

Mechanisms of Ketogenic Diet Action

Susan A. Masino¹ and Jong M. Rho^{2,*}

Within the past two decades, interest in understanding the therapeutic mechanisms of ketogenic diet (KD) action has grown steadily. Expanded knowledge about underlying mechanisms has yielded insights into the biochemical basis of brain function, both normal and pathologic. Metabolic changes likely related to the KD's anticonvulsant properties include – but are not limited to – ketosis, reduced glucose, elevated fatty acid levels, and enhanced bioenergetic reserves. Direct neuronal effects induced by the KD may involve ATP-sensitive potassium (K_{ATP}) channel modulation, enhanced purinergic (i.e., adenosine) and GABAergic neurotransmission, increased brain-derived neurotrophic factor (BDNF) expression consequent to glycolytic restriction, attenuation of neuroinflammation, as well as an expansion in bioenergetic reserves and stabilization of the neuronal membrane potential through improved mitochondrial function. Importantly, beyond its utility as an anticonvulsant treatment, the KD may also exert neuroprotective and anti-epileptogenic properties, heightening the clinical potential of the KD as a disease-modifying intervention. As dietary treatments are already known to evoke a wide array of complex metabolic changes, future research will undoubtedly reveal a more complex mechanistic framework for KD action, but one which should enable improved formulations offering comparable or superior efficacy with fewer side-effects, not only for epilepsy but perhaps a broader range of neurological disorders.

INTRODUCTION

The ketogenic diet (KD) is a high-fat, low-carbohydrate, adequate protein diet that has been employed as a treatment for medically-refractory epilepsy for over 90 years.¹ This “alternative” therapy was originally designed to mimic the biochemical changes associated with fasting, a treatment reported anecdotally over millennia to control seizure activity. The hallmark features of KD treatment are the production of ketone bodies (principally β -hydroxybutyrate, acetoacetate and acetone) – products of fatty acid oxidation in the liver – and reduced blood glucose levels (Figure 1). Ketone bodies provide an alternative substrate to glucose for energy utilization, and, in the developing brain, also constitute essential building blocks for the biosynthesis of cell membranes and lipids.

Author Affiliations: 1 Neuroscience Program and Psychology Department, Trinity College, Hartford, CT (USA). 2 Departments of Pediatrics and Clinical Neurosciences, Alberta Children's Hospital, University of Calgary Faculty of Medicine, Calgary, Alberta (Canada).

* For correspondence: Susan A. Masino, PhD, Life Sciences Center, Trinity College, 300 Summit St., Hartford, CT 06106, Phone: 860-297-2557, Fax: 860-297-2538, susan.masino@trincoll.edu

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Throughout much of the past century, the popularity of the KD has waxed and waned. Initial enthusiasm was fueled by dramatic success rates, reported entirely in uncontrolled studies, but was quickly supplanted by new antiepileptic drugs (such as phenytoin) that became available in the 1930's. Clinicians found it more convenient to administer a drug than supervise a regimen requiring scrupulous attention to foodstuffs and avoidance of anti-ketogenic carbohydrates. Notwithstanding the prolonged stigma of being a fad therapy and one without a credible scientific basis, the KD experienced a major resurgence in the late 1990's, mostly as a consequence of serendipitous media attention and the continued failure of even newer antiepileptic drugs to offer significantly enhanced clinical efficacy.

Today, the KD is acknowledged as a proven therapy for epilepsy.² The growing number of clinical KD treatment centers throughout the world serves as a testament to the notion that irrespective of cultural and ethnic differences that define dietary and nutritional practices, a fundamental shift from carbohydrate-based consumption to fatty acid oxidation results in similar clinical effects. Despite such broad use, surprisingly little is understood about its underlying mechanisms of action. This may be due to the inherently complex interplay between the network dynamics of the human brain (particularly in the disease state and during development), and the myriad biochemical and physiological changes evoked by consumption of dietary substrates. It has not been straightforward to determine cause-and-effect relationships in this bewildering context, and one cannot be certain whether specific molecular and cellular alterations observed are relevant or simply represent epiphenomena. This knowledge gap has hindered efforts to develop improved or simplified treatments (such as a "KD in a pill"³) that obviate the strict adherence to protocol that the KD requires. However, research efforts have been intensifying over the past decade, and recent investigations have provided new insights and molecular targets.

Herein we outline the most prominent mechanisms underlying KD action. We introduce these mechanisms chronologically as they were proposed, integrate these ideas with more recent findings, and examine the evidence for the broad neuroprotective properties of the KD – the latter, if validated, would highlight the clinical potential of the KD as a broadly encompassing disease-modifying intervention.⁴⁻⁷ Whether the cellular mechanisms responsible for the clinical utility of the KD for human epilepsies are identical to those observed in animal models, or whether they overlap with the mechanisms that afford clinical benefits for other neurological conditions, remains to be determined. An integration of older and newer ideas regarding underlying mechanisms of KDs might represent the ideal scientific strategy to eventually unlock the secrets of this metabolism-based therapy.

HISTORICAL AND CLINICAL PERSPECTIVES

Early Hypotheses of KD Action

Initial studies into the mechanisms underlying KD action focused on concepts of acidosis, dehydration and increased ketone concentrations – largely because these were the readily apparent ideas stemming from clinical implementation and observations.⁸ Mild dehydration was postulated as necessary, possibly to maximize concentrations of ketones which were believed to render anticonvulsant effects. As discussed later, however, peripheral ketone concentrations alone (whether measured in urine or blood) are not tightly correlated with seizure control, and there is no evidence that dehydration or fluid restriction is necessary for clinical efficacy.⁹ Nonetheless, because ketone metabolism generates protons and pH-lowering metabolic products, decreased pH (i.e., acidosis) was also considered initially to be a key aspect of the KD. However, there is no clear evidence that a KD significantly lowers brain pH, let alone that a decrease in pH – however small – may be associated with its anticonvulsant activity.¹⁰

Despite these negative results, however, it is possible that the KD may induce dynamic and differential pH changes in local microdomains; this possibility has yet to be assayed during KD treatment. Indeed, local

