence cellular energy metabolism by augmenting adenine nucleotide concentration through the action of this enzyme.

Short-term creatine (3 g) treatment in humans increases plasma creatine concentrations from 76 μM to 500–735 μM and is associated with a beneficial effect on glucose metabolism in diabetes patients [35]. Creatine supplementation has been shown to reduce blood lipids in hyperlipidemic subjects [36]. In addition, oral creatine supplementation reduced markers of DNA damage and lipid peroxidation after resistance training [37]. Currently metformin and dietary intervention are the only treatments for a fatty liver that are recommended by clinical practitioners [38]; metformin is a general effect drug that is not specific to lipid metabolism in the liver. If creatine is effective in reducing hepatic TG in humans then it may be of benefit as a therapy in conditions that involve fat accumulation in the liver.

In summary, creatine treatment lowered TG synthesis and accumulation in McA cells while increasing fatty acid oxidation and TG secretion. These changes appear to be at least partially mediated by PPARα signaling and do not appear to involve changes in lipase activity or activation of AMPK. These findings are consistent with our earlier report [12] and clearly show that creatine can directly affect lipid metabolism in liver cells.

Table 1
Creatine increases the abundance of mRNA of lipid trafficking, oxidative and lipogenic genes as determined by qPCR.

	Control	Creatine	*p* value
Lipid trafficking			
cd36	1.00 ± 0.18	1.74 ± 0.08*	<0.0001
fatp1	1.03 ± 0.13	1.27 ± 0.16*	0.03
lfabp	0.70 ± 0.11	1.30 ± 0.19*	<0.001
mtp	0.76 ± 0.15	1.04 ± 0.06*	<0.01
apob	0.63 ± 0.23	0.98 ± 0.08*	0.01
apoa1	1.05 ± 0.13	0.95 ± 0.07	0.17
Lipid synthesis			
lipin1	1.01 ± 0.19	3.16 ± 0.34*	<0.0001
dgat1	1.07 ± 0.09	1.10 ± 0.06	0.63
dgat2	1.06 ± 0.13	1.33 ± 0.13*	0.01
gpam	0.97 ± 0.14	1.26 ± 0.07*	<0.01
agpat2	1.11 ± 0.16	1.32 ± 0.10*	0.04
agpat3	0.89 ± 0.11	1.04 ± 0.07*	0.03
FA oxidation			
pparα	1.10 ± 0.16	3.36 ± 0.13*	<0.0001
pgc-1α	0.58 ± 0.11	0.59 ± 0.09	0.95
cpt1α	0.93 ± 0.13	1.37 ± 0.08*	<0.001
mcad	0.76 ± 0.12	1.07 ± 0.05*	<0.001
lcad	0.87 ± 0.11	1.21 ± 0.06*	<0.001
vlcad	1.03 ± 0.07	1.20 ± 0.05*	<0.01
acox1	0.92 ± 0.14	1.22 ± 0.08*	<0.01

Relative abundance of mRNA was calculated using the $\Delta\Delta C_T$ method. Values are expressed as mean ± S.E.
Abbreviations: acylglycerolphosphate acyltransferase 2 (agpat2); acylglycerolphosphate acyltransferase 3 (agpat3); apolipoprotein A1 (apoa1); apolipoprotein B (apob); acyl-CoA oxidase (acox1); cluster of differentiation/fatty acid translocase (cd36/FAT); carnitine palmitoyltransferase 1a (cpt1a); diacylglycerol acyltransferase 1 (dgat1); diacylglycerol acyltransferase 2 (dgat2); fatty acid transport protein 1 (fatp1); mitochondrial glycerol-3-phosphate acyltransferase (gpam); phosphatidate phosphatase 1 (lipin1); long-chain acyl-CoA dehydrogenase (lcad); liver fatty acid binding protein (lfabp); medium-chain acyl-CoA dehydrogenase (mcad); microsomal triglyceride transfer protein (mtp); peroxisome proliferation activating receptor α (pparα/ppara); peroxisome proliferation activating receptor gamma coactivator 1α (pgc-1α); very long-chain acyl-CoA dehydrogenase (vlcad).

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