Neurochemical Research (2023) 48:54–61
https://doi.org/10.1007/s11064-022-03726-6

BRIEF COMMUNICATION

β-Hydroxybutyrate and Medium-Chain Fatty Acids are Metabolized by Different Cell Types in Mouse Cerebral Cortex Slices

Jens V. Andersen[1] · Emil W. Westi[1] · Elliott S. Neal[2] · Blanca I. Aldana[1] · Karin Borges[2]

Received: 6 April 2022 / Revised: 6 July 2022 / Accepted: 11 August 2022 / Published online: 23 August 2022

Abstract

Ketogenic diets and medium-chain triglycerides are gaining attention as treatment of neurological disorders. Their major metabolites, β-hydroxybutyrate (βHB) and the medium-chain fatty acids (MCFAs) octanoic acid (C8) and decanoic acid (C10), are auxiliary brain fuels. To which extent these fuels compete for metabolism in different brain cell types is unknown. Here, we used acutely isolated mouse cerebral cortical slices to (1) compare metabolism of 200 µM [U-^{13}C]C8, [U-^{13}C]C10 and [U-^{13}C]βHB and (2) assess potential competition between metabolism of βHB and MCFAs by quantifying metabolite ^{13}C enrichment using gas chromatography-mass spectrometry (GC–MS) analysis. The ^{13}C enrichment in most metabolites was similar with [U-^{13}C]C8 and [U-^{13}C]C10 as substrates, but several fold lower with [U-^{13}C]βHB. The ^{13}C enrichment in glutamate was in a similar range for all three substrates, whereas the ^{13}C enrichments in citrate and glutamine were markedly higher with both [U-^{13}C]C8 and [U-^{13}C]C10 compared with [U-^{13}C]βHB. As citrate and glutamine are indicators of astrocytic metabolism, the results indicate active MCFA metabolism in astrocytes, while βHB is metabolized in a different cellular compartment. In competition experiments, ^{12}C-βHB altered ^{13}C incorporation from [U-^{13}C]C8 and [U-^{13}C]C10 in only a few instances, while ^{12}C-C8 and ^{12}C-C10 only further decreased the low [U-^{13}C]βHB-derived ^{13}C incorporation into citrate and glutamine, signifying little competition for oxidative metabolism between βHB and the MCFAs. Overall, the data demonstrate that βHB and MCFAs are supplementary fuels in different cellular compartments in the brain without notable competition. Thus, the use of medium-chain triglycerides in ketogenic diets is likely to be beneficial in conditions with carbon and energy shortages in both astrocytes and neurons, such as GLUT1 deficiency.

Keywords Astrocytes · MCFA · Ketone bodies · Octanoic acid · Decanoic acid · Epilepsy

Abbreviations

βHB	β-Hydroxybutyrate
C8	Octanoic acid
C10	Decanoic acid
GC–MS	Gas chromatography-mass spectrometry
GS	Glutamine synthetase
MCFA	Medium-chain fatty acid
MCT	Monocarboxylate transporter
TCA	Tricarboxylic acid

✉ Jens V. Andersen
jens.andersen@sund.ku.dk

✉ Karin Borges
k.borges@uq.edu.au

[1] Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark

[2] School of Biomedical Sciences, Faculty of Medicine, The University of Queensland, Brisbane, Australia

Introduction

Ketogenic diets, and more recently medium-chain triglycerides, are becoming popular and increasingly researched for treatment of neurological disorders, especially epilepsy and cognitive impairment [1, 2]. Medical ketogenic diets have been used for over 100 years for the treatment of medication-resistant epilepsy in children and lately also in adults [3, 4]. Many versions of ketogenic diets exist today. These diets are designed to increase the plasma levels of the ketone bodies acetoacetate and β-hydroxybutyrate (βHB) while keeping blood glucose in a low but normal range [5]. This is typically achieved by consuming predominantly natural fats and low amounts of protein and carbohydrates. Caloric restriction and/or inclusion of medium-chain triglycerides can be used to increase ketone body levels, but there is also evidence of other beneficial effects of medium-chain triglycerides. These include inhibition of seizure generation and improved cognition in the context of "healthy regular" diets, without the restriction of

carbohydrates (reviewed in [6]). Medium-chain triglycerides typically contain high amounts of the medium-chain fatty acids (MCFAs) octanoic acid (C8) and decanoic acid (C10), which are released after lipase action in the digestive system. The levels of these MCFAs increase in blood shortly after intake, and can then, at least partially, be converted into ketone bodies in the liver [7]. It is currently unknown whether, apart from ketone body production, MCFAs have value as auxiliary fuels to the brain.

