Medium chain triglyceride ketogenic diet in neurological and metabolic disorders

Katrin Augustin BSc[1], Aziza Khabbush PhD[2], Sophie Williams PhD[3], Simon Eaton PhD[2], Michael Orford PhD[2], J Helen Cross PhD[4], Simon J R Heales PhD[2*], Matthew C Walker PhD[3*], Robin S.B. Williams PhD[1*]

[1]Centre for Biomedical Sciences, School of Biological Sciences, Royal Holloway University of London, Egham, TW20 OEX, UK; [2]Clinical and Molecular Genetics Unit, University College London Institute of Child Health, London, WC1N 3JH, UK. [3]Department of Clinical and Experimental Epilepsy, Institute of Neurology, University College London, London, WC1N 3BG, UK; [4]Neurosciences Unit, UCL Institute of Child Health, London, WC1N 1EH, UK. *These authors contributed equally

Corresponding author

Robin S B Williams
Centre for Biomedical Sciences, School of Biological Sciences, Royal Holloway University of London, Egham, TW20 OEX, UK
Robin.Williams@rhul.ac.uk

Glossary

Aβ: amyloid β, a small peptide involved in Alzheimer's disease pathology
AD: Alzheimer's disease
AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, a key glutamate receptor that is targeted by epilepsy treatments
Decanoic acid: a medium chain fatty acid of ten carbons
GBM: glioblastoma multiforme, an aggressive form of brain tumour
GluA1-4: subunits of AMPA receptors
Ketones: D-β-hydroxybutyrate (BHB) and acetoacetate (ACA)
Ketogenic: generating ketones
MCT: medium-chain triglyceride
Medium chain fatty acids: a fatty acid of 6-12 carbons in length, often derived from MCT
Octanoic acid: a medium chain fatty acid of eight carbons
PPARγ: peroxisomal proliferator-activated receptor gamma
PTZ: pentelenetetrazol an epileptogenic compound use to generate seizures

Abstract

The ketogenic diet has been used for almost 100 years as a non-pharmacological treatment for refractory epilepsy; the generation of ketones was proposed to be a key mechanism by providing neurons with an energy source that is more efficient than glucose, resulting in beneficial downstream metabolic changes. However, *in vitro* and *in vivo* studies have challenged the central role of ketones as medium chain fatty acids, which are part of a commonly used ketogenic diet, the medium chain triglyceride (MCT) ketogenic diet, have been demonstrated to directly inhibit AMPA receptors (key excitatory neurotransmitter receptors), and to change cell energetics through mitochondrial biogenesis. Through these mechanisms, medium chain fatty acids are likely to block seizure onset and raise seizure threshold, These mechanisms may also play roles in the ketogenic diet's potential in other therapeutic areas, such as reducing neurodegeneration in Alzheimer's disease, proliferation and spread of cancer, and insulin resistance in type 2 diabetes. Analyzing medium chain fatty acids in future ketogenic diet studies will provide further insights into their importance in other forms of the ketogenic diet. Moreover, the results of these studies may facilitate the development of new pharmacological and dietary therapies.

Introduction

The ketogenic diet, a high-fat, low-carbohydrate diet, was developed nearly one century ago as a treatment for epilepsy to mimic the metabolic profile of fasting by reducing blood glucose and increasing ketone levels, as starvation had long been observed to reduce the frequency of seizures. In the 1920's and 1930's, the ketogenic diet became an established treatment for epilepsy[1], but rapidly lost favor following the development of phenytoin and the subsequent growth in antiepileptic drug development. However, there was a resurgence of interest in the diet in the 1990's for drug-resistant epilepsy in children in whom it is increasingly being used. Despite its long and burgeoning use, the mechanisms underlying its efficacy in epilepsy have remained unclear. Recent advances, however, have resulted in a paradigm shift in our understanding of the putative mechanisms underlying such diets, and have paved the way for novel dietary and drug therapies.

The ketogenic diet exists in two main forms. The "classic" ketogenic diet provides 60-80% of dietary energy through long chain fats (comprising 16-20 carbons)[2]. This diet is particularly stringent (there is a very low carbohydrate content) and consequently is difficult to maintain. So an alternative medium-chain triglyceride (MCT) ketogenic diet was developed[2], where fats are provided though triglycerides comprising ~60% octanoic (an eight carbon fatty acid) and ~40% decanoic acid (a ten carbon fatty acid). In contrast to the classic ketogenic diet, only about 45% of dietary energy is provided by these medium chain fats (so allowing a larger carbohydrate component) [2], and more rapid metabolism of the shorter fatty acid results in more efficient ketone generation.

The MCT ketogenic diet is currently used world-wide to treat drug-resistant epilepsy, mainly in children[1], but also in adults[3,4]. In addition, both the classical and MCT ketogenic diets have garnered increased interest as potential treatments for other diet-sensitive disorders, including Alzheimer's disease,[5-7] cancer,[8-12]and diabetes,[13,14] As with epilepsy, the main therapeutic mechanism was assumed to occur through the replacement of carbohydrates by ketones as an alternative energy source[15]. However, despite the efficacy of the ketogenic diet in epilepsy, several studies have shown a poor correlation between plasma ketone levels and seizure control[16], and ketones do not acutely block seizure activity in an in vitro model.[17] Indeed, one study has shown seizure control in the absence of ketosis.[18] These observations challenge the view that ketones alone have a role in seizure control and raise the question of the roles of other components, in particular, the high fat content. Several studies have indicated that medium chain fats provided in the MCT ketogenic diet, can have a direct action on seizure activity and mitochondrial function. The aim of this review is to summarize the most recent advances in our understanding of the mechanisms of action of the MCT ketogenic diet, in relation to epilepsy and other disorders.

