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The Metabolic Basis of Epilepsy

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Abstract

The brain is a highly energy-demanding organ and requires bioenergetics adaptability to balance normal activity with pathophysiologic fueling of spontaneous recurrent seizures, the hallmark feature of the epilepsies. For decades, it has been accepted that recurrent or prolonged seizures can permanently alter neuronal circuitry and cause excitotoxic injury and aberrant inflammation. Further, pathological changes in bioenergetics and metabolism have been considered downstream consequences of epileptic seizures that may begin at the synaptic level. Less appreciated is the fact that primary derangements in cellular or mitochondrial metabolism, in reverse fashion, can result in seizure genesis, and lead to spontaneous recurrent seizures. Increasingly, however, basic-translational research has demonstrated that the relationships between brain metabolism and epileptic seizures are both complex, bidirectional and produce a vicious cycle that compounds the deleterious consequences. From a therapeutic standpoint, metabolism-based treatments such as the high-fat, anti-seizure ketogenic diet have become mainstream, and metabolic substrates and enzymes (such as ketone bodies, certain fatty acids and adenosine) have become attractive molecular targets to prevent seizures and to aid in recovery processes. Moreover, given that metabolism is critical for epigenetic as well as inflammatory changes, the thesis that epileptogenesis – the molecular and cellular processes that produce a chronic epileptic brain – can be both negatively and positively influenced by metabolic changes is rapidly gaining ground. Here, we present the current evidence supporting the view that brain metabolism is both a pathophysiological and therapeutic paradigm for epilepsy.

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

JMR has been a paid consultant to Aquestive Pharmaceuticals, Danone Nutricia, Mallinckrodt, Eisai Pharma, and Zogenix. JMR has also served on the Scientific Advisory Board of The Charlie Foundation for Ketogenic Therapies (Santa Monica, California, USA). DB is a co-founder of PrevEp Inc. and JMR is the Chief Medical Officer for Path Therapeutics, Inc.

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Epilepsy is a common neurological disorder that occurs throughout the age-span and has a worldwide prevalence of approximately 1% of the general population[1]. Spontaneous recurrent seizures (SRS), the defining hallmark of epilepsy, have been linked to increased neuronal excitability and hypersynchrony from various causes – genetic and secondary brain insults, modified further by environmental influences[1]. Irrespective of the etiology, there is a high energy need to prevent the generation of seizures (ictogenesis), to provide energy to sustain prolonged seizure activity, and to recover from seizures and repair damage. Indeed, without sufficient ATP – the universal currency of life and mostly produced by mitochondria[2] – it would be impossible to regenerate membrane potentials needed to enable epileptiform activity [3]. Therefore, metabolic treatments such as a ketogenic diet, which increase fuel to the brain in the form of ketone bodies are uniquely suited to prevent seizures and to aid in recovery after a seizure.

In this light, it has long been understood that SRS and prolonged seizures such as status epilepticus induce secondary aberrations in cellular bioenergetics and metabolism[4,5]. These include glutamate- and free radical-mediated injury, neuroinflammation[6–8], and widespread disruptions in brain homeostatic mechanisms. However, less appreciated is the fact that inherent defects in brain energy metabolism, whether through mutations in genes encoding mitochondrial proteins or metabolic substrates and enzymes constituting major biochemical pathways[5,9], redox instability[10] or epigenetic changes[11], can independently precipitate seizures. Indeed, irrespective of the actual inciting events or causes, seizure activity is characterized by a vicious cycle of metabolic derangements and excitotoxic damage to the brain[12,13], further compounding the fundamental mechanisms that generate aberrant network excitability and instability.

The mainstay of epilepsy therapeutics is the growing armamentarium of anti-seizure medications (ASMs). For the most part, ASMs are believed to target cellular membrane-bound ion channels and transporters to reduce excitation and/or increase inhibitory neurotransmission at the synaptic level[14]. While ASMs have been shown to control SRS in most patients with epilepsy (but not without potentially significant side-effects), approximately one-third remain medically intractable[1]. The sobering reality is that despite decades of basic-translational epilepsy research and the advent of dozens of new drugs approved for clinical use, this refractory population remains unchanged[15]. This may be due in part because traditional drug development for epilepsy has historically utilized acutely provoked seizure models that may not faithfully recapitulate the pathophysiologic changes seen in chronic epileptic brain and has focused mostly on molecular targets localized to the neuronal cell membrane[16]. That said, some of the current pharmacological agents in clinical use and under development appear to modulate novel mechanisms such as binding to the synaptic vesicle protein 2A (SV2A) and inhibition of cholesterol 24-hydroxylase[17,18]. And preclinical development of ASMs has steadily included chronic epilepsy models in

the screening process[16]. Importantly though, it is only recently that metabolic targets have drawn significant attention in the field of epilepsy experimental therapeutics[18].

The century-old high-fat, low-carbohydrate ketogenic diet, the most established metabolism-based treatment for epilepsy, provides the strongest evidence that targeting brain bioenergetics and metabolism can mitigate seizure activity – notably, in patients who fail to respond to ASMs[19–21]. Successful variations of the ketogenic diet, such as the medium-chain triglyceride diet[22], modified Atkins diet[23], and the low-glycemic index treatment[24], have provided further evidence-based validation of metabolic and dietary therapies for the spectrum of epilepsies encountered in clinical practice[25]. And while the fundamental mechanisms underlying the anti-seizure efficacy of such treatments remain unclear, there is increasing evidence that metabolic approaches abstracted from these diets can afford neuroprotective (and perhaps even disease-modifying) effects[26]. In this review, we summarize the literature supporting the growing recognition that disruptions in cellular metabolism can be both a cause and consequence of epileptic seizures, hence affording a broad opportunity to exploit this growing science for innovative therapeutic strategies.

Brain Energy Metabolism

The brain is a highly energy-dependent organ. At rest, it requires approximately 20% of oxygen and 25% of glucose relative to the rest of the body, although the brain comprises only 2–3% of total body weight[27]. Neurons have an exceedingly high energy demand due to many cellular housekeeping functions such as synthesis and degradation of macromolecules, maintenance of cytoskeletal dynamics and axoplasmic transport, as well as other costly bioenergetic functions related to the high level of action potential signaling, synaptic activity, and plasticity changes[2,28,29]. Although the brain is clearly energy demanding, it does not have an adequate energy reserve; hence, it must rely on a variety of exogenous energy sources to maintain normal function – mostly via transport of substrates through the blood-brain-barrier[30,31]. That said, while astrocytes are known to store glycogen as a source of glucose-6-phosphate, a main substrate for glycolytic ATP production[32], this is wholly insufficient to meet the immediate energy needs of the brain and brain glycogen may act more as an emergency energy reserve and may also serve unique signaling functions between neurons and glia[32]. Glucose is an obligate source of energy for the brain[33], but other fuels such as lactate, ketone bodies and medium-chain fatty acids can be used when the availability of glucose is restricted[34–36].

Astrocytes rely on both glycolysis and oxidative metabolism through the tricarboxylic acid (TCA) cycle to produce ATP[37]. In contrast, neurons are much more dependent on the immediate availability of glucose that is extracted from the capillaries via glucose transporters (GLUT3, in particular)[33,38]. And while neuronal oxidative metabolism can generate much needed ATP for their high energy needs, neurons might maintain viability and long-term function by accessing some metabolic fuel from astrocytes. Specifically, there is some evidence that astrocytes and neurons control neurometabolic coupling by furnishing lactate according to the astrocyte-neuron lactate shuttle (ANLS) hypothesis[39–41] (Fig. 1). Lactate cannot passively diffuse across the blood brain barrier[30], and since it cannot be directly utilized for energy production, it must be first transported via monocarboxylic

transporters (MCTs) into cells[42], and then be subsequently converted enzymatically to pyruvate by lactate dehydrogenase (LDH) which exists in multiple isoforms – LDH1 being primarily expressed in neurons and LDH5 in astrocytes (Fig. 1). However, the ANLS hypothesis is not without some controversy, as there is evidence that oxidative metabolism of lactate in neurons may not always be important for synaptic neurotransmission[33,43,44] and the directionalities of lactate transport under different physiological conditions remain unclear. Moreover, when abnormally increased neuronal activity occurs during epileptic seizures, neurons may rely more on their own aerobic glycolysis than astrocyte-derived lactate[44]. Nevertheless, to better understand cerebral blood flow, metabolism, and metabolic coupling among different cell types, it is important to examine the interdependent relationships between neurons, astrocytes and the microcirculation – elements that comprise at a unitary level the neurovascular unit (Fig. 1). Finally, whether in neurons, glial cells or the vascular endothelium, mitochondria are the most important determinant of bioenergetics capacity, without which there can be no normal – or even pathological – function[2,45]. Any compromise of mitochondrial function can induce dysregulation of intracellular calcium, increased oxidative stress, and ultimately apoptotic cell death.

Often neglected in the epilepsy field, glia clearly play a major role in seizure prevention, but also seizure genesis, controlling the ionic balance between intracellular and extracellular compartments, and modulating synaptic neurotransmission[37]. During normal brain activity astrocytes play an important role in preventing neuronal hyperexcitability and seizures by buffering K^+ and by regulating glutamate uptake[37]. Astrocytes can restore ionic and neurotransmitter homeostasis that is disrupted during seizure activity through various mechanisms, the most important of which are re-uptake of glutamate from the synaptic cleft and buffering of extracellular potassium through glial end-feet processes that link to the brain microvasculature[30,37]. Importantly, in an *in vitro* stem cell model, re-uptake of glutamate into astrocytes triggered enhanced glycolysis, which in turn generated more pyruvate that was converted to lactate and subsequently transported to neurons via the ANLS[46]. Moreover, it is increasingly recognized that astrocytes comprise large networks of highly inter-connected cells, mostly through gap junction channels (connexin43 and Cx30) which enable the bi-directional exchange of ions, nutritional metabolites, second messengers, amino acids, peptides, nucleotides, and even RNA[37]. Hence, astrocytes are uniquely poised to modulate network activity – electrically and metabolically. In short, astrocytes, being able to use glycogen as fuel, play a critical role in maintaining energy metabolism but also excessive neuronal firing as seen during epileptic seizures. Indeed, there are growing lines of evidence implicating astrocytes in seizure genesis and epileptogenesis[37,47–49].

