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ORIGINAL ARTICLE

SH-SY5Y cells undergo changes in peroxisomal metabolism when exposed to decanoic acid

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Abstract

Medium-chain fatty acids (MCFAs), particularly decanoic acid (C10) and octanoic acid (C8), have garnered attention in recent years for their potential antiepileptic properties. A previous study from our laboratory demonstrated that C10 targets the PPARγ nuclear receptor, increasing the activity of the antioxidant enzyme catalase and thereby possibly modulating peroxisomal content. Here, we examined markers of peroxisomal content and activity in response to C10 and C8 exposure in neuronal-like SH-SY5Y cells. SH-SY5Y were treated with 250 mM C10 or C8 for a period of 6 days. Following this, biochemical markers of peroxisomal content and function were assessed, including acyl-coA oxidase activity, peroxisomal gene expression and peroxisomal VLCFA β-oxidation. Our findings revealed that C10 treatment augments acyl-CoA oxidase 1 (ACOx1) activity by 129% in comparison to control cells. An exploration into genes related to peroxisomal biosynthesis showed 23% increased expression of PEX11α upon C10 exposure, implying peroxisomal proliferation. Furthermore, it was observed that C10 exposure not only elevated ACOx1 activity but also enhanced peroxisomal β-oxidation of docosanoic acid (C22). Our findings bolster the premise that C10 functions as a peroxisome proliferator, influencing peroxisomal content and function. Further investigations are required to fully understand the mechanistic details as to how this may be beneficial in epilepsy and the potential implications with regards to peroxisomal disease.

KEYWORDS
C8, C10, MCT diet, metabolism, peroxisome, seizures

1 | INTRODUCTION

While the ketogenic diet (KD) is recognised as a potent intervention for drug-resistant epilepsy, the exact mechanisms driving its antiseizure effects remain elusive (Neal et al., 2009). Initial research suggested that the accumulation of ketone bodies may stimulate seizure reduction (Rho et al., 2002); however, plasma ketone body levels correlate poorly with seizure control (Likhodii et al., 2000). In addition, there are several disadvantages associated with the diet, including metabolic acidosis, severe

Abbreviations: ACOX1, acyl CoA oxidase 1; AT, aminotriazole; C10, decanoic acid; C22, docosanoic acid; C8, octanoic acid; CAT, catalase; DCFH, diacetyldichlorofluorescein; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulphoxide; FBS, foetal bovine serum; GCMS, gas chromatography mass spectrometry; KD, ketogenic diet; MCFA, medium chain fatty acid; MCFA, very long chain fatty acid; MCT, medium chain triglyceride; PPARγ, peroxisome proliferator activated receptor gamma; RRID, Research Resource Identifier.

Simon Heales and Simon Eaton contributed equally to this work.

gastrointestinal disturbances and hypoglycaemia, particularly in paediatric patients (Tong et al., 2022). The medium-chain triglyceride (MCT) version of the KD allows for a degree of liberalisation in carbohydrate restriction, which has been attributed to the ketogenic potential of the MCT KD (Kossoff et al., 2009; Neal, 2012). However, there is a growing body of evidence suggesting that the medium chain fatty acids (MCFA) octanoic acid (caprylic acid; C8) and decanoic acid (capric acid; C10), components of the MCT diet, play a role in the observed seizure reduction observed on the diet (Jancovski et al., 2021).

