Neuronal Decanoic Acid Oxidation is Markedly Lower than that of Octanoic Acid: a Mechanistic Insight into the Medium-Chain Triglyceride Ketogenic Diet

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SUMMARY

Objective: The medium chain triglyceride ketogenic diet contains both octanoic (C8) and decanoic (C10) acids. The diet is an effective treatment for pharmacoresistant epilepsy. Whilst the exact mechanism for its efficacy is not known, it is emerging that C10, but not C8, interacts with targets that can explain anti-seizure effects, e.g. peroxisome proliferator-activated receptor-γ (PPARγ) (eliciting mitochondrial biogenesis and increased antioxidant status) and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor. For such effects to occur, significant concentrations of C10 are likely to be required in the brain.

Methods: In order to investigate how this might occur, we measured the β-oxidation rate of ^{13}C-labelled C8 and C10 in neuronal SH-SY5Y cells using isotope-ratio mass spectrometry. The effects of carnitine palmitoyl transferase I (CPT1) inhibition, with the CPT1 inhibitor etomoxir, on C8 and C10 β-oxidation were also investigated.

Results: Both fatty acids were catabolised, as judged by $^{13}CO_2$ release. However, C10 was β-oxidised at a significantly lower rate; 20% of C8. This difference was explained by a clear dependence of C10 on CPT1 activity, which is low in neurons, whereas 66% of C8 β-oxidation was independent of CPT1. In addition, C10 β-oxidation was decreased further in the presence of C8.

Significance: It is concluded that, since CPT1 is poorly expressed in the brain, C10 is relatively spared from β-oxidation and can accumulate. This is further facilitated by the presence of C8 in the MCT ketogenic diet, which has a sparing effect upon C10 β-oxidation.

Key Words: CPT1, epilepsy, drug-resistance, fatty acids, capric acid, caprylic acid

INTRODUCTION

The medium-chain triglyceride ketogenic diet (MCT KD) is frequently used as an effective treatment against drug-resistant epilepsy in children. A high fat, low carbohydrate regimen, the MCT KD induces ketone body production, pushing the body into ketosis thereby maintaining near normal plasma glucose levels due to provision of an alternative fuel source**.** During these shifts in metabolism, seizures are controlled in patients. Although ketone bodies have been postulated to play a therapeutic role, seizure control is poorly correlated with ketone body levels[1,2]. Thus, despite the relative success of the diet, the precise mechanism of action of the MCT KD remains unknown. However, there is a growing interest in the potential effects on seizures of the medium-chain octanoic (C8) and decanoic (C10) acids, which are known to accumulate in the plasma of patients receiving the MCT KD[3,4].

Despite both these fatty acids being present in the MCT KD and in patient plasma, evidence is now emerging to suggest that C10, and not C8, may be responsible for the beneficial effects associated with the MCT KD. Thus C10, and not C8, has previously been reported by us to elicit PPARγ-dependent increases in neuronal mitochondrial content, complex I and catalase activity[5]. These findings, coupled with the direct inhibitory effect of C10 on AMPA receptors[6], provide mechanistic evidence whereby C10 could elicit seizure control. The anti-seizure properties of C10 are further supported by observations*, in vivo,* that report that mice fed with a C10-enriched chow display significantly improved seizure tolerance and brain antioxidant capacity[7–11]. In studies conducted by Wlaź et al., C10 levels were also determined in the brains of mice following C10-enriched feeding. Interestingly, the concentration of C10 in mouse brain was found to average at 240-250μM, corresponding with therapeutic levels

reported in patient plasma on the MCT KD, as well as the optimum concentration (250μM) for mitochondrial proliferation in our previous studies[5,12].

Currently, MCT oils used in the MCT KD are varying mixtures of C8 and C10, the ratios of which differ from product to product[13]. This diet is generally favoured due to its ability to provide energy and generate ketones with a lower total fat intake compared to long-chain triglyceride-based diets thus improving palatability[14,15]. However, as medium-chain fatty acids are usually rapidly oxidised or converted to ketone bodies, how can sufficient levels of C10 accumulate in the brain to permit the fatty acid to exert its effects on targets, such PPARγ and the AMPA receptor? To address this question, we here assessed the rates of β-oxidation of C8 and C10 in the human SH-SY5Y neuroblastoma cell line, i.e. following on from our previous work[5]. A well-characterised cell line, SH-SY5Y cells exhibit a range of neuron-like properties, providing a suitable model for this study. Furthermore, as C10 appears to be a key component with regards to understanding the mechanisms of the MCT KD, we also investigated whether C8 exerts any sparing effect concerning C10 β-oxidation.