Metabolism of the MCT ketogenic diet

Dietary triglycerides (the main form of dietary fat in the body) are hydrolyzed in the gut and intestines by lipases that preferentially hydrolyze medium-chain over long-chain esters[19] (Figure 1). Medium-chain triglycerides are hydrolyzed to medium-chain fatty acids (fatty acids with 6-12 carbons), which are then absorbed directly through the gut wall, and transferred to the liver where they are rapidly degraded in first-pass metabolism[19]. The liver metabolises these medium chain fatty acids through β-oxidation, which is mainly directed towards the generation of three major ketones, β-hydroxybutyrate, acetoacetate, and acetone (collectively called 'ketone bodies'). These ketones as well as those fats that escape metabolism are distributed through the circulatory

system in blood. The brain is thought to be dependent primarily on glucose as an energy source, and secondarily on hepatically-derived ketone bodies. However, medium chain fatty acids are able to cross the blood-brain barrier[20,21], reach brain concentrations that are >50% that of plasma fatty acids[20] and provide an alternative energy source for astrocytes. Evidence indicates that medium chain fatty acids have direct and differing effects on astrocyte energy metabolism. Octanoic acid seems to undergo β-oxidation in astrocytes more easily than does decanoic acid, and so more readily produces ketones, whereas decanoic acid preferentially stimulates glycolysis, producing lactate[22] which neurons are able to use as an energy source. Decanoic acid could promote the proposed astrocyte-neuron lactate shuttle, which has been proposed to be the main energy source for neurons; however the importance of this shuttle *in vivo* has been challenged.[23] In addition, neurons are also capable of β-oxidation of medium chain fatty acids at low rates, but octanoic acid is preferentially oxidized (over decanoic) suggesting a key metabolic role in the regulation of medium chain fat levels.[24]

The MCT ketogenic diet and epilepsy

Ketones and seizures

Under normal dietary conditions, ketones (acetoacetate, beta-hydroxybutyrate, and acetone) are found in blood plasma at very low levels, but their concentration increases under fasting conditions up to a total of 9 mM/L and can be taken up by brain, crossing the blood-brain barrier via monocarboxylate transporters[25]. Under fasting conditions, ketones can provide the energy source for cells, and have been considered the key mechanism of action of the ketogenic diet[15,26]. Patients with mutations of the glucose transporter, GLUT1, which plays a critical role in glucose transport from the systemic circulation to the brain, respond well to both classical and MCT ketogenic diets because ketones are thought to replace the energy supply normally provided by glucose.[27] There is evidence that glucose supplementation diminishes the anticonvulsant effects of the ketogenic diet in a mouse model of epilepsy, so that both fat administration and carbohydrate restriction in the ketogenic diet may be important in seizure control.[28] It is also likely that ketone bodies influence amino acid metabolism, either directly as substrates or indirectly, resulting in changes to GABA and glutamate concentrations.[29] But do ketones have any direct effects on synaptic transmission or intrinsic neuronal excitability or can they directly or indirectly modify neuronal or network excitability ? Neither β-hydroxybutyrate, nor acetoacetate affect ionotropic GABAergic (GABA(A) receptor mediated) or glutamatergic (AMPA and NMDA receptor mediated) currents at therapeutically relevant concentrations.[30] Acetone and β-hydroxybutyrate only affect GABA(A) receptors and glycine receptors at toxic levels (>100 mM).[31] Nevertheless, there is a suggestion that ketones can compete with chloride at the vesicular glutamate transporter, so decreasing vesicular glutamate content and consequently glutamatergic transmission.[32] In addition, high concentrations of acetoacetate (10 mM) have been shown to inhibit voltage-dependent Ca^{2+} channels (VDCCs) in pyramidal cells of the hippocampus.[33] However, ketones at high concentrations (10 mM) have no direct effects on *in vitro* seizure-like activity induced in *ex vivo* hippocampal slices by applying the GABA(A) receptor antagonist, PTZ[17], or exposing them to low external magnesium.[17] The evidence, therefore, despite a possible effect on glutamatergic transmission does not support a direct action of ketones on seizure activity.

Ketones can, however, have indirect effects on neuronal and network excitability, and have anti-seizure effects in some in vivo seizure models.[34-36] Switching from glucose to ketones results in a hyperpolarization of neurons and a reduction in neuronal excitability. One indirect mechanism could be the reduction in ATP production from glucose oxidation, opening ATP-sensitive potassium (K_{ATP})channels,[37]; in particular the ketone β-hydroxybutyrate has been proposed to modify seizures through K_{ATP} channels (and GABA(B) receptor signaling) in a *Drosophila* seizure model.[38] Other possible indirect mechanisms include inhibition of the mitochondrial permeability transition pore, which has been implicated in mitochondrial dysfunction and neuronal death,[34,35] and inhibition of adenosine kinase so increasing adenosine levels, and activating the inhibitory adenosine A1 receptors[34,35]. Moreover, ketones have been implicated in epigenetic effects that could be disease modifying in chronic epilepsy, possibly through an action on adenosine metabolism.[39,40] Overall, there is mixed evidence that ketones can have an effect on seizure activity, and it is most likely that this occurs through indirect metabolic effects.