There have been several proposed mechanisms through which C10 and C8 may lead to the seizure reduction seen on the MCT KD. These include binding to and inhibiting the AMPA receptor (Chang et al., 2016). Overactivation of AMPA receptors has been linked to excitotoxicity and the propagation of seizure activity. By binding to and inhibiting these receptors, C10 may reduce excessive excitatory neurotransmission, thereby lowering the likelihood of seizure occurrence. It has been demonstrated that C10, but not C8, binds to the PPARγ nuclear receptor (Malapaka et al., 2012). This interaction has been shown to stimulate alterations in cellular metabolism—particularly that of the mitochondria. SH-SY5Y cells exposed to C10 for a period of 6 days were demonstrated to have increased activities in several mitochondrial markers, including the activities of citrate synthase and mitochondrial respiratory chain complex I (Hughes et al., 2014). While C8 was not found to elicit any biological effects through targeting either the AMPA receptor (Chang et al., 2016) or PPARγ (Hughes et al., 2014), it has been demonstrated that co-administration of C10 and C8 in SH-SY5Y cells leads to a slower oxidation rate of C10, allowing it to accumulate (Khabbush et al., 2017). This 'sparing' mechanism, where C8 is preferentially oxidised in neuronal cells, is suggested to provide a route through which C10 can accumulate in vivo to levels needed in previous in vitro studies to stimulate biological effects—>200 μM for PPARγ activation (Malapaka et al., 2012). Indeed, a recent study demonstrated in the *Scn1a*$^{R1407X/+}$ mouse model of Dravet syndrome that mice fed a diet of C10/C8 in an 80:20 ratio reached the highest levels of C10 in the brain (approximately 200 μM), which was also mirrored in increases in mitochondrial content and improvements in antioxidant status (Jancovski et al., 2021). Further, a recent human study indicated that dietary supplementation of C10 and C8 in an 80:20 ratio led to significant increases in plasma levels of C10, but not ketones, that correlated significantly with seizure reduction (Schoeler et al., 2021).

Interestingly, the activity of catalase (CAT), a peroxisomal marker, has also been demonstrated to be increased in response to C10 exposure in both SH-SY5Y cells and *Scn1a*$^{R1407X/+}$ mouse brain (Hughes et al., 2014; Jancovski et al., 2021). The mitochondria and peroxisome are closely linked in numerous cellular functions, such as energy metabolism via fatty acid oxidation, reactive oxygen species modulation, phospholipid biosynthesis and both organelles are impacted by mitochondrial and peroxisomal disease (Demarquoy, 2015). Therefore, for any alterations in mitochondrial content, as seen in previous studies, a corresponding shift in peroxisomal content may be essential to preserve the balance in the critical cellular processes jointly managed by these organelles. Peroxisomes also play a role in several major processes in neuronal development and homeostasis, including myelin sheath formation (Kassmann, 2014), glutamatergic signalling (Sasabe et al., 2014) and neuronal migration (Janssen et al., 2003). The importance of the peroxisome to CNS health is highlighted by peroxisomal disorders such as Zellweger's syndrome, in which patients present with severe neuronal phenotypes, such as seizures, hypotonia and early death (Kheir, 2011). In view of this, the aim of this study is to investigate the effects of C10 and C8 on peroxisomal biosynthesis and metabolism to further our understanding of how the peroxisome may contribute to the beneficial effects of MCT supplementation in drug-resistant epilepsy.

2 | METHODS

2.1 | Materials

Unless specified otherwise, all reagents were sourced from Sigma Aldrich (Poole, UK). The following products: DMEM/F-12 (1:1 ratio, with 4.5 g/L glucose; Cat no. 11540566), heat-treated foetal bovine serum (FBS; Cat no. 10500064), trypsin–EDTA (2.5 g/L; Cat no. 11560626) and Trypan Blue solution (0.4%; Cat no. 11538886) were acquired from Fisher Scientific Ltd. (Leicestershire, UK). The DNeasy Blood and Tissue kit was obtained from Qiagen (Manchester, UK; Cat no. 69506). Products including TRIzol reagent (Cat no. 11588616), RNase away spray (Cat no. 17894594) and RNase-free 1.5-mL microfuge tubes were purchased from Thermo-Fisher Scientific (Loughborough, UK). iQ SYBR Green Supermix was purchased from Bio-rad Laboratories Inc. (Hertfordshire, UK). The SensiFast cDNA synthesis kit was from Bioline (London, UK). Stable isotope-labelled fatty acids were sourced from CDN Isotopes (Quebec, Canada) and Cambridge Isotopes (Andover, USA). The SH-SY5Y neuroblastoma cell line (RRID—CVCL_0019; not listed as a commonly misidentified cell line by ICLAC) was sourced from the European Collection of Cell Cultures under Public Health England in Salisbury. Image figures were created with BioRender.com.