METHODS

Materials

Unless otherwise stated, all reagents were purchased from Sigma-Aldrich (Poole, UK). Dulbecco's Modified Eagle's Medium (DMEM)/Ham's Nutrient Mixture (1:1), heat-inactivated foetal bovine serum (FBS) and 2.5 g/L trypsin-EDTA were purchased from ThermoFisher Scientific (Paisley, UK). Trypan Blue (0.4%) solution was purchased from Bio-Rad Laboratories Ltd (Hemel Hempstead, UK). Human neuroblastoma cell line, SH-SY5Y, was obtained from the European Collection of Cell Cultures (Public Health England, Salisbury, UK). Stable isotope-

labelled compounds, [U-^{13}C]glucose, [1-^{13}C]decanoic acid ([1-^{13}C]C10) and [1-^{13}C]octanoic acid ([1-^{13}C]C8) were purchased from Cambridge Isotopes Laboratories, Inc. via CK Isotopes (Ibstock, UK). [U-^{13}C]palmitic acid ([U-^{13}C]C16) was purchased from Larodan (Malmö, Sweden) and used as a 5:1 complex (molar ratio) with fatty acid-free bovine serum albumin.

Cell culture

SH-SY5Y cells were utilised between passages 20-24 for all experimental procedures. Cells were cultured in 1:1 DMEM/F12 medium, containing 17.5mM glucose and supplemented with 100ml/L heat-inactivated FBS and 10ml/L 200mM L-glutamine. All cells were maintained in a humidified atmosphere at 37°C and 5% CO_2. Cells were subcultured by washing with 10ml/flask Mg^{2+}/Ca^{2+}-free Dulbecco's phosphate-buffered saline (DPBS), lifted with 2ml 0.25% trypsin-EDTA and suspended in 8ml culture medium to inactivate the trypsin. The cell suspension was transferred to a falcon tube and centrifuged for 4 minutes at 500xg. The supernatant was removed and cells suspended in a known volume of fresh culture medium. Cells were counted using the Trypan Blue exclusion test for viability. A known volume of the cell suspension was mixed 1:1 with 0.4% Trypan Blue solution and counted with a Bio-Rad TC20™ Automated Cell Counter (Hemel Hempstead, UK). Cells were then seeded at a density of 1x10^4 cells/cm^2 in 6-well plates, made up to a final volume of 2ml with fresh medium and cultured for 5 days prior to all experiments. The same starting number of cells was used for each investigation. Medium was refreshed every two days.

Glucose, C10 and C8 β-oxidation in SH-SY5Y cells

All experimental procedures were carried out with the use of a specifically formulated DMEM medium, containing a final concentration of 15mM HEPES, 2.9mM sodium bicarbonate, 2mM L-glutamine, 0.5mM sodium pyruvate and 21.5µM phenol red. A stock solution of 134.3mM [U-^{13}C]glucose was prepared by dissolving glucose in DMEM solution and then frozen at -20°C in aliquots. Stock solutions of 50mM [1-^{13}C]decanoic acid ([1-^{13}C]C10) and 50mM [1-^{13}C]C8 were prepared in DMSO, sterile-filtered and then stored in aliquots at -20°C.

On day 5 of culture, complete growth medium was removed and cells washed once with DPBS. Cells were then incubated with 2ml of the DMEM formula, supplemented with 10% FBS and 3mM D-glucose, for 20h at 37°C and 5% CO_2. After 20h, the medium was removed and cells washed once with DPBS. To each well, 3ml of DMEM was then added containing 3mM [U-^{13}C]-glucose and vehicle control DMSO, or 3mM D-glucose with either 250µM [1-^{13}C]C10 or 250µM [1-^{13}C]C8. Wells were then sealed with a 3ml layer of heavy mineral oil in order to prevent the loss of $^{13}CO_2$ through gas exchange between the medium and the atmosphere[16]. Using this approach we were able to demonstrate negligible (<0.1%) loss of label from the medium. Cells were incubated at 37°C for 6 hours, with 100µl of medium sampled from each well at hourly intervals. Sampled medium were immediately stored in rubber-sealed Exetainer™ vials (Labco Ltd, Ceredigion, UK) and kept at -20°C until analysis.

C10 and C8 co-incubation

The effects of co-incubating C10 and C8 on C10 β-oxidation in SH-SY5Y cells were also explored. Additional stock solutions of 25mM C8 and 100mM [1-^{13}C]C10 were prepared in DMSO, sterile-filtered and stored in aliquots at -20°C. Cells were cultured and incubated for 20h in 10% FBS-supplemented DMEM medium containing 3mM D-glucose, as previously described. Medium was then removed and cells washed. Medium was then replaced with 3ml DMEM containing either 3mM [U-^{13}C]glucose and vehicle control DMSO, or 3mM unlabelled D-glucose. In each D-glucose-supplemented well, cells were treated with either a final concentration of 250µM [1-^{13}C]C10, or 250µM [1-^{13}C]C10 plus 62.5µM C8, at a fixed total volume. Wells were then sealed with heavy mineral oil, cells incubated and medium sampled as previously outlined.