For entry into the brain, βHB depends on monocarboxylate transporters (MCTs) while MCFAs appear to diffuse passively across cell membranes [6, 8, 9]. Studies have suggested that MCFAs are metabolized by astrocytes, as metabolism of C8 supports astrocyte respiration and leads to enrichment in glutamine [10–12], but MCFAs may also to some extent support neuronal metabolism [13, 14]. Glutamine is a marker of astrocytic metabolism as glutamine synthetase (GS) is almost exclusively expressed in astrocytes [15]. There is currently no knowledge about cellular metabolism of C10 in vivo, as the solubility of this MCFA is too low for such experiments where high concentrations are needed. Neurons appear to be the primary cellular compartment of ketone body metabolism, as they express MCT2 with high affinity for βHB, while the affinity of the astrocytic MCT1 for βHB is tenfold lower [8, 16]. This has been confirmed by several functional studies in awake mice and humans, concluding that ketone body metabolism was similar to that of glucose taking place largely in the neuronal compartment [17–19]. Similarly, ketone bodies were found to be metabolized to a greater extent in cultured neurons as compared to cultured astrocytes [11]. However, cultured astrocytes can metabolize ketone bodies [20, 21], and in anesthetized rats, mostly astrocytic metabolism has been reported [22]. In support of this notion, ^{13}C-enriched ketone bodies give rise to significant labeling in glutamine [17, 23, 24] indicating active ketone body metabolism by astrocytes. However, it remains unknown to which extent MCFAs may compete with the metabolism of ketone bodies in the brain. This question is difficult to answer in vivo, as MCFAs can be turned into ketone bodies by the liver. More knowledge regarding this question is needed, as it is unclear to which extent and how the addition of medium-chain triglycerides to ketogenic diets may improve brain metabolism in both the healthy and diseased brain. To address this issue, we applied acutely isolated cerebral cortical slices to assess the metabolism of [U-^{13}C]C8, [U-^{13}C]C10 and [U-^{13}C]βHB and how these substrates may compete for cellular metabolism.

Materials and Methods

Materials

The ^{13}C enriched compounds [U-^{13}C]octanoic acid ([U-^{13}C]C8, CLM-3981-PK, 98%), [U-^{13}C]decanoic acid ([U-^{13}C]C10, CLM-9950-PK, 98%) and [U-^{13}C] β-hydroxybutyrate ([U-^{13}C]βHB, CLM-3853-PK, sodium salt, 97%) were all from Cambridge Isotope Laboratories (Tewksbury, USA). Octanoic acid (^{12}C-C8, C2875), decanoic acid (^{12}C-C10, C1875) and (*R*)-β-hydroxybutyrate (^{12}C-βHB, 54,920) were from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of the purest grade available from regular commercial sources.

Brain Slice Incubations

Animal experiments were approved by the Danish National Ethics Committee and performed in accordance with the European Convention (ETS 123 of 1986) and comply with the ARRIVE guidelines. Six male NMRI mice (Envigo, Cambridgeshire, United Kingdom) of 12 weeks of age were used for incubations of acutely isolated cerebral cortical brain slices as previously described [25]. Briefly, one mouse was euthanized at a time by cervical dislocation, decapitated and the brain transferred to ice-cold artificial cerebrospinal fluid (ACSF) containing in mM: NaCl 128, $NaHCO_3$ 25, D-glucose 10, KCl 3, $CaCl_2$ 2, $MgSO_4$ 1.2, KH_2PO_4 0.4, pH = 7.4. The cerebral cortices were dissected and sliced (350 µm) on a McIlwain tissue chopper (The Vibratome Company, O'Fallon, MO, USA). The cerebral cortical slices were kept just below the surface of 37 °C oxygenated (5% CO_2/95% O_2) ACSF and pre-incubated for 60 min. Subsequently, the medium was exchanged for ACSF containing the stable ^{13}C enriched compounds [U-^{13}C]C8, [U-^{13}C]C10 or [U-^{13}C]βHB (all 200 µM) supplemented with 5 mM unlabeled D-glucose and incubated for additional 60 min. To further investigate the metabolic relationship between C8/C10 and βHB, a competition assay was performed in which slices were incubated in the presence of either [U-^{13}C]C8 or [U-^{13}C]C10 ± unlabeled ^{12}C-βHB and [U-^{13}C]βHB ± unlabeled ^{12}C-C8 or ^{12}C-C10 (all 200 µM). Competing metabolism of an unlabeled substrate (^{12}C) will dilute the ^{13}C accumulation from the ^{13}C enriched substrate. The incubations were terminated by transferring slices into ice-cold 70% ethanol. The slices were subsequently sonicated and centrifuged (4,000 g × 20 min) and the supernatant was removed and lyophilized before further analysis. A concentration of 200 µM was chosen for all substrates, based on reported MCFA blood levels after feeding of

medium-chain triglycerides or ketogenic diets with and without medium-chain triglycerides (in the range of 100–500 µM) [7]. Blood βHB levels are in similar ranges after consuming medium-chain triglycerides [7], while in people on ketogenic diets, βHB levels vary greatly and may approach levels found in ketoacidosis. The applied concentration of βHB in this study is slightly lower than that found in non-fasted human plasma and brain [17], but higher than that of hippocampal extracellular fluid in mice fed a ketogenic diet [26].

Metabolic Mapping Using Gas Chromatography-Mass Spectrometry (GC–MS) Analysis

[U-^{13}C]C8, [U-^{13}C]C10 and [U-^{13}C]βHB all enter cellular metabolism as ^{13}C enriched acetyl CoA leading to ^{13}C enrichment of TCA cycle intermediates and connected amino acids. The metabolite ^{13}C enrichment from metabolism of [U-^{13}C]C8, [U-^{13}C]C10 and [U-^{13}C]βHB was determined by GC–MS analysis as previously described [27]. Briefly, slice extracts were reconstituted in water, acidified, extracted twice with ethanol and the metabolites were derivatized using *N*-tert-butyldimethylsilyl-*N*-methyltrifluoroacetamide. Samples were analyzed by GC (Agilent Technologies, 7820A, J&W GC column HP-5 MS) coupled to MS (Agilent Technologies, 5977E). The isotopic enrichment was corrected for the natural abundance of ^{13}C by analyzing standards of the unlabeled metabolites of interest. Data is presented as the molecular carbon labeling (MCL), which is the weighted average percentage of all the isotopologues of a metabolite, providing a measurement of the overall ^{13}C accumulation [28]. Part of the ^{13}C enrichment data ([U-^{13}C]C8/[U-^{13}C]C10 of **Fig. 1**) has previously been presented