2.2 | Cell culture

SH-SY5Y cells were stored in liquid nitrogen and were used at passages 20–24 (Hughes et al., 2014). Cells were cultured in 75 cm^2 flasks in a medium containing DMEM/F-12 Ham's 1:1 mixture, 17.5 mM glucose, 10% heat inactivated foetal bovine serum and 10 mL/L 200 mM L-Glutamine and cultured at 37°C and 5% CO_2. Cells were harvested from the flask via trypsinization. Cells were treated with either 250 μM C10 or C8 for a period of 6 days control cells were treated with vehicle (DMSO). After the 6-day period, cells were harvested, and peroxisomal markers were assessed.

2.3 | Measurement of acyl-CoA oxidase activity

The acyl-CoA oxidase 1 (ACOx1; EC 1.3.3.6) activity assay was adapted from (Small et al., 1985). The reaction was adapted for the Tecan Infinite/F200 plate reader. Each 200 μL reaction contained 4 μL cell homogenate, 4 μL horseradish peroxidase, type VI (HRP; 20 mg/mL), 4 μL 2.6 mM diacetyldichlorofluorescein (DCFH), 10 μL 1 M aminotriazole and 172 μL 11 mM potassium phosphate buffer, pH 7.4. The reaction was started upon the addition of 30 μM palmitoyl-CoA and followed at 30°C for 1.5 h at a wavelength of 502 nm. Activity is derived using the Beer-Lambert law and the DCFH molar extinction coefficient (91 000 M-1.cM-1; Small). Results are presented in nmol/hour/mg protein.

2.4 | qPCR-based analysis of alterations in peroxisomal gene expression

mRNA was extracted from SH-SY5Y cells and cDNA was synthesised. Specific primers were designed to target cDNA GAPDH, PEX11α and ACOX1 using Primer3 software (Table S1). Changes in expression of the peroxisomal genes PEX11α and ACOX1 were analysed, relative to expression of GAPDH, in C10 treated SH-SY5Y cells. Gene expression was assessed by RT-qPCR using the Applied Biosystems StepOnePlus Real-time PCR system. Each reaction consisted of 1 μL diluted cDNA, 250 nM Primers, 12.5 μL iQ SyBr Green Supermix and nuclease-free water to 20 μL. The reaction course consisted of an initial 5-min stage at 95°C, followed by a 44-cycle stage of 45 s at 95°C, to 60°C for 15 s, followed by a 3-min holding stage at 60°C. Changes in the expression of target genes were related to the expression levels of the nuclear housekeeping gene GAPDH. Relative gene expression was calculated using the ΔΔCT method (Livak & Schmittgen, 2001). Statistical significance was calculated utilising ΔCt data.

2.5 | GC–MS based analysis of peroxisomal β-oxidation

The GCMS-based analysis of peroxisomal β-oxidation is adapted from the assay described (van de Beek et al., 2017). To assess the impact of C10 and C8 exposure on peroxisomal β-oxidation of C22, a physiologically important substrate for peroxisomal metabolism, SH-SY5Y cells were cultured and cell treatment was carried out as above. After the 6-day period, cells were harvested and reseeded at a density of 1×10^3/mL SH-SY5Y cells. Using C22 as substrate, we analysed the alterations in levels of chain-shortened products (C20, C18 and C16) down to C14 as below this point (C8-C12), MCFA is transported to the mitochondria for further β-oxidation. Fatty acids were deuterium-labelled on the ω-carbon, meaning no labelling was lost through β-oxidation and chain shortened fatty acids could be distinguished from unlabelled endogenous fatty acids. Cells were incubated with 30 μM C22-d_3 for a period of 72 h, then harvested as above. Prior to analysis, samples were thawed and kept on ice. One hundred microlitres of sample/cell media/labelled fatty acid standard were added to individual glass vials, along with 100 μL of internal standard (20 μM C16-d_{31} in ethanol). All samples were then acid hydrolysed in 1 mL of ACN/37% HCL (4:1) for 2 h at 90°C. Fatty acids were then extracted in 2 mL of hexane and centrifuged at 1855 *g* for 5 min. The upper phase was then moved to a fresh glass vial and dried under nitrogen. Samples were derivatised in 10 μL triethylamine and 50 μL 10% pentafluorobenzyl bromide in acetonitrile for 15 min. Following this, the samples were dried down again under nitrogen. Finally, the dried samples were redissolved in 100 μL of ethyl acetate, vortexed and transferred to glass autosampler insert vials. Labelled fatty acids were analysed using a Thermo-Scientific Trace GC Ultra, DSQII mass spectrometer, Triplus sample injector, Zebron ZB-1 fused silica column, 15 m × 0.25 mm × 0.1 μm film thickness (Phenomenex) and XCalibur V 3.0.63 software. Two microlitres of sample were injected into the GC system and data are collected and analysed using XCalibur V 3.0.63 software. Helium flow rate: 0.8 mL/min, inlet temperature: 250°C, methane flow rate: 2 mL/min and split ratio: 1/12.