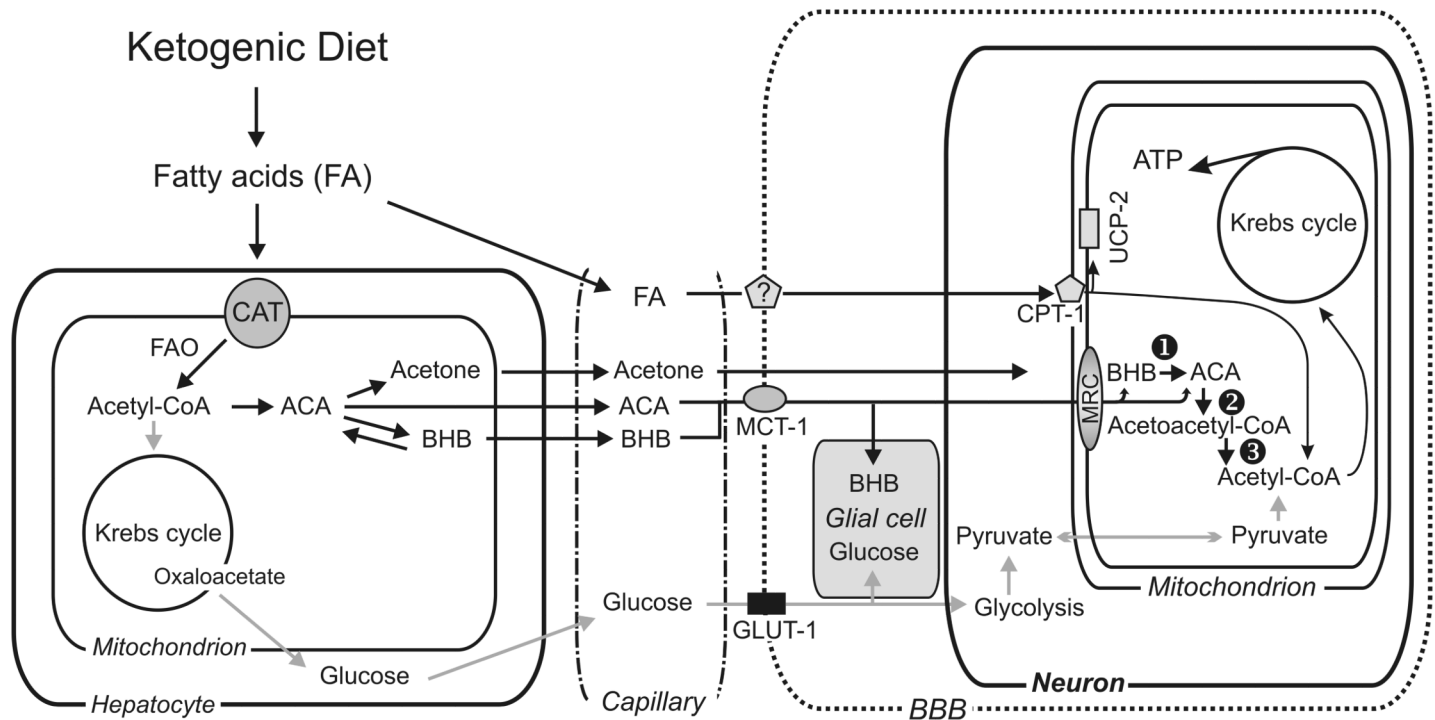


Figure 1. Metabolic pathways involved in ketogenic diet (KD) treatment. In the liver, fatty acids are ordinarily converted into acetyl-CoA which enters the tricarboxylic acid (TCA) cycle. When fatty acid levels are elevated and exceed the metabolic capacity of the TCA cycle, acetyl-CoA is shunted to ketogenesis. Two acetyl-CoAs can combine through a thiolase enzyme to produce acetoacetyl-CoA, which is a precursor for the synthesis of acetoacetate (ACA) and β -hydroxybutyrate (BHB). Acetone, the other major ketone body, is produced primarily from spontaneous decarboxylation of ACA, and can be eliminated as a volatile substrate through the lungs and kidneys. In the blood, ACA and BHB are transported from the vascular lumen to the brain interstitial space, and to both glia and neurons, by monocarboxylic acid transporters (MCTs). MCT-1 is the principal carrier localized to the vascular endothelium. Within neurons, both ACA and BHB are transported directly into mitochondria, and then converted to acetyl-CoA through several enzymatic steps. BHB is converted to ACA through D- β -hydroxybutyrate dehydrogenase, and ACA undergoes subsequent conversion to acetoacetyl-CoA through a succinyl-CoA transferase enzyme. Finally, acetoacetyl-CoA-thiolase converts acetoacetyl-CoA to two acetyl-CoA moieties which then enter the TCA cycle. Abbreviations: CAT (carnitine-acylcarnitine translocase), FAO (fatty acid oxidation), ACA (acetoacetate), BHB (β -hydroxybutyrate), MCT-1 (monocarboxylate transporter-1), GLUT-1 (glucose transporter-1), BBB (blood-brain barrier), CPT-1 (carnitine palmitoyl transferase), UCP (uncoupling protein), ATP (adenosine triphosphate), ① (3-hydroxybutyrate dehydrogenase), ② (succinyl-CoA:3-oxoacid CoA transferase), ③ (mitochondrial acetoacetyl-CoA thiolase), MRC, mitochondrial respiratory complex. Reprinted with permission from Kim dY, Rho JM, The ketogenic diet and epilepsy. *Curr Opin Clin Nutr Metab Care* 2008; 11:113–20.

compartments have been shown to exhibit differential pH regulation during neuronal activity,^{11,12} and recent work has highlighted novel pH-related anticonvulsant mechanisms. For example, acidosis *in vivo* reduced seizure activity via activation of a depolarizing acid-sensing ion channel 1a (ASIC1a) localized to hippocampal interneurons.¹³ Other studies showed that decreased intracellular pH during periods of increased neuronal excitability releases adenosine and decreases excitatory synaptic transmission and bursting in *in vitro* hippocampal slices.¹⁴ Thus, pH-related mechanisms may offer new prospects for anticonvulsant therapies, and techniques enabling higher resolution studies of pH dynamics *in vivo* and within neuron-glia microdomains may resolve permanently whether the KD acts in part by changing pH - which can influence proton-sensitive ion channels such as N-methyl-D-aspartate (NMDA) receptors^{15,16} and specific GABA_A receptor isoforms.^{17–19}

Clinical Insights

From decades of clinical experience, it is been observed that almost any diet resulting in ketonemia and/or reduced blood glucose levels can produce an anticonvulsant effect. Comparable clinical efficacy has been seen using KDs comprised of either long-chain triglycerides (LCTs)^{20,21} or medium-chain triglycerides (MCTs).^{22–25} Within the last decade, two additional variations of these KDs have emerged²⁶: the modified Atkin's diet (MAD)^{27–30} and the low-glycemic index treatment (LGIT).^{31–33} The former allows for more liberal carbohydrate consumption and does not significantly restrict protein intake compared to the classic KD, whereas the latter was developed to mirror reduced glucose levels during KD treatment, and as such is based on a fundamental adherence to foods with low glycemic indices. The glycemic index is a value that describes the extent to which a carbohydrate is absorbed and elevates blood glucose, and lower indices correlate with slower insulin responses compared to glucose. Interestingly, LGIT does not induce the prominent ketosis seen with classic KDs and the Atkin's diet.

Thus, at present, there are three primary dietary therapies for epilepsy: the traditional KD (a variation of which is the MCT diet), the MAD, and the LGIT. All three diets have been increasingly studied and are being used currently for both children and adults in centers worldwide. Importantly, available evidence indicates that dietary composition *per se* does not appear to affect the anticonvulsant efficacy of the diet, as long as there is a degree of sustained ketosis and/or calorie restriction.^{34–39} And, although there are patients with epilepsy who respond dramatically within days of initiating the KD, maximum efficacy is not generally achieved for several days or weeks after initiation, suggesting that longer-term adaptive metabolic and/or genetic mechanisms may be recruited.⁴⁰ These adaptations are likely generalized throughout the epileptic brain, irrespective of underlying pathology or genetic predisposition to seizures, because the KD is an effective treatment for diverse epileptic conditions.^{41,42} Table 1 provides a general context for the subsequent discussion, comparing and contrasting key parameters observed clinically and in experimental models.

TABLE 1. Ketogenic Diet: Clinical Correlates and Experimental Observations.

	<i>Clinical Correlate</i>	<i>Observation in Animal Models</i>
Seizure type	KD is effective against many seizure types and epilepsy syndromes	KD is effective in models employing a wide variety of seizure paradigms
Age range	Children extract and utilize ketones from blood more efficiently than older individuals	Younger animals respond better to the KD
Calorie restriction	Associated with seizure reduction	Increases seizure threshold
Diet type	Classic and MCT KDs are equally efficacious	Classic and MCT diets both increase seizure threshold
Ketosis	Ketosis is necessary but not sufficient for seizure control	A threshold level of ketosis is necessary but not sufficient to explain anti-seizure effects
Fat	Practical concerns limit the ketogenic ratio; possible role of fat chain length and degree of saturation (e.g., PUFAs)	Improved efficacy with higher ketogenic ratios; uncertain if type of fat is a critical variable
Latency to KD effectiveness	Seizures may be seen during the pre-diet fast or after a latency of days to weeks	Several days
Reversal of protective effect when KD discontinued	Rapid (hours)	Rapid (hours)

Abbreviations: KD, ketogenic diet; MCT, medium chain triglycerides; PUFAs, polyunsaturated fatty acids. Adapted with permission from reference ⁵⁹.