Preparation of [U-^{13}C]-palmitic acid

[U-^{13}C]palmitic acid was neutralised with 0.35M NaOH and made to a concentration of 17.5mM in water, before heating to 70°C until fully dissolved. Fatty acid-free bovine serum albumin (BSA) was then dissolved to a concentration of 3.5mM in water at 37°C. Swirling gently, [U-^{13}C]-palmitate ([U-^{13}C]-C16) was then slowly added 1:1 to BSA at 37°C, forming an 8.75mM [U-^{13}C]C16:BSA complex (5:1 molar ratio fatty acid:BSA). An additional stock solution of 1.75mM BSA in water was also prepared. Both solutions were aliquoted and stored at -20°C until further use.

CPT1 inhibition assay

A stock solution of 50mM etomoxir was prepared in sterile cell culture grade water and stored in aliquots at -20°C. On day 5 of culture, growth medium was replaced with 2ml DMEM formula containing 3mM D-glucose and 10% FBS. Cells were incubated with or without the

presence of 50µM etomoxir, for 20h at 37°C and 5% CO_2. Medium was then removed and replaced with 3ml DMEM, containing 3mM [U-^{13}C]glucose and vehicle control DMSO or 3mM D-glucose with either 250µM [1-^{13}C]C10, [1-^{13}C]C8 or [U-^{13}C]C16, with 50µM etomoxir also added to previously exposed cells. Wells were then sealed with heavy mineral oil, with cells incubated and medium sampled as previously described.

Measurement of $^{13}CO_2$ release

Samples were thawed at room temperature and 100µl 1M hydrochloric acid was injected through the septum into each vial to release CO_2 from the medium. Vials were centrifuged for 30 seconds at 500xg. Samples were then analysed on a GasBench II coupled to a Thermo Delta-XP isotope-ratio mass spectrometer (Thermo-Finnigan, Bremen, Germany). Ten repeat injections were carried out per sample, with $^{13}CO_2/^{12}CO_2$ ratios measured against Vienna Pee Dee Belemnite (VPDB) using a calibrated CO_2 reference gas. Following this, $^{13}CO_2/^{12}CO_2$ ratios were then converted to mole percent excess using absolute molar ratio of ^{13}C to ^{12}C (0.0111796) in VPDB. The change in mole percent excess was then converted to pmol CO_2 generated using the volume of medium and concentration of bicarbonate (2.9mM) present, which was then corrected for the number of labelled carbon atoms (1 for C8 and C10, 6 for glucose and 16 for palmitate) to obtain pmol substrate oxidised.

Cell viability

Cells were incubated for 20h at 37°C and 5% CO_2 with 50µM etomoxir in DMEM medium supplemented with 10% FBS, and then a further 6h at 37°C with 50µM etomoxir in DMEM medium without FBS. Cells were then lifted from wells with 1ml 0.25% trypsin-EDTA, suspended in 4ml culture medium and centrifuged for 4 minutes at 500xg. Supernatant was

removed and cells suspended in 1ml of fresh culture medium. Viability of cells was then tested using the Trypan Blue exclusion test.

Statistical analysis

Data are expressed as mean ± SD, with n number indicating the number of independent experiments carried out. Statistical analysis between two groups was performed using a Student's t-test, whilst analysis of three groups or more was performed with one-way ANOVA, followed by a post-hoc Tukey's test for multiple comparisons. Variables were considered to be statistically significant when $p < 0.05$.

RESULTS

β-oxidation rates of Glucose, C8 and C10 in SH-SY5Y cells

^{13}C-labelled compounds permit the measurement of cellular β-oxidation rates of glucose, C8 or C10 via CO_2 release emanating from pyruvate dehydrogenase activity and/or the TCA cycle. $^{13}CO_2$ release over 6 hours was used to determine and quantify the rate of cellular β-oxidation of each compound. Cells were treated with 3mM ^{13}C-labelled glucose, in order to replicate physiological levels observed in patients under the MCT KD. ^{13}C-labelled C10 and C8 were added separately to a final concentration of 250µM, this being the concentration of C10, previously determined by us[5], for optimum effects upon mitochondria and antioxidant status. Furthermore, this replicates the brain concentration achieved following peripheral C10 administration[9,10]. Unlabelled 3mM glucose was used in the presence of ^{13}C-labelled C10 and C8.

$^{13}CO_2$ release, for each molecule studied, was linear for the 6 hour incubation. As expected, the rate of glucose oxidation was markedly faster than that of either C8 or C10 (Fig. 1A). However, C8 and C10 were found to be differentially oxidised in these cells, with C10 β-oxidation significantly lower than that of C8, by approximately 80% (Fig. 1A). This suggests that C8 may be preferentially oxidised in SH-SY5Y cells.