Temperature profile:

Temperature (°C)	Rate (°C/min)	Hold (min)
150	-	
270	10	
310	40	1

Detection mode—Selected ion monitoring:

Compound	m/z
C24-d_3	370
C22-d_3	342
C20-d_3	314
C18-d_3	286
C16-d_3	258
C14-d_3	230
C12-d_3	202
C16-d_{31} (internal standard)	286

2.6 | Total protein

Total protein content of analysed cell samples was assessed using the Bradford protein assay (Bradford, 1976).

2.7 | Statistical analysis

Statistical evaluations were conducted using GraphPad Prism 8 software (Version 10.2.1, San Diego, CA, USA). Values are presented as mean ± standard deviation (SD) or as stated. An unpaired Student's *t*-test was employed for individual comparisons, while a one-way ANOVA followed by a Tukey's post hoc test was used when

comparing multiple groups. A result was deemed statistically significant with a p-value less than 0.05. The normality of the data was assessed via the Shapiro-Wilk test; the data were deemed to pass normality testing if $p > 0.05$. No tests for outliers were conducted.

3 | RESULTS

3.1 | Exposure to C10, but not C8, lead to an increase in ACOx1 activity

To determine whether C10 or C8 influence peroxisomal metabolism, ACOx1 activity was assessed after a 6-day treatment with either a vehicle control (DMSO), 250 μM C10 or C8. No difference in ACOx1 activity was observed between the untreated and the vehicle-treated SH-SY5Y cells. However, a marked increase in ACOx1 activity was seen post-C10 exposure (3.33 ± 0.84 nmol/hour/mg protein), compared with control cells (1.45 ± 0.4 nmol/hour/mg; $p = 0.0009$, $F = = 14.49$, Df = 15) and C8-treated (1.46 ± 0.59 nmol/hour/mg; $p = 0.0009$, $F = 14.49$, Df = 15) cells (Figure 1); there was no difference in ACOx1 activity between the C8-treated and the control cells (Figure 1).

3.2 | Treatment with C10 lead to an increased expression of a marker of peroxisomal biogenesis

Building on the observation that C10 exposure may induce enhanced peroxisomal metabolism, we examined the expression levels of two peroxisomal marker proteins, PEX11α and ACOX1, to assess peroxisomal biogenesis. There was a highly significant increase in PEX11α abundance levels compared with control cells ($p = 0.005$, $t = 4$, Df = 20) and a non-significant increase in ACOX1 relative abundance ($p = 0.182$, $t = 1$, Df = 20), equivalent to a median 1.23-fold increase in PEX11α expression and a 1.19-fold increase in ACOX1 (Figure 2).

3.3 | Alterations in ACOx1 activity and peroxisomal gene expression corresponded to enhanced peroxisomal β-oxidation

We then sought to determine the effect of C10 and C8 exposure on peroxisomal β-oxidation of C22 in SH-SY5Y cells. Cells exposed to C10 for 72 h showed a significant increase in the formation of C18-d_3 ($p = 0.0482$, $F = 5.713$, Df = 14), C16-d_3 ($p = 0.0015$, $F = 14.99$, Df = 14) and C14-d_3 ($p = 0.0016$, $F = 14.66$, Df = 14) relative to control cells (Figure 3b–d). Conversely, no significant change in the formation of chain-shortened products was seen in cells exposed to C8 when compared to controls. This suggests that C10 exposure amplifies the entire peroxisomal β-oxidation process. No intermediates shorter than C14 were detected, suggesting complete mitochondrial oxidation of chain-shortened fatty acids.