Pathophysiology of Epileptic Seizures

Epileptic seizures are generally divided into two clinical groups according to their presumed site of origin and pattern of spread[1]. Focal onset seizures are believed to arise from a specific locus in the brain, and the clinical manifestations are a consequence of perturbations in the function(s) ordinarily ascribed to that area. These can at times secondarily generalize to the entire cortex via adjacent spread or through commissural pathways. In contrast, primary generalized seizures manifest at their onset through disruption and enhancement of bilateral

and reciprocal thalamocortical connections that are modulated by subcortical structures via multiple ascending pathways[50]. While this framework is at times viewed as overly simplistic and not reflective of the complex brain dynamics and connectivity required to generate seizures, it is a clinically useful dichotomy that has endured the test of time.

There are numerous mechanisms that have been implicated in ictogenesis. Although much is understood about the electro-clinical phenomenology in epilepsy, fundamentally we know very little about the mechanisms that directly initiate seizure activity and that terminate seizures after variable durations[51], and in response to circadian and environmental factors. Where do focal onset seizures originate and are the pathways and structures traditionally implicated in propagation necessary and sufficient? Why does seizure activity eventually stop – even without pharmacological intervention? Are endogenous control mechanisms recruited in response to seizures adequate to do so? The answers to these fundamental questions continue to remain elusive. However, what is clear from the above discussion is that the energy required to sustain epileptic seizure activity is wholly dependent on adaptations in cerebral blood flow and metabolic coupling within the tri-partite neurovascular unit.

For decades, the simple conceptual notion has been that overall central excitatory-inhibitory imbalance precipitates seizure activity. While this makes intuitive sense, the mechanisms that induce network instability cannot all be reduced to a matter of increased excitation and/or reduced inhibition. As an example, increased GABAergic interneuron function has been shown to ‘paradoxically’ induce network excitability and seizures[52–54], and there are ephaptic (i.e., non-synaptic) mechanisms[55–58] that can regulate neuronal synchrony in either direction. The broad pathophysiological mechanisms elucidated over the past quarter century include ion channel dysfunction (primarily due to genetic defects in various subunit proteins resulting in either loss-of-function or gain-of-function)[59], structural brain pathologies such as focal cortical dysplasia and other malformations of cortical development[60,61] or secondary changes to post-natal brain insults such as hypoxic-ischemic encephalopathy or traumatic brain injury, cellular loss and synaptic re-organization, neurogenesis, and BBB dysfunction[30,31,62], among many others. Molecular disruptions in cell signaling pathways such as mTOR (mammalian target of rapamycin)[63], have also been implicated, as well as neuroinflammation from intrinsic and extrinsic causes[6,7], stress, and hormones. Additionally, developmental changes in neurotransmitter subunit expression, immaturity of homeostatic mechanisms affecting ionic gradients and transport all have been linked to increased seizure propensity, especially in the developing brain[64]. More recently, the gut microbiota has been implicated in both the pathogenesis of seizures and the therapeutic efficacy of the ketogenic diet[65], further strengthening the notion that a systems biology and whole organismal approach is necessary to better understand the pathogenesis of epilepsy[66,67] – particularly in the context of systemic metabolism and inflammation. Finally, relevant to the current review, inborn errors of metabolism, particularly those that impair normal mitochondrial physiology and respiratory chain function, can manifest prominently with epileptic seizures[9]. Given the ever-expanding list of seizure-modifying factors, we are learning that virtually any pathophysiological mechanism involving the brain – and which induces stress – can elevate the risk of seizures[68,69]. In support of this, there are an increasing number of studies reporting seizures or epilepsy arising from

unexpected mechanisms or genes[59,70,71], and clinically, epilepsy is recognized as a fairly common co-morbidity of many neurological disorders. Conversely, there are innovative molecular approaches that are being taken to mitigate epileptogenesis[64,72] and identify relevant biomarkers[73].

What is the human evidence that physiological and metabolic changes are potentially a cause and/or consequence of seizure activity? Previous studies, utilizing functional and biochemical imaging techniques such as positron emission tomography (PET), functional magnetic resonance imaging (fMRI) and magnetic resonance spectroscopy (MRS) have revealed significant alterations in cerebral blood flow and metabolism in the epileptic brain[74–76]. Human PET studies utilizing 2-deoxy-2[^{18}F]fluoro-D-glucose (^{18}F-DG) have shown significant increases in focal or regional blood glucose utilization during seizure activity[77], and although this has traditionally been interpreted as evidence of a seizure focus, hypermetabolism *per se* may reflect other pathologies such as brain changes associated with brain plasticity[77]. Concomitant EEG monitoring during ^{18}F-DG uptake can be used to distinguish between local seizure foci and more general plasticity changes. Interestingly, during the time-span of epileptogenesis, children affected with Sturge-Weber syndrome were characterized by interictal hypermetabolism as opposed to ictal hypermetabolism[78]. In line with those findings, a follow-up MR-spectroscopy-based study demonstrated *in vivo* evidence that interictal hypermetabolism is likely based on increased glutamate release in the affected brain regions[79]. However, ^{18}F-DG studies have also shown interictal hypometabolism in human TLE subjects[80], which might indicate an energy crisis as a functional basis for impaired astrocytic K^+-buffering and glutamate uptake, and hence a lowering of seizure thresholds. Animal studies, by contrast, have consistently shown increased metabolism during epileptic seizures. Complementing these observations, single-photon computed emission tomography (SPECT) studies have shown dynamic changes in cerebral perfusion, during both interictal and ictal periods[76]. Functional MRI (fMRI), which measures blood-oxygenation levels as a surrogate for neuronal function and connectivity during cognitive tasks has also proved useful in studying both normal and epileptic brain[76]. While fMRI has mostly been used to conduct language mapping for epilepsy surgery, there is a growing list of applications, including a broader assessment of neuronal functioning (information processing), brain network connectivity and activity, and even treatment outcomes. fMRI itself is not a technique to directly interrogate brain metabolism, but it can be a valuable tool to dissect functional brain network properties in complementary fashion with MRS, PET, SPECT, and of course, the gold standard electrocephalography (EEG), which is utilized both non-invasively and invasively. Each of these techniques play distinct roles in the clinical armamentarium and given their intrinsic differences in spatial and temporal resolution – and ultimately what the data generated signify, they are often used in combination to delineate the functional and biochemical properties of the epileptic brain.

Despite the numerous scientific and technological advances that have been made over the past few decades, there is an important caveat with respect to an accurate mechanistic understanding of the epileptic brain. As is the case throughout much of science and medicine, it is often tempting to assign causality to any identifiable alteration in the epileptic brain that could theoretically enhance neuronal excitation toward seizure genesis. However, researchers often discover that it is not straightforward to identify the critical mediators

and pathways that are directly responsible for ictogenesis and epileptogenesis. Ultimately, it should be emphasized that seizures reflect a complex array of perturbations occurring at multiple hierarchical levels of biochemical, molecular, cellular structure and function, and remain unpredictable consequences of neuronal and glial network activity.

Seizure-Induced Impairment of Metabolic Homeostasis

To truly appreciate the crucial role of metabolism in epilepsy and its pathophysiology, it is important to acknowledge the existence of bi- and multi-directional molecular interactions, which add layers of complexity to the many vicious cycles implicated thus far in the processes of epileptogenesis[62,64,81]. Broadly stated, seizures by themselves cause an impairment in metabolic homeostasis, and a derailment of key metabolic and biochemical functions contributes to the increased likelihood of seizure generation. In this section, we discuss how epileptic seizures impact cellular metabolism. Acute seizures utilize glucose to fuel heightened energy demand and lead to the preferential formation of lactate rather than acetyl-coA[82]. Given the availability of glucose, glycolytic flux increases during seizure activity but decreases during the interictal periods – changes which are believed to underlie the phenomena of ictal hypermetabolism and interictal hypometabolism, both long considered the metabolic hallmarks of human and experimental epilepsies[83,84]. Whereas glycolysis is a less efficient process for ATP production, in comparison to the mitochondrial metabolism of acetyl-CoA, an increase in the glycolytic rate by a factor of 10 to 30 is adequate to generate immediate energy to sustain seizure activity. A shift toward glycolysis under aerobic conditions during seizures is supported by the increased production of lactate,[85] mimicking the Warburg effect described in cancer cells which are intriguingly characterized by metabolic derangements mirrored in the epileptic brain[86]. Increased ictal glucose metabolism may support plasticity processes in the brain, which include gliosis, neurogenesis, and axonal spouting[87]. Mechanistically, glucose is not only used as a substrate for the generation of ATP, but is also a metabolic precursor for neurotransmitters and neuromodulators including acetylcholine, glutamate, GABA, D-serine, glycine, and D-aspartate[87]. Together, alterations in key energy metabolites such as glucose, neurotransmitters, and neuromodulators are likely responsible for profound plasticity changes in the brain. A major metabolic consequence of increased ictal glycogen and glucose utilization is the formation of lactate from pyruvate through LDH (Fig. 1). The hypothesis that seizure-induced lactate formation enables epileptic activity is supported by findings that LDH inhibitors provide robust anti-ictogenic effects[88]. LDH inhibition is likely to interfere with glycolysis by limiting the availability of NAD^+ and supporting the oxidative metabolism of pyruvate in mitochondria.

