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Chronic acetyl-L-carnitine alters brain energy metabolism and increases noradrenaline and serotonin content in healthy mice

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ABSTRACT

Acetyl-L-carnitine (ALCAR), the short-chain ester of carnitine, is a common dietary supplement readily available in health food stores, claimed to improve energy levels and muscle strength. ALCAR has numerous effects on brain and muscle metabolism, protects against neurotoxic insults and may be an effective treatment for certain forms of depression. However, little is known about the effect of chronic ALCAR supplementation on the brain metabolism of healthy mice. Here, we investigated ALCAR's effect on cerebral energy and neurotransmitter metabolism after supplementing the drinking water of mice with ALCAR for 25 days, providing a daily dose of about 0.5 g/kg. Thereafter the animals were injected with [1-^{13}C]glucose, and ^{13}C incorporation into and levels of various metabolites were quantified in extracts of the hippocampal formation (HF) and cortex using ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectroscopy and high performance liquid chromatography (HPLC). Increased glucose levels were detected in both regions together with a decreased amount of [3-^{13}C]lactate, but no alterations in incorporation of ^{13}C derived from [1-^{13}C]glucose into the amino acids glutamate, GABA and glutamine. These findings are consistent with decreased metabolism of glucose to lactate but not via the TCA cycle. Higher amounts of the sum of adenosine nucleotides, phosphocreatine and the phosphocreatine/creatine ratio found in the cortex of ALCAR-treated mice are indicative of increased energy levels. Furthermore, ALCAR supplementation increased the levels of the neurotransmitters noradrenaline in the HF and serotonin in cortex, consistent with ALCAR's potential efficacy for depressive symptoms. Other ALCAR-induced changes observed included reduced amounts of GABA in the HF and increased *myo*-inositol. In conclusion, chronic ALCAR supplementation decreased glucose metabolism to lactate, resulted in increased energy metabolite and altered monoamine neurotransmitter levels in the mouse brain.

1. Introduction

Acetyl-L-carnitine (ALCAR), the short-chain ester of carnitine, is endogenously produced within mitochondria and peroxisomes and is involved in the transport of acetyl-moieties across the membranes of these organelles. Therefore, ALCAR can affect lipid, carbohydrate and amino acid, as well as energy metabolism. Since the early 1990s, much attention has been directed towards the possible role of ALCAR as a therapeutic agent in aging, disorders of the brain and its mechanism of action (reviewed by Jones et al. (2010)). Plasma and CSF concentrations of ALCAR increase after oral administration, and the compound is transported across the blood brain barrier by the organic cation/carnitine transporter OCTN2 (Kido et al., 2001; Parnetti et al., 1992). Carnitine transporters from the OCTN family are present on both neurons and astrocytes (Januszewicz et al., 2010, 2009). In rat brain cells, the acetyl moiety of ALCAR may be used for the biosynthesis of acetylcholine (Dolezal and Tucek, 1981), fatty acids (Ricciolini et al., 1998), and amino acids (Scafidi et al., 2010). Acute ALCAR administration altered rat brain energy homeostasis by increasing phosphocreatine and decreasing lactate and inorganic phosphate levels (Aureli et al., 1990), stimulating glycogen synthesis (Aureli et al., 1998), and regionally increasing [^{14}C]2-deoxyglucose labeling measured by autoradiography in certain brain regions (Ori et al., 2002). Also, chronic ALCAR increased [^{14}C]2-deoxyglucose labeling in similar regions of the rat brain (Freo et al., 2009), as well as the

Abbreviations: ALCAR, acetyl-L-carnitine; HF, hippocampal formation; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; GABA, γ-aminobutyric acid; 5-HIAA, 5-hydroxyindoleacetic; 5-HT, serotonin; HVA, homovanillic acid; MDMA, 3,4-methylenedioxymethamphetamine; NA, noradrenaline; NAA, N-acetylaspartate; NMR, nuclear magnetic resonance; PDH, pyruvate dehydrogenase; TCA, tricarboxylic acid.

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enzyme activities of the sodium potassium and the calcium magnesium ATP-ases by about 30% (Villa et al., 2011).

ALCAR has both antioxidant and anti-apoptotic properties (Ishii et al., 2000; Liu et al., 1993) and can protect against various neurotoxic insults such as excessive glutamate (Forloni et al., 1994) and amyloid-ß exposure (Virmani et al., 2001). Treatment with ALCAR improved neurological outcome and energy metabolism in various animal models of ischemia (Aureli et al., 1994; Rosenthal et al., 1992) and aging (reviewed by Ames and Liu (2004), Jones et al. (2010)). In light of such promising findings in animal models, ALCAR has been tested in several clinical trials for various disorders. Small beneficial clinical effects have been reported for Alzheimer's disease in a meta-analysis of double blind randomized controlled clinical trials (Montgomery et al., 2003). However, a Cochrane review found no convincing effects (Hudson and Tabet, 2003). There is some evidence from small clinical trials with 24–67 patients that ALCAR can decrease symptoms of depression in the elderly (Garzya et al., 1990; Tempesta et al., 1987), fibromyalgia patients (Rossini et al., 2007) and patients with minimal hepatic encephalopathy (Malaguarnera et al., 2011).

