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Review

The mechanisms mediating the antiepileptic effects of the ketogenic diet, and potential opportunities for improvement with metabolism-altering drugs

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ABSTRACT

The ketogenic diet (KD) is increasingly being used to treat patients with intractable epilepsy. Despite decades of research, the reason for its success, when anticonvulsants have failed, is still not completely understood. There are, however, many candidate mechanisms which can be grouped into those that alter neuronal excitability at the synapse, and those that confer neuroprotection. The molecular underpinning of these mechanisms centres on the shift from glucose- to lipid-based energy generation that accompanies a high fat/low carbohydrate diet. Here we describe how changes in the relative abundances of energy substrates (ketone bodies), intermediates of glycolysis and fat metabolism, and metabolic end products (ATP, reactive oxygen species) underlie many of the antiepileptic effects of the KD. We propose that the success of the KD for treating epilepsy lies in the large variety of antiepileptic mechanisms that it confers. Different subsets of the mechanisms may be clinicallyrelevant in different patients. We extend this to suggest that the broad benefits of the KD could therefore be achieved by pharmacologically promoting the production of ketone bodies in the liver as they represent a key mediator that is common to all of the proposed mechanisms.

1. Introduction

The use of the ketogenic diet (KD) as a therapy for epilepsy developed from observations that fasting could reduce seizures, a strategy that was in use as far back as ancient Greece [1]. Fasting continued as a treatment up to the 1920s, but work around that time also revealed that both starvation and high fat/low carbohydrate diets increase plasma levels of the ketone bodies acetoacetate and β-hydroxybutyrate [2]. It was also proposed that these metabolic products may benefit children with neurological disorders [3]. In 1928 a study by Barborka [4] confirmed the effectiveness of the KD for treating epilepsy, as seizures in adults receiving the diet were completely controlled or improved in 56 out of 100 patients. From then on the KD was a common epilepsy treatment until it was gradually superseded by anticonvulsant drugs, so that by the 1980s it was rarely used. However, there has been a resurgence of interest and usage of the KD for epilepsy since the turn of the century, particularly for the ~30% of patients with intractable epilepsy. New variants of the KD have been developed with slightly increased carbohydrate or protein content and/or medium chain triglycerides rather than the longer chain triglycerides of the traditional KD. Nonetheless all the variants are essentially high fat/low carbohydrate diets that induce the production of ketone bodies (KBs) in the liver through fatty acid catabolism. The KBs then enter the bloodstream and are taken up by organs including the brain where they are further metabolised in mitochondria to generate energy. Currently the KD is commonly used in children who have not improved after trialling two to three anti-epileptic drugs, although recent studies have confirmed its effectiveness in adults [5,6]. Patients typically undergo a three month trial to see if the KD is effective, and if so they can remain on it for several years until they are seizure-free, significantly improved or stop using it due to the difficulties in complying with such a restrictive regime. As for a reason why a high fat diet is beneficial for a neuronal disorder, one possible explanation is that KBs are a major fuel source for brain development *in utero* and in infancy [1,7]. Perhaps the similarities between development and repair mean that the reinstatement of KB-based brain metabolism

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creates an environment in which brain cells can repair the structural damage associated with epileptogenesis.

Despite the success of the KD in reducing seizures, and also the considerable amount of research into the cellular and molecular changes that the diet induces, no single mechanism has been identified that explains its efficacy. The question, "How does the ketogenic diet reduce seizures" is an unusually challenging one. Finding the answer is complicated by both the extreme variability of the causes of epilepsy as well as the extensive changes in physiology induced by the diet. The shift in energy generation from carbohydrate sources to lipid essentially rewires many fundamental biological systems, and as with all therapies that alter metabolism, both the molecular end products *and* intermediates in a pathway can be potentially therapeutic. Furthermore, research that goes deeper into the molecular changes induced by the KD, rather than supporting or refuting existing hypotheses, often ends up providing yet more potentially seizure-reducing mechanisms [1,8]. Finally, translation of discoveries in animal models into clinical studies is complicated by the huge inter-individual variability in the underlying causes of epilepsy. This means that rejection of a proposed mechanism would require its examination in multiple groups that represent the various forms of epilepsy (potentially at multiple ages, and/or severities), which is beyond the scope of most research projects.

However, despite these challenges, several KD-induced changes have been identified that are beneficial to neuronal structure and/or function. They can be broadly grouped into changes that alter neurotransmission at synapses, and those that improve neuronal structure and homeostasis, though there are many overlapping molecules and processes. Recent reviews have described these in more detail [1,8,9].