ANIMAL MODELS

Acute Models

The bulk of the existing experimental literature pertaining to the KD involves studies in which various high-fat treatments are implemented prior to acute provocation with either electrical or chemoconvulsant stimulation in rodents. In these studies, animal diets have modeled the classic LCT diet and conform closely to either a 4:1 or approximately 6:1 ketogenic ratio of fats to carbohydrates plus protein (by weight). In general, irrespective of precise dietary formulation – as long as ketosis is seen, reflecting a shift from primarily glycolysis to intermediary metabolism – anticonvulsant effects have been observed. Indeed, whether seizures are provoked by corneal electroshock, hydration electroshock, maximal electroshock, pentylenetetrazol (PTZ), bicuculline, semicarbazide, kainate, fluorothyl, or 6 Hz stimulation, chronic pre-treatment with a KD appears to render anticonvulsant effects.^{43–53} It should be noted that the KD is not anticonvulsant in all acute animal models, particularly in mice,^{54–56} and its effects may be of limited duration⁴⁴ or can even exacerbate (maximal) seizures.^{37,46,47,57,58} But while such results may raise concerns for the validity of the studies as a whole, one must recall that the KD is not universally effective in patients with medically refractory epilepsy.^{20,21,41}

Further complicating interpretation of the animal literature, the highly variable methodologies used (e.g., use of calorie restriction, age at initiation and duration of therapy, dietary ratios and formulations, timing of treatment, mode of seizure induction, etc.) have made cross-comparisons nearly impossible.^{59,60} Regarding observations in rodent models, anticonvulsant efficacy of 4:1 or 6:1 KDs may be confounded by the fact that they did not control for intake of vitamins, minerals and antioxidants. Highlighting this cautionary note, when a balanced KD was utilized in the PTZ, kainate or fluorothyl models, anticonvulsant efficacy was not actually observed.⁵⁵ Thus, acute animal studies highlight a number of problems with these experimental approaches and have challenged our ability to directly translate animal research to the human epileptic condition.

Chronic Animal Models

Published animal studies, in attempting to replicate the clinical experience, suggest that dietary effects in controlling brain excitability extend beyond species boundaries, but they have done little to enhance our knowledge of underlying mechanisms of action. Rather, they raise more issues and highlight the necessity of investigating KD effects in a more clinically relevant model – namely, a chronic model characterized by early-onset, medically-refractory epilepsy that is responsive to a clinically validated formulation of the KD.⁶¹ That said, it is also important to recognize that species differences, particularly with regard to fatty acid metabolism and blood-brain-barrier properties, may dictate incongruity of experimental results.

Muller-Schwarze and colleagues⁶² provided the first evidence that a KD can retard epileptogenesis in a chronic animal model. In this study, rats were first subjected to kainate-induced status epilepticus, and then treated with a KD. Seizure frequency and duration, recorded after the latent period, were both significantly lower in the KD-treated group compared to controls. Further, there was a significant reduction in the extent of mossy fiber sprouting in the KD-fed group. In another model of chronic epilepsy, the KD was shown to prolong lifespan in succinic semialdehyde dehydrogenase (SSADH)-deficient (*Aldh5a1*^{-/-}) mice which are characterized by GABA deficiency, recurrent seizures and early demise.⁶³ Interestingly, KD treatment restored spontaneous inhibitory synaptic currents to control levels, effects that were later attributed to an increase in the number of mitochondria in hippocampus, as well as a restoration of reduced hippocampal ATP levels compared to controls.⁶⁴

Comparable to induced models, the KD has also been shown to be effective in genetically-determined epilepsy models. The EL mouse is a seizure-susceptible inbred strain believed to represent a model of multifactorial idiopathic partial epilepsy with secondary generalization.⁶⁵ Environmental stimulation such as repetitive handling can induce seizures in EL mice and facilitate epileptogenesis beginning at P30. Generalized seizures generally manifest by the second postnatal month and persist throughout later life.⁶⁶ When fed a 4.75:1 KD

formula over a 10-week period, seizure susceptibility scores were significantly reduced compared to controls after 3 weeks, but this difference disappeared by week 7.⁶⁷ These transient results were similar to what had been reported earlier in the kindling model.⁴⁴

More recently, effects of a 6.3:1 KD were investigated in *Kcna1*-null mice lacking the gene encoding the delayed rectifier potassium channel α subunit, Kv1.1. The frequency of spontaneous recurrent seizures was significantly reduced in KD-fed mice compared to wild-types⁶⁸ This observation is of particular interest due to the facts that: (1) *Kcna1*-null mice exhibit progressive histological changes in the hippocampus similar to that observed in human epileptic tissues and in many animal models of temporal lobe epilepsy,⁶⁹ and (2) the *Kcna1* gene is one of only few epilepsy genes in a developmental animal model that has a homologue in a human epileptic condition.^{70,71}

MECHANISTIC STUDIES IN THE EARLY RENAISSANCE ERA

Ultimately, to produce anticonvulsant effects, the KD must reduce neuronal excitability and/or synchrony. The essential currency of neuronal excitability – both normal and aberrant – is the complex array of primarily voltage-gated and ligand-gated ion channels that determine the firing properties of neurons and mediate synaptic transmission. At present, there appears to be at least six important mechanisms through which the currently available antiepileptic drugs exert their anticonvulsant action,^{72–74} and the vast majority of molecular targets are ion channels and transporters localized to plasmalemmal membranes. In this light, a fundamental question pertaining to KD effects at the cellular and molecular levels has been whether any of the metabolic substrates (e.g., ketone bodies) elaborated by this “non-pharmacological” intervention can interact with ion channels known to regulate neuronal excitability. Broadly speaking, this does not appear to be the case. Given this tantalizing situation, there has been intense recent interest in how metabolic changes induced by a KD translate in a causal manner to a reduction in neuronal excitability and/or synchrony. Most of the postulated mechanisms discussed below are considered in the context of multiple lines of evidence, from human data as well as *in vivo* and *in vitro* model systems.

Ketone Bodies

Perhaps the most obvious and potentially important clinical observation pertaining to the mechanistic underpinnings of the KD is the prominent ketonemia seen in patients. At face value, it would seem to be a relatively straightforward matter to establish a cause-and-effect relationship between the degree of ketonemia and seizure control. Further, it should be no surprise that since the 1930's, researchers have asked time and again whether ketone bodies might themselves exert anticonvulsant effects.^{49,75–78} Despite intense scrutiny, this matter remains unresolved. It is well known that seizure control gradually improves within the first few weeks of initiating the KD, as serum ketone levels steadily increase. Interestingly, seizure control can be lost abruptly when ketosis is broken, usually through ingestion of carbohydrates,²² again implicating ketone body action. Further, blood β -hydroxybutyrate (BHB) levels can appear to correlate directly with seizure control in children placed on a KD,^{20,21,79} but the relationship is inconsistent.⁴¹ The threshold BHB level for seizure control appears to be a blood concentration of 4 mmol/L for children successfully maintained on the KD for 3 or 6 months,⁷⁹ and investigators have used such clinical observations to model KD conditions in both animal models and *in vitro* studies. In general, blood levels are better correlated with seizure control than urinary levels; the latter are obtained through dipstick assessments that grossly reflect only acetoacetate levels.^{79,80}