Most psychiatric treatment is centered on altering monoamine neurotransmitter homeostasis. There are few reports on the effect of ALCAR on monoamine metabolism in the brain and they are restricted to rats: acute ALCAR pretreatment before 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) injection prevented mitochondrial damage and also loss of serotonin (5-HT) (Alves et al., 2009). In the control group acute ALCAR only increased the level of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA), but not 5-HT, in one of six investigated brain regions, namely the prefrontal cortex. In a model of attention deficit hyperactive disorder, chronic ALCAR treatment restored the noradrenaline (NA) level and the serotonin turnover ratio (5-HIAA/5-HT) in specific brain regions towards the values of normal young rats. In normal young rats the only change was an increased serotonin turnover ratio in the cingulate cortex (Adriani et al., 2004).

In summary, there is a lack of studies on the effect of chronic ALCAR administration on brain metabolism in healthy non-elderly animals other than rats. Specifically, this is the case for monoamine neurotransmitters; information undoubtedly important for understanding, evaluating and exploiting chronic ALCAR as a therapeutic agent. Here, we give a comprehensive metabolomic analysis which includes; glucose, energy, amino acid and monoamine neurotransmitter metabolism after chronic ALCAR supplementation in mice, showing for the first time increases of noradrenaline and serotonin levels in the healthy brain.

2. Materials and methods

2.1. Chemicals

[1-^{13}C]glucose (99% ^{13}C) and D_2O (99.9%) were purchased from Cambridge Isotope Laboratories (Woburn, MA, USA), ethylene glycol from Merck (Darmstadt, Germany), and acetyl-L-carnitine from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were of the purest grade available from local commercial sources.

2.2. Animals

The Norwegian National Animal Research Authority and the local ethics committee approved the experimental procedures. Fourteen male four week old NMRI mice with an average weight of 25 ± 0.7 g (n = 14; Taconic, Ejby, Denmark) were used in the experiment. Nine mice were used in the intervention group and five as controls. All animals were maintained under standard laboratory conditions on a 12/12 h light/dark cycle (lights on at 7AM), at a constant temperature of 22 °C and a humidity of 60%. Animals were housed in individual cages with free access to food and water. The mice were acclimatized to the above conditions for 1 week before the start of the experiments.

In accordance with previous studies, mice received drinking water containing ALCAR (1.5 g/L, pH adjusted to 6) ad libitum (Hagen et al., 2002; Mollica et al., 2001) drinking an average of 9.9 ± 0.6 ml per day (calculated by weighing the water bottles), which provides a daily ALCAR dose of 496 ± 21 mg/kg body weight. Control mice receiving filtered tap water drank 7.8 ± 1.2 ml per day. There was no difference in the weight gain between the groups. Both groups received saline injections (10 ml/kg i.p.) three times a week until the last day, due to the fact that they were part of a bigger study examining the kindling epilepsy model (data not shown). After 25 days, mice were injected with 543 mg/kg [1-^{13}C]glucose (i.p., using a 0.3 M solution) and 15 min later were subjected to microwave fixation of the head at 4 kW for 1.7 s (Model GA5013, Gerling Applied Engineering, Modesto, CA, USA), a time point that ensures substantial label incorporation without washout (Hassel et al., 1995). The brains were removed and the cortices and the HFs were dissected and stored at −80 °C till extraction. The tissue samples were homogenized in 0.7% perchloric acid using a Vibra Cell sonicator (Model VCX 750, Sonics & Materials, Newtown, CT, USA), potassium perchlorate precipitated by neutralization with 1 M KOH, and supernatants containing metabolites lyophilized.

2.3. High performance liquid chromatography (HPLC)

To determine the total amount of glutathione, the samples were analyzed using a Hewlett Packard 1100 System (Agilent Technologies, Palo Alto, CA, USA) with fluorescence detection, after derivatization with o-phtaldialdehyde and a standard curve derived from a standard solution of glutathione (Geddes and Wood, 1984). The components were separated with a ZORBAX SB-C18 column (4.6 × 150 mm, 3.55 micron, Agilent) using 50 mM sodium phosphate buffer (pH 5.9) with 2.5% tetrahydrofurane and methanol/tetrahydrofurane (98.75%/1.25%) as eluent.