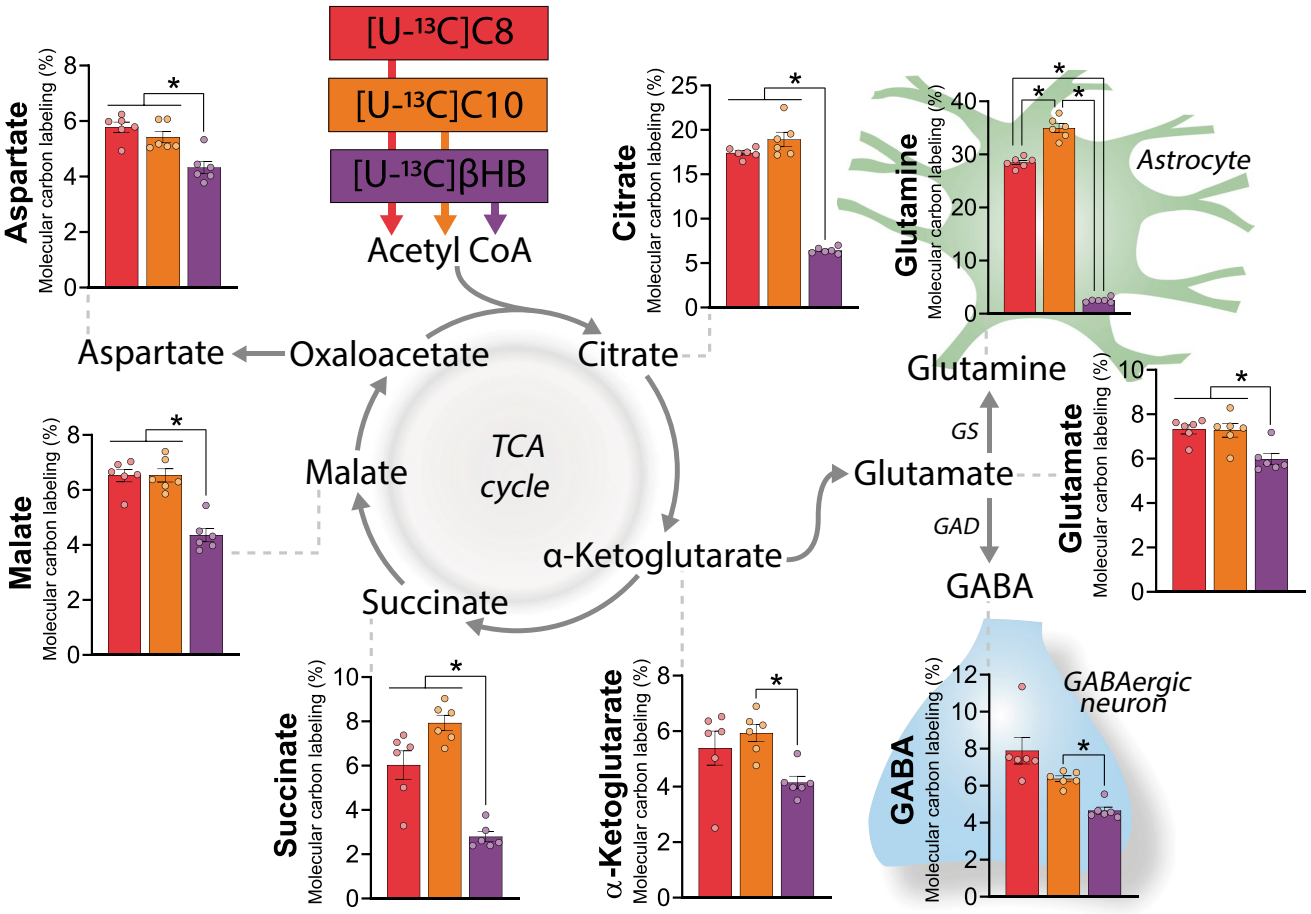
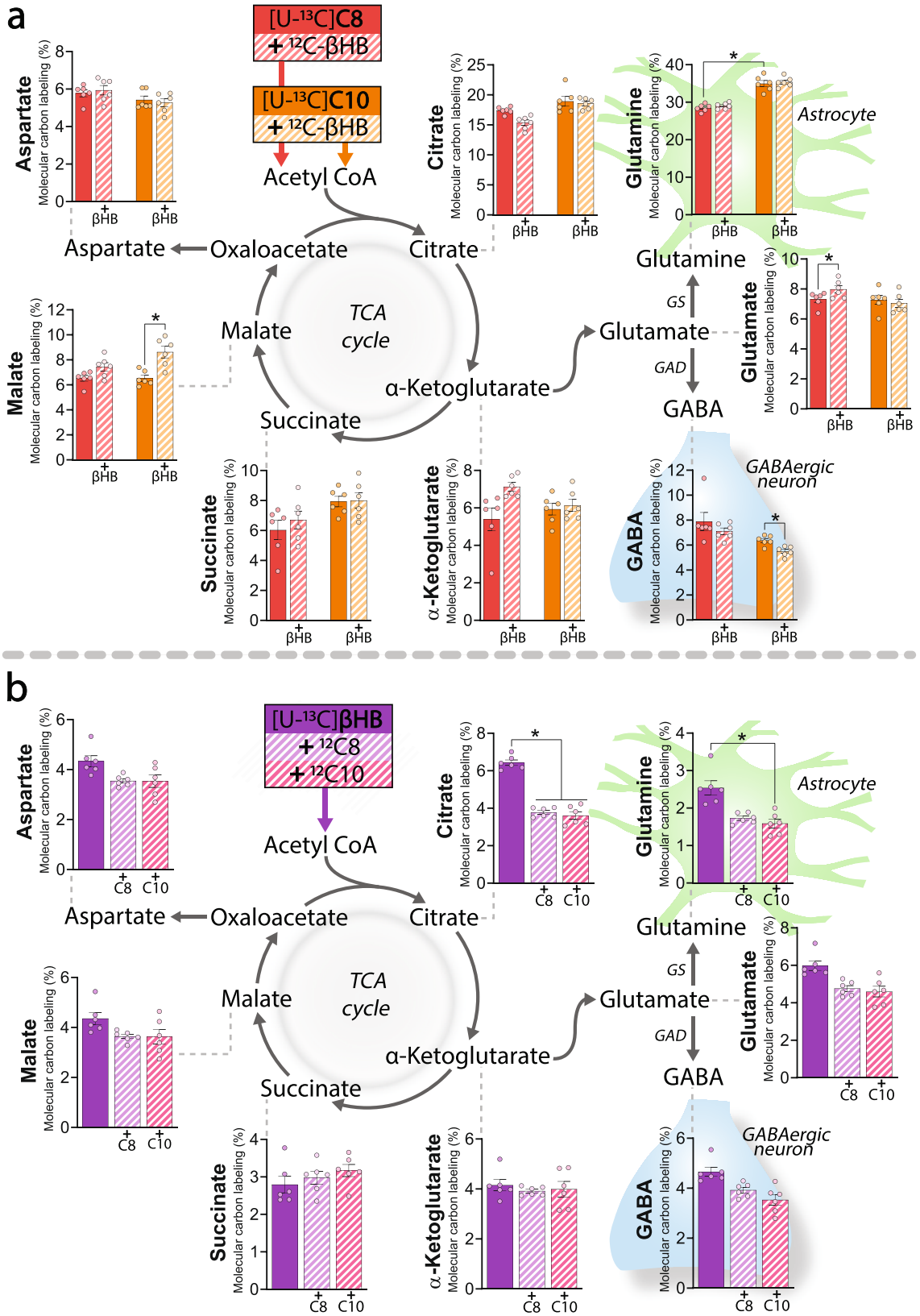