Effect of co-incubation of C8 and C10 on β-oxidation

Current MCT KD preparations are composed of a mixture of C8 and C10 at varying ratios. In light of this, we examined the effects on [1-^{13}C]C10 β-oxidation when SH-SY5Y cells were treated with 62.5μM unlabelled C8, reflecting the move towards a more C10-enriched formula, i.e. with C8 compromising only 20% of the total fatty acids. Despite the relatively low concentration, C8 addition was found to significantly impair the β-oxidation rate of C10 by 29% (Fig. 1B).

C10 β-oxidation following CPT1 inhibition

To determine the mechanisms behind the differential β-oxidation of C8 and C10, the potential role of the carnitine shuttle was explored. Whilst long chain fatty acids require this system, medium chain fatty acids are generally considered to be able to enter the mitochondrial matrix in a carnitine-independent manner[17]. Whilst this may be the case for C8, there are reports to suggest that C10, in contrast to C8, may require the carnitine system for complete mitochondrial β-oxidation[18]. Carnitine palmitoyltransferase I (CPT1) is responsible for transferring fatty acyl groups to carnitine and is the rate-limiting step in carnitine-dependent β-oxidation in mitochondria[19–21]. To evaluate the potential role of CPT1 in C8 and C10 β-oxidation, the well-characterised CPT1 irreversible inhibitor etomoxir was used[22–24]. Through

dose-response experiments, [U-^{13}C]palmitic (C16) acid, which is well-known to depend on CPT1 for mitochondrial β-oxidation, was used as a positive control to allow us to determine the maximal concentration (50 μM) of etomoxir that could be used to inhibit CPT1 without affecting cell viability (100μM etomoxir caused cell death as judged by trypan blue exclusion). With the conditions employed, β-oxidation of [U-^{13}C]C16 was reduced by 99%, suggesting complete irreversible inhibition of CPT1 (Table 1). Moreover, etomoxir was observed to have no effect on viability of the SH-SY5Y cells used (data not shown). Under the same conditions, C10 β-oxidation was found to be reduced by 95% in the presence of etomoxir (Table 1), whereas C8 β-oxidation was only inhibited by 34%.

DISCUSSION

There is growing interest in the mode of action of the MCT KD and, in particular, with regards to the effects of the constituent medium-chain fatty acid, C10. In contrast to C8, C10 appears to have a number of biological targets that can explain the anti-seizure properties of the diet. However, critical for C10 to exert its beneficial effects is attainment of a sufficiently high concentration within the brain. Existing data, including those generated from our own laboratory, suggest concentrations of up to 250μM C10 are required to facilitate an increase in mitochondrial biogenesis, increased antioxidant capacity and AMPA receptor inhibition[5,6,12]. However, such a concentration appears achievable, as oral administration of C10 to mice leads to brain concentrations of up to 250μM[9,10]. Medium-chain fatty acids are catabolised by β-oxidation resulting in acetyl CoA formation. This can be further metabolised to generate ketones and/or enter the TCA cycle. Since medium-chain fatty acids are able to cross the blood brain barrier[25–27] and the enzymes of β-oxidation are reported to be present in neuronal cells[28,29], we have in this study evaluated the ability of neuronal-like SH-SY5Y cells

to β-oxidise C8 and C10. We hypothesised that C10 would be relatively spared, which may result in a degree of accumulation of this fatty acid to occur.

The use of ^{13}C-labelled substrates allows for the metabolism of substrates to be compared, i.e. by capturing and quantifying the labelled CO_2 released by the cells. Using this approach, metabolism of glucose was considerably greater than that for C8 or C10. This was expected, given that it is well established that glucose is the main fuel for neuronal energy metabolism[30–33]. Whilst showing lower rates of oxidation than glucose, both C8 and C10 were β-oxidised to some extent by the cells. However, our observation that C10 β-oxidation was markedly lower than that of C8 suggests that these two fatty acids are processed differently by SH-SY5Y cells. To ascertain the potential cause for this difference, we considered the involvement of the carnitine shuttle, as C10 is reported to utilise this system for maximal β-oxidation to occur[18]. Furthermore, C10 oxidation is impaired in inherited disorders of carnitine metabolism[34]. This reliance of C10 upon the carnitine shuttle is further supported here by our finding that CPT1 inhibition, by etomoxir, markedly impairs C10 β-oxidation, whereas C8 β-oxidation appeared mainly CPT1-independent. Whilst etomoxir is a very well-characterised CPT1 inhibitor, as reflected by its ability to almost completely abolish C16 β-oxidation, it should be noted that it may have other targets, e.g. by acting as a PPARα agonist. Thus, relatively prolonged exposure (5 days) has been suggested to lead to up-regulation of fatty acid oxidation[35]. However, in our acute study, the converse was apparent, i.e. in keeping with CPT1 inhibition. Our finding that CPT1 inhibition markedly impairs C10 β-oxidation, whereas C8 β-oxidation was mainly CPT1-independent, supports the need for this system in neuronal type cells, i.e. if maximal catabolism of the fatty acid is to occur.