To quantitate the levels of monoamines NA, 5-HT and dopamine (DA) and acid metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA), an Agilent 1200 system with an electrochemical detector (Coulochem III, ESA, Sunnyvale, CA, USA) was used. Components were separated with an Eclipse XDB-C18 column (4.6 × 150 mm, 5 micron, Agilent) with an aqueous mobile phase (0.90 mL/min) containing 90 mM NaH_2PO_4, 50 mM citric acid, 0.1 mM EDTA, 0.5 mM octanesulfonic acid and 7% methanol. A standard curve derived from standard solutions of monoamines was run repeatedly at 15 samples intervals for quantitation.

2.4. ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectroscopy

Lyophilized samples were dissolved in 200 µL D_2O containing 0.1% ethylene glycol as an internal standard for quantitation and re-adjusted to pH 6.5–7.5 Spectra were recorded at 25 °C using a BRUKER DRX-600 for HF and a BRUKER DRX-500 spectrometer for cortex samples (both BRUKER Analytik GmbH, Rheinstetten, Germany). ^{1}H-NMR spectra were acquired on the same instruments with the following parameters: pulse angle of 90°, spectral width of 32 K data points and the number of scans was 128 and 1024 for cortical and HF extracts respectively. Acquisition time was 1.36 s and relaxation delay 10 s. Water suppression was achieved by applying a low-power pre-saturation pulse at the water frequency. Proton decoupled ^{13}C-NMR spectra were obtained using a 30° pulse angle and 30 kHz spectral width with 64 K data points employing an acquisition time of 1.08 s and a

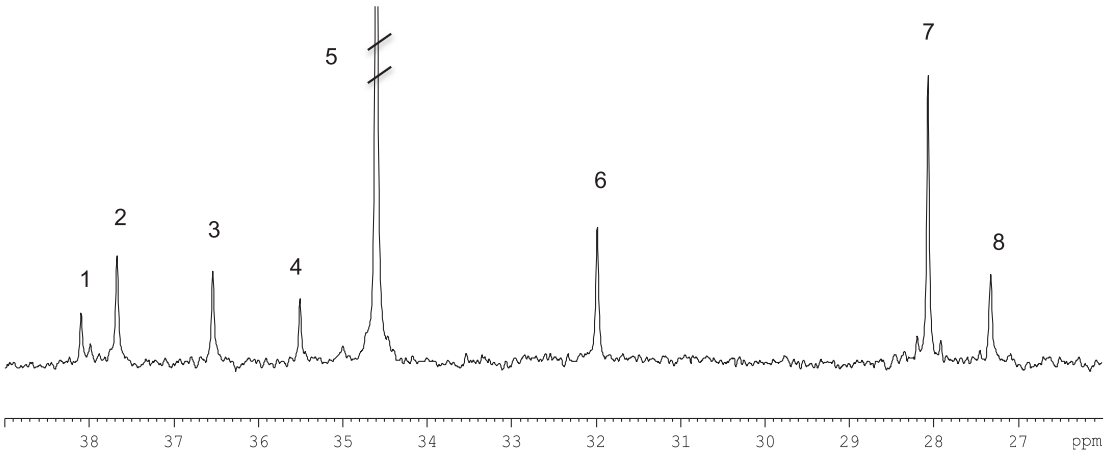

Fig. 1. Typical ^{13}C-NMR-spectrum of a tissue extract of cerebral cortex from a ALCAR-treated mouse. Peak assignment: 1: creatine C-3; 2: aspartate C-3; 3: taurine C-3; 4: GABA C-2; 5: glutamate C-4 (peak is truncated); 6: glutamine C-4; 7: glutamate C-3; 8: glutamine C-3.

relaxation delay of 0.5 s. The number of scans needed to obtain an appropriate signal to noise ratio was typically 25,000 for cortex and 35,000 for HF samples.

Relevant peaks in the spectra were identified and integrated using XWIN NMR software (BRUKER BioSpin GmbH, Rheinstetten, Germany). The total amounts and ^{13}C labeling of metabolites were quantified from the areas under the peaks using ethylene glycol as an internal standard. Correction for natural abundance, nuclear Overhauser enhancement and relaxation effects relative to the internal standard were applied to all relevant resonances. For a typical ^{13}C-NMR spectrum see Fig. 1.

2.5. Labeling patterns from metabolism of [1-^{13}C]glucose

The metabolism of [1-^{13}C]glucose is described in Fig. 2. Via glycolysis, [1-^{13}C]glucose is converted to [3-^{13}C]pyruvate, which can be further converted to [3-^{13}C]alanine, [3-^{13}C]lactate or [2-^{13}C]acetyl-CoA. [2-^{13}C]acetyl-CoA may enter the TCA cycle through condensation with oxaloacetate to form citrate. Eventually the TCA cycle intermediate α-[4-^{13}C]ketoglutarate is formed, which is the precursor of [4-^{13}C]glutamate. Thereafter, [4-^{13}C]glutamate may be transformed to [4-^{13}C]glutamine in astrocytes due to the astrocyte specific localization of glutamine synthetase (Norenberg and Martinez-Hernandez, 1979) and to [2-^{13}C]GABA in GABAergic neurons. If α-[4-^{13}C]ketoglutarate is further converted in the TCA cycle it gives rise to different labeling patterns in these amino acids (Alvestad et al., 2008). In astrocytes [3-^{13}C]pyruvate can be converted to [3-^{13}C]oxaloacetate via pyruvate carboxylase (Patel, 1974) which can lead to the formation of [2-^{13}C]glutamate, [2-^{13}C]glutamine and [4-^{13}C]GABA.