2. The ketogenic diet changes energy metabolism in the liver and the brain

In a carbohydrate rich diet, glucose is the preferred substrate for energy production in the brain. Glucose is initially catabolised in the cytoplasm of a neuron or glial cell through the process of glycolysis which produces ATP and NADH. The latter can be used in the mitochondrial electron transport chain (ETC) to create the proton gradient across the inner mitochondrial membrane which is used in oxidative phosphorylation to produce more ATP. The end product of glycolysis is pyruvate which enters the mitochondria and is converted to acetyl-CoA. This central molecule of energy metabolism can then combine with oxaloacetate to enter the tricarboxylic acid (TCA) cycle and produce more NADH that can be used in the ETC.

All of the variants of the KD instigate shifts in body-wide energy metabolism away from glucose-based and towards fat-based energy generation. The ingested fatty acids are metabolised in liver mitochondria into KBs which are then released into the blood stream and are taken up by multiple organs including the brain. In the mitochondria of neurons and glial cells KBs are catabolised to acetyl-CoA, which can then enter the TCA cycle for energy generation (producing NADH and ultimately ATP), or it can be used in lipogenesis to produce fatty acids. A consequence of the increased dependence on mitochondria for energy generation with of the KD is that the numbers of that organelle increase in neurons and glia. This is one proposed contributor to the observation that ketones and the KD lead to high levels of ATP production [10,11].

With the KD the reduction in the use of glucose as an energy source is compounded as not only is there simply less dietary carbohydrate, but glycolysis is additionally inhibited by shifts in the mitochondrial ratios of acetyl-CoA/CoA and $NADH/NAD^+$ [12,13].

The antiepileptic effects of the KD are thought to be due to changes in the levels of the molecules or enzymes involved in energy metabolism. It should be noted that it isn't just the end-products glucose- versus fat-based energy metabolism that are candidates for antiepileptic effects, as proposed mechanisms also involve the KBs themselves, and the intermediates of their catabolism. The links between several of these molecules and neuronal excitability and/or neuroprotection form the basis of our understanding of the antiepileptic mechanism of the KD and are described in the next section (Fig. 1).

3. Summary of mechanisms that may explain the anti-seizure effects of the ketogenic diet

The cellular state associated with seizures is increased neuronal excitability which involves an increase in action potentials which are in turn induced by depolarization of the cell membrane at a synapse. The polarity at a synapse is regulated by inhibitory and excitatory neurotransmitters which control ion pumps and channels that facilitate the influx and efflux of ions such as Na^+, K^+, Cl^- and Ca^{2+}. Accordingly potential anti-seizure mechanisms of the KD are linked to changes in the amounts of neurotransmitters as well changes that influence neural membrane polarity. The major excitatory neurotransmitter glutamate can be synthesised from the TCA cycle intermediate α-ketoglutarate. Glutamate can then either be converted to the major inhibitory neurotransmitter GABA, or be transaminated to aspartate in a reaction that requires another TCA cycle compound, oxaloacetate. As oxaloacetate is required for energy generation, there is a relative increase in the amount of glutamate that is converted to GABA [14]. Some human and rat studies support this anti-seizure mechanism by showing that a KD leads to increased GABA and reduced glutamate in the brain [15,16]. Another potential player is the inhibitory neurotransmitter neuropeptide Y (NPY) which would be increased by ketogenesis or starvation [17], and is known to be antiepileptic [18].

The increase in ATP generation by the KD provides fertile ground for theories to explain the anti-seizure effects as a multitude of cellular processes require ATP. The ATP requirement of several types of ion channels and transporters is a leading candidate. Perhaps the best example of the influence of ATP as an anti-seizure mechanism is the export of ATP from neurons which is then converted to adenosine in the synapse which activates adenosine A1 receptors (A_1R) leading to activation of potassium channels thereby leading to hyperpolarization of the cell membrane and a reduction in excitability (Fig. 1) [19].

ATP and energy metabolism are intimately connected to another process through which the KD is antiepileptic – neuroprotection. Neuroprotective mechanisms are proposed to both increase the seizure threshold and to reduce the damage to the brain that is generated by seizures. As mentioned above the KD is known to increase the number of mitochondria in brain cells [20,21]. This is expected to be due to the requirement for mitochondria for energy generation from fats as well as the reduction in glycolysis (which doesn't require mitochondria). At the onset of the KD the existing mitochondria will experience a high workload which reduces the efficiency of ATP production leading to the production of reactive oxygen species (ROS). Increased ROS levels in seizures are actually a contributor to the cell damage and death, so the KD-induced increase contradicts the observed benefits of the KD. However this state of increased oxidative stress is temporary as the increased ROS induces mitochondrial biogenesis to deal with the increased workload, and also induces anti-ROS mechanisms such as glutathione. The end result is neurons and glia that have an improved cellular infrastructure for energy generation and reducing oxidative stress