To further explore the potential common metabolic fates of C8 and C10, we looked at the effect of the presence of C8 on C10 β-oxidation. Even in the presence of a relatively low concentration of C8, C10 β-oxidation was significantly inhibited. This observation may suggest that in the MCT KD, the presence of C8 has a sparing effect to further limit the catabolism of C10 thus facilitating its accumulation in the brain and the manifestation of its anti-seizure effects. However, it is possible that other mechanisms could also contribute to the differential metabolism of C8 and C10, e.g. varying degrees of cellular uptake for the two fatty acids. Further studies, involving cellular fractionation following C8 and/or C10 exposure may therefore be informative

To summarise, the data presented here suggest that in a neuronal cell line, C10 and C8 are differentially oxidised. Thus, C8 may be preferentially metabolised and provide energy, whereas, because of its requirement of the carnitine shuttle, C10 is metabolised at a lower rate and can consequently accumulate (Fig. 2). CPT1 activity in brain is low, and although there is also a brain-specific isoform of CPT1 (CPT1c), this isoform has not been found to demonstrate any significant enzymatic activity[21,36–39]. This hypothesis is further supported by the recent observation that reports, in astrocytes, that C8 is ketogenic whilst C10 is not[40].

In conclusion, the fatty acid components of the MCT KD are differentially metabolised by neuronal-like cells. The carnitine dependence and sluggish metabolism of C10 provides an explanation for how critical concentrations may occur and permit interaction with key anti-seizure targets. In contrast, C8 may be preferentially metabolised and have two key effects: sparing of C10 by inhibiting C10 β-oxidation and acting as a fuel source for brain energy metabolism.

Whilst this study provides further mechanistic insight into the MCT KD, relevance to understanding the classical KD is not immediately clear. However, the possibility exists that progressive (mitochondrial/peroxisomal) oxidation of the long-chain fatty acid components occurs, leading to C10 formation and eventual neuronal C10 accumulation. Further work is clearly needed to test this hypothesis. Additional alternative mechanisms could also be responsible for efficacy of the classical KD.

KEY POINTS

- C10 β-oxidation is markedly lower than that of C8 in neuronal cells.
- C10 β-oxidation is CPT1-dependent, whilst C8 is only partially so.
- The presence of C8 significantly reduces C10 β-oxidation.
- Low β-oxidation rates of C10 may lead to accumulation in brain and, consequently, anti-seizure effects.

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ETHICAL PUBLICATION STATEMENT

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

REFERENCES

1. Likhodii SS, Musa K, Mendonca A, et al. Dietary fat, ketosis, and seizure resistance in rats on the ketogenic diet. *Epilepsia*. 2000;41:1400-1410.
2. Thavendiranathan P, Mendonca A, Dell C, et al. The MCT ketogenic diet: effects on animal seizure models. *Exp Neurol*. 2000;161:696-703.
3. Haidukewych D, Forsythe WI, Sills M. Monitoring octanoic and decanoic acids in plasma from children with intractable epilepsy treated with medium-chain triglyceride diet. *Clin Chem*. 1982;28:642-645.
4. Sills M, Forsythe W, Haidukewych D. Role of octanoic and decanoic acids in the control of seizures. *Arch Dis Child*. 1986;61:1173-1177.
5. Hughes SD, Kanabus M, Anderson G, et al. The ketogenic diet component decanoic acid increases mitochondrial citrate synthase and complex I activity in neuronal cells. *J Neurochem*. 2014;129:426-433.
6. Chang P, Augustin K, Boddum K, et al. Seizure control by decanoic acid through direct AMPA receptor inhibition. *Brain*. 2016;139:431-433.
7. Sengupta A, Ghosh M. Comparison of native and capric acid-enriched mustard oil effects on oxidative stress and antioxidant protection in rats. *Br J Nutr*. 2012;107:845-849.
8. Sengupta A, Ghosh M, Bhattacharyya DK. Antioxidative Effect of Rice Bran Oil and Medium Chain Fatty Acid Rich Rice Bran Oil in Arsenite Induced Oxidative Stress in Rats. *J Oleo Sci*. 2014;63:1117-1124.
9. Wlaź P, Socała K, Nieoczym D, et al. Anticonvulsant profile of caprylic acid, a main

constituent of the medium-chain triglyceride (MCT) ketogenic diet, in mice. *Neuropharmacology*. 2012;62:1882-1889.