Given the alternative metabolic fates of glycolysis-derived pyruvate (lactate vs. mitochondrial oxidation), the impact of seizures on mitochondrial function is of paramount importance. Several enzymes involved in the mitochondrial TCA cycle, which converts pyruvate into carbon dioxide, are known to be compromised by acute seizures (e.g., status epilepticus or SE) or chronic seizure activity in epilepsy. Reduced activity of pyruvate dehydrogenase, alpha ketoglutarate dehydrogenase, 2-oxoglutarate dehydrogenase, and aconitase[89–93], combine to reduce the flux of metabolites through the TCA cycle in the epileptic brain[94]. In addition, the multimeric protein complexes of the electron transport

chain (ETC) which enable oxidative phosphorylation – such as complex I – are known to be impaired in human temporal lobe (TLE) epilepsy and rodent models of epilepsy[95–97]. Mitochondrial dysfunction based on those alterations is directly supported by findings from experimental SE-induced epileptogenesis models, in which oxygen consumption rates transiently increase within minutes of SE onset, return to baseline during the seizure-free latent period and finally decrease during the chronic epilepsy phase[98]. Those experimental data on the role of mitochondria in epilepsy are directly supported by clinical findings based on rare inherited mitochondrial disorders associated with epilepsy[99].

High levels of oxygen and reactive oxygen species (ROS) are known to be detrimental to mitochondrial health. NADPH oxidase 2, also known as cytochrome b(558) subunit beta or Cytochrome b-245 heavy chain, is a superoxide-generating enzyme expressed in mitochondria and the plasma membrane, which has been shown to play a major role in the generation of SE-induced ROS[92,100,101]. Seizure-induced increases in mitochondrial O_2 and H_2O_2 production can be attributed to higher substrate utilization and electron transfer to O_2 during interictal periods, to an overload of calcium, and to the inhibition of ETC complexes, all of which combine to transfer electrons to oxygen. Seizures also cause inactivation of the mitochondrial antioxidant superoxide dismutase 2 via decreased activity of Sirtuin 3 or altered activities of peroxide detoxification as additional mechanisms contributing to seizure-induced oxidative stress. The generation of ROS can result in the inhibition of complex I of the ETC, leading to ROS-induced ROS production, thus forming a pathologically regenerative cycle driving increased oxidative stress, which results in further free radical damage to cellular macromolecules – a major cause and consequence of extended seizure activity[102]. Seizure-induced neuronal death can directly be attributed to oxidative stress and the formation of reactive aldehydes, hydroxyl radicals, and redox active iron, which induce damage to mitochondrial DNA, proteins, and lipids[10,103]. In line with increased oxidative stress as a pathological hallmark of epilepsy, a depletion of the endogenous antioxidant glutathione and the formation of oxidized glutathione disulfide has been demonstrated in both human epilepsy and in rodent models of acquired epilepsy[94,104–106]. Notwithstanding the above observations and our traditional understanding of the pathological effects of ROS, it is increasingly understood that ROS may possess signaling properties at low concentrations and can modulate GABAergic neurotransmission through both pre- and post-synaptic mechanisms[107].

While ROS promote neurotoxicity, seizure-induced disruption of calcium homeostasis may also contribute to the detrimental effects of seizures. Excitotoxic neuronal death is primarily driven by excessive activation of N-methyl-D-aspartate (NMDA) receptors and the subsequent intraneuronal accumulation of toxic levels of calcium. This calcium overload in turn promotes the generation of ROS or reactive nitrogen species, which as outlined above compromise mitochondrial function, cause metabolic impairment and activate necrotic and apoptotic pathways, which are all calcium-dependent processes. Calcium imaging during an ictal event consistently demonstrates increased calcium influx in almost all neurons and astrocytes[108]. Mitochondria are also crucially involved in the control of calcium homeostasis. Mitochondrial calcium homeostasis depends on mitochondrial calcium uniporter, rapid mitochondrial calcium uptake, and mitochondrial ryanodine receptors[109]. Changes in calcium concentration in the mitochondrial matrix in turn regulate the

activity of the mitochondrial ETC which is required for ATP production[110,111]. Through this mechanism, an increase in seizure-induced calcium fluxes can directly compromise mitochondrial function.

Metabolic and Epigenetic Changes in Epilepsy

From a metabolic viewpoint, the regulation of energy homeostasis has been crucial for the evolution of all living systems. A rheostat is needed to conserve energy in case of excessive energy consumption or depletion of energy supplies. A relatively simple system to regulate energy homeostasis is based on the ATP-adenosine balance with adenosine acting as a retaliatory metabolite[112]. From this evolutionary perspective, an epileptic seizure represents excessive energy consumption entailing the degradation of ATP into adenosine, with adenosine being used as an innate feedback rheostat to conserve energy. Therefore, a massive seizure-induced release of adenosine[113] acts as the brain's endogenous anticonvulsant and seizure terminator[51,114]. Because adenosine is also a building block of RNA and regulator of the S-adenosylmethionine-dependent transmethylation pathway[115,116], an energy crisis would also affect RNA synthesis by lowering ATP, and DNA methylation by increasing adenosine. Both processes would combine to reduce gene transcription globally and to conserve energy.

In contrast to the organization of living systems, with a metabolic base and subsequent layers of added complexity (Fig. 2), conventional pharmacotherapy development starts with 'druggable' targets at the top of the pyramid. For example, benzodiazepines were almost discovered by chance in 1957 leading to the subsequent characterization of the 'benzodiazepine receptor' in the CNS in 1977[117]. It was later revealed that the benzodiazepine binding site was in fact an integral part of the $GABA_A$ receptor complex[117]. Drug-driven therapy development therefore led to a major focus on G-protein coupled receptors (GPCRs), ion channels, and protein kinases, which still form the mainstay of CNS therapeutics today, and which do not target mechanisms at the base of the evolutionary pyramid. Methods to exploit gene regulation therapeutically are still in its infancy and the therapeutic potential of epigenetic, biochemical, and metabolomics approaches constitutes a new and lesser explored frontier in therapy development. If the metabolic base of the pyramid depicted in Fig. 2 is disrupted, it becomes obvious that a traditional pharmacological treatment approach would have limitations.

It is now abundantly clear that metabolic, biochemical, and epigenetic alterations play a major role in the pathophysiology of acquired epilepsies[118]. Among those alterations, maladaptive changes in adenosine metabolism constitute a central mechanism, linking metabolism with neuronal excitability and gene expression. As a neuromodulator, adenosine exerts a wide range of well-characterized functions based on the activation of a class of four G-protein coupled adenosine receptors (A_1, A_{2A}, A_{2B}, A_3)[119–121]. Together, those receptors control neuronal excitability, neuroprotection, synaptic plasticity, and inflammatory responses. Adenosine is under metabolic control of adenosine kinase (ADK), which exists in a cytoplasmic form ADK-S (regulation of tissue levels of adenosine) and a nuclear form (ADK-L), which controls adenosine metabolism in the cell nucleus[122]. ADK-L drives the flux of methyl groups through the transmethylation pathway, whereby

increased ADK-L drives global hypermethylation of DNA (Fig. 3). Increasing adenosine through various methods leads to reduced global 5-methylcytosine (5mC), an effect that is independent of adenosine receptors. Importantly, cultured cell lines engineered to overexpress ADK-L have increased global 5mC levels as compared to ADK-S cells or ADK-deficient cells[123].

Metabolic and Mitochondrial Epilepsies

The mechanisms described above strongly support a metabolic and mitochondrial basis of epilepsy and consequently, metabolic and mitochondrial epilepsies have received increasing attention. Metabolic epilepsy in humans can usually be linked to inborn errors of metabolism, which typically present during infancy or childhood. Those genetically defined aberrations in metabolism provide valuable insights into the etiology of seizures and epilepsy.

A classic example is pyridoxine-dependent epilepsy which presents with recurrent, drug-refractory neonatal seizures, and arises from an inborn error of lysine catabolism. Notably, pyridoxine-dependent epilepsy responds to therapeutic pyridoxine supplementation. Pyridoxine deficiency compromises the function of pyridoxal 5′-phosphate, the active form of vitamin B6, a coenzyme required for the synthesis and metabolism of amino acids. A deficiency in pyridoxal 5′-phosphate thereby leads to decreased GABA concentrations in the brain, as one of the underlying ictogenic mechanisms[124].

Of note, too much GABA can also trigger seizures as exemplified by mutations in succinic semialdehyde dehydrogenase deficiency, which is an enzyme of the GABA degradation pathway. Succinic semialdehyde dehydrogenase deficiency causes developmental delay, autism, epilepsy, hypotonia, and extrapyramidal movement disorders[125]. In addition, the disruption of lysine catabolism results in the accumulation of toxic intermediary substrates such as α-aminoadipic semialdehyde, which increases oxidative stress.

Pyridoxine dependent epilepsy can also be caused by mutations in the PROSC gene, which encodes a pyridoxal 5′-phosphate binding protein[126]. In a related but distinct condition affecting pyridoxine metabolism, pyridox(am)ine 5′-phosphate oxidase deficiency affects the key enzyme responsible for the conversion of pyridoxine to its active metabolite pyridoxal phosphate[127]. Clinically, neonatal seizures result, which are unresponsive to pyridoxine, and which are associated with hypoglycemia, lactic acidosis, and encephalopathy[128].

Cerebral folate deficiency can have multiple etiologies and is characterized by low levels of 5-methyltetrahydrofolate (the active metabolite of folate) in the brain, but normal folate metabolism in peripheral organs[129]. The homocysteine-to-methionine conversion requires the enzyme methionine synthetase, methyl-tetrahydrofolate as a methyl donor, and vitamin B12 as a co-factor. Folate deficiency thereby leads to a disruption of methylation reactions and reduced methionine synthesis, resulting in DNA hypomethylation as a possible epigenetic mechanism triggered by a metabolic defect[130].