4 | DISCUSSION

In prior studies, it was observed that C10 targets the nuclear receptor PPARγ, enhancing the activity of the antioxidant enzyme CAT (Hughes et al., 2014). CAT is mainly located within the peroxisome (Walton, 2012) and PPARγ ligands have been demonstrated to induce peroxisomal biogenesis in neural cells (Diano et al., 2011; Zanardelli et al., 2014). This suggests that C10-mediated increases in CAT activity may be a product of increased peroxisomal biogenesis. The aim of this study was to delve deeper into C10's impact on peroxisomal metabolism.

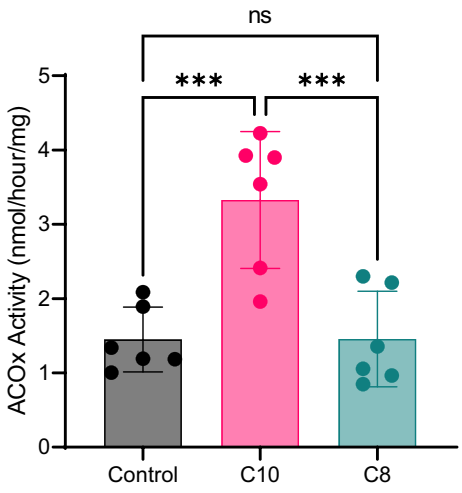

FIGURE 1 Analysis of ACOx1 activity in control, C10 and C8-treated SH-SY5Y cells. Following exposure to 250 μM C10 for a period of 6 days, there was a 129% increase in ACOx1 activity ($p < 0.001$). There was no observable difference in C8-treated cells. Data are expressed as mean ± SD. $n = 6$ independent cell culture preparations per treatment group.

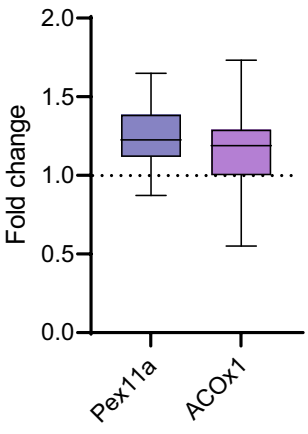

FIGURE 2 qPCR analysis of peroxisomal gene expression. There was a 1.23-fold increase in relative *PEX11α* expression in C10-treated SH-SY5Y compared with control ($p = 0.005$ for ΔC_t) and a 1.19-fold increase in relative ACOX1 expression ($p = 0.182$ for ΔC_t). Data are expressed as median, IQR and range. $n = 11$ independent cell culture preparations per group.