Fig. 1 The medium-chain fatty acids octanoic acid and decanoic acid, but not β-hydroxybutyrate, are primarily metabolized in astrocytes in mouse cerebral cortical slices. Molecular carbon labelling (MCL) of TCA cycle intermediates and amino acids after incubation with [U-^{13}C]C8 (red), [U-^{13}C]C10 (orange) or [U-^{13}C]βHB (purple). All ^{13}C-labelled substrates were applied separately at concentrations of 200 µM in addition to 5 mM D-glucose. Mean ± SEM, n = 6 from individual animals, repeated measures 1-way ANOVA with Bonferroni post hoc test results indicated by *p < 0.05. βHB: β-hydroxybutyrate, C8: octanoic acid, C10: decanoic acid, GAD: glutamate decarboxylase GS: glutamine synthetase


a
[U-13C]C8
+ 12C-βHB
[U-13C]C10
+ 12C-βHB
Acetyl CoA
Aspartate
Oxaloacetate
Citrate
Malate
TCA cycle
α-Ketoglutarate
Succinate
Glutamate
GS
GAD
GABA
Glutamine
Astrocyte
GABAergic neuron
Molecular carbon labeling (%)
βHB
b
[U-13C]βHB
+ 12C8
+ 12C10
C8
C10

◂**Fig. 2** Low competition between metabolism of the medium-chain fatty acids octanoic acid and decanoic acid and β-hydroxybutyrate in mouse cerebral cortical slices. **a** Molecular carbon labelling (MCL) of TCA cycle intermediates and amino acids after incubation with [U-^{13}C]C8 (red) and [U-^{13}C]C10 (orange) in the absence (solid columns) and presence of unlabeled ^{12}C-βHB (striped columns). **b** Molecular carbon labelling of TCA cycle intermediates and amino acids after incubation with [U-^{13}C]βHB in the absence (solid purple columns) and presence of unlabeled ^{12}C-C8 (light purple striped columns) and ^{12}C-C10 (pink striped columns). All substrates were provided at concentrations of 200 µM in addition to 5 mM D-glucose. Metabolism of an unlabeled substrate (^{12}C) will dilute the ^{13}C accumulation and hereby lead to a reduction in the molecular carbon labelling. Mean ± SEM, n = 6 from individual animals, repeated measures 1-way ANOVA with Bonferroni post hoc test results indicated by *p < 0.05. βHB: β-hydroxybutyrate, C8: octanoic acid, C10: decanoic acid, GAD: glutamate decarboxylase GS: glutamine synthetase

[29], and has been re-analyzed with focus on the metabolic relationship between C8/C10 and βHB. The data from competition experiments with βHB and results from [U-^{13}C]βHB metabolism are new, but were obtained at the same time and in the same experimental set-up as the previously published [U-^{13}C]C8 and [U-^{13}C]C10 data [29].