Medium chain fats as a direct mechanism for seizure control

Research on medium-chain triglycerides within the MCT ketogenic diet has provided important insights into the roles for fatty acids in seizure control. The efficacy of decanoic acid in seizure control has been shown in *in vitro* experiments, where seizure-like activity is induced in hippocampal slices with PTZ, or perfusion with artificial CSF containing no magnesium.[17] Importantly, in these studies, decanoic acid blocked seizure-like activity within 30 minutes of application, within replenishing (perfusate) conditions and under conditions (high glucose) in which ketone generation is unlikely to occur[17]. Decanoic acid also reduces seizure thresholds in a range of *in vivo*

animal models of acute seizures including both the 6 Hz test (a model of drug resistant seizures) and the maximal electroshock test (a model of tonic-clonic seizures) although it is not active against PTZ-induced seizures (proposed to be a model of absence seizures)[17,20]. These experiments support a direct role of decanoic acid in seizure control.

An important step forward in understanding the role for decanoic acid in seizure control was the discovery that decanoic acid can act as a selective antagonist of AMPA receptors (Figure 2), demonstrated by direct inhibition of these receptors *in vitro*.[17] AMPA receptors, are composed of four subunits, each containing an amino terminal and ligand binding extracellular domain, and three transmembrane domains. These receptors are key components in the generation of seizures[41], are blocked by micromolar concentrations of decanoic acid.[17] The mean concentration of decanoic acid in blood plasma from patients with epilepsy that receive the MCT ketogenic diet is around 157 μM[42]. Decanoic acid rapidly and easily crosses the blood brain barrier after ingestion in rodent models[20]. It is therefore likely that, in patients with epilepsy on the MCT ketogenic diet, decanoic acid would reach sufficient concentrations in the brain to reduce excitation and thereby provide seizure protection. This decanoic acid-dependent AMPA receptor inhibition is likely to be receptor isoform specific, shows enhanced inhibition during synaptic activation (when neurons are depolarised), and is non-competitive to glutamate[17], and thus might provide a strong basis for therapeutic efficacy. Interestingly, direct inhibition of AMPA receptor activity has been well established as an effective therapeutic mechanism in focal seizures and generalized tonic-clonic seizures and a recently licensed antiepileptic drug perampanel acts directly on AMPA receptors but at a different site from decanoic acid.[43,44] Thus, the effects of decanoic acid seen in *in vivo* models are therefore likely to be a direct result of AMPA receptor inhibition.

Octanoic acid is the more abundant fatty acid in the MCT ketogenic diet supplement, and is found in epileptic patient blood plasma at around 310 μM.[42] A range of animal studies have investigated its role in seizure control. In one series of experiments, acute oral dosing of rodents with increasing levels of octanoic acid increased the threshold for induction of myoclonic and clonic convulsions in a rat model.[21] Octanoic acid also significantly increased the seizure threshold in the 6 Hz seizure model, through an adenosine receptor dependent manner under reduced glucose levels.[45] However, using the same seizure model, this therapeutic effect was not seen in animals that received dietary octanoic acid-containing triglycerides[16], when glucose levels were not controlled. Octanoic acid has no inhibitory activity at AMPA receptors at concentrations found in patients on the MCT ketogenic diet[17] suggesting the potential anti-seizure effect is more likely to occur through indirect effects on adenosine receptors. However, novel branched octanoic acid derivatives, such as 5-methyloctanoic acid provide both *in vitro* and *in vivo* seizure control and AMPA receptor inhibition.[17,46,47]

Medium chain fats as an indirect mechanism for seizure control.

An alternative mechanism for the effect of the MCT ketogenic diet on epilepsy arises from beneficial effects upon brain energy metabolism. The diet causes alterations in glycolysis and/or mitochondrial function, where increasing ATP availability leads to an increase in seizure threshold.[48] Although long-chain fatty acids can uncouple mitochondria so potentially decreasing ATP production and lowering seizure threshold (although mitochondrial uncoupling can also have a paradoxical neuroprotective effect), medium-chain fatty acids are much less likely to have a physiological role as uncouplers.[19] Clinical studies into the effects of ketogenic diets in mitochondrial disorder patients report marked improvements in seizure control.[49,50] This may be partly due an action of decanoic acid on the peroxisomal proliferator-activated receptor gamma (PPARγ)[51,52], resulting in enhanced mitochondrial function by stimulating mitochondrial biogenesis and increasing mitochondrial complex I activity.[51] Decanoic acid is a recognized PPARγ agonist and PPARγ agonists elicit neuronal mitochondrial biogenesis (Figure 3).[53-55] Similar results have been shown in an *in vivo* model, where the dietary treatment of rats with decanoic acid-containing triglycerides increased brain mitochondrial function and ATP synthesis capacity,[16] and one study confirmed a synergistic effect of PPARγ agonists with the ketogenic diet in an *in vivo* seizure model.[56] This mechanism of increased brain mitochondrial function appears to be specific to decanoic acid and unlikely to be shared by octanoic acid, the other major component of the MCT supplement. Octanoic acid does not activate PPARγ[53], nor does it enhance levels of mitochondria *in vitro*[51] and octanoic acid-containing triglycerides do not enhance mitochondria function *in vivo*.[16] In addition, decanoic acid does not affect glycolytic enzymes suggesting limited contribution to its anticonvulsant properties.[16] The increased activity of decanoic acid in these studies, in comparison to octanoic acid, suggests a role for dietary decanoic acid providing seizure control from the MCT ketogenic diet.[16,20].