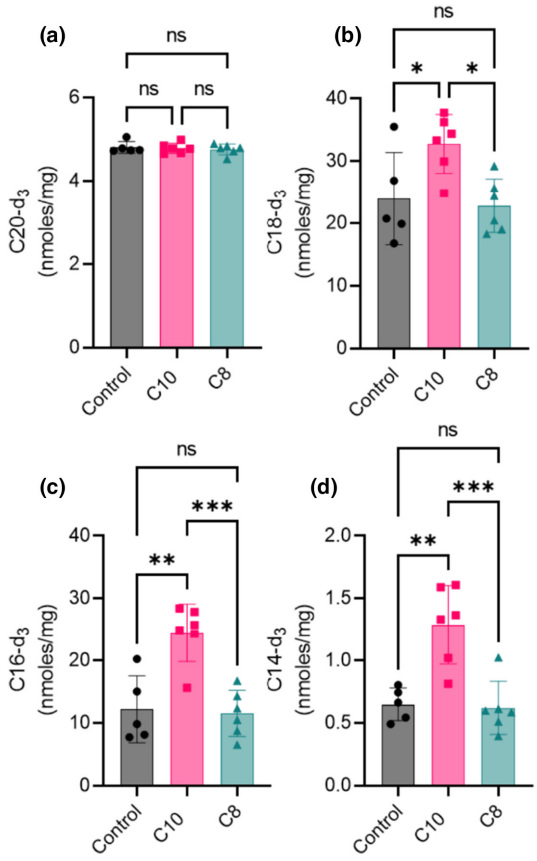

FIGURE 3 Effect of C10 and C8 on peroxisomal β-oxidation of C22 in SH-SY5Y cells. There was found to be no significant difference in levels of (a) C20-d_3. The amounts of (b) C18-d_3 ($p=0.0482$), (c) C16-d_3 ($p=0.0015$) and (d) C14-d_3 ($p=0.0016$) were found to be significantly increased in cells treated with C10 but not C8. Data are expressed as mean ± SD. $n=5$ in the control group and $n=6$ independent cell culture preparations in both C10 and C8 treatment groups.

The enzyme ACOx1 initiates the first reaction in the peroxisomal fatty acid β-oxidation pathway and is a recognised marker for peroxisome-proliferator responses (Lee et al., 1997). Analysis of its activity in this study revealed comparable ACOx1 activity to that observed in mouse cerebral cortex studies (Sato et al., 1996). Under the same conditions that we previously evaluated the effect of C10 on mitochondrial content (Hughes et al., 2014), we observed a significant increase in the activity of ACOx1. This, coupled with our previous finding of a PPARγ dependent increase in CAT activity, suggests increased peroxisomal biogenesis in neuronal-like cells in response to C10 exposure.

To better comprehend the potential influence of C10 exposure on peroxisomal content/metabolism, we examined changes in the expression of two genes, PEX11α and ACOX1, associated with peroxisomal biosynthesis. PEX11α is known to play a role in peroxisomal biosynthesis. This gene was selected for study as its expression has been shown to be increased in response to agents known to induce peroxisomal biogenesis, such as phenylbutyrate (Schrader et al., 2012), which is also a known PPARγ agonist. Our data reveals that PEX11α expression is enhanced following C10 exposure, suggesting peroxisomal proliferation. Expression of ACOX1, the gene encoding acyl-CoA oxidase 1, was elevated, but not significantly, despite the significant increase in enzyme activity. Post-transcriptional control of acyl-CoA oxidase 1 is poorly understood, although regulation of activity via succinylation/desuccinylation may regulate hydrogen peroxide production (Chen et al., 2018). Whilst GAPDH is a widely used housekeeping gene for these types of studies, the effects of MCFA treatment on GAPDH mRNA levels have not, to the best of our understanding, been investigated. Therefore, it is possible that the effects of C10 on ACOX1 mRNA are being masked by alterations in GAPDH mRNA. Further work will investigate other housekeeping genes in this assay. A further point to consider is the half-life of ACOX1 mRNA and total protein levels, which may provide insight into the discrepancy between qPCR and enzyme assay findings.

Taken together, the data on acyl-CoA oxidase activity and PEX11α mRNA levels (this work) together, with previous data showing increased catalase activity (Hughes et al., 2014), suggest that peroxisomal biogenesis occurs following exposure to C10. To determine whether biogenesis is associated with increased peroxisomal function, we established a GC–MS-based assay to measure peroxisomal β-oxidation of C22. Our results revealed a significant increase in chain shortened product formation in cells that had been pretreated with C10 compared to control (vehicle) and C8-treated cells. C10-treated cells reported higher levels of C18-, C16- and C14-d_3 formation after 72 h compared to control and C8-treated cells; however, the levels of C14-d_3 formation were minimal compared to the longer chains. This suggests that C10 exposure not only increases the activity of ACOx1 but also the entire peroxisomal β-oxidation pathway.

As with prior studies (Hughes et al., 2014; Jancovski et al., 2021), we observe no alterations in cellular metabolism in response to C8 exposure. It has been demonstrated that C10, but not C8, is capable of binding to and activating the PPARγ receptor (Malapaka et al., 2012). This potentially explains the differential effect on peroxisomal content and metabolism reported here. However, C8 has been observed to play an important 'sparing' role in terms of preventing the metabolism of C10 when used in an 80:20 ratio (C10:C8; Khabbush et al., 2017), allowing for C10 to accumulate to biologically relevant levels. Future work will investigate the impact of optimising the effects of C10 exposure on peroxisomal content with the addition of C8.