Nonketotic hyperglycinemia results in glycine encephalopathy, which is a rare, inborn defect in the glycine cleavage enzyme complex, and which is characterised by profound accumulation of glycine in the CSF[131]. Glycine acts as obligatory co-agonist of the NMDA receptor by binding to its strychnine-insensitive glycine binding site[132]. Because this recognition site is normally not saturated[133], an increase in extracellular glycine can potentiate impulse-dependent NMDA receptor activation, which might be the underlying mechanism for seizure generation in this condition.

Glucose transporter 1 deficiency syndrome is characterized by impaired glucose transport into the brain[134], which results in infantile-onset refractory seizures, developmental delay, intellectual impairment, and movement disorders[134]. Interestingly, there is an increased frequency of seizures before meals and providing ketones as an alternative energy fuel via the ketogenic diet can be an effective treatment strategy.

A direct link between metabolism and cellular excitability is exemplified in patients with mutations in ATP-sensitive potassium (K_{ATP}) channels, a type of potassium channel involved in the regulation of neuronal excitability of neurons and the physiology pancreatic beta cells[135–137]. Those channels normally open under conditions of low glucose or low ATP/ADP ratios, and through K^+ efflux keeps the post-synaptic neuron hyperpolarized. Mutations in this channel directly impair the brain's ability to respond to a metabolic challenge by shutting down brain activity – in this scenario clinically, diabetes and seizures result. Metabolic epilepsies can also arise from mutations that lead to the accumulation of toxic compounds such ammonia in urea cycle disorders. Hyperammonemia can cause irreversible neurologic damage[138] and increases neuronal excitability likely through excessive NMDA receptor activation[139].

Mitochondrial dysfunction is known to precipitate a wide spectrum of neurological manifestations[140], whereas 'mitochondrial epilepsy' is a unique subset of epilepsy characterized by refractoriness to treatment and poor prognosis in some patients[141]. Patients with mitochondrial epilepsy are characterized by distinct neuropathological changes, which include a loss of expression of mitochondrial complex I and complex IV subunits in neurons and reactive astrocytes[142,143]. Based on these observations, a brain slice model of mitochondrial epilepsy was recently developed, one that relies on the pharmacological inhibition of mitochondrial complexes I and IV as well as specific inhibition of the astrocytic TCA cycle enzyme, aconitase[142]. In this model, astrocytes play a key role in the generation of epileptiform discharges – specifically, the astrocytic GABA-glutamate-glutamine cycle is affected, thus compromising the regulation of GABAergic mediated inhibition[142]. Findings from this study suggest that glutamine is a critical mediator of the interactions between astrocytic and neuronal compartments controlling the epileptiform network in this *in vitro* model of mitochondrial epilepsy. In addition, mutations that perturb mitochondrial tricarboxylic acid cycle function, such as nonsense and missense mutations in the citrate transporter SLC13A5, can lead to early infantile epileptic encephalopathies[93] Together, the metabolic and mitochondrial epilepsies demonstrate that altered metabolism and energy homoeostasis can have a direct impact on neuronal excitability and provide a rationale for the development of metabolism-based treatments. The myriad metabolic factors

altered in many inborn errors of metabolism resulting in synaptic dysfunction are broadly grouped and depicted in Fig. 4.

Metabolism-Based Treatments

The signature metabolic therapy for medically intractable epilepsy is the ketogenic diet, which was formulated a century ago to mirror the key biochemical changes associated with fasting – something anecdotally observed over millenia to help control seizures in individuals with epilepsy, but without significant caloric deprivation[19]. The ketogenic diet is a high-fat, low-carbohydrate and adequate protein diet that has been shown in prospective controlled studies to be effective against medically intractable epilepsy. As the name implies, the ketogenic diet – like fasting – induces prominent ketonemia through enhanced fatty acid oxidation which elevates blood levels of the principal ketone bodies beta-hydroxybutyate and acetoacetate[19,34,35]. In clinical practice, patients are treated with dietary formulations adhering to ketogenic/anti-ketogenic ratios of 3:1 to 4:1 ratio by weight of fats to carbohydrate plus protein, which results in serum beta-hydroxybutyate levels in the very low millimolar range (typically 2–5 mM, but this is often variable – as low as 0.4 mM and as high as 10 mM)[19,144].

Over the years, clinicians have used variations of the ketogenic diet to address common side-effects and theoretically increase ketosis (the medium-chain triglyceride diet)[22], to enhance palatability (the modified Atkins diet)[23], or have employed diets that capitalize on another seminal biochemical change induced by the ketogenic diet – i.e., relative hypoglycemia (the low-glycemic index treatment)[145]. Clinicians have reported that these variations of the ketogenic diet are all effective in a small majority of patients with treatment-resistant epilepsy, yielding significantly improved seizure control (>50% reduction in up to 60% of individuals, especially in very young infants[20,25,146,147]). There is also growing evidence that these diets can be successfully implemented in adult patients[148]. The traditional ketogenic diet and the medium-chain triglyceride diet appear comparable in terms of clinical effectiveness[21], but it is unclear whether the modified Atkins diet and low-glycemic index treatment show similar response rates as there are yet no rigorously controlled prospective head-to-head comparisons[25,149,150].

Given the remarkable clinical efficacy of the ketogenic diet and its variants, there has been growing scientific interest in elucidating the mechanisms of these metabolism-based treatments[14,151]. At present, despite a multiplicity of innovative mechanisms that have been advanced in the literature, and the surprising finding that specific metabolic substrates and enzymes can act like anti-seizure medications or modulate neuronal excitability in unexpected ways (Fig. 5), how diets render such clinical effects remains unclear. Dietary therapies are not without their challenges with respect to compliance and tolerability, so efforts continue to better understand the basic mechanisms of ketogenic diet action, such that insights gleaned from basic-translational studies can uncover novel targets – or indeed paradigms – for experimental therapeutics. In this regard, use of specific fuels such as ketone bodies (as readily administered esters) or medium-chain fatty acids are paving the way for novel therapeutic approaches based on the study of ketogenic diet mechanisms[152,153].

The major proposed mechanisms of metabolic therapies can currently be grouped into a few broad categories (Fig. 6): 1) restoration of impaired bioenergetics and mitochondrial function, notably modulation of TCA cycle and respiratory chain activity[154–156], as well as improved redox regulation and antioxidant capacity[102,103,106,157]; 2) synaptic regulation of both excitatory and inhibitory neurotransmission via metabolic substrates and enzymes[88], at both pre-synaptic and post-synaptic levels[158,159], and involving γ-aminobutyric acid type A ($GABA_A$), glutamate, and adenosine receptors[159] as well as K_{ATP} channels[160]; 3) glycolytic restriction with 2-deoxy-D-glucose[161] and regulation of glycolysis by apoptotic factors[162]; or augmentation of the pentose phosphate pathway by fructose-1,6-bisphosphate (a glycolytic intermediate)[163]; 4) signaling pathways involved in cellular metabolism such as the mTOR pathway[164,165]; 5) direct and indirect anti-inflammatory actions on neurons and BBB permeability[31,166–168]; and 6) epigenetic effects on DNA[169] and histones[166] which form the basis for disease-modifying and antiepileptogenic effects of the diet. A summary of proposed mechanisms underlying neuroprotective effects of various dietary treatments is depicted in Fig. 7. Beyond this, an exhaustive discussion of proposed mechanisms of dietary therapies is beyond the scope of the present review, and the reader is referred to recent references for additional details[14,26,151,170,171]. What should be emphasized at the outset though is that the mechanisms underlying the clinical effects of the ketogenic diet are multiple, parallel, overlapping and potentially synergistic. There is no single mechanism that can fully explain these effects. In this regard, the same can be said for the majority of ASMs[14,18].

From a therapeutic standpoint, detailed basic-translational studies of dietary therapies for epilepsy have not only strengthened the view that epilepsy in many ways can be considered a metabolic disease, but research has presented a few novel approaches that can theoretically be exploited for clinical development. A few examples illustrating the potential of certain metabolic substrates and enzymes as treatments for epilepsy are provided here. One example is 2-deoxy-D-glucose, which inhibits phosphoglucose isomerase, a key glycolytic enzyme, and which has been shown to possess relatively broad anti-seizure properties, to retard kindling (a model of epileptogenesis)[172], and mitigate post-traumatic epilepsy[173]. However, the protective effects of 2-deoxy-D-glucose may not be simply due to inhibition of glycolysis as other mechanisms have been implicated[161]. Current clinical efforts – in part encouraged by strong safety data – are being directed toward the treatment of acute repetitive seizures and status epilepticus[18]. And more recently, a medium-chain triglyceride product has been shown to be effective in pediatric patients with drug-resistant epilepsy[174].

As further proof of principle, the anti-epileptogenic potential of transient focal adenosine delivery was first tested in a rat model of systemic kainic acid induced epilepsy[123]. Young male rats were treated with the excitotoxin kainic acid to trigger epilepsy, and adenosine-releasing silk was implanted 9 weeks later into the lateral brain ventricles. Strikingly, epilepsy progression in the adenosine-group was completely suppressed and this effect was maintained far beyond the expiration of adenosine-release from the polymer – for at least 12 weeks with a reduction in seizure frequency by more than 70%. In addition, the progression of mossy fiber sprouting was also attenuated in the adenosine-treated rats. Mechanistically, the transient delivery of adenosine restored normal 5mC in the hippocampus long-term. A DNA methylation array analysis identified genes whose methylation status changed during

epilepsy development and was restored back to normal after adenosine therapy. Among the target genes identified was the polymerase PolD and various DNA interacting proteins including zinc finger proteins[123]. Taken together, these findings suggest that transient therapeutic adenosine augmentation can restore normal DNA methylation patterns, thereby preventing epilepsy progression (Fig. 3). In this regard, it is important to note that adenosine has been strongly implicated in the mechanism of ketogenic diet action, and that the ketogenic diet has been shown to reverse DNA methylation changes in a chronic animal model of epilepsy[169].