10. Wlaź P, Socała K, Nieoczym D, et al. Acute anticonvulsant effects of capric acid in seizure tests in mice. *Prog Neuro-Psychopharmacology Biol Psychiatry*. 2015;57:110-116.
11. Tan KN, Carrasco-Pozo C, McDonald TS, et al. Tridecanoin is anticonvulsant, antioxidant, and improves mitochondrial function. *J Cereb Blood Flow Metab*. 2016;37:2035-2048.
12. Kanabus M, Fassone E, Hughes SD, et al. The pleiotropic effects of decanoic acid treatment on mitochondrial function in fibroblasts from patients with complex I deficient Leigh syndrome. *J Inherit Metab Dis*. 2016;39:415-426.
13. Rogawski MA. A fatty acid in the MCT ketogenic diet for epilepsy treatment blocks AMPA receptors. *Brain*. 2016;139:304-316.
14. Neal EG, Chaffe H, Schwartz RH, et al. The ketogenic diet for the treatment of childhood epilepsy: a randomised controlled trial. *Lancet Neurol*. 2008;7:500-506.
15. Neal EG, Chaffe H, Schwartz RH, et al. A randomized trial of classical and medium-chain triglyceride ketogenic diets in the treatment of childhood epilepsy. *Epilepsia*. 2009;50:1109-1117.
16. Will Y, Hynes J, Ogurtsov VI, et al. Analysis of mitochondrial function using phosphorescent oxygen-sensitive probes. *Nat Protoc*. 2006;1:2563-2572.
17. Papamandjaris A, MacDougall D, Jones P. Medium chain fatty acid metabolism and energy expenditure: obesity treatment implications. *Life Sci*. 1998;62:1203-1215.
18. Parini R, Invernizzi F, Menni F, et al. Medium-chain triglyceride loading test in carnitine-acylcarnitine translocase deficiency: Insights on treatment. *J Inherit Metab*

Dis. 1999;22:733-739.

19. McGarry J, Brown N. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. *Eur J Biochem*. 1997;244:1-14.
20. Eaton S. Control of mitochondrial β-oxidation flux. *Prog Lipid Res*. 2002;41:197-239.
21. Wolfgang MJ, Kurama T, Dai Y, et al. The brain-specific carnitine palmitoyltransferase-1c regulates energy homeostasis. *Proc Natl Acad Sci U S A*. 2006;103:7282-7287.
22. Selby PL, Sherratt HSA. Substituted 2-oxiranecarboxylic acids: a new group of candidate hypoglycemic drugs. *Trends Pharmacol Sci*. 1989;10:495-500.
23. Ratheiser K, Schneeweiß B, Waldhäusl W, et al. Inhibition by etomoxir of carnitine palmitoyltransferase I reduces hepatic glucose production and plasma lipids in non-insulin-dependent diabetes mellitus. *Metabolism*. 1991;40:1185-1190.
24. Spurway TD, Pogson CI, Sherratt HSA, et al. Etomoxir, sodium 2-[6-(4-chlorophenoxy)hexyl]oxirane-2-carboxylate, inhibits triacylglycerol depletion in hepatocytes and lipolysis in adipocytes. *FEBS Lett*. 1997;404:111-114.
25. Olendorf W. Blood Brain Barrier Permeability to Lactate. *Eur Neurol*. 1971;6:49-55.
26. Olendorf W. Carrier-mediated blood-brain barrier transport of short-chain monocarboxylic organic acids. *Am J Physiol*. 1973;224:1450-1453.
27. Spector R. Fatty acid transport through the blood-brain barrier. *J Neurochem*. 1988;50:639-643.
28. Yang S-Y, He X-Y, Schulz H. Fatty acid oxidation in rat brain is limited by the low activity of 3-ketoacyl-coenzyme A thiolase. *J Biol Chem*. 1987;262:13027-13032.
29. Reichmann H, Maltese W, DeVivo D. Enzymes of Fatty Acid Beta-Oxidation in Developing Brain. *J Neurochem*. 1988;51:339-344.
30. Dienel GA, Hertz L. Glucose and Lactate Metabolism During Brain Activation. *J*

Neurosci Res. 2001;66:824-838.

31. Mergenthaler P, Lindauer U, Dienel G, et al. Sugar for the brain: the role of glucose in physiological and pathological brain function. *Trends Neurosci*. 2013;36:587-597.
32. Hall CN, Klein-Flugge MC, Howarth C, et al. Oxidative Phosphorylation, Not Glycolysis, Powers Presynaptic and Postsynaptic Mechanisms Underlying Brain Information Processing. *J Neurosci*. 2012;32:8940-8951.
33. Lundgaard I, Li B, Xie L, et al. Direct neuronal glucose uptake heralds activity-dependent increases in cerebral metabolism. *Nat Commun*. 2015;6:6807.
34. Chalmers R, Stanley CA, English N, et al. Mitochondrial carnitine-acylcarnitine translocase deficiency presenting as sudden neonatal death. *J Pediatr*. 1997;131:220-225.
35. Portilla D, Dai G, Peters JM, et al. Etomoxir-induced PPARalpha-modulated enzymes protect during acute renal failure. *Am J Physiol Ren Physiol*. 2000;278:F667-675.
36. Price NT, van der Leij FR, Jackson VN, et al. A Novel Brain-Expressed Protein Related to Carnitine Palmitoyltransferase I. *Genomics*. 2002;80:433-442.
37. Dai Y, Wolfgang MJ, Cha SH, et al. Localization and effect of ectopic expression of CPT1c in CNS feeding centers. *Biochem Biophys Res Commun*. 2007;359:469-474.
38. Wolfgang MJ, Cha SH, Millington DS, et al. Brain-specific carnitine palmitoyl-transferase-1c: Role in CNS fatty acid metabolism, food intake, and body weight. *J Neurochem*. 2008;105:1550-1559.
39. Lee J, Wolfgang MJ. Metabolomic profiling reveals a role for CPT1c in neuronal oxidative metabolism. *BMC Biochem*. 2012;13:23.
40. Thevenet J, De Marchi U, Domingo JS, et al. Medium-chain fatty acids inhibit mitochondrial metabolism in astrocytes promoting astrocyte-neuron lactate and