2.6. Data analysis

Statistics were performed using the 2-tailed unpaired Student's *t*-test, and $p \leqslant 0.05$ was regarded as significant. Data are presented as mean ± SD. Due to technical problems, the number of analyses varied between analytical methods.

3. Results

3.1. Glucose-derived and related metabolites

Injection of [1-^{13}C]glucose led to labeling of many metabolites as shown in a typical ^{13}C-NMR spectrum from cerebral cortex

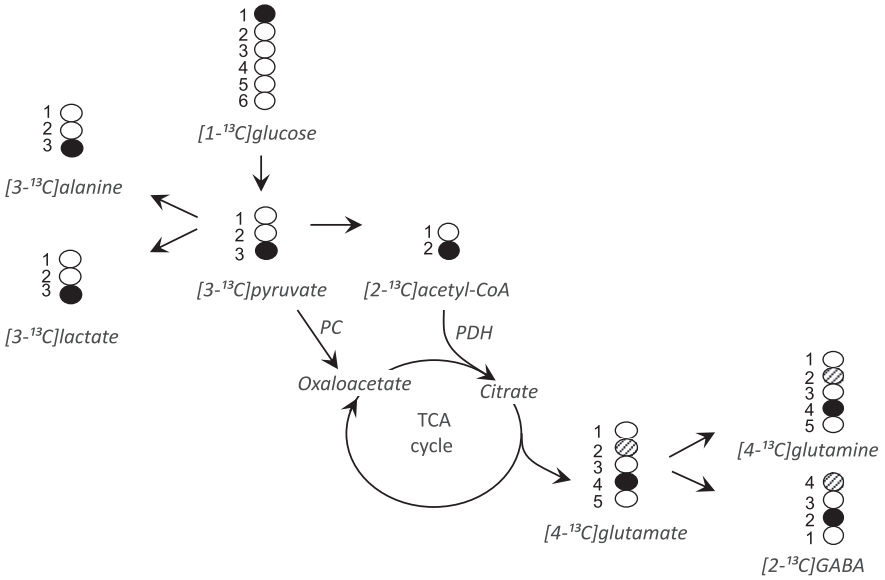

Fig. 2. Schematic presentation of isotopomers derived from [1-^{13}C]glucose via [2-^{13}C]acetyl-CoA. Black circles indicate ^{13}C labeling from pyruvate dehydrogenase (PDH) and hatched circle ^{13}C labeling from pyruvate carboxylase (PC). Only the first turn of the TCA cycle is illustrated.