Subsequently, it was shown that the transient and systemic use of the ADK inhibitor 5-iodotuber-cidin (to increase adenosine) is antiepileptogenic in the intrahippocampal kainic acid mouse model of temporal lobe epilepsy[175]. Three days after kainic acid induced status epilepticus in mice, 5-iodotubercidin or vehicle was injected for a restricted time-span of 5 days. Six weeks after the status epilepticus, 94% of mice in the kainic acid/vehicle-injected control group developed robust seizures. In marked contrast, 59% of kainic acid/5-iodotubercidin-treated mice showed almost no seizure activity. These findings are in line with the anti-epileptogenic activity of adenosine that is mediated through an epigenetic mechanism[123]. Importantly, histopathological analysis demonstrated characteristic granule cell dispersion in KA/vehicle-injected control animals, which was prevented by 5-ITU[175]. Thus, the transient inhibition of ADK holds promise as a therapeutic strategy for epilepsy prevention.

Specifically, the nuclear and epigenetically active isoform ADK-L is a unique molecular and therapeutic target that links metabolism with developmental and epigenetic functions. It was previously demonstrated that a ketogenic diet leads to a reduction of ADK-L[176], a potential mechanism which could explain the epigenetic and disease modifying properties of metabolic treatments[169,177]. Consistent with this function and therapeutic significance of ADK-L, efforts are currently under way to develop novel small molecule compounds to target ADK-L as potential therapeutic agents for epilepsy prevention[178].

Additional strategies are currently pursued for the prevention of human epilepsy based on network pharmacology and the repurposing of FDA-approved drugs[179,180]. Those approaches include the antibiotic ceftriaxone[180], which increases astroglial glutamate uptake via the glutamate transporter GLT-1. Interestingly, through interaction with the adenosine A_{2A} receptor, adenosine has been shown to regulate GLT-1 expression and function[181,182] as a possible mechanism through which maladaptive changes in adenosine metabolism during epileptogenesis could influence a downstream target amenable to correction by an existing FDA-approved drug.

In a different vein, ever since the 1930's, investigators have long been interested in examining whether ketone bodies exert direct anti-seizure effects [183,184]. Despite numerous studies in preclinical models, whether ketones are directly responsible for the anti-seizure effects of the ketogenic diet remains unclear, since peripheral ketone levels have not consistently correlated well with seizure control in patients with epilepsy[183,185]. Nevertheless, the growing evidence that beta-hydroxybutyrate can indirectly modulate K_{ATP} channels, act as endogenous ligands for free fatty acid receptors[186], inhibits histone

deacetylases[166], regulate the mitochondrial permeability transition pore[187], prevent NLRP3 inflammasome assembly[167] – among many other actions[188,189] – provides a compelling scientific rationale for exploring ways to induce ketosis without dietary manipulation, such as administrating ketone esters which obviate the challenges inherent in administering ketone salts to achieve low millimolar concentrations in the blood[152].

Fatty acids have also drawn great attention in the study of ketogenic diet mechanisms given their important role as the key constituent of ketogenic diets described above. It has long been known that polyunsaturated fatty acids can directly inhibit voltage-gated sodium and calcium ion channels[190], but perhaps more importantly they can activate the transcription factor peroxisome proliferator-activated receptor (PPAR) alpha which regulates genes involved in the beta-oxidation of fatty acids and more generally energy homeostasis[191]. Moreover, the ketogenic diet has been shown to increase PPAR gamma expression, which then leads to anti-inflammatory and anti-oxidant actions[192]. However, despite strong pre-clinical data, whether polyunsaturated fatty acid ingestion affords anti-seizure effects remain unclear[193,194]. More recently, medium-chain triglycerides, notably decanoic acid (a C10 medium-chain triglyceride) was shown to inhibit post-synaptic AMPA receptors[195] much like the ASM perampanel[196], but also increased mitochondrial content in neurons and inhibited mTORC1 activity independent of glucose and insulin signaling[165]. Indeed, medium chain triglycerides may represent a potential new metabolic treatment for epilepsy across several species, including humans[197]. For example, a prospective open-label feasibility study of a medical food containing a specific ratio of decanoic acid and octanoic acid revealed beneficial effects on seizure frequency in children with drug-resistant epilepsy[174]. Furthermore, triheptanoin – the triglyceride of heptanoic acid which promotes anaplerosis (the replenishment of substrates involved in the TCA cycle) – induced anti-seizure effects in both animal models[198] and patients[199] with epilepsy. Finally, ketogenic diet therapy led to an increase in adenosine[176,177], which not only reduced seizure thresholds[200] but also exerted potent disease-modifying and antiepileptogenic effects[116,175]. Collectively, whether certain ketone preparations, adenosine-laden delivery vehicles or fatty acid formulations ultimately prove feasible and effective for epilepsy therapeutics remains to be determined.

An intriguing question that has emerged from the epilepsy and metabolism literature is whether any current ASMs used in clinical practice affect metabolic pathways, actions that may in part explain their efficacy against epileptic seizures. Metabolic targets are not believed to be relevant to ASM mechanisms, with one notable exception. Stiripentol, an ASM that is believed primarily to allosterically modulate $GABA_A$ receptors, appears to exert anti-seizure effects via inhibition of lactate dehydrogenase[88] (Fig. 5). This important intersection between traditional ASMs and brain metabolism (notably, the ANLS hypothesis summarized in Fig. 1) presents a broad opportunity to integrate concepts in both these areas for therapeutic innovation. Related to this is the notion that ASMs may act in part by reducing the energy needs of the epileptic brain, such that the spared energy can be redirected for normal neuronal signaling. Given that synaptic activity is highly energy demanding, the direct inhibitory effects of ASMs on neurotransmission may yield an important secondary benefit.

A more recent paradigm-shifting discovery has been the observation that the ketogenic diet may provide anti-seizure effects by modulating the gut microbiome – i.e., through specific changes in the abundance and composition of bacterial species that secondarily affect the blood-brain metabolome (e.g., decreasing gamma glutamyl amino acids) and influences brain levels of key neurotransmitters such as GABA and glutamate[65]. The gut microbiome, which has been increasingly linked to neurological and psychiatric disorders, now stands as a powerful integrator of metabolism and inflammation through the brain-gut axis.

Conclusions/Perspective

From both teleological and evolutionary perspectives, it is clear that cellular metabolism is one of the most important biological frameworks within which epileptic seizures arise. Indeed, without the fundamental substrates, enzymes and biochemical pathways that collectively constitute metabolic homeostasis or disruption in the brain, both normal and abnormal neuronal and glial activity would not be possible. In this sense and in a reductionist manner, epilepsy can be considered a metabolic disease, or more accurately, the heterogeneous epilepsies may represent electroclinical manifestations of complex perturbations in physiological networks fueled by basic biochemical processes. In recent years, this notion has been amplified by basic-translational as well as human studies revealing the metabolic underpinnings of seizure genesis and epileptogenesis. Moreover, the field of metabolism-based treatments for epilepsy has exploited our growing knowledge of brain metabolism to advance both empiric and highly reasoned approaches toward expanding therapeutic options. Additionally, paradigms for novel drug discovery have begun to explore the utility of metabolic readouts in various model systems[142,201–204], and technical advances have made it possible for investigators to interrogate cellular metabolism in unprecedented ways – whether through the development of molecular biosensors of specific molecules or biochemical flux, or molecular imaging techniques that provide new insights into the complex inner workings of cells, with ramifications for cellular membrane physiology, epigenetic changes and inflammation[3,205]. As drug discovery for epilepsy has been hampered for decades by a true lack of understanding of the mechanisms responsible for drug refractoriness[206] and given the sobering reality of unaltered treatment outcomes over many decades[15], renewed and expanded attention to the metabolic basis of epilepsy might yield new avenues for greater therapeutic gain.

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KEY POINTS

- Epileptic seizures induce widespread derangements in cellular and mitochondrial metabolism, as well as cerebral flood flow
- Primary defects in genes encoding mitochondrial proteins and/or metabolic substrates and enzymes can result in neuronal and glial network excitability
- Brain metabolic homeostasis and function should be viewed as an interplay between the cerebral circulation, glia and neurons
- Epilepsy can be viewed as a metabolic disease, and primordial mechanisms evoked by compounds such as adenosine may be highly relevant to seizures and epileptogenesis
- Metabolism-based treatments such as the high-fat ketogenic diet and its variants can help restore metabolic homeostasis and lead to seizure control
- The metabolic underpinnings of epilepsy provide a rational explanation for its syndromic nature and plethora of symptoms, and explain why metabolic therapies can treat epilepsy comprehensively
- As dietary therapies for epilepsy often control seizures in the medically intractable population, experimental therapeutics based on metabolic targets should be increasingly explored