Keith first reported that acetoacetate (ACA) protected against thujone-induced seizures in rabbits,⁷⁵ an observation that was later confirmed in an audiogenic seizure susceptible mouse model.⁴⁹ Later, Likhodii and colleagues provided direct evidence that acetone could block induced seizures in multiple animal models of seizures and epileptogenesis.⁷⁶ They demonstrated that acetone, when injected intraperitoneally, yielded plasma and cerebrospinal fluid (CSF) concentrations consistent with doses used to suppress seizures. In support of this,

clinical investigators found that acetone was detectable in the brains of fully controlled KD-treated epileptic patients using proton magnetic resonance spectroscopy, and was estimated to be present in concentrations of approximately 0.7 mM.⁸¹ Curiously, while *in vivo* experiments have demonstrated the acute anticonvulsant properties of ACA and acetone, there are as yet no convincing data indicating that the major ketone body, BHB, can exert similar effects. Intriguingly, however, recent work suggests that metabolizing BHB rather than glucose reduces the availability of glutamate, which could then contribute to anticonvulsant and potentially neuroprotective effects.⁸²

Condering the other ketone bodies, the link between ACA and acetone is particularly tight; ACA is spontaneously decarboxylated to acetone, and as such, it was speculated that ACA's acute anticonvulsant effects might be due to immediate conversion to acetone and subsequently to its many downstream derivatives.⁸³ But more recent work has clearly demonstrated that the anticonvulsant activity of acetone is not dependent upon its metabolites,⁸⁴ so the exact mechanism of acetone's rather broad-spectrum anticonvulsant activity remains unknown. It has been proposed that S-D-lactoylglutathione, an intermediate of acetone metabolism, might activate voltage-gated potassium channels and thereby hyperpolarize the neuronal cell membrane,⁸⁵ but there is as yet no direct evidence. And there is yet another report that acetone and BHB enhanced inhibitory glycine receptors, whereas BHB alone was able to enhance GABA_A-receptor mediated currents – but all of these actions were observed at highly supratherapeutic (i.e., anesthetic) concentrations, not seen during KD treatment.⁸⁶

Investigations involving potential anticonvulsant compounds would not be complete without the use of cellular electrophysiological techniques, and thus it was of interest whether ketone bodies would affect any of the principal ion channels that are the primary targets of clinically used antiepileptic drugs. Surprisingly, such studies were initiated little more than a decade ago. Thio and colleagues⁸⁷ reported that acute application of low millimolar concentrations of BHB or ACA did not affect synaptic transmission in normal rat hippocampus, and did not affect GABA_A receptors, ionotropic glutamate receptors, or voltage-gated sodium channels over a wide concentration range (300 μM – 10 mM). The fact that ACA was ineffective in their hands was indeed surprising, especially given its clear anticonvulsant effects when administered *in vivo*.^{49,76}

Recently, however, Ma and colleagues⁷⁷ found that BHB and ACA – at physiological concentrations – reduced the spontaneous firing of GABAergic neurons in rat substantia nigra pars reticulata (SNr; a subcortical structure that influences seizure propagation) by opening cellular membrane-bound ATP-sensitive potassium (K_{ATP}) channels. K_{ATP} channels, a type of inwardly rectifying potassium channel (Kir6) that is activated when intracellular ATP levels fall, were long considered logical candidates for linking metabolic changes to cellular membrane excitability.⁸⁸ Despite the intuitive appeal of this observation, an inherent discrepancy remains to be reconciled. Consistently, studies have shown that the KD can increase levels of ATP and other bioenergetic substrates through enhanced mitochondrial respiration.^{89–93} Because high ATP levels block K_{ATP} channel activity, it is unclear how opening of these channels is achieved by infusion of ketone bodies in the SNr.

Yet another intriguing link between ketone bodies and neuronal excitability was recently reported. Juge and colleagues demonstrated that ACA inhibits vesicular glutamate transporters (VGLUTs), which are required for exocytotic release of the excitatory neurotransmitter glutamate, specifically by competing with an anion-dependent regulatory site on presynaptic vesicles.⁷⁸ These investigators demonstrated that ACA decreased the quantal size of excitatory neurotransmission at hippocampal synapses, and suppressed glutamate release and seizures evoked by the convulsant 4-aminopyridine in rats. This novel finding may be a plausible explanation for the acute *in vivo* effects of ACA observed nearly eight decades earlier,⁷⁵ but ACA is reported to have other actions as well, notably on mitochondria.^{92,94} The other caveat is that ACA is highly unstable, and undergoes spontaneous decarboxylation to acetone which may have other actions. Further, in the presence of BHB dehydrogenase, ACA is interconverted to the major ketone body BHB. Thus, it appears that seemingly straightforward metabolic substrates are players in a more complex arena.

In summary, the available evidence thus far fails to strongly support a primary mechanistic role for ketone bodies in the clinical efficacy of the KD, as no compelling molecular target has been identified and linked to attenuation of spontaneous seizures in a chronic epilepsy model. At this juncture, it is unlikely that there is only one relevant action of any of the primary ketone bodies on neuronal activity. If ketones are indeed fundamentally required for the anticonvulsant efficacy of the KD, they are more likely contributory to other parallel (and possibly synergistic) effects of the diet. Of the various hypotheses proposed, the most likely candidate mechanisms are membrane hyperpolarization through activation of potassium channels, increased GABAergic neurotransmission, or a reduction in vesicular glutamate release. Ketones have been shown to reduce brain glucose consumption,⁹⁵ and reduced glucose is another key hallmark of a KD discussed in more detail below.

Age-dependence of Ketone Utilization

Multiple studies and anecdotal observations have suggested that the KD is most effective in immature animals or infants and children,^{34,45,54,58,96} and is perhaps due to greater fatty acid oxidation of breast milk which is high in fats, more efficient extraction of ketone bodies from the blood, and an early age-dependent surge in the expression of the monocarboxylic acid transporters, MCT1 and MCT2.^{97,98} If indeed the degree of ketonemia is a major determinant of seizure control, one would predict that younger patients would respond better than older ones. Clinical studies to date would tend to support this notion, although it is becoming clearer that adolescents and adult patients with epilepsy also benefit from KD treatment.^{99,100} In contrast, there is abundant laboratory evidence suggesting that the anticonvulsant effects of KDs are not age-dependent.⁴⁰

There is recent controversy involving ketone bodies aimed straight at the heart of a scientific dogma – that is, the notion that γ -aminobutyric acid (GABA)-mediated responses in the early developing brain are excitatory, not inhibitory.^{101,102} Zilberter and colleagues reported that the addition of ketones and other metabolic substrates such as lactate to artificial cerebrospinal fluid (aCSF) – which traditionally contains glucose as the sole energy substrate – resulted in an age-dependent hyperpolarizing shift of the GABA reversal potential in both hippocampus and neocortex, and a significant reduction in the generation of giant depolarizing potentials (GDPs) which have long been regarded as the hallmark of spontaneous neonatal network activity *in vitro*.^{103,104} This striking finding suggests that in the immature brain, GABAergic neurotransmission might actually be inhibitory *in situ* as there is a greater preponderance of energy substrates (such as ketone bodies, lactate, and pyruvate) than at later ages.^{105–107} However, these provocative findings have quickly been challenged by other groups who found that: (1) the depolarizing GABA action in neonatal hippocampal slices is not due to deficiencies in energy metabolism;¹⁰⁸ and (2) physiological plasma concentrations of BHB, lactate and pyruvate failed to affect the depolarizing actions of GABA in immature rat pups (postnatal days 4–7), and only non-physiological concentrations of pyruvate (5 mM) reduced the driving force for GABA_A receptor-mediated currents and blocked GDPs.¹⁰⁹ Clearly, while this controversy remains unsettled at present, the general notion that bioenergetic substrates may exert profound cellular electrophysiological effects is becoming increasingly appreciated.