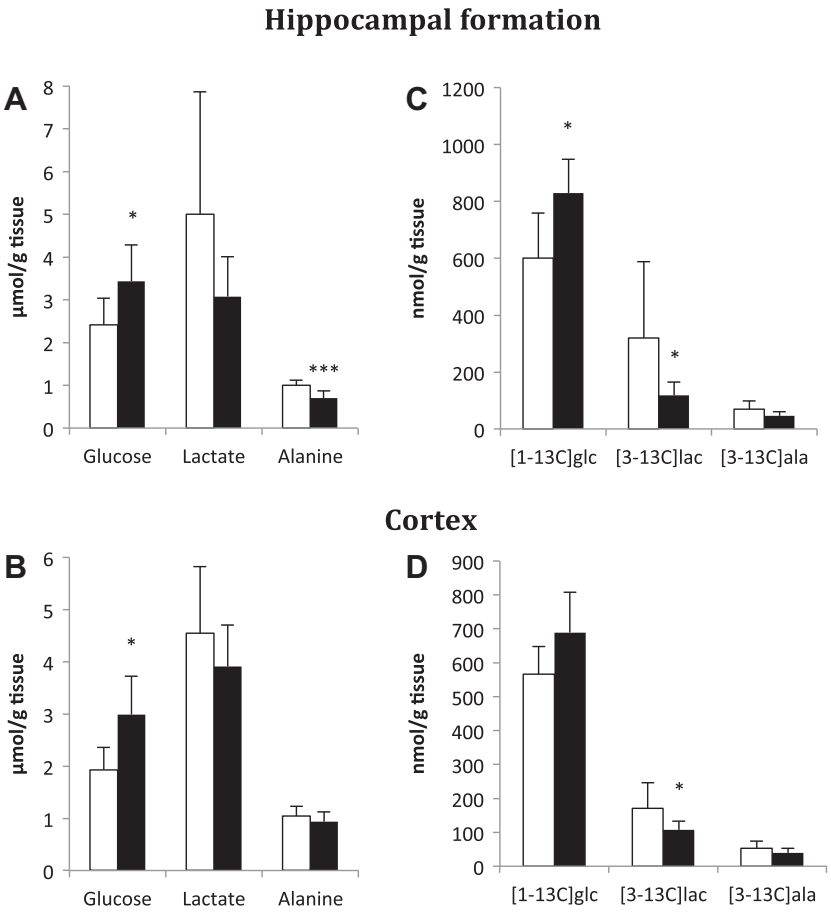

Fig. 3. Glucose metabolism in brain extracts of hippocampal formation and cortex from control (white bars) and ALCAR-treated mice (black bars). Levels of glucose, lactate and alanine quantified by ^{1}H-NMR spectroscopy (A and B) and [1-^{13}C]glucose, [3-^{13}C]lactate and [3-^{13}C]alanine obtained by ^{13}C-NMR spectroscopy are shown (C and D). Data represent mean ± SD of four control mice and nine ALCAR-treated mice for hippocampal formation, and five control mice and nine ALCAR-treated mice for cortex. $^{*}p \leqslant 0.05$, $^{***} \leqslant 0.005$, statistically significantly different from control group, analyzed by Student's *t*-test.

extract of an ALCAR-treated mouse (Fig. 1). Only the part of the spectrum containing the C-4 and C-3 glutamate and glutamine, C-2 aspartate and C-2 and C-3 GABA peaks is shown. The labeling patterns of metabolites labeled from [1-^{13}C]glucose via [2-^{13}C]acetyl-CoA from the first turn of the TCA cycle are depicted in Fig. 2. The C-2 and C-3 positions are labeled in the second and subsequent turns of the TCA cycle (not shown).

The total and labeled amounts of glucose, lactate, alanine and amino acids were quantified using ^{1}H- and ^{13}C-NMR spectroscopy. In the HF the total amounts of glucose and alanine were increased by 43% ($p = 0.05$) and decreased by 31% ($p = 0.004$), respectively after 25 days of ALCAR treatment (Fig. 3). Furthermore, lactate content in the ALCAR treated group compared to control was decreased by 38% ($p = 0.08$). In the cortex the glucose content was increased by 55 % ($p = 0.01$), but amounts of lactate and alanine remained unchanged. The levels of [3-^{13}C]lactate were lower in both brain regions investigated by 63% ($p = 0.04$) in the HF and 37% ($p = 0.04$) in the cortex. Moreover, [1-^{13}C]glucose content was 38% higher in the HF of mice treated with ALCAR ($p = 0.01$) and a similar trend was seen in cortex (22 % increase, $p = 0.06$). The amounts of [3-^{13}C]alanine were not significantly changed in either brain region.

Following ALCAR administration the amounts of GABA were significantly decreased in the HF by 32 % ($p = 0.01$), but were unchanged in the cortex (Fig. 4), consistent with regional specific metabolic alterations by ALCAR as previously described (Freo et al., 2009; Ori et al., 2002). The amounts of glutamate and glutamine were unaffected in both brain regions. The same was true for the levels of [4-^{13}C]glutamate, [4-^{13}C]glutamine and [2-^{13}C]GABA, the [1-^{13}C]glucose-derived metabolites labeled in the first turn of the TCA cycle via [2-^{13}C]acetyl-CoA. Consequently, the percent ^{13}C enrichment of glutamate and glutamine was unaltered. The same was the case for GABA since the individual values for [2-^{13}C]GABA content in the ALCAR treated animals were smaller than those in the control group, even though this difference was not significant ($p = 0.30$). No alterations in the pyruvate carboxylation to dehydrogenation ratios were observed between control and ALCAR treated animals (results not shown).

3.2. Other metabolites

ALCAR treatment significantly increased the levels of several energy metabolites in the cortex, such as the sum of adenosine nucleotides AMP, ADP, and ATP by 23% ($p = 0.03$), phosphocreatine by 66% ($p = 0.04$), and *myo*-inositol by 30% ($p = 0.02$, Table 1). The ratio of phosphocreatine/creatine (PCr/ratio) was about twofold higher in the cortex of mice treated with ALCAR ($p = 0.03$). The amounts of creatine, succinate, glutathione, choline-containing compounds and N-acetylaspartate (NAA) were unchanged in both brain regions investigated. The levels of nicotinamide adenine dinucleotide (NAD) were not quantifiable in the HF and were unaffected in the cortex.

3.3. Monoamine neurotransmitters

ALCAR administration resulted in a significant 25% increase in the level of NA in the HF ($p = 0.03$), but not in cortex (Fig. 5).