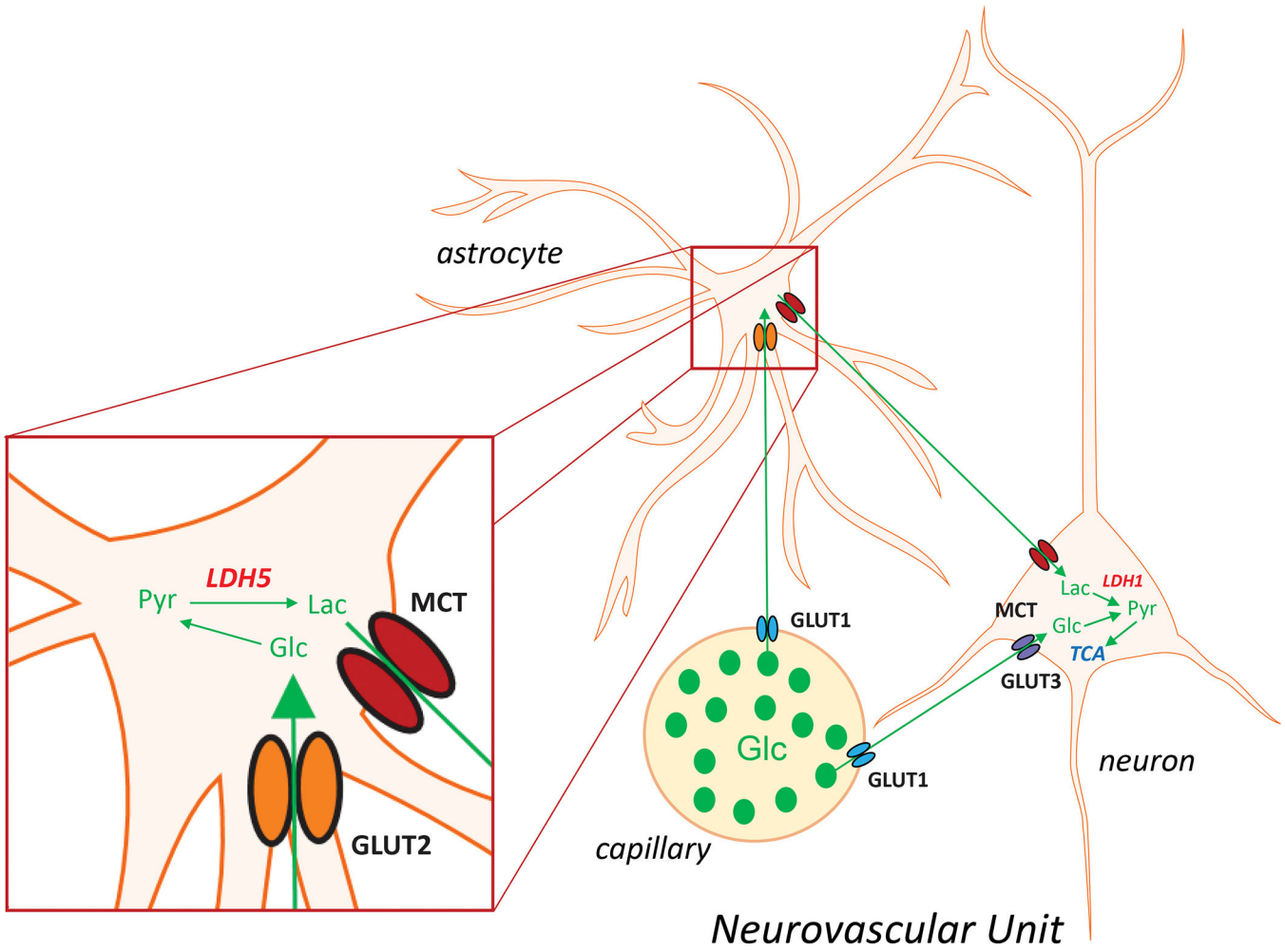

Figure 1: Astrocyte-Neuron Lactate Shuttle

Astrocytes control neurometabolic coupling. Glucose is taken up from the peripheral circulation by glucose transporters (GLUTs) differentially localized to different elements of the tri-partite neurovascular unit. GLUT1 is the main astrocyte glucose transporter, whereas in neurons the principal isoforms are GLUT2 and GLUT3. Glucose is metabolized to pyruvate within the glycolytic pathway, and then converted to lactate via lactate dehydrogenase (LDH) which exists as distinct isoforms as well, with LDH1 and LDH5 being prominently expressed in neurons and astrocytes, respectively. Lactate cannot passively diffuse down its concentration gradient through the blood-brain-barrier; hence, it must be transported via monocarboxylic acid transporters (MCTs). Our current conceptual understanding is that the lactate generated in astrocytes is transported into neurons, is subsequently converted to pyruvate, which then enters the tricarboxylic acid (TCA) cycle to generate energy.

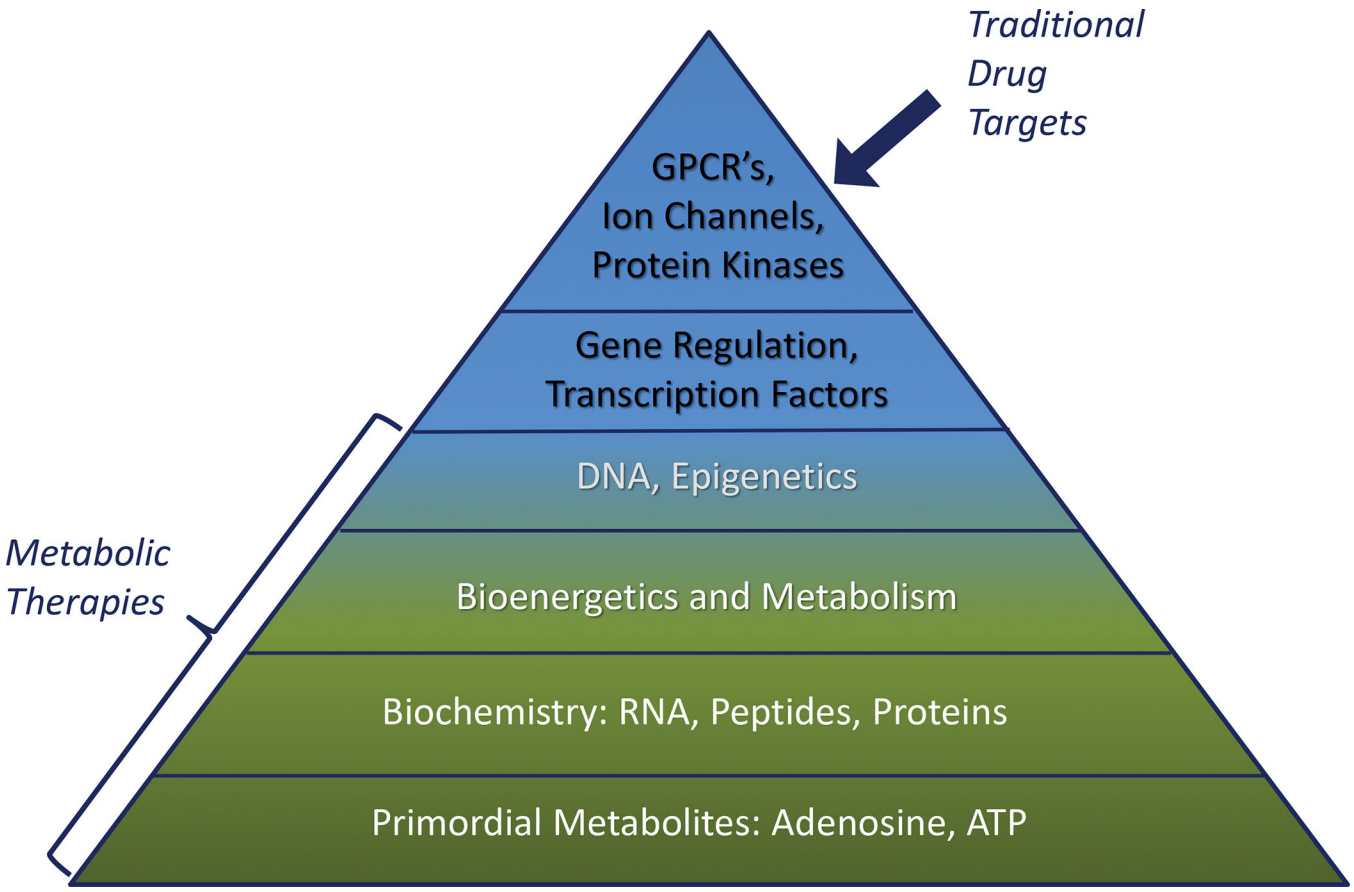

Figure 2: Epilepsy Treatment Targets in the Pyramid of Life

Traditional drug targets are only found at the top of the pyramid, whereas metabolic therapies are uniquely positioned to reset fundamental self-regulatory mechanisms that form functional homeostatic systems. Primordial metabolites such as adenosine and ATP are essential components of complex biochemical networks and represent the foundation for simple metabolism-based regulatory systems to enable energy homeostasis. The evolution of life required the advent of RNA, peptides and proteins, which established the framework for bioenergetics and metabolism. This was followed later by DNA and gene regulatory mechanisms such as epigenetic modifications or use of non-coding RNAs. Transcription factors provided further refinement to these mechanisms. Finally, regulatory systems based on G protein-coupled receptors (GPCRs), ion channels, and protein kinases evolved. From this schema, it becomes evident that if fundamental mechanisms at the base of the pyramid are disrupted, disease can result. Modified from Boison D, The Biochemistry and Epigenetics of Epilepsy: Focus on Adenosine and Glycine, Frontiers in Molecular Neuroscience 2016; 9:26. doi: 10.3389/fnmol.2016.00026.