GABAergic Inhibition

An enduring hypothesis regarding the mechanisms KD action involves enhancement of GABA-mediated inhibition. Facilitation of GABAergic neurotransmission has long been accepted as a critical mechanism of action for a variety of clinically effective antiepileptic drugs, and thus there is an intrinsic appeal to invoking this mechanism. With respect to ketone bodies, however, GABA_A receptors do not appear to be the primary targets in this regard. The indirect evidence comes from acute animal studies in which the KD is found to be most effective against seizures evoked by the GABAergic antagonists (i.e., PTZ, bicuculline, picrotoxin and γ -butyrolactone), whereas it fails to block acute seizures provoked by kainic acid, strychnine (a glycine receptor antagonist), and maximal electroshock (MES; involving voltage-dependent sodium channels).⁴⁸

More direct evidence comes from electrophysiological recordings conducted *in vivo*, demonstrating that the KD increased paired-pulse inhibition and elevated maximal dentate activation threshold in rats, consistent with an elevated seizure threshold via enhancement of GABAergic inhibition.³⁷ In a related study, caloric restriction - which results in mild ketosis - enhanced the expression of both isoforms of glutamic acid decarboxylase (GAD65 and GAD67, the biosynthetic enzymes for GABA) in the tectum, cerebellum, and temporal cortex of rats, suggesting increased GABA levels.¹¹⁰ However, increased GAD expression might actually reflect decreased GABA production¹¹¹⁻¹¹³ so the ramifications of these findings to neuronal inhibition remain unclear.

At a neurochemical level, Yudkoff and colleagues have proposed that in the ketotic state, a major shift in brain amino acid handling results in a reduction of aspartate relative to glutamate (the precursor to GABA synthesis), and a shift in the equilibrium of the aspartate amino-transferase reaction.¹¹⁴⁻¹¹⁷ This adaptation in the metabolism of the excitatory neurotransmitter glutamate (i.e., a decrease in the rate of glutamate transamination to aspartate) would be predicted to increase the rate of glutamate decarboxylation to GABA, the major inhibitory neurotransmitter, because more glutamate would be available for the synthesis of both GABA and glutamine.^{114,115,118} An increase in brain GABA levels would then be expected to dampen seizure activity (Figure 2). But is there any evidence that the KD increases GABA levels in seizure-prone areas of the brain? The experimental data thus far are inconsistent or are reflective of changes outside of critical areas such as the hippocampus, thalamus and neocortex.^{10,91,114,119} Two clinical studies have reported significant increases in GABA levels following KD treatment,^{120,121} however, further substantiating this view. More recent work has demonstrated that BHB decreases GABA degradation, and thus could increase the available pool of GABA.¹²² Collectively, whereas both laboratory and clinical data support a role for increases in GABA levels and presumably increased inhibition via GABA receptors as a potential mechanism underlying KD action, it remains unclear why a KD can be effective in stopping seizures in patients who have failed to respond to GABAergic drugs.

Norepinephrine and Neuropeptides

One of the more intriguing observations regarding KD action involves the noradrenergic system. Several lines of evidence support the notion that increases in noradrenergic tone result in anticonvulsant activity. For example, norepinephrine (NE) re-uptake inhibitors prevent seizures in genetically epilepsy-prone rats (GEPRs),¹²³ agonists of noradrenergic receptors are generally anticonvulsant,¹²⁴ and ablation of the locus coeruleus (the origin of both ascending and descending noradrenergic innervation) contributes to the ontogeny of self-sustaining status epilepticus (SSSE) in rats.¹²⁵ Along these lines, it is of significant interest that mice lacking the ability to produce NE (i.e., dopamine β -hydroxylase deficient mice) do not exhibit an increased resistance to fluorothyl seizures when treated with a KD,¹²⁶ whereas similar treatment renders protective effects in control wild-types. These data indicate that NE is required for the anticonvulsant effect of KD, at least in the fluorothyl seizure threshold model. It was later shown that a KD increased basal NE levels in hippocampus nearly two-fold,¹²⁴ further supporting this mechanistic hypothesis. Increased NE release would also be predicted to promote co-release of anticonvulsant orexigenic peptides such as neuropeptide-Y (NPY) and galanin.¹²⁴ However, there is no evidence for enhanced transcription of either of these peptides in the brain after KD treatment, suggesting that neither NPY nor galanin contribute significantly to the anticonvulsant actions of a KD.¹²⁷

Another peptide potentially important in mediating KD effects is leptin, an endogenous substrate that helps regulate energy homeostasis. Leptin is part of the hormonal system that limits energy intake and expenditure and is intimately involved in appetite regulation.¹²⁸ Less appreciated, however, is the fact that it can exert modulatory effects on neuronal excitability and suppress seizure activity.^{129,130} In an important translational study, leptin was shown to attenuate focal or generalized seizures in rodents, possibly through alteration of AMPA receptor-mediated synaptic transmission.¹³¹ Since the KD causes a rise in leptin levels,¹³² it is possible that the KD's mechanism, at least in part, may relate to a leptin-associated reduction in synaptic excitability.