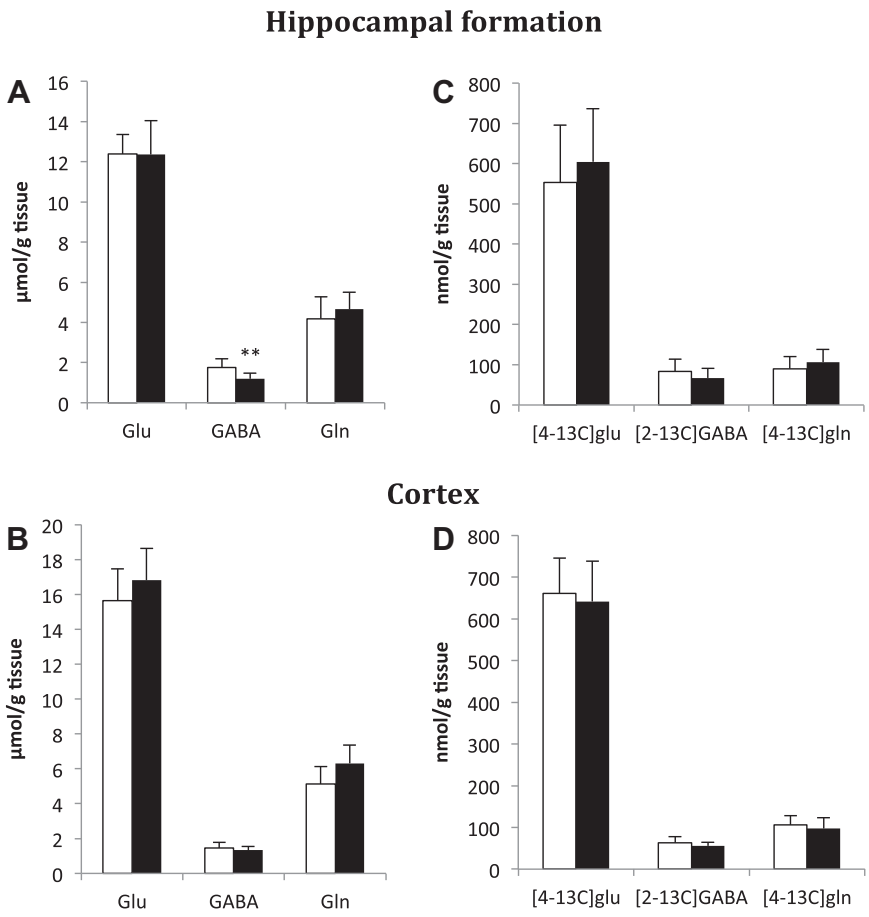

Fig. 4. Amounts of amino acids glutamate, GABA and glutamine (A and B) and ^{13}C-labeled amino acids derived from [1-^{13}C]glucose (C and D) in brain extracts of hippocampal formation and cortex from control (white bars) and ALCAR-treated mice (black bars). Data represent mean ± SD of five control mice and nine ALCAR-treated mice, $^{**}p \leqslant 0.01$, statistically significantly different from control group.

Table 1
Amounts (µmol/g tissue) of metabolites in brain extracts of hippocampal formation and cortex from control and ALCAR-treated mice.

	Hippocampal formation		Cortex	
	Control	ALCAR	Control	ALCAR
AMP + ADP + ATP	1.64 ± 0.97	1.83 ± 1.14	2.48 ± 0.50	3.07 ± 0.35*
Creatine	8.64 ± 0.93	8.02 ± 0.96	9.57 ± 2.06	8.52 ± 1.08
Phosphocreatine	1.62 ± 1.20	2.02 ± 0.72	1.15 ± 0.61	1.91 ± 0.59*
Creatine + phosphocreatine	10.26 ± 1.18	10.05 ± 0.97	10.72 ± 2.45	10.43 ± 0.74
PCr/Cr ratio	0.19 ± 0.15	0.26 ± 0.11	0.12 ± 0.06	0.23 ± 0.09*
NADH	n.d.	n.d.	0.29 ± 0.07	0.36 ± 0.08
Succinate	0.55 ± 0.28	0.63 ± 0.12	0.41 ± 0.10	0.49 ± 0.18
Glutathione	1.30 ± 0.24	1.35 ± 0.30	1.16 ± 0.15	1.32 ± 0.15
Choline[a]	1.72 ± 0.06	1.57 ± 0.18	1.78 ± 0.13	1.82 ± 0.14
Myo-inositol	10.12 ± 2.32	11.04 ± 2.13	5.86 ± 0.80	7.60 ± 1.40*
N-acetylaspartate	5.51 ± 0.34	5.50 ± 0.67	7.36 ± 0.50	7.79 ± 0.97

All metabolite levels were quantified using ^{1}H-NMR spectroscopy, with the exception of glutathione (HPLC) and *myo*-inositol (^{13}C-NMR spectroscopy). Abbreviations: Choline[a]: choline-containing compounds; NADH: nicotinamide adenine dinucleotide; n.d: not determined; PCr/Cr ratio: phosphocreatine/creatine ratio. Data represent mean ± SD of five control mice and nine ALCAR-treated mice, and were analyzed with the Student's *t*-test. $^{*}p \leqslant 0.05$, statistically significant difference from control mice.