ketone body shuttle systems. *FASEB J*. 2016;30:1913-1926.

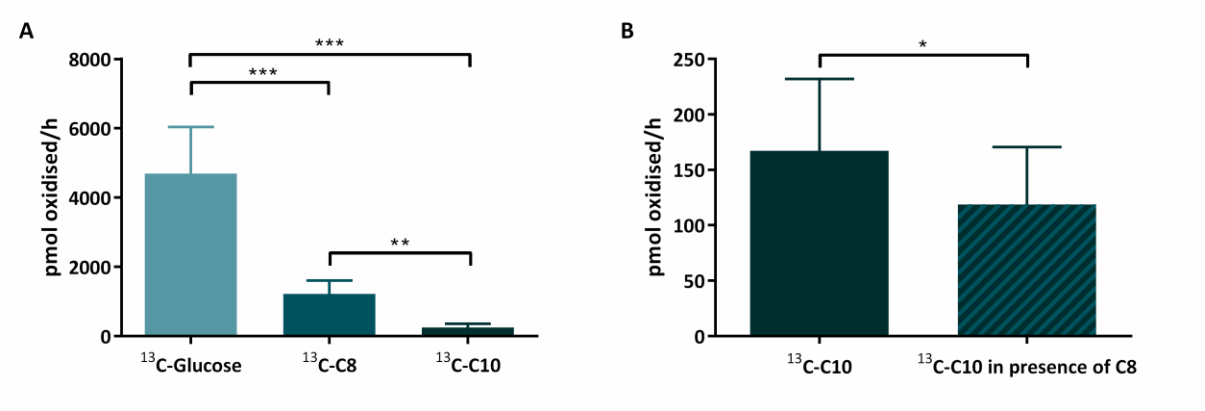

Figure 1.

*A) Absolute oxidation rates of ^{13}C-labelled 3mM glucose, 250µM C8 and 250µM C10 in SH-SY5Y cells per hour. The oxidation rate of [1-^{13}C]C10 was found to be significantly lower than that of [U-^{13}C]glucose (***p < 0.001) and [1-^{13}C]C8 (**p < 0.01). B) Effect of C8 co-incubation on* ***ß-oxidation*** *of C10 in SH-SY5Y cells. The* ***ß-oxidation*** *rate of 250µM [1-^{13}C]C10 was significantly reduced (*p = 0.0262) in the presence of 62.5µM unlabelled C8. Data are expressed as mean ± SD of 4 or 5 independent experiments (n=4, n=5), each performed in 4 replicate wells.*

	Oxidation rate (pmol/h)	
Compound	**- Etomoxir**	**+ Etomoxir**
[1-^{13}C]C8	1139 ± 336	750 ± 200**
[1-^{13}C]C10	198 ± 75	10 ± 28***
[U-^{13}C]C16	224 ± 105	2 ± 5***

Table 1.

Effects of CPT1 inhibition on the ***ß-oxidation*** *rates of C8 and C10 in SH-SY5Y cells. Cells were pre-treated for 20h with the irreversible CPT1 inhibitor etomoxir (50µM) and then incubated for 6h with ^{13}C-labelled 250µM C8, C10, or C16:BSA in the presence of 50µM etomoxir. Control cells were processed identically but were pre-treated and incubated in the absence of etomoxir. CPT1 inhibition resulted in a significant*

*reduction (***p < 0.001 compared with untreated cells) in C10* ***β-oxidation*** *by 95%. The* ***β-oxidation*** *rate of C8 was also significantly reduced by 34% (**p = 0.006 compared to untreated cells). Results are expressed as mean ± SD of 5 independent experiments (n=5), each performed in duplicate. CPT1 inhibition was confirmed with a significant 99% inhibition of [U-^{13}C]C16 oxidation (***p < 0.001 compared to untreated cells). Results are expressed as mean ± SD of 3 independent experiments (n=3), each performed in duplicate.*