In conclusion, the data presented here further supports the suggestion indicating that C10 may also act as a peroxisome proliferator in the SH-SY5Y cell line. Cells treated with 250 μM C10 for a period of 6 days resulted in increases in ACOx1 activity and expression of the peroxisomal biosynthesis gene *PEX11α*, indicative of peroxisomal biosynthesis. This increase in peroxisomal content is matched with improved function, with C10-treated cells exhibiting increased rates of peroxisomal β-oxidation of VLCFA compared to control and C8-treated cells. Considering previous work indicating mitochondrial biogenesis in response to C10 treatment, this improvement

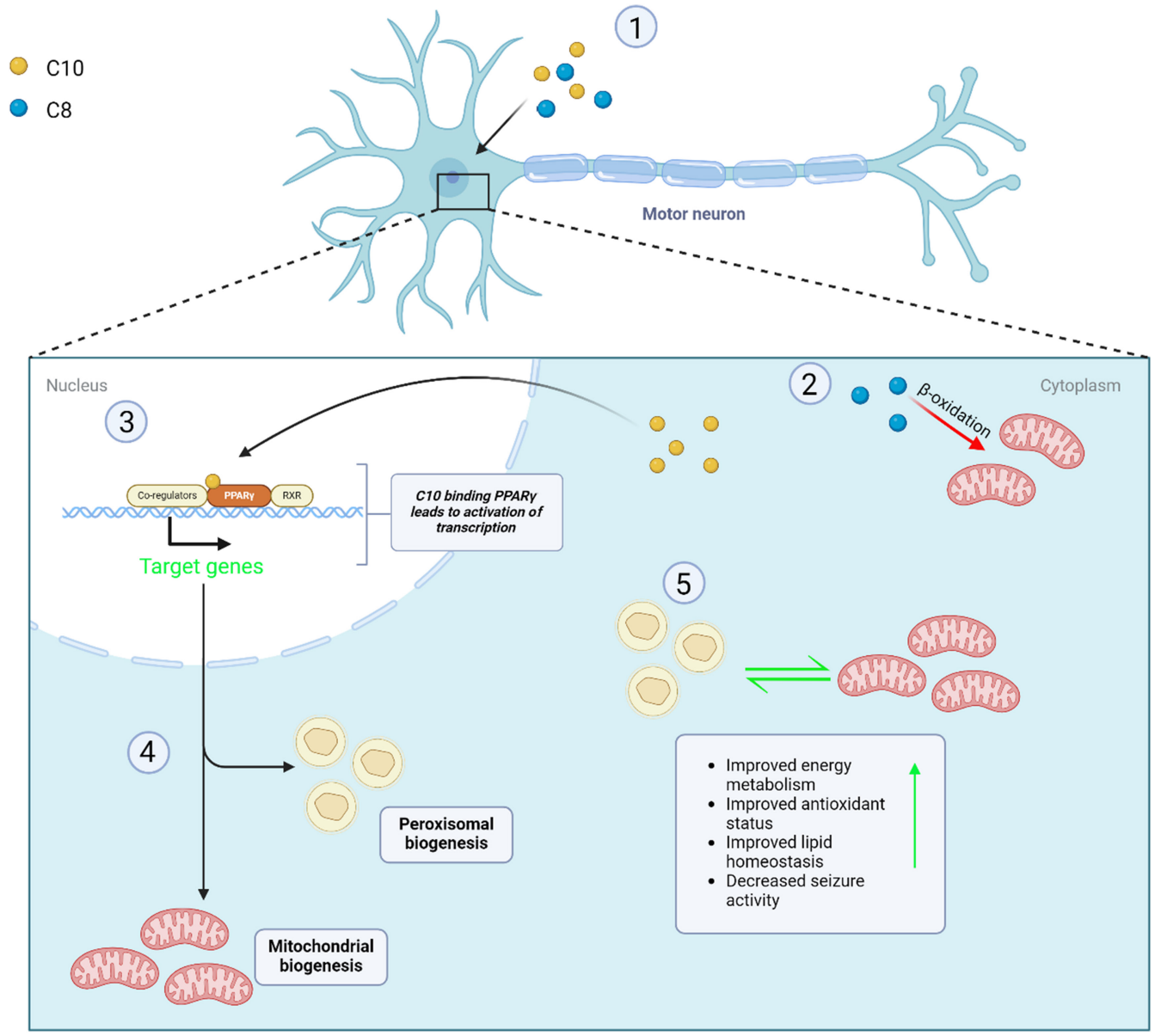

FIGURE 4 Overview of the proposed beneficial effects of C10 and C8 on neuronal function. (1) C10 and C8 accumulate in the brain and enter neuronal cells. (2) C8 is preferentially oxidised by mitochondria over C10, allowing for the intracellular accumulation of C10. (3) C10 interacts with the nuclear receptor PPARγ, leading to the upregulation of target genes and, subsequently, (4), increased mitochondrial and peroxisomal biogenesis. (5) this increase in cellular mitochondrial and peroxisomal content is thought to have several beneficial effects on neuronal function, including improved energy metabolism, antioxidant status and lipid homeostasis.

in peroxisomal content and function is an important finding, as an imbalance between mitochondrial and peroxisomal metabolism could lead to undesirable outcomes including lipolysis and increased cellular oxidative stress levels. Increases in CAT activity (Koepke et al., 2007) and peroxisomal β-oxidation (Liepinsh et al., 2013) have been demonstrated to improve mitochondrial function and protect the organelle from oxidative damage, which may provide protection from seizure propagation in epilepsy. Further, following seizure activity, there is an increase in lipid peroxidation in the brain (Júnior et al., 2009). An increase in peroxisomal content following C10 treatment may provide a mechanism of clearance of these deleterious substances before secondary damage occurs in the brain. Through the modulation of these important pathways—energy metabolism, oxidative stress, etc.