Although the discovery of these direct and indirect mechanisms has yet to be widely adopted, their identification is likely to trigger an increasing interest in fatty acids as a therapeutic mechanism of the diet. Monitoring plasma

fatty acid levels (especially medium chain fatty acids) in clinical studies relating to the MCT diet may provide a corollary from this. Further research will be needed to examine the complex interactions in the brain between medium chain fatty acids, ketones and the role of both components in therapeutic function.

The MCT ketogenic diet in other diseases

In addition to the established role drug resistant epilepsy treatment, the MCT ketogenic diet is increasingly being considered as a potential treatment for a range of other indications.

Alzheimer's disease

The ketogenic diet might be a potential treatment for Alzheimer's disease since it may function to combat metabolic changes underlying the disease.[5,6] Reduced uptake and metabolism of glucose has been strongly linked to progressive cognitive and motor degeneration as cells starve due to inefficient glycolysis.[7] This association has provided a rationale for using the ketogenic diet as a therapeutic treatment, where ketones present an alternative energy source[15] that replenishes glycolytic and tricarboxylic acid cycle intermediates[57]. One *in vitro* study has also shown that the direct application of the ketone β-hydroxybutyrate in relevant concentrations protects hippocampal neurons from amyloid β (Aβ) toxicity.[58] In another study, 20 patients with a diagnosis of Alzheimer's disease or mild cognitive impairment, received a single oral dose of MCT, but only those without the ApoE4 allele showed enhanced short-term cognitive performance with a range of tests, indicating that ApoE4 genotype may influence response to dietary treatments.[59] In addition, both classical and MCT ketogenic diets improve motor function, but not cognition, in a transgenic mice model of amyloid deposition.[60] Three studies (including two randomized control trials) have reported that treatment with an MCT diets benefitted only patients with mild forms of Alzheimer's disease but not those that were genetically predisposed with an ApoE4 mutation which is strongly associated with an increased risk of developing Alzheimer's disease.[61-63]

There is strong evidence that Aβ increases AMPA receptor currents and triggers subunit internalization; this directly links glutamate receptor hyperactivity to neurotoxicity and memory loss in Alzheimer's disease. Aβ has been shown to interact with β adrenergic receptors which regulate gene expression and the activity of receptors including AMPA-type glutamate receptors via the cAMP/PKA signaling cascade.[64,65] Phosphorylation of AMPA receptor GluA1 subunits by PKA has been shown to increase channel opening probability which results in augmented calcium entry into the cell, leading to neurotoxicity.[66] A study[67] has shown that the addition of Aβ to neuronal cultures causes neurotoxicity by strengthening calcium-dependent AMPA-receptor generated currents. This suggests that Aβ induced excitotoxicity could contribute to the widespread neuronal death in Alzheimer's disease. In addition to ketones providing energy to glucose resistant neurons, the MCT ketogenic diet might also improve neuronal survival through the inhibition of AMPA receptors by decanoic acid. There is evidence that Aβ treatment triggers the internalization of GluA2 subunits, the only AMPA receptor subunit type that confers calcium impermeability.[68,69] Internalization of GluA2 could therefore further increase total post-synaptic calcium influx, which could further increase inflammation and neurotoxicity. It needs to be noted, however, that it has been suggested that Aβ-induced internalization of AMPA receptor subunits could be sufficient to reduce LTP and therefore be linked to memory loss in Alzheimer's disease.[70] Indeed, patient studies have shown that loss of GluA2 precedes pathological marker (tangle) development in the brain.[71] This effect would be augmented if the remaining postsynaptic subunits were to be blocked by AMPA receptor antagonists. More research is needed to determine a role for the MCT ketogenic diet and AMPA receptor antagonists in the treatment of Alzheimer's disease.