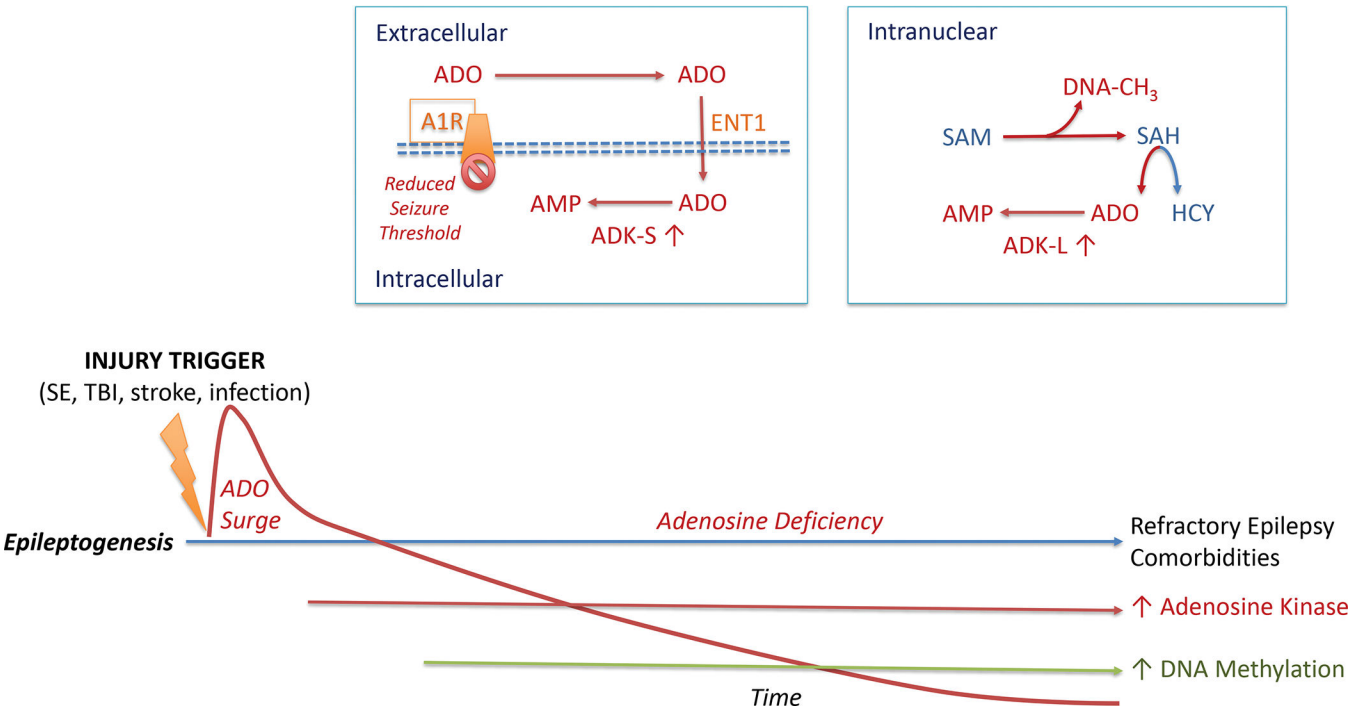

Figure 3: Adenosine Metabolism and Epileptogenesis

The epileptogenic process leading to acquired epilepsy can be initiated through a variety of injurious triggers including status epilepticus (SE), traumatic brain injury (TBI), stroke, or an infection of the brain. Such precipitating events lead to a biphasic response of the adenosine system: an acute neuroprotective adenosine (ADO) surge followed by progressive adenosine deficiency during epileptogenesis. Chronic adenosine deficiency is driven by maladaptive overexpression of the key adenosine metabolizing enzyme (ADK), which also drives an increase in DNA methylation, an epigenetic hallmark of acquired epilepsies. Extracellular adenosine is regulated by intracellular metabolism of the cytoplasmic isoform of adenosine kinase (ADK-S), which drives the uptake of adenosine through the equilibrative nucleoside transporter 1 (ENT1)). Metabolism-driven cellular uptake of adenosine leads to reduced adenosine A_1 receptor (A1R) binding, which is a mechanistic explanation why a pathological increase in ADK-S reduces seizure thresholds. In the cell nucleus, adenosine is coupled to the S-adenosylmethionine (SAM)-dependent transmethylation pathway, which determines DNA methylation (DNA-CH_3). The reaction product S-adenosylhomocysteine (SAH) is hydrolized into homocysteine (HCY) and adenosine. Metabolism of adenosine to AMP via the nuclear isoform of adenosine kinase (ADK-L) drives the flux of methylation reactions through this pathway. Thereby, increased ADK-L drives increased DNA methylation. It is important to note that this figure is not drawn to scale. The acute adenosine surge takes place during the first 24 hours after an injurious event, whereas all subsequent steps of the epileptogenic cascade occur over a time-span of days to weeks to months (or longer) after a precipitating event.

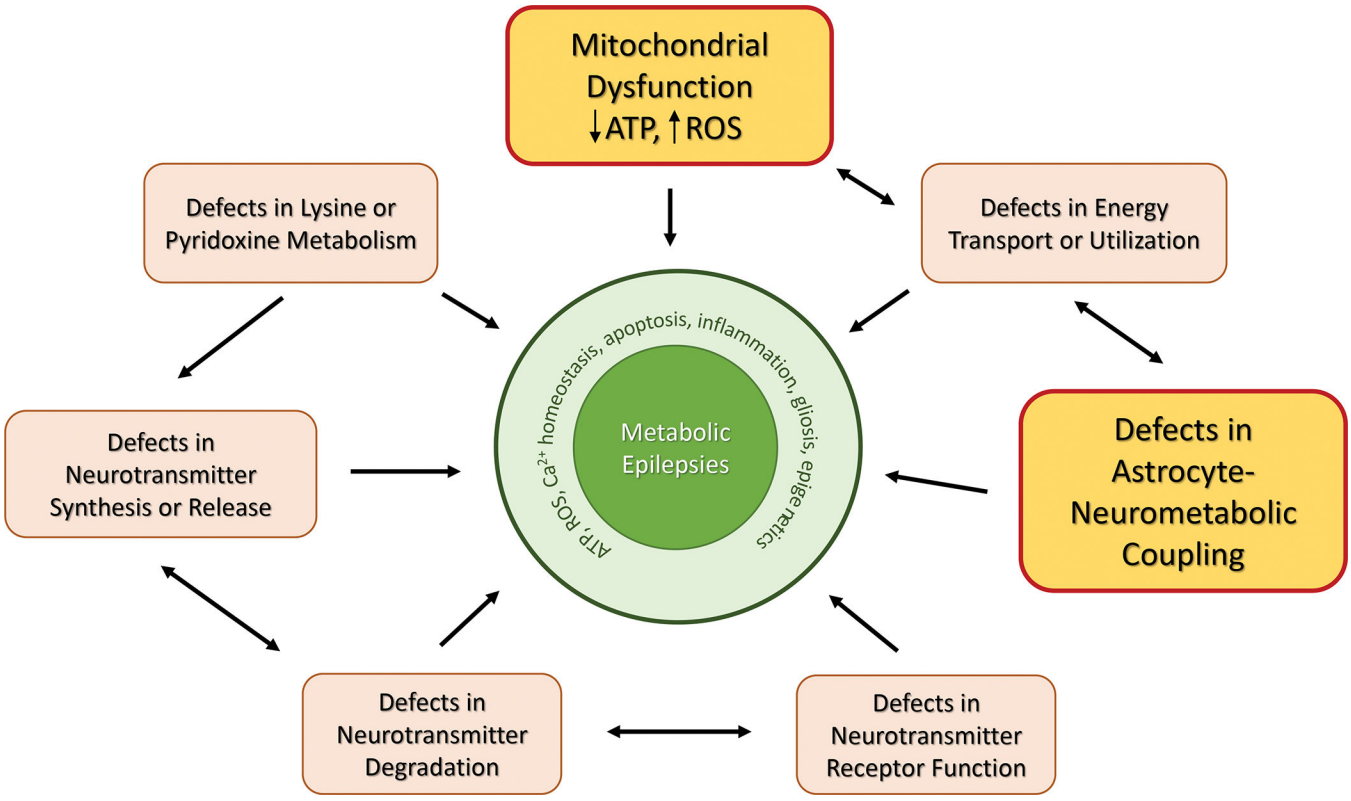

Figure 4: Principal Mechanisms Involved in the Pathogenesis of Metabolic Epilepsies

Metabolic epilepsies are complex syndromes characterized by seizures, co-morbidities and developmental impairment. The complex pathology of metabolic epilepsies can be explained by congenital defects leading to an imbalance of neurotransmitter function on the levels of synthesis, degradation, transport, and receptor binding. More importantly, congenital or acquired defects in mitochondrial function and astrocyte-neurometabolic coupling combine to trigger major defects in energy transport or utilization as a potential and preventable (e.g., through metabolic therapies) cause for metabolic epilepsies.