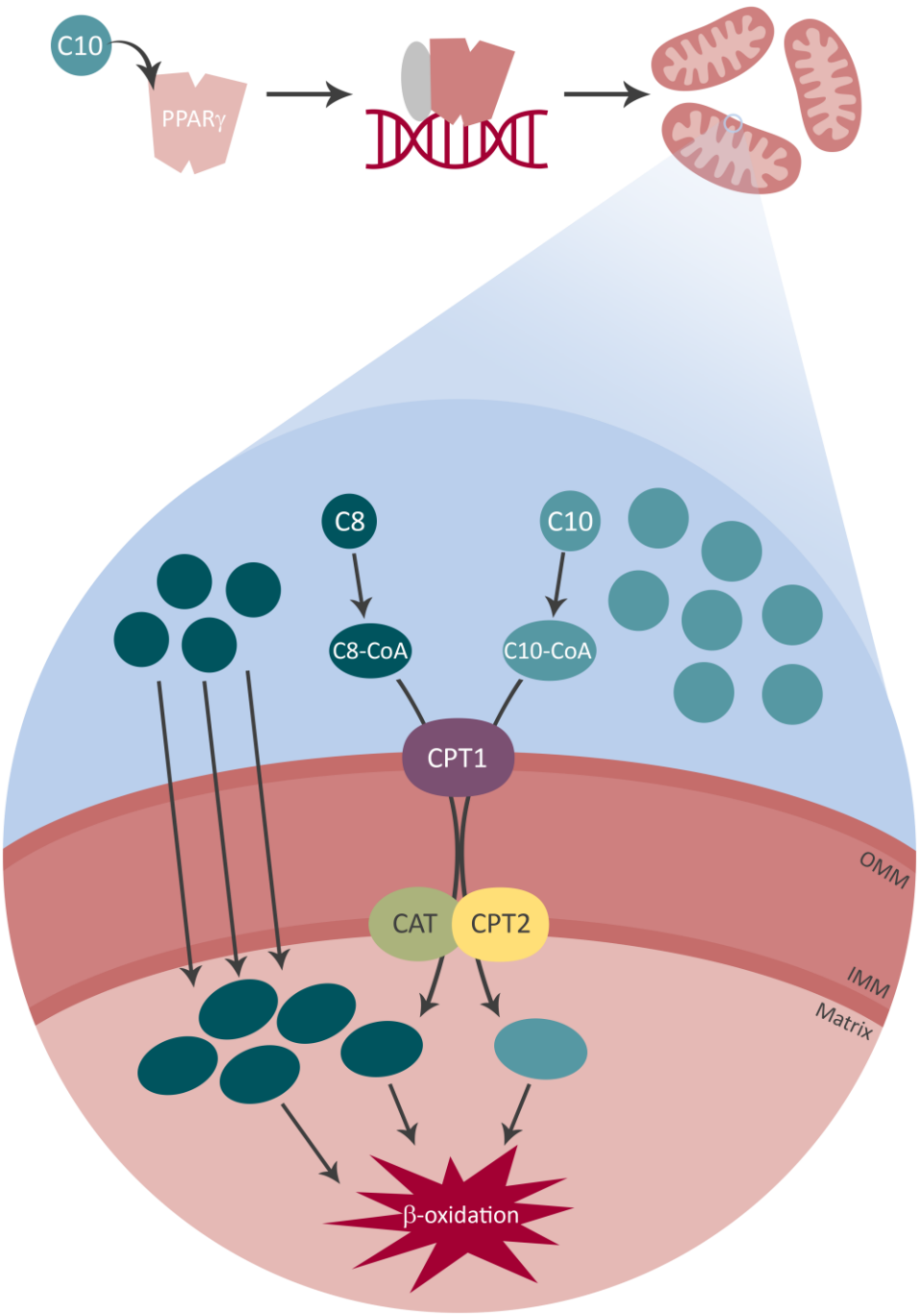

Figure 2.

C10 and C8 have differential effects with regards to potential seizure control, e.g. C10 acting as a PPARγ agonist, eliciting improvement in mitochondrial function. For this to occur, a sufficient neuronal concentration of C10 is required. It is proposed that due to its reliance on the carnitine

shuttle, β-oxidation of C10 is relatively slow, permitting accumulation of this fatty acid. In contrast, C8 oxidation may proceed largely in the absence of the carnitine system, contributing to β-oxidation and cellular energy metabolism. In addition, C8 may inhibit any C10 β-oxidation, further contributing to its accumulation.

C8 acyl-coenzyme A (C8-CoA); C10 acyl-coenzyme A (C10-CoA); carnitine palmitoyltransferase I (CPT1); carnitine-acylcarnitine translocase (CAT); carnitine palmitoyltransferase II (CPT2), Outer mitochondrial Membrane (OMM), Inner mitochondrial membrane (IMM).