Mitochondrial dysfunction has also been implicated in the pathogenesis of Alzheimer's disease. With a high demand for energy, the brain is rendered dependent on mitochondria, leaving it sensitive to aberrant changes in mitochondrial function. Structural abnormalities of mitochondria, imbalances in mitochondrial fission and fusion, and defective electron transport chain activity have been observed in Alzheimer's disease models.[72] Moreover, evidence suggests that Aβ accumulation is associated with toxic effects against mitochondria, including impaired energy homeostasis and electron transport chain complex activity, particularly of cytochrome c oxidase[72], disrupted mitochondrial structure and dynamics[73], and increased mitochondrial oxidative stress.[72,74] With mitochondria intrinsically linked to cell signaling, mitochondrial damage consequentially leads to cell death and may potentially be responsible for the synaptic degeneration observed in Alzheimer's disease. However, very few studies have investigated the therapeutic effects of the MCT ketogenic diet in light of mitochondrial function, although one in vitro study has reported the attenuation of deleterious Aβ-induced effects on cortical neurons treated with coconut oil (containing high levels of MCT), observing increased cell survival and improved mitochondrial structure and size.[75] Whilst the mechanisms of these observed effects remain unknown, there remains a potential for the role of medium chain fatty acids in this context. In particular, decanoic acid, which has the ability to improve mitochondrial function[51,76] may prove beneficial in the amelioration of Aβ-

induced mitochondrial damage. In addition, role of decanoic acid as an antioxidant[51,77] and as a PPARγ activator[78] may provide molecular mechanisms underlying the improved mitochondrial function.

Cancer

Ketogenic diets have gained considerable interest as an adjunctive therapy in the treatment of cancer, with data from both different animal models[8] and observational patient studies[9-12], although evidence for clinical efficacy from randomized controlled trials is lacking. Cancer cells are often highly dependent on glucose as a substrate, relying on anaerobic glycolysis to provide ATP, known as the Warburg effect[79]; this dependence on glucose is utilized in tumor imaging using positron emission tomographic uptake of fluorodeoxyglucose. The commonly accepted mechanism by which the ketogenic diet may aid in cancer therapy is that the lowering of circulating glucose, and the inability of tumors to use ketone bodies, results in reduced tumor growth or tumor regression.[80,81] Whilst this theory remains the most accepted explanation for a mechanism of the ketogenic diet, several studies have suggested that the effect on tumor growth may not be solely via a decrease in glucose levels.[9,12,82] Indeed, many tumors preferentially use glutamine as a substrate rather than glucose but whether a ketogenic diet has any effect on such tumors is unknown.

A link between the MCT ketogenic diet, AMPA receptors, and cancer treatment comes from studies demonstrating that human glioblastoma cells express increased levels of AMPA receptors[83], and inhibition of AMPA receptors suppresses migration and proliferation of glioblastoma multiforme cells[84] and other cancer cells[85]. Furthermore, the recently licensed AMPA receptor-specific inhibitor Perampanel, which binds at a different site to decanoic acid (Figure 2)[17], has been shown to be a potentially chemotherapeutically active adjuvant in a single case study of glioblastoma multiforme cells treatment.[86] These studies thus suggest that AMPA receptor inhibition through decanoic acid[17] might provide an adjunctive cancer treatment.

Diabetes

Diabetes can be broadly split into type 1 diabetes, in which the pancreas does not produce enough insulin due to a combination of genetic and environmental factors, and type 2 diabetes in which lifestyle choices including obesogenic diets rich in carbohydrates and saturated fats, together with lack of exercise, lead to hyperglycaemia and insulin resistance.[87] Dietary interventions, including the MCT ketogenic diet have been investigated as new therapeutic approaches, mainly in type 2 diabetes mellitus due to its increased incidence. In a number of studies, MCT ketogenic diets have been found to reduce serum lipid levels and improve lipid profiles, decrease body fat and reduce total body weight in both animals[13] and humans[14] and to increase energy expenditure.[88] MCTs also reduce insulin resistance and improve glucose tolerance in animal models and in patients with Type 2 diabetes.[13,89] Although the exact mechanism of these overall effects remains unknown, these studies suggest a beneficial role of MCTs in the treatment of type 2 diabetes and associated glucose-sensitive metabolic disorders (eg, XX +ref). Ketogenic diets in patients with type 1 diabetes is more limited and the literature consists of case reports of patients with type 1 diabetes and poorly controlled epilepsy, or anecdotal reports. A major concern about implementation of any ketogenic diet in patients with diabetes, especially type 1, is the potentially life-threatening complication of diabetic ketoacidosis as a lack of insulin promotes fatty acid oxidation and ketosis.

Mitochondrial dysfunction has also been postulated to play a role in insulin resistance and, consequently, the pathology of diabetes. Patients with Type 2 diabetes have been found to exhibit impaired mitochondrial activity[90], with alterations in function and morphology[91], as well as increased reactive oxygen species levels[92], linked to insulin resistance. Genetic variations and alterations in gene expression of PPARγ coactivator-1[93], the master regulator of mitochondrial biogenesis, have also been proposed to play a role in the pathogenesis of diabetes. In light of these findings, a role for decanoic acid as a PPARγ agonist may provide a therapeutic effect in treatment of diabetes. Thus, increasing mitochondrial content through decanoic acid treatment, in conjunction with improved mitochondrial function and increased antioxidant capacity, could form a vital defence against the deleterious effects of mitochondrial dysfunction in diabetes.