—C10 elicits several beneficial effects on neuronal function and health, suggesting that MCFA treatment may be useful in neurological conditions where alterations in these pathways are pathogenic, such as Alzheimer's and Parkinson's disease (Jo et al., 2020; Figure 4). The works presented here indicate a possible use for C10 administration in peroxisomal disorders. Recent investigations have demonstrated the potential for glitazones, such as pioglitazone, as therapeutic strategies in x-linked adrenoleukodystrophy via PPARγ agonism (Monternier et al., 2022). However, glitazones, including pioglitazone, are associated with numerous undesirable side effects, including weight gain, bone loss and an

increased risk of congestive heart failure in at-risk individuals (Shah & Mudaliar, 2010). Long-term administration of MCT with a high concentration of C10 has been demonstrated to have a good safety profile with limited undesirable side effects (Schoeler et al., 2021). In addition, a study using a mouse model of Refsum disease demonstrated that PPAR activation led to alterations in liver metabolism of fatty acids—ω-oxidation and dicarboxylic acid β-oxidation—but led to no observable aberrations in the brain (Khalil et al., 2022). This suggests that C10 has potential as a glitazone alternative in the treatment of some peroxisomal disorders via PPARγ agonism. Further work will be undertaken to determine the efficacy of C10 in this area.

AUTHOR CONTRIBUTIONS

Tomas Baldwin: Conceptualization; methodology; data curation; formal analysis; writing – original draft. **Peter Clayton:** Conceptualization; writing – review and editing. **Tricia Rutherford:** Conceptualization; writing – review and editing. **Simon Heales:** Conceptualization; methodology; writing – original draft; writing – review and editing. **Simon Eaton:** Conceptualization; methodology; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

T.B. has received support from Vitaflo during the conduct of the study. T.R. is employed by Vitaflo International, a for-profit company that develops innovative foods for special medical purposes (FSMPs). S.H. and S.E. have received grants from Vitaflo during the conduct of the study. A patent relating to this work has been submitted by UCL Business PLC and Vitaflo. None of the other authors have any conflicts of interest to disclose. 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.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/jnc.16185.

DATA AVAILABILITY STATEMENT

Data is available upon reasonable request to the authors.

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SUPPORTING INFORMATION

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