Moreover, 5-HT concentrations were significantly increased in the cortex by 20% ($p = 0.005$), with a similar trend in the HF, although without statistical significance (22%, $p = 0.09$). The serotonin turnover rate (5-HIAA/5-HT) was decreased in the cortex from 0.49 to 0.37 ($p = 0.04$), a potential reason for the increase in serotonin levels (Table 2). The following parameters measured were not affected by ALCAR treatment; (1) DA and 5-HIAA amounts in both brain regions and (2) neither DOPAC and HVA levels nor the ratios of DA metabolites to DA, HVA/DA and DOPAC/DA, measures of DA turnover, in cerebral cortex. The amounts of DOPAC and HVA in the HF were below our detection level for quantitation.

4. Discussion

The most important findings of this work are (1) alterations in glucose and lactate metabolism, (2) increases in high energy phosphates and (3) *myo*-inositol, as well as (4) increases in the levels of

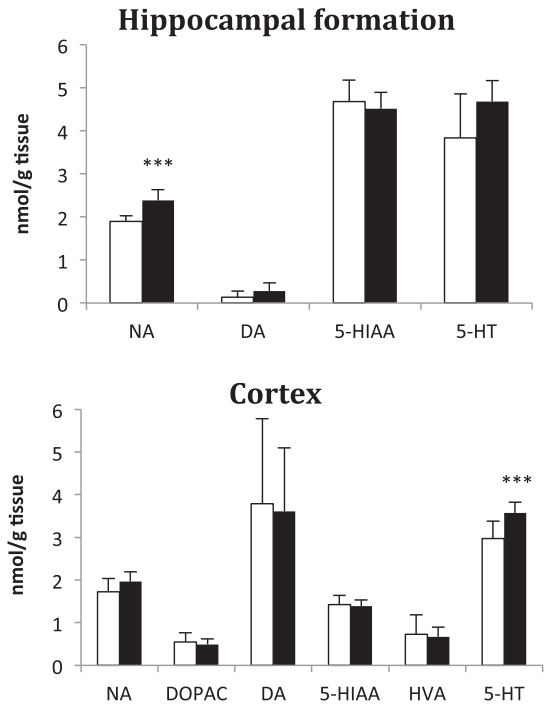

Fig. 5. Amounts (nmol/g tissue) of monoamines and their metabolites in brain extracts of hippocampal formation and cortex from control (white bars) and ALCAR-treated mice (black bars) quantified using HPLC. Abbreviations: Noradrenaline (NA), 3,4-dihydroxyphenylacetic acid (DOPAC), dopamine (DA), 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA), serotonin (5-HT). Data represent mean ± SD of five control mice and seven ALCAR-treated mice for hippocampal formation, and five control mice and nine ALCAR-treated mice for cortex. $^{***}p \leqslant 0.005$ statistically significantly different from control group, analyzed with the Student's *t*-test.

Table 2
"Turnover" of dopamine and serotonin in brain extracts of hippocampal formation and cortex from control and ALCAR-treated mice.

	Hippocampal formation		Cortex	
	Control ($n = 5$)	ALCAR ($n = 7$)	Control ($n = 5$)	ALCAR ($n = 9$)
DOPAC/DA	n.d.	n.d.	0.16 ± 0.04	0.15 ± 0.02
HVA/DA	n.d.	n.d.	0.15 ± 0.06	0.20 ± 0.04
5-HIAA/5-HT	1.31 ± 0.43	0.98 ± 0.16	0.49 ± 0.09	0.37 ± 0.05*

The ratios of monoamine neurotransmitters to their metabolites were calculated as a measure for their turnover rates calculated using data from HPLC. Abbreviations: n.d. not determined. Data represent mean ± SD, and were analyzed with the Student's *t*-test. $^{*}p \leqslant 0.05$, statistically significant difference from control mice.

some monoamine neurotransmitters in hippocampus and/or cortex after chronic ALCAR supplementation in mice. The implications of our results are discussed below in relation to previous findings.