Conclusion and future directions

The MCT ketogenic diet is widely considered to function through the generation of ketones, in the treatment of a range of disorders including epilepsy, cancer, Alzheimer's disease, and diabetes. However, the underlying mechanisms of the diet are still largely unknown. The recent discovery of roles for medium chain fats, provided in the diet, in the direct inhibition of a key neurotransmitter receptor (the AMPA receptor), and through regulating cellular energy through PPARγ activation and mitochondrial biosynthesis have provided alternative therapeutic mechanisms to explore. Understanding the role of AMPA, PPAR and mitochondrial biosynthesis, in

relation to MCT ketogenic diet-responsive disorders may provide new therapeutic targets, and facilitate the development of new pharmacological and dietary treatments such as altered fatty acid with MCTs, or chemical modification of fats to reduce first-pass metabolism clearance. Since the proposed mechanism of AMPA receptor inhibition, PPAR activation and mitochondrial biosynthesis provides a rationale for efficacy in other conditions, further clinical studies are necessary to validate the use of the MCT ketogenic diet in treatment in these disorders (Table 1). In addition, further clinical studies are necessary to either decrease or mitigate potential adverse effects of ketogenic diets, such as the low grade acidosis resulting from elevation in β-hydroxybutyric and acetoacetic acids.[94] Furthermore, it remains to be elucidated if other ketogenic diets, such as the classical diet, are also associated with elevated levels of medium chain fatty acids, and monitoring of these components in clinical studies will help to explore these mechanisms. Validation of these and other targets of fats provided in the diet may both improve and widen the use of the diet, in both children and adults, for the treatment of epilepsy, cancer, Alzheimer's disease, diabetes, and other disorders.

Search strategy and selection criteria

We selected references by searching PubMed for manuscripts published in English between Month/Day 2010 and Sept 18st 2017, using the term "ketogenic diet" or "medium chain triglyceride" and assorted combinations of the following terms: "epilepsy", "seizures", "antiepileptic drugs", "dementia", "neurodegenerative disease", "Alzheimer's disease", "diabetes", "cancer", and "tumor". We examined the reference lists within original research and review articles for additional references. We finalised the reference list on the basis of originality and relevance to the scope of this Review.

Declaration of interests

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Contributions

All authors contributed equally to the preparation and writing of the manuscript. All authors approved the final version.

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Figure 1: Breakdown and circulation of dietary medium chain trigylcerides. (1) The consumption of MCT (containing decanoic acid (ten carbon) and octanoic acid (eight carbon)) as a supplement in the MCT ketogenic diet. (2) Medium chain fatty acids (decanoic acid and octanoic acid) are liberated from the triglycerides in the intestine, transferred to the liver, where (3) the majority of these medium chain fatty acids are broken down to three ketone bodies (BHB, ACA and acetone). (4) Both fatty acids and ketones are transported through the circulation to the brain. (5) Transport of fatty acids and ketones across the blood brain barrier leads to neuronal exposure as the site of action for the treatment of epilepsy.

Figure 2: Schematic representation of the relation between AMPA receptors and decanoic acid. Schematic representation of AMPA receptors that occur as heterotetramers. Individual subunits comprise a large extra-cellular amino (NH_2) terminal domain and ligand binding domain (for glutamate), three transmembrane domains (M1, M3 and M4) and reentry loop (M2). The proposed site for decanoic acid, on the M3 domain, is distinct to that of perampanel at the linker regions (S1-M1 and S2-M4) to the M1 and M4 domains. The carboxy terminal (HOOC) resides on the cytoplasmic side of the membrane.

Figure 3: Schematic representation of the activation of peroxisome proliferator-activated receptor gamma (PPARγ) signaling through decanoic acid. Decanoic acid (DA) binds the PPARγ to bind target DNA (with the retinoid X receptor (RXR) to elevate gene transcription, where enhanced gene expression is thought to trigger

mitochondrial biogenesis. This effects leads to elevated tricarboxylic acid (TCA) cycle and complex 1 activity, and complex 1 activity, resulting in optimal ATP availability.

Table 1:

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Figure 1

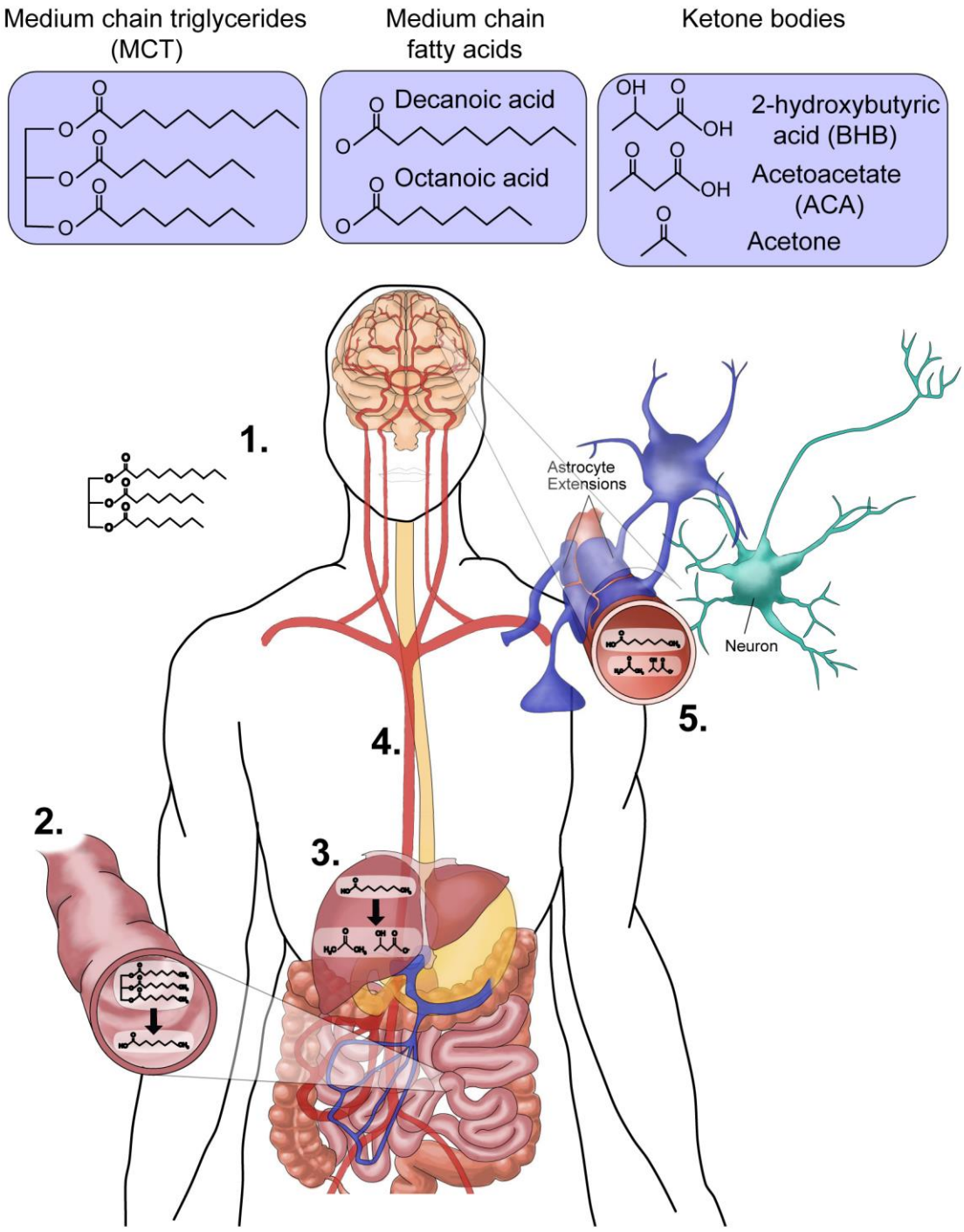

Medium chain triglycerides (MCT)
Medium chain fatty acids
Decanoic acid
Octanoic acid
Ketone bodies
2-hydroxybutyric acid (BHB)
Acetoacetate (ACA)
Acetone
1.
2.
3.
4.
5.
Astrocyte Extensions
Neuron

Figure 2

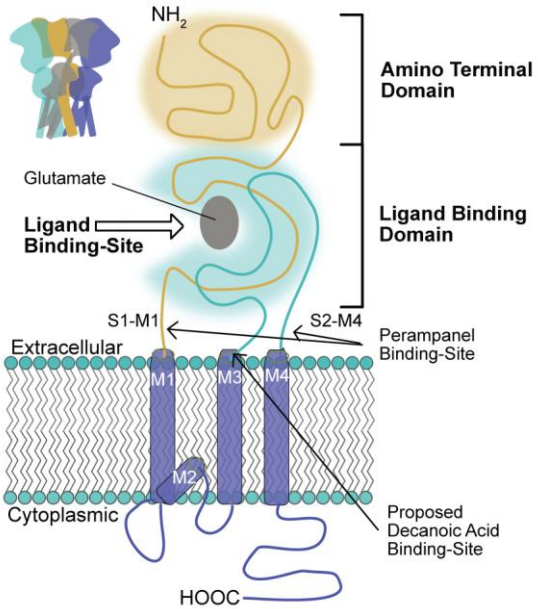
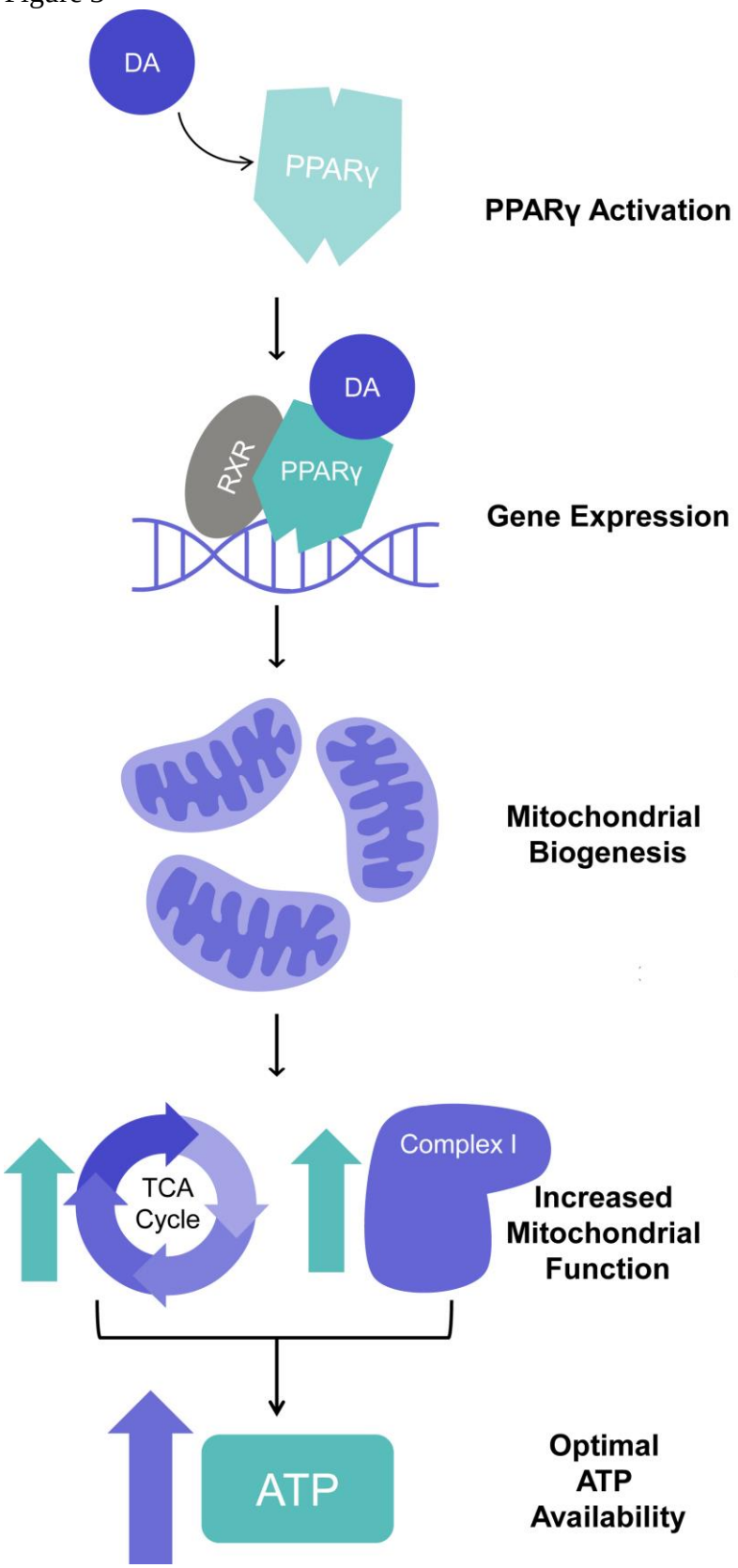