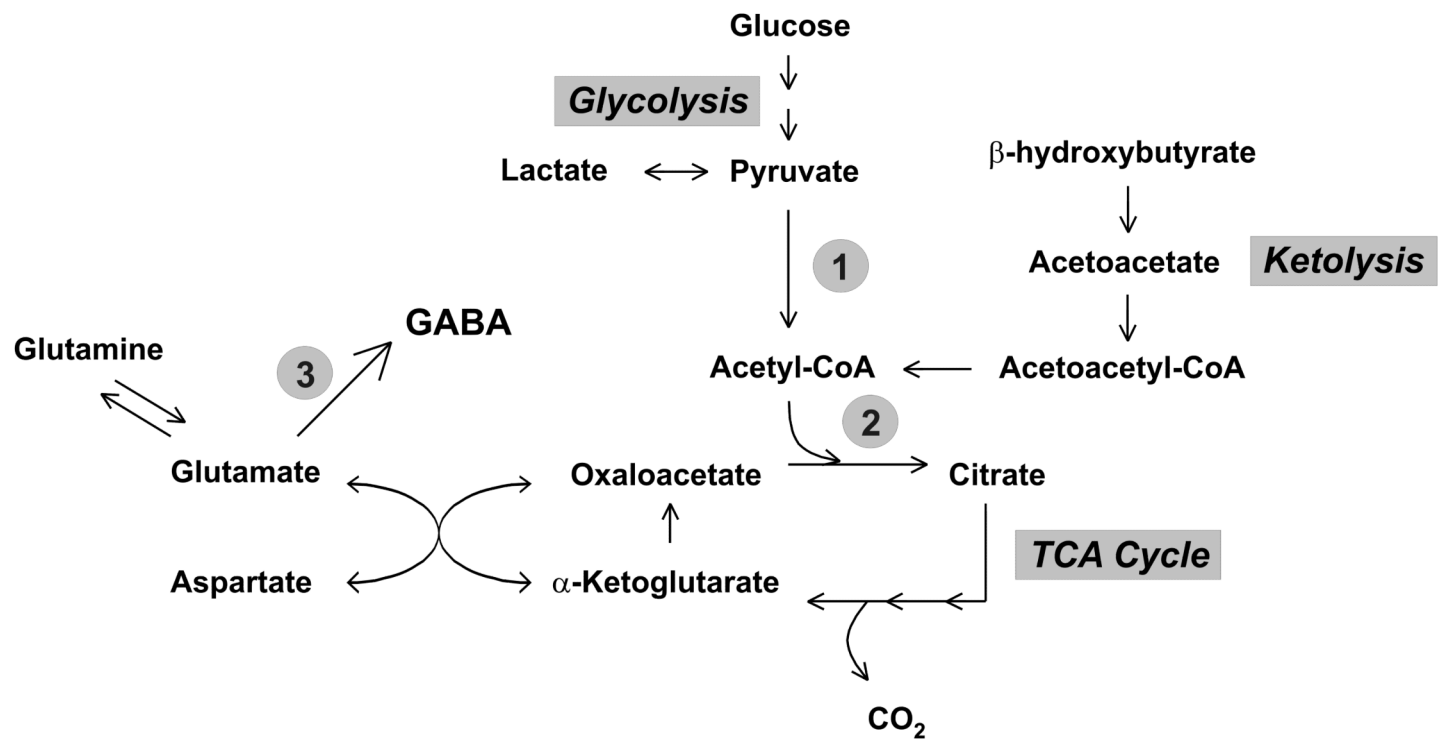


Figure 2. The metabolic inter-relationships between brain metabolism of glutamate, ketone bodies and glucose. In ketosis, 3-OH-butyrate (β -hydroxybutyrate) and acetoacetate contribute heavily to brain energy needs. A variable fraction of pyruvate (1) is ordinarily converted to acetyl-CoA via pyruvate dehydrogenase. In contrast, all ketone bodies generate acetyl-CoA which enters the tricarboxylic acid (TCA) cycle via the citrate synthetase pathway (2). This step involves the consumption of oxaloacetate, which is necessary for the transamination of glutamate to aspartate. Oxaloacetate is then less available as a reactant of the aspartate aminotransferase pathway, which couples the glutamate-aspartate interchange via transamination to the metabolism of glucose through the TCA cycle. Less glutamate is converted to aspartate, and thus more glutamate is available for synthesis of GABA (3) through glutamic acid decarboxylase (GAD). Adapted with permission from Yudkoff et al. *The Ketogenic Diet: Interactions with Brain Amino Acid Handling*, in *Epilepsy and the Ketogenic Diet*, Stafstrom CE & Rho JM (Eds), Humana Press, Totowa, NJ, 2004.

Polyunsaturated Fatty Acids

Of the various fats that constitute KDs, polyunsaturated fatty acids (PUFAs) by far have received the most attention. Docosahexaenoic acid (DHA, C22:6 ω 3), arachidonic acid (AA, C20:4 ω 6), or eicosapentaenoic acid (EPA, C20:5 ω 3) are the main PUFAs believed to profoundly affect cardiovascular function and health. It is well known that PUFAs can directly inhibit voltage-gated sodium channels and L-type calcium channels,¹³³⁻¹³⁵ and can activate certain classes of potassium channels.^{136,137} Further, DHA and EPA have been shown to decrease neuronal excitability and bursting in hippocampus.¹³⁸

Intriguingly, there is clinical evidence that the KD elevates key PUFA levels in the blood.¹³⁹ In this study – in which each patient served as his/her own control – AA increased 1.6- to 2.9-fold, DHA increased 1.5- to 4.0-fold, and the rise in total serum AA correlated with improved seizure control. A later thought-provoking study revealed that cerebrospinal fluid taken from epileptic patients on the KD promoted opening of voltage-gated Shaker-type potassium channels expressed in *Xenopus* oocytes, and these authors surmised that the DHA, EPA, and linoleic acid might be responsible for this effect.¹⁴⁰

Laboratory investigations linking PUFAs to KD action appear discordant. Several studies have found that PUFAs are anticonvulsant in rodents,^{133,137,141-143} yet other published investigations report no such effects.^{144,145} Interestingly, KDs with widely differing effects on tissue lipids and fatty acid profiles can confer similar degrees of seizure protection,¹⁴⁶ suggesting that specific fatty acids might not be the key mediators of the KD's clinical

effects. This latter study is consistent with the notion that a general metabolic shift toward fatty acid oxidation is a fundamental requirement.

Clinical studies are similarly inconclusive. One early study of five patients found that dietary supplementation with a “PUFA spread” (specifically, 5 gm of 65% omega-3 PUFAs once daily) suppressed seizures in those who tolerated the treatment.¹⁴⁷ Later, a randomized, placebo-controlled study involving a larger sample size failed to find similar effects when a PUFA supplement consisting of EPA plus DHA (2.2 mg/day in a 3:2 ratio) was administered over a 12-week treatment period.¹⁴⁸ Another study found no correlation between changes in fatty acid levels and seizure response when a KD was supplemented with omega-3 fatty acids in 25 children with epilepsy.¹⁴⁹ And more recently, a retrospective analysis revealed that AA levels were significantly decreased in epileptic patients treated successfully with the KD as compared to non-responders.¹⁵⁰ Although it would be reasonable to dismiss the PUFA hypothesis given the clinical data thus far, it is likely that proper controlled trial design has yet to be implemented. Further, while there are a number of clinical research variables that were not considered, factors such as PUFA load, duration of treatment, and potentially, degree of ketosis might be highly important in demonstrating significant effects.

There are a few additional aspects of PUFA biology worth mentioning. PUFAs are natural activators of fatty acid receptors, specifically peroxisome proliferator-activated receptors (PPARs), and there is evidence that activation of brain-localized PPARs can influence seizure activity.^{151–153} In this regard, PPAR α could act as both a sensor and effector of KD action.¹⁵⁴ Further, nuclear translocation of activated PPARs inhibits pro-inflammatory transcription factors,¹⁵¹ and, as inflammation is increasingly recognized as a core contributor to seizures and epileptogenesis,¹⁵⁵ a KD (or a similar metabolic formulation) might constitute a rational treatment approach.¹⁵⁶

METABOLIC MECHANISMS

The earliest demonstration that the KD enhances energy substrates was provided by DeVivo and colleagues¹¹⁹ who showed significant increases in brain bioenergetic substrates such as ATP, creatine and phosphocreatine in normal adult rats fed a high-fat diet for three weeks. The metabolic data examined in this study indicated an overall increase in the cerebral energy reserve and energy charge which the authors believed could account for the neuronal stability that accompanied the chronic ketosis. A later study confirmed the elevation in brain adenine nucleotides as a consequence of KD treatment.¹⁵⁷ However, the most compelling demonstration that the KD profoundly affects energy metabolism was by Bough and colleagues who used microarray and electron microscopic techniques to examine patterns of gene expression in the hippocampus of rats fed either a KD or control diet.⁹¹ They quantified a robust up-regulation of transcripts encoding metabolism enzymes and mitochondrial proteins, and an increased number of mitochondrial profiles. Importantly, and consistent with increased energy reserves, hippocampal slices from KD-fed animals were highly resistant to the metabolic stress induced by low glucose conditions. Additional studies support the beneficial effects of a KD on mitochondrial energy metabolism.^{64,158} Figure 3 highlights some of the effects of the KD on mitochondrial function.

As intriguing as these studies are, how exactly would enhanced energy reserves lead to a stabilization of synaptic functioning, membrane potential and diminished seizure activity? One interpretation of the neuronal consequences of these bioenergetic changes is that neurons are better able to maintain ionic gradients and resting membrane potential, and thus resist depolarizing influences. The most likely way this stabilization is accomplished is through the Na⁺/K⁺ ATPase pump; specifically, KD-induced elevations in ATP concentrations might enhance and/or prolong the activation of the Na⁺/K⁺-ATPase, perhaps via an increase in the delta-G' of ATP hydrolysis.¹⁵⁹ To date, however, there are no published studies directly testing this sodium-pump hypothesis in epileptic brain.