Experimental Design and Statistical Analysis

Data is presented as means ± standard error of the mean (SEM), with individual data points shown. In total 6 mice were used (n = 6) and each individual data point represents a biological replicate (i.e. obtained from an individual animal). For each condition 2 cerebral cortical slices from a single mouse were used. Since all conditions were tested in different slices from each individual mouse, statistical analysis was performed for each metabolite by repeated measurements one-way ANOVA across all five conditions. If a significant main effect in the ANOVA was found, this was followed by a Bonferroni post hoc multiple comparisons test, with relevant comparisons selected a priori. $p < 0.05$ was considered statistically significant and a single asterisk indicates $p < 0.05$ for comparisons in the post hoc tests.

Results

Metabolism of [U-^{13}C]βHB into TCA Cycle Intermediates and Amino Acids is Lower Compared to [U-^{13}C]C8 and [U-^{13}C]C10, Specifically for Glutamine and Citrate

As reported previously, there were no changes in the intracellular pool sizes of amino acids after incubation of the mouse cerebral cortex slices with 200 µM [U-^{13}C]C8, [U-^{13}C]C10 or [U-^{13}C]βHB (Table S1 in [29]), indicating that the addition of substrates does not alter the levels of these metabolic pools. We determined the ^{13}C incorporation into TCA cycle metabolites (citrate, α-ketoglutarate, succinate, and malate) as well as connected amino acids (aspartate, glutamate, glutamine and GABA) (**Fig. 1**). [U-^{13}C]C8 and [U-^{13}C]C10 gave rise to similarly high ^{13}C enrichments in most metabolites, except for glutamine which showed an increased enrichment with [U-^{13}C]C10 vs. [U-^{13}C]C8 (23% increase, $p = 0.0005$). Most strikingly, metabolism of [U-^{13}C]βHB led to substantially lower ^{13}C enrichments in most metabolites when compared to the MCFAs as substrates. Notably, the ^{13}C enrichments in glutamate, a commonly used indicator for neuronal metabolism, was only slightly lower with βHB (MCL 6.0%), when compared to the MCFAs (C8: MCL 7.3%, $p = 0.030$, C10: MCL 7.3%, $p = 0.019$). In contrast, the ^{13}C enrichments in the astrocytic metabolic markers, glutamine and citrate, were much higher for both [U-^{13}C]C8 and [U-^{13}C]C10 than with [U-^{13}C]βHB, indicating that the MCFAs are metabolized in astrocytes, while βHB is not. Specifically, ^{13}C enrichments in glutamine were 11.2–13.8-fold higher with [U-^{13}C]C8 and [U-^{13}C]C10 (MCL 28% and 35% respectively, both $p < 0.0001$) compared with [U-^{13}C]βHB (MCL 2.5%). ^{13}C enrichments in citrate were 2.7–2.9-fold higher with [U-^{13}C]C8 (MCL 17.4%, $p < 0.0001$) and [U-^{13}C]C10 (MCL 18.9%, $p = 0.0001$) relative to [U-^{13}C]βHB (MCL 6.4%). Furthermore, compared to [U-^{13}C]βHB the ^{13}C enrichment was higher in succinate (C8: $p = 0.027$, C10: $p < 0.0001$), malate (C8: $p = 0.0057$, C10: $p = 0.0031$) and aspartate (C8: $p = 0.019$, C10: $p = 0.025$) when the brain slices were provided [U-^{13}C]C8 and [U-^{13}C]C10. In GABA ($p = 0.002$) and α-ketoglutarate ($p = 0.003$), slight increases in ^{13}C enrichment were only observed when comparing metabolism of [U-^{13}C]C10 to [U-^{13}C]βHB.

βHB and MCFAs do not Compete for Cellular Metabolism

In the competition experiment, addition of 200 µM ^{12}C-βHB to 200 µM [U-^{13}C]C8 or [U-^{13}C]C10 did not affect the incorporation of ^{13}C into most of the measured metabolites, indicating low cellular competition between βHB and MCFAs for oxidative metabolism (**Fig. 2a**). However, ^{12}C-βHB had subtle effects on the [U-^{13}C]C10-derived ^{13}C enrichment into malate ($p = 0.0453$) and GABA ($p = 0.0078$), and of [U-^{13}C]C8 in glutamate ($p = 0.0469$). Similarly, the presence of ^{12}C-C8 and ^{12}C-C10 only diluted [U-^{13}C]βHB-derived ^{13}C incorporation into citrate (C8: $p = 0.0002$, C10: $p = 0.0008$) and ^{12}C-C10 of glutamine ($p = 0.0237$), but not into any other measured metabolites (**Fig. 2b**).

Discussion

βHB and MCFAs are Metabolized in Different Cellular Compartments Optimizing Energy Supply to both Astrocytes and Neurons