4.1. Glucose metabolism

In this study we found that chronic dietary supplementation with ALCAR increased the amount of glucose in both cerebral cortex and HF. Similarly, Ori et al. (2002) and Freo et al. (2009) reported that acute and chronic ALCAR administration increased [^{14}C]2-deoxy-D-glucose labeling in various brain regions. Also, ALCAR treatment did not alter serum concentrations of glucose (Aureli et al., 1998; Freo et al., 2009; Ori et al., 2002) or the uptake of [^{18}F]fluoro-2-deoxy-D-glucose into rat brain slices (Tanaka et al., 2003). This indicates that ALCAR does not alter brain glucose uptake. In agreement with the reported reduced amounts of lactate in the normal adult and aged rat brain after acute ALCAR treatment (Aureli et al., 1990), we also found decreased [3-^{13}C]lactate levels in mice, but no changes in the concentrations of TCA cycle-derived ^{13}C-labeled glutamate, glutamine or GABA. Increased glucose concentration and unchanged TCA cycle activity have also been observed by Nilsen et al. (2011) in mice with reduced α-ketoglutarate dehydrogenase complex activity. Taken together our results indicate that ALCAR administration reduced glucose metabolism to lactate without changing glucose metabolism via the TCA cycle, which lead to higher brain glucose concentrations. Similarly, acute ALCAR treatment counteracted production of lactate in rat and dog models of ischemia (Aureli et al., 1994; Rosenthal et al., 1992).

4.2. Energy metabolites

The levels of the sum of AMP + ADP + ATP, phosphocreatine and the PCr/Cr ratio were significantly increased in the cortex in our mice supplemented with ALCAR compared to control. The fact that the sum of creatine + phosphocreatine was unaltered indicates that the increase in phosphocreatine reflects a larger reservoir of high-energy phosphates. Furthermore, the increased amount of the sum of AMP + ADP + ATP may reflect increased concentration of ATP (see studies below). We could not distinguish the phosphorylation state of adenosine due to resonance overlap in the ^{1}H-NMR spectra. Our findings are in line with several studies demonstrating that ALCAR treatment increased the levels of phosphocreatine and reduced the amount of free organic phosphate in the adult and old rat brain (Aureli et al., 1990), elevated the amounts of phosphocreatine and ATP in a rat model of ischemia (Aureli et al., 1994), prevented ATP depletion in neuroblastoma cells exposed to beta-amyloid (Dhitavat et al., 2002), and ameliorated the decrease of ATP in rat hippocampus after ischemia (Al-Majed et al., 2006). Taken together, the data indicate that ALCAR treatment improves the capacity of the brain to produce high-energy phosphates and reduces anaerobic glucose metabolism. This potential neuroprotective ability may prove to be beneficial in conditions with disturbed energy metabolism.

4.3. Myo-inositol

The level of *myo*-inositol was increased in the cortex of mice supplemented with ALCAR in the present study. Likewise, ALCAR has been shown to prevent *myo*-inositol depletion in a streptomycin induced rat model of diabetic neuropathy (Nakamura et al., 1998; Stevens et al., 1996). *Myo*-inositol is important for the synthesis of PIP2, IP3, DAG and complex signaling phospholipids. If *myo*-inositol is increased due to a decreased synthesis of these molecules, it would have widespread functional consequences. It is also an important osmolyte in the brain and is the most abundant form of inositol (Fisher et al., 2002). *Myo*-inositol has been found to be particularly enriched in astrocytes (Brand et al., 1993). Thus, the increased content of this metabolite appears to reflect an effect of ALCAR on this cell type. The effect on *myo*-inositol suggests that ALCAR can have a positive influence in disorders where water homeostasis is altered.

4.4. Monoamine neurotransmitters

ALCAR supplementation enhanced the amounts of NA and 5-HT in healthy mice. An increased level of NA was detected in the HF, whereas the amount of 5-HT was increased in the cortex, accompanied by a decreased 5-HIAA/5-HT ratio, the latter reflecting reduced serotonin turnover. It is of interest in this context that NA increases oxidative metabolism in cultured astrocytes and freshly dissociated astrocytes express the relevant receptor subtypes (Hertz et al., 2010). In several small trials, ALCAR was found to

be beneficial for patients with depressive disorders and related conditions (Martinotti et al., 2011; Pettegrew et al., 2002; Soczynska et al., 2008; Zanardi and Smeraldi, 2006). The reported increase of 5-HT and NA levels presented here validate further study of these neurotransmitters, their metabolism and receptors in relation to ALCAR and depressive disorders, and may provide rationale for an antidepressant effect of ALCAR.

5. Summary

In conclusion, we report that ALCAR supplementation in healthy mice resulted in improved energy metabolism and sparing of glucose in both HF and cortex. The amounts of the monoamines NA and 5-HT were increased in the HF and cortex respectively. These new insights warrant further studies of ALCAR in clinical settings especially for diseases known to involve energy deficits or monoamine neurotransmitter derangements.

6. Author disclosure

OB. Smeland, TW. Meisingset, K Borges and U. Sonnewald have no conflicts of interest.

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