There is an alternate and complementary hypothesis linking increased energy substrate availability with membrane hyperpolarization. Kawamura and colleagues evaluated the electrophysiological effects of reduced glucose (a consistent finding in patients successfully treated with the KD) in CA3 hippocampal pyramidal

neurons – importantly, under conditions of adequate or enhanced ATP levels, but without the addition of ketone bodies – using whole-cell recording techniques.¹⁶⁰ These investigators found that glucose restriction led to ATP release through pannexin hemichannels localized on CA3 neurons. The increased extracellular ATP, upon rapid degradation by ectonucleotidases to adenosine, resulted in activation of adenosine A1 receptors which were shown to be coupled to opening of plasmalemmal K_{ATP} channels. This study highlights a novel mechanism of metabolic autocrine regulation, involving close cooperativity among pannexin hemichannels, adenosine receptors and K_{ATP} channels, and provides an elegant link to an earlier study demonstrating ketone-mediated attenuation of spontaneous neuronal discharge in SNr.⁷⁷ It should be noted that the potential contribution of adenosine is plausible, given the well-substantiated role of this endogenous purine in suppressing cellular excitability.¹⁶¹ Consistent with the involvement of adenosine, this latter group recently found evidence that the KD suppresses electrographic seizures *in vivo* in mice with spontaneous seizures, through a mechanism involving A1 receptors.¹⁶²

It is important to note that the observation that reduced glucose might contribute to seizure control is not recent. Calorie restriction in rodents was shown to reduce seizure susceptibility, and lowered levels of blood glucose correlated with inhibition of epileptogenesis in a genetic model.^{35,163} When examining the interplay of glucose levels and ketosis, metabolic control theory would argue that glucose restriction might be more important than ketonemia.¹⁶⁴ Clinically, the link between blood glucose levels and seizure control has yet to be proven, and skepticism regarding relative hypoglycemia as the major contributor to KD action is supported by the incongruity of animal data.¹⁶⁵

Another potentially important consequence of reduced glucose has been highlighted through the use of 2-deoxy-D-glucose (2DG), a glucose analog that inhibits glycolysis by blocking phosphoglucose isomerase. 2DG has been shown to be a potent anticonvulsant and antiepileptic agent in several animal models, including kindling, audiogenic seizures in Fring's mice, 6-Hz corneal stimulation,^{6,166} as well as *in vitro* and decreases synaptic transmission via adenosine *in vitro*.¹⁶⁷ The acute effects of 2DG may be mediated through a number of different downstream mechanisms, but one intriguing possibility is a decrease in the endogenous phosphorylation of GABA_A receptors (which renders them dysfunctional) by the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase.^{168,169} Perhaps the most compelling effect of 2DG in the kindling model is decreasing the expression of brain-derived neurotrophic factor (BDNF) and its principal receptor, TrkB, via induction of the transcription factor NRSF (neuron restrictive silencing factor), a master negative regulator of neuronal genes. This fascinating study reveals a biochemical interruption of glycolysis that can result in downstream physicochemical modulation of transcription, yielding a powerful effect in retarding epileptogenesis.

While glucose inhibition may exert pleiotropic effects in animal seizure models, similar anticonvulsant actions have been achieved by diversion of glucose to the pentose phosphate pathway (PPP). Fructose-1,6-diphosphate (FDP), a glycolytic intermediate, has been shown to exert acute anticonvulsant activity in several seizure models in adult rats including kainate, pilocarpine, pentylentetrazole, and kindling.^{170,171} Indeed, FDP was more effective as an anticonvulsant than 2DG, KD, or valproate in these studies. The precise mechanisms through which FDP produces anticonvulsant effects remain unclear, but it is conceivable that this substrate may exert an antioxidant action because the NADPH generated through the PPP reduces glutathione.¹⁷²

Another fascinating dietary approach toward epilepsy treatment is based on the observation that seizures cause a deficiency in tricarboxylic acid cycle (TCA) intermediates (especially α -ketoglutarate and oxaloacetate). It has been hypothesized that “refilling” these deficient compounds (a process called “anaplerosis”) might oppose seizure generation. In support of this idea, the anaplerotic substrate triheptanoin was recently studied in both acute and chronic seizure models.¹⁷³ Mice fed triheptanoin exhibited delayed development of corneal kindled seizures and triheptanoin feeding increased PTZ seizure threshold in chronically epileptic mice that had undergone status epilepticus 3 weeks before PTZ testing.¹⁷³ Therefore, like 2DG, anaplerotic compounds appear

to alter both acute and chronic seizure susceptibility. Anaplerosis represents a novel approach that expands the potential metabolic modifications that could be anticonvulsant or antiepileptic. Taken together, results from studies of KDs, 2DG, FDP, and anaplerosis suggest that shifts in the activity of certain metabolic pathways might be exploited to develop novel treatments for epilepsy.

KETOGENIC DIET AND NEUROPROTECTION

While a detailed discussion of the expanding literature on the neuroprotective properties of the KD is beyond the scope of this chapter, a brief discussion is warranted as such actions are intimately related to epileptogenesis and seizure propensity. Further, the neuroprotective potential of the KD is increasingly being explored in a number of neurological disorders (see below). The reader is referred to recent reviews for more details on this subject.^{4,174–176}

Ketone bodies and PUFAs – metabolic substrates that are both elevated in epileptic patients treated with the KD – have been shown to exert neuroprotective activity in neurodegenerative conditions associated with impaired mitochondrial function.¹⁷⁵ Ketone bodies appear not only to raise ATP levels in seizure-prone areas such as hippocampus,¹⁷⁷ but also diminish reactive oxygen species (ROS) production through increases in NADH oxidation⁹² and inhibition of mitochondrial permeability transition.¹⁷⁸

Other than effects on voltage-gated ion channels, PUFAs - through induction of PPAR α and its co-activator PGC-1 (peroxisome proliferator-activated receptor γ coactivator-1) - induce the expression of mitochondrial uncoupling proteins, which are homodimers spanning the inner mitochondrial membrane and enable a proton leak from the intermembrane space to the mitochondrial matrix. The net effect of this action is to reduce ATP synthesis, reduce calcium influx into the mitochondrial matrix, dissipate heat, and reduce ROS production.^{179,180} The protective consequences of UCP activation have been detailed in a number of published reports.¹⁸⁰ One study demonstrated that dietary enhancement of UCP expression and function in immature rats protected against kainate-induced excitotoxicity, most likely by decreasing ROS generation.¹⁸¹ Further work demonstrated that mice maintained on a high-fat KD demonstrated an increase in the hippocampal expression and activity of all three brain-localized mitochondrial UCPs (UCP2, UCP4 and UCP5) and exhibited a significant reduction in ROS generation in mitochondria isolated from the same brain region.⁹⁰ Thus, it appears that a prominent neuroprotective mechanism of KD action involves a reduction in mitochondrial free radical production, which would decrease oxidative stress, and potentially neuronal injury.

FUTURE DIRECTIONS

To date, research efforts aimed at elucidating the mechanisms of KD action have not yielded simple answers. It is becoming increasingly apparent that the relevant mechanisms are likely diverse and operate in a coordinated and potentially synergistic fashion. The ongoing quest is challenged by the inherent difficulties in understanding neuronal network activity, let alone metabolism in the context of disease states, and importantly, in the intact patient or animal. Despite these issues, the research reviewed herein provides a clearer link between metabolism and neuronal excitability, and further validates the emerging field of neurometabolism, especially as it relates to epilepsy.

Based on the foregoing discussion, there are broader clinical implications posed by the KD. As the mechanisms underlying the neuroprotective activity of the KD are fundamental to many disease processes, it should be no surprise that diet can profoundly influence brain function, and in a growing number of instances exert protective and potentially disease-modifying effects.^{4,175,182} As examples, the KD (or various formulations of its key metabolic substrates) have been found to ameliorate a range of clinical disorders and/or experimental models such as autism and Rett syndrome,^{183,184} pain and inflammation,¹⁸⁵ traumatic brain injury,^{186–188} neurodegenerative diseases such as Alzheimer's and Parkinson's disease,^{189,190} brain cancer,^{191–193} prostate