In our brain slice experiments, both βHB and MCFAs were substrates of the TCA cycle, however, there was low competition between the cellular metabolism of MCFAs and βHB. This illustrates that metabolism of these two substrate types occurs in different cellular compartments. The high MCL in glutamine and citrate when applying [U-^{13}C]C8 and [U-^{13}C]C10 as substrates indicate astrocytic metabolism [25], which may suggest that βHB is primarily metabolized in non-astrocytic cells, i.e. neurons. On the other hand, the reduced ^{13}C incorporation into citrate and glutamine from metabolism of [U-^{13}C]βHB in the presence of ^{12}C-C8/C10 indicates that, although low, βHB is metabolized to some extent in astrocytes. Interestingly, coenzyme A (CoA) derivatives of both C8 and C10 are able to inhibit citrate synthase [30], which may account for some of the reduced citrate labeling of [U-^{13}C]βHB-derived carbons in the presence of ^{12}C-MCFAs. However, our results are consistent with previous reports that ketone bodies are mainly metabolized in non-astrocytic cells [17, 31, 32], while MCFAs are primarily metabolized by astrocytes [10–12]. Thus, when adding medium-chain triglycerides to regular or ketogenic diets, breakdown of medium-chain triglycerides to MCFAs, and to some extent ketone bodies, is expected to provide fuels for both neurons and astrocytes. This is important, as both cell types are involved in most energy expensive processes in the brain, namely, glutamate signaling and potassium homeostasis, which are pivotal for maintaining neuronal signaling functional and balanced [33]. Energy shortages in either astrocytes or neurons are likely to derail these essential functions and may result in the generation of epileptic seizures and other neurological defects. For example, patients in which glucose transport into the brain is reduced due to deficient function of glucose transporter 1 (GLUT1 deficiency syndrome), often suffer from epileptic seizures and paroxysmal abnormal movements [34]. To treat GLUT1 deficiency syndrome, ketogenic diets are typically used to provide auxiliary fuel to the brain. However, many patients do not tolerate or show little improvement with these high fat diets. Our data indicate that medium-chain triglycerides, in addition to ketogenic diets, or potentially by themselves, are likely to be beneficial, especially when GLUT1 splice variants expressed in astrocytes are perturbed. Note that in addition to serving as fuel in several cell types, both MCFAs and ketones bodies have been found to support GABA synthesis and have antioxidant, anti-inflammatory and other beneficial effects [29, 35, 36], which may contribute to the anticonvulsant effects.

βHB Metabolism is Likely to be Higher in Vivo

Metabolism of [U-^{13}C]βHB gave rise to lower MCL in most metabolites in cerebral cortical slices when compared to [U-^{13}C]C8 and [U-^{13}C]C10. The fact that C8 and C10 have at least double the number of carbon atoms compared to βHB needs to be taken into consideration, as it may, to some extent, explain the substrate differences in ^{13}C enrichment. It could be argued that the MCL should be adjusted according to the total ^{13}C content of the substrates. However, such a correction is not easily applied as the acetyl CoA units of C8 are not metabolized at the same rate [37]. The differences in MCL between βHB and the MCFAs can also be explained by the fact that cellular βHB uptake requires MCT activity whereas MCFAs are able to diffuse across cell membranes [38, 39]. The K_m of MCT1 for βHB is 12.5 mM, in endothelial cells and astrocytes, and 1.2 mM for the neuronally expressed MCT2 [16]. As we used 200 µM βHB in our experiments, the low MCT affinities for βHB are expected to limit its uptake, particularly in astrocytes. Furthermore, given that glucose and βHB are metabolized in the same cellular compartment, the 5 mM of unlabeled glucose may have competed to a higher extent with metabolism of [U-^{13}C]βHB than MCFAs in the brain slices. However, during ketogenic diet feeding in mice, cerebral glucose and βHB levels were found to be 1.3 mM and 50 µM, respectively [26], amounting to 4% of βHB relative to glucose, which is the same βHB to glucose ratio used in the present study.

One limitation of our experimental set-up is that the blood brain barrier (BBB) is not involved in βHB uptake in cerebral cortical slices. The in vivo metabolism of βHB appears to be mostly limited by uptake at the BBB, where MCT1 is expressed in endothelial cells and astrocytes [40]. Under physiological conditions, when βHB enters the brain via the BBB, it is potentially metabolized to a greater extent by astrocytes, whose end feet are part of the barrier. This is reflected by similar ^{13}C enrichments in glutamate and glutamine in vivo during ^{13}C-glucose and ^{13}C-ketone body infusion [17, 23]. It should also be considered that blood βHB levels can reach 1–5 mM with ketogenic diets in humans and rodents [2]. Such elevated βHB levels, in combination with lower glucose levels induced by a ketogenic diet, further increase βHB metabolism in vivo. Elevating levels of blood ketone bodies, induced by starvation or during ketogenic diet use, linearly increased cerebral βHB uptake and metabolism, while glucose metabolism was proportionally reduced, with ketone bodies contributing up to a third of the brain energy needs [5, 41]. Under such conditions, MCTs were found to be expressed at higher levels and capillary density was increased [42, 43]. Interestingly, C8 metabolism has been

shown to stimulate astrocyte βHB synthesis in vitro [13, 37, 44]. It has been proposed that the formed βHB may be transferred from astrocytes to neurons, hereby constituting a ketone body shuttle [45]. The present study does not support a very high activity of this shuttle, as most of the ^{13}C enrichment from [U-^{13}C]C8 was found in the astrocytic metabolic markers citrate and glutamine, which is supported by in vivo observations [12]. However, such a shuttle would be in line with the observed neuronal preference for βHB metabolism. Further studies are needed to investigate cerebral βHB and MCFA metabolism in vivo and other effects after prolonged usage of ketogenic diets and/or MCFAs.

Conclusions

Increased understanding of functional, biochemical and molecular changes of the therapeutic use of MCFAs and ketogenic diets is expected to help optimize their anticonvulsant and neuro-protective effects. More research is needed to better understand MCFA- and ketonemia-induced changes in brain metabolism and other non-metabolic effects.

Author Contributions JA Investigation, Methodology, Formal Analysis, Visualization, Writing—Reviewing and Editing. EW Investigation, Methodology. EN Writing—Reviewing & Editing. BA Supervision, Methodology, Resources. KB Conceptualization, Writing—Original draft, Resources.

Funding This work was supported by The Scholarship of Peter & Emma Thomsen (personal financial support to both JVA & EWW), the Hørslev Foundation (awarded to BIA) and a grant from the Australian National Health and Medicine Research Council (1186025, awarded to KB).