NH2
Amino Terminal Domain
Glutamate
Ligand Binding-Site
Ligand Binding Domain
S1-M1
S2-M4
Perampanel Binding-Site
Extracellular
M1
M3
M4
M2
Cytoplasmic
Proposed Decanoic Acid Binding-Site
HOOC

Figure 3


DA
PPARγ
PPARγ Activation
DA
RXR
PPARγ
Gene Expression
Mitochondrial Biogenesis
TCA Cycle
Complex I
Increased Mitochondrial Function
ATP
Optimal ATP Availability

Identifier	Study title	Intervention(s)	Condition/study population	Lead centre	Expected date of completion*
NCT03075514	Ketogenic Diets as an Adjuvant Therapy in Glioblastoma: A Randomised Pilot Trial	Modified KD vs. MCT KD	Glioblastoma	University of Liverpool, UK	March 2018
NCT02825745	Use of Betashot in Children and Adults With Epilepsy	MCT based emulsion	Epilepsy	National Hospital for Neurology and Neurosurgery and Great Ormond Street Hospital for Children, UK	December 2017
NCT02516501	Impact of a Ketogenic Diet Intervention During Radiotherapy on Body Composition	MCT based emulsion	Neoplasms	Schweinfurt, Germany	June 2018
NCT02021526	Triheptanoin (C7 Oil), a Food Supplement, for Glucose Transporter Type I Deficiency	Normal diet + C7 oil vs. C7 oil as part of KD	Glucose Transporter Type I Deficiency	University of Texas Southwestern Medical Center, USA	June 2019
NCT02426047	Medium Chain Triglycerides as an Adjunct to the Modified Atkins Diet for Women With Catamenial Epilepsy	Modified Atkins diet plus MCT based emulsion	Epilepsy	Johns Hopkins University, USA	March 2018
NCT02912936	A Medium Chain Triglyceride Intervention for Patients With Alzheimer Disease	MCT milk vs. olive oil milk	Alzheimer's Disease	University of British Columbia, Canada	February 2018
NCT02679222	Comparing the Ketogenic Effect of Coconut Oil and Different MCTs	Different MCT supplements	Healthy adults	Université de Sherbrooke, Canada	December 2016
NCT02709356	Medium Chain Triglycerides and Brain Metabolism in Alzheimer's Disease	Different MCT emulsions	Alzheimer's disease / healthy elderly people	Université de Sherbrooke, Canada	July 2017
NCT02409927	Effect of MCT Emulsification on Ketogenesis in Human Adults	Different MCT preparations	Healthy adults	Université de Sherbrooke, Canada	September 2014
NCT02551419	Proof of Mechanism of a New Ketogenic Supplement Using Dual Tracer PET (Positron Emission Tomography)	MCT milk vs. olive oil milk	Adults with Mild Cognitive Impairment	Université de Sherbrooke, Canada	June 2018

* Final data collection date for primary outcome measure

Table 1: Current clinical trials using medium-chain triglyceride ketogenic diets. Clinicaltrials.gov was search using the terms ‘medium-chain’ AND ‘ketogenic’ in October 2017