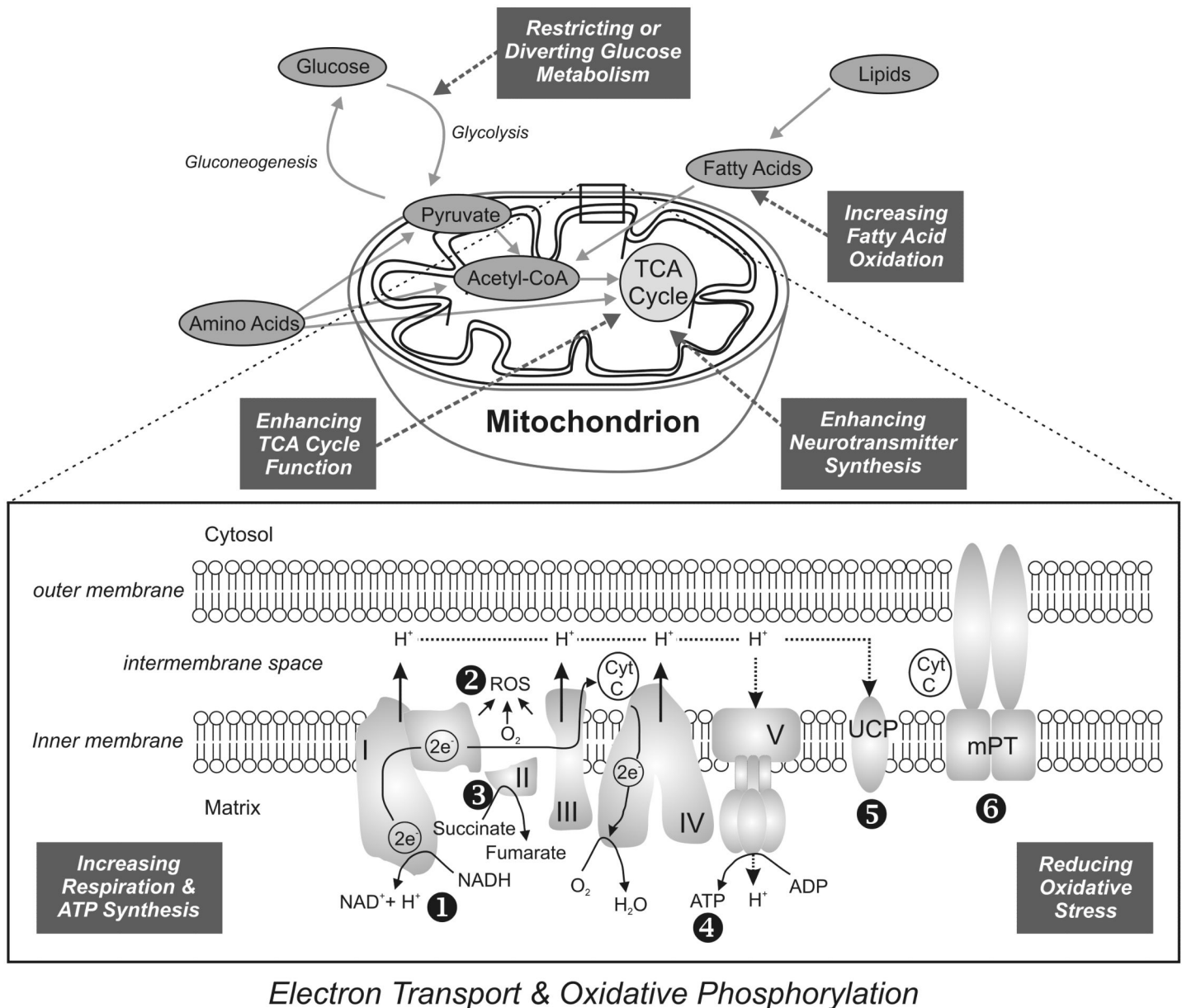


Figure 3. Major changes in important biochemical pathways reported to exert anticonvulsant and anti-epileptogenic effects in experimental models (*shadowed boxes*; also see text). Below, Putative interactions between mitochondrial respiratory complexes (MRCs) and KD-related metabolites. First **1**, either acetoacetate (ACA) or β -hydroxybutyrate (BHB) can oxidize the NADH couple. Second **2**, ketone bodies (KB) can decrease mitochondrial reactive oxygen species (ROS) generation. Third **3**, KB can protect neurons against MRC I & II inhibitors. Also, the KD elevates seizure threshold in epileptic patients with impaired MRC function. Fourth **4**, either the KD or KB can enhance ATP production. Fifth **5**, fatty acids can activate mitochondrial uncoupling proteins (UCPs). Finally **6**, KB can elevate the threshold for mitochondrial permeability transition (mPT) activation. The bulk of experimental evidence supports the hypothesis that activation of mKATP channels decreases ROS formation, likely by diminishing the proton-motive force ($\Delta\psi$) across the mitochondrial inner membrane (via transmembrane flux of potassium), and subsequently attenuating electron flux across the MRC, in a manner similar to mitochondrial uncoupling. Abbreviation: Cyt C, cytochrome c.

cancer,¹⁹⁴ diabetes^{195,196} and obesity.¹⁹⁷ It should be noted that some of these disorders are co-morbid with each other, thus presenting the opportunity to help ameliorate multiple diseases with a single therapy.

Of the many potential uses of the KD, brain cancer is perhaps one of the most compelling targets given the rising interest in identifying metabolic targets for intervention.¹⁹⁸ The theoretical basis for using the KD to treat cancer is that tumorigenesis relies heavily on glucose, whereas normal cells retain metabolic flexibility and can

use ketones for fuel.^{192,198,199} A calorically-restricted KD or other glycolysis-limiting treatment forces higher ketone production, putting maximal stress on the tumor cells and minimal stress on the normal cells.^{191,192,198} Paradoxically, some aspects of the current standard of care for brain cancer may support tumor growth.²⁰⁰

Neurodegenerative diseases are universally associated not only with mitochondrial dysfunction but also inflammation, and inflammation is a hallmark of virtually every chronic disease process throughout the body. In addition to reducing pain and inflammation,¹⁸⁵ the KD appears to enhance motor and cognitive functioning in a model of multiple sclerosis.²⁰¹ Further evidence is provided by recent reports of the KD mitigating MPTP-induced neurotoxicity and microglial activation,¹⁹⁰ and encephalopathy and seizures in forms of fever-induced epilepsy.²⁰² Ultimately, reducing inflammation could be one of the most important disease-modifying effects of a KD.

In addition to inflammation, disrupted sleep and circadian rhythms are common co-morbidities with many diseases. Recent evidence in humans and animal models shows that a KD can improve sleep in children with epilepsy²⁰³ and circadian rhythmicity in epileptic *Kcna1*-null mice.⁶⁸ It is well-known that circadian disruption is associated with epilepsy, psychiatric disorders, and the prevalent metabolic syndrome.^{204–207} Normalization of circadian rhythms alone could yield enormous clinical benefits.²⁰⁸

CONCLUSIONS

The evidence for a KD as a successful epilepsy treatment is clear. Multiple retrospective, multi-center and randomized prospective studies document consistent and significant clinical benefits. The true efficacy of dietary treatments for epilepsy may be underestimated as the KD is rarely used as a first-line therapy. Certainly, by the time the KD is initiated to thwart medically-refractory epilepsy, in some instances the severity of the epileptic condition may be too difficult to overcome. But remarkably, the KD works in the majority of patients who failed to respond to numerous antiepileptic drugs. A detailed understanding of key KD mechanisms could offer a meaningful adjuvant or ultimately the development of a “diet in a pill”.³ But while clinical applications of metabolism-based therapy appear to be growing rapidly, there is a continuing need to develop modified diet formulations with improved efficacy and tolerability (as well as palatability), and to identify new pharmacological targets for drug discovery.

It is often stated that no single mechanism is likely to explain the clinical effects of antiepileptic drugs, and certainly the same could be said for the KD. The challenge of finding key mediators of KD action is made ever more difficult by the intrinsic complexity of metabolic activity within neurons and glia, which, to be relevant to the epileptic condition, must be interpreted at network levels. In this chapter, we have reviewed a number of seemingly disparate variables proposed to collectively exert anticonvulsant (and potentially neuroprotective) effects. The important interrelationships are summarized in Figure 4. The fact that a fundamental modification in diet can have such profound, therapeutic effects on neurological disease underscores the importance of elucidating mechanisms of KD action. In summary, mounting interest and insight into the mechanisms of KD action have laid a promising foundation for metabolic therapy as an emerging strategy for neurological disorders.

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