Data Availability All data of this study is available from the corresponding authors upon request.

Declarations

Conflict of interest The authors have no conflict of interest to declare.

References

1. Cunnane SC, Trushina E, Morland C, Prigione A, Casadesus G, Andrews ZB, Beal MF, Bergersen LH, Brinton RD, de la Monte S, Eckert A, Harvey J, Jeggo R, Jhamandas JH, Kann O, la Cour CM, Martin WF, Mithieux G, Moreira PI, Murphy MP, Nave KA, Nuriel T, Oliet SHR, Saudou F, Mattson MP, Swerdlow RH, Millan MJ (2020) Brain energy rescue: an emerging therapeutic concept for neurodegenerative disorders of ageing. Nat Rev Drug Discov 19:609–633
2. Rho JM, Boison D (2022) The metabolic basis of epilepsy. Nat Rev Neurol 18(6):333–347
3. Liu H, Yang Y, Wang Y, Tang H, Zhang F, Zhang Y, Zhao Y (2018) Ketogenic diet for treatment of intractable epilepsy in adults: a meta-analysis of observational studies. Epilepsia Open 3:9–17
4. Masino SA, Rho JM (2019) Metabolism and epilepsy: Ketogenic diets as a homeostatic link. Brain Res 1703:26–30
5. Courchesne-Loyer A, Croteau E, Castellano CA, St-Pierre V, Hennebelle M, Cunnane SC (2017) Inverse relationship between brain glucose and ketone metabolism in adults during short-term moderate dietary ketosis: A dual tracer quantitative positron emission tomography study. J Cereb Blood Flow Metab 37:2485–2493
6. Han FY, Conboy-Schmidt L, Rybachuk G, Volk HA, Zanghi B, Pan Y, Borges K (2021) Dietary medium chain triglycerides for management of epilepsy: New data from human, dog, and rodent studies. Epilepsia 62:1790–1806
7. St-Pierre V, Vandenberghe C, Lowry CM, Fortier M, Castellano CA, Wagner R, Cunnane SC (2019) Plasma ketone and medium chain fatty acid response in humans consuming different medium chain triglycerides during a metabolic study day. Front Nutr 6:46
8. Achanta LB, Rae CD (2017) β-Hydroxybutyrate in the brain: one molecule, multiple mechanisms. Neurochem Res 42:35–49
9. Halestrap AP, Meredith D (2004) The SLC16 gene family-from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. Pflugers Arch 447:619–628
10. Cremer JE, Teal HM, Heath DF, Cavanagh JB (1977) The influence of portocaval anastomosis on the metabolism of labelled octanoate, butyrate and leucine in rat brain. J Neurochem 28:215–222
11. Edmond J, Robbins RA, Bergstrom JD, Cole RA, de Vellis J (1987) Capacity for substrate utilization in oxidative metabolism by neurons, astrocytes, and oligodendrocytes from developing brain in primary culture. J Neurosci Res 18:551–561
12. Ebert D, Haller RG, Walton ME (2003) Energy contribution of octanoate to intact rat brain metabolism measured by 13C nuclear magnetic resonance spectroscopy. J Neurosci 23:5928–5935
13. Thevenet J, De Marchi U, Domingo JS, Christinat N, Bultot L, Lefebvre G, Sakamoto K, Descombes P, Masoodi M, Wiederkehr A (2016) Medium-chain fatty acids inhibit mitochondrial metabolism in astrocytes promoting astrocyte-neuron lactate and ketone body shuttle systems. Faseb j 30:1913–1926
14. Khabbush A, Orford M, Tsai YC, Rutherford T, O'Donnell M, Eaton S, Heales SJR (2017) Neuronal decanoic acid oxidation is markedly lower than that of octanoic acid: A mechanistic insight into the medium-chain triglyceride ketogenic diet. Epilepsia 58:1423–1429
15. Norenberg MD, Martinez-Hernandez A (1979) Fine structural localization of glutamine synthetase in astrocytes of rat brain. Brain Res 161:303–310
16. Enerson BE, Drewes LR (2003) Molecular features, regulation, and function of monocarboxylate transporters: implications for drug delivery. J Pharm Sci 92:1531–1544
17. Pan JW, de Graaf RA, Petersen KF, Shulman GI, Hetherington HP, Rothman DL (2002) [2,4–13 C2]-beta-Hydroxybutyrate metabolism in human brain. J Cereb Blood Flow Metab 22:890–898
18. Cremer JE (1971) Incorporation of label from D- -hydroxy(14 C) butyrate and (3–14 C)acetoacetate into amino acids in rat brain in vivo. Biochem J 122:135–138
19. Cremer JE, Heath DF (1974) The estimation of rates of utilization of glucose and ketone bodies in the brain of the suckling rat using compartmental analysis of isotopic data. Biochem J 142:527–544
20. McKenna MC, Tildon JT, Stevenson JH, Boatright R, Huang S (1993) Regulation of energy metabolism in synaptic terminals and cultured rat brain astrocytes: differences revealed using aminooxyacetate. Dev Neurosci 15:320–329
21. Valdebenito R, Ruminot I, Garrido-Gerter P, Fernández-Moncada I, Forero-Quintero L, Alegría K, Becker HM, Deitmer JW, Barros

LF (2016) Targeting of astrocytic glucose metabolism by beta-hydroxybutyrate. J Cereb Blood Flow Metab 36:1813–1822
22. Künnecke B, Cerdan S, Seelig J (1993) Cerebral metabolism of [1,2–13C2]glucose and [U-13C4]3-hydroxybutyrate in rat brain as detected by 13C NMR spectroscopy. NMR Biomed 6:264–277
23. Zhang Y, Zhang S, Marin-Valencia I, Puchowicz MA (2015) Decreased carbon shunting from glucose toward oxidative metabolism in diet-induced ketotic rat brain. J Neurochem 132:301–312
24. Andersen JV, Christensen SK, Nissen JD, Waagepetersen HS (2017) Improved cerebral energetics and ketone body metabolism in db/db mice. J Cereb Blood Flow Metab 37:1137–1147
25. McNair LF, Kornfelt R, Walls AB, Andersen JV, Aldana BI, Nissen JD, Schousboe A, Waagepetersen HS (2017) Metabolic characterization of acutely isolated hippocampal and cerebral cortical slices using [U-13C]glucose and [1,2–13C]acetate as substrates. Neurochem Res 42:810–826
26. Samala R, Klein J, Borges K (2011) The ketogenic diet changes metabolite levels in hippocampal extracellular fluid. Neurochem Int 58:5–8
27. Walls AB, Bak LK, Sonnewald U, Schousboe A, Waagepetersen HS (2014) Metabolic mapping of astrocytes and neurons in culture using stable isotopes and gas chromatography-mass spectrometry (GC-MS). In: Hirrlinger J, Waagepetersen HS (eds) Brain energy metabolism neuromethods, vol 90. Humana Press, New York
28. Andersen JV, Christensen SK, Westi EW, Diaz-delCastillo M, Tanila H, Schousboe A, Aldana BI, Waagepetersen HS (2021) Deficient astrocyte metabolism impairs glutamine synthesis and neurotransmitter homeostasis in a mouse model of Alzheimer's disease. Neurobiol Dis 148:105198
29. Andersen JV, Westi EW, Jakobsen E, Urruticoechea N, Borges K, Aldana BI (2021) Astrocyte metabolism of the medium-chain fatty acids octanoic acid and decanoic acid promotes GABA synthesis in neurons via elevated glutamine supply. Mol Brain 14:132
30. Lai JC, Liang BB, Zhai S, Jarvi EJ, Lu DR (1994) Brain mitochondrial citrate synthase and glutamate dehydrogenase: differential inhibition by fatty acyl coenzyme a derivatives. Metab Brain Dis 9:143–152
31. Achanta LB, Rowlands BD, Thomas DS, Housley GD, Rae CD (2017) β-Hydroxybutyrate boosts mitochondrial and neuronal metabolism but is not preferred over glucose under activated conditions. Neurochem Res 42:1710–1723
32. Jiang L, Mason GF, Rothman DL, de Graaf RA, Behar KL (2011) Cortical substrate oxidation during hyperketonemia in the fasted anesthetized rat in vivo. J Cereb Blood Flow Metab 31:2313–2323
33. Andersen JV, Markussen KH, Jakobsen E, Schousboe A, Waagepetersen HS, Rosenberg PA, Aldana BI (2021) Glutamate metabolism and recycling at the excitatory synapse in health and neurodegeneration. Neuropharmacology 196:108719
34. Wang P, Pascual JM, De Vivo DC (2018) Glucose transporter type 1 deficiency syndrome. In: Adam MPA, Ardinger HH, Pagon RA (eds) GeneReviews. University of Washington, Seattle
35. Masino SA, Rho JM (2012) Mechanisms of ketogenic diet action. In: Noebels JL, Avoli M, Rogawski MA (eds) Jasper's basic mechanisms of the epilepsies. Bethesda
36. Yudkoff M, Daikhin Y, Nissim I, Lazarow A, Nissim I (2001) Brain amino acid metabolism and ketosis. J Neurosci Res 66:272–281
37. Auestad N, Korsak RA, Morrow JW, Edmond J (1991) Fatty acid oxidation and ketogenesis by astrocytes in primary culture. J Neurochem 56:1376–1386
38. Schönfeld P, Wojtczak L (2016) Short- and medium-chain fatty acids in energy metabolism: the cellular perspective. J Lipid Res 57:943–954
39. Kamp F, Hamilton JA (2006) How fatty acids of different chain length enter and leave cells by free diffusion. Prostaglandins Leukot Essent Fatty Acids 75:149–159
40. Halestrap AP (2013) The SLC16 gene family – Structure, role and regulation in health and disease. Mol Aspects Med 34:337–349
41. Zhang Y, Kuang Y, Xu K, Harris D, Lee Z, LaManna J, Puchowicz MA (2013) Ketosis proportionately spares glucose utilization in brain. J Cereb Blood Flow Metab 33:1307–1311
42. Puchowicz MA, Xu K, Sun X, Ivy A, Emancipator D, LaManna JC (2007) Diet-induced ketosis increases capillary density without altered blood flow in rat brain. Am J Physiol Endocrinol Metab 292:E1607-1615
43. Leino RL, Gerhart DZ, Duelli R, Enerson BE, Drewes LR (2001) Diet-induced ketosis increases monocarboxylate transporter (MCT1) levels in rat brain. Neurochem Int 38:519–527
44. Sonnay S, Chakrabarti A, Thevenet J, Wiederkehr A, Christinat N, Masoodi M (2019) Differential metabolism of medium-chain fatty acids in differentiated human-induced pluripotent stem cell-derived astrocytes. Front Physiol 10:657
45. Guzmán M, Blázquez C (2001) Is there an astrocyte-neuron ketone body shuttle? Trends Endocrinol Metab 12:169–173