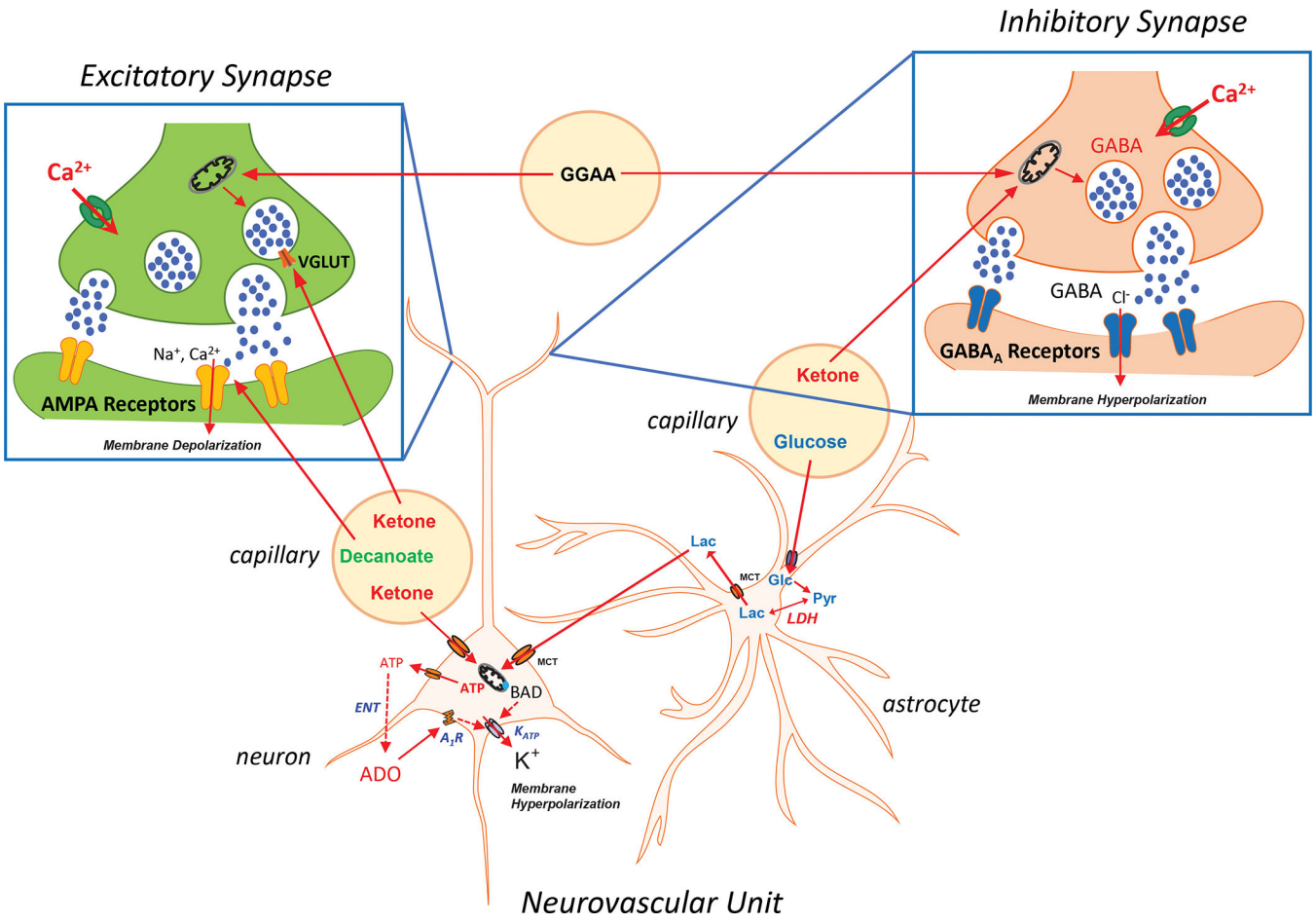

Figure 5: Metabolic Substrates and Enzymes Regulating Synaptic Neurotransmission and Neuronal Excitability

Schematic of the neurovascular unit comprising the neuron, astrocyte and microvasculature, and expanded views of prototypic excitatory and inhibitory central synapses – showing presynaptic and postsynaptic terminals. Ketone bodies can directly compete with the chloride anion regulatory site of vesicular glutamate transporters (VGLUTs) to prevent presynaptic release of glutamate. Decanoate, a C10 medium chain triglyercide, can inhibit post-synaptic AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors much like the anti-seizure medication perampanel. Ketones can also serve as a substrate for glutamate production in inhibitory presynaptic terminals, which increases the synthesis of γ-aminobutyric acid (GABA) via glutamic acid decarboxylase, thereby enhancing inhibitory neurotransmission. Additionally, ketones enhance mitochondrial ATP production. When ATP is released into the extracellular space via pannexin channels, it is degraded to adenosine through an equilibrative nucleotidase (ENT). Adenosine in turn can bind to inhibitory adenosine type 1 receptors (A1Rs) in an autocrine fashion to limit membrane excitability. Ketones, likely through this mechanism, can indirectly activate ATP-sensitive potassium (K_{ATP}) channels which induce membrane hyperpolarization. Moreover, increased membrane activity may deplete ATP levels in proximity or subjacent to K_{ATP} channels, which would decrease the ATP/ADP ratio and activate these inhibitory channels. K_{ATP} channels can also be indirectly regulated by BAD (Bcl2 associated agonist of cell death),

a protein that serves a dual function as a pro-apoptotic factor and modulator of glycolysis. Glucose (Glc) transported into astrocytes produces pyruvate (Pyr) which can be converted to lactate (Lac) via lactate dehydrogenase (LDH); inhibition of LDH – as reported using the anti-seizure medication stiripentol – through yet unclear mechanisms affords anti-seizure effects (see Fig. 1 which details some aspects of the astrocyte-neuron lactate shuttle). Finally, ketogenic diet-induced changes in the gut microbiome has been shown to decrease gamma glutamyl amino acids (GGAA) in the blood metabolome, resulting in secondary changes in glutamate and GABA production – specifically, an increase in the GABA/glutamate ratio. Abbreviations: MCT, monocarboxylic acid transporter; ADO, adenosine.

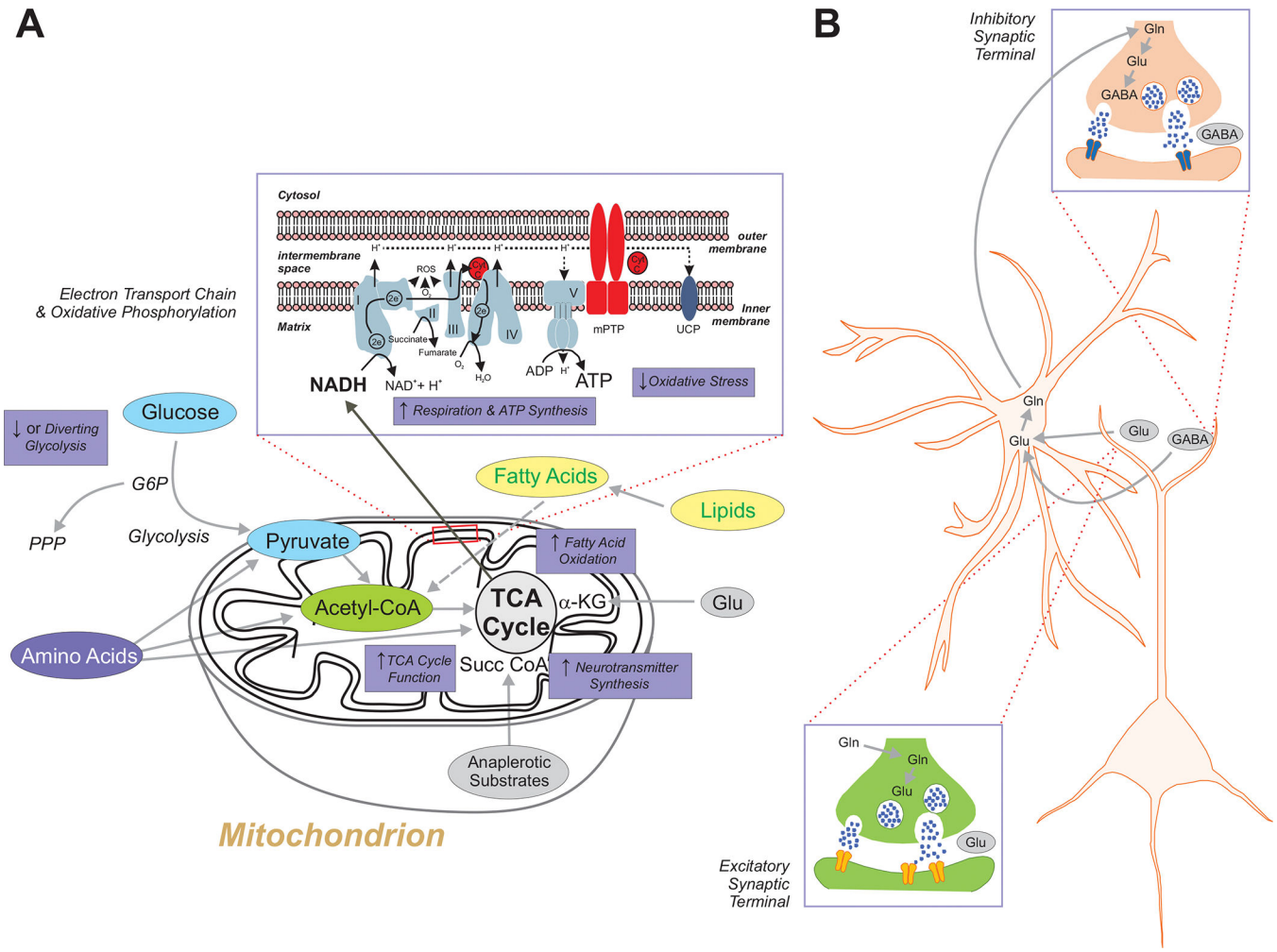

Figure 6: Biochemical Pathways and Mitochondria

A. The biochemical effects of metabolic anti-seizure treatments have been linked in one way or another to the control of seizures or epilepsy in various experimental models. Inhibition of glycolysis with 2-deoxy-D-glucose results in seizure control in multiple animal models. Additionally, the glycolytic intermediate fructose-1,6-bisphosphate has been shown to exert broad anti-seizure effects, possibly by augmenting the pentose phosphate pathway. All amino acids can be converted for energy utilization if required, and can be glucogenic, ketogenic or both depending on where they enter certain biochemical pathways. For example, alanine, glycine and tryptophan can be converted to pyruvate, leucine and tryptophan to acetyl-coA, and histidine and glutamate into alpha-ketoglutarate of the tricarboxylic acid (TCA) cycle. Fatty acids derived from lipids can undergo fatty acid oxidation within the mitochondrial matrix and generate acetyl-CoA which can be condensed into acetoacetyl-CoA which is a precursor for the synthesis of acetoacetate and beta-hydroxybutyrate. Ketones are not only utilized for ATP production, but they can also be used for the synthesis of neurotransmitters such as glutamate and GABA. Certain fatty acids also enhance mitochondrial uncoupling protein (UCP) activity which partially dissipates the proton gradient across the inner mitochondrial membrane and as a result reduces reactive oxygen species (ROS) and reactive nitrogen species (RNS) production.

Triglycerides such as triheptanoin provide anaplerotic substrates that when subsequently metabolized to succinyl CoA in mitochondria can also render anti-seizure effects. Finally, whether through the ketogenic diet (ketogenic diet) or ketone bodies, or through oxidative metabolism, mitochondrial respiratory chain function is enhanced – with resultant increases in oxygen consumption and adenosine triphosphate (ATP) production, and a reduction in oxidative stress. Inset: detailed view of the mitochondrial respiratory chain, and the five respiratory chain complexes denoted in Roman numerals. **B**. The glutamine-glutamate cycle between neurons and astrocytes is critical for the presynaptic biosynthesis of GABA, as well as providing glutamate that is converted by glutamate dehydrogenase to α-ketoglutarate which enters the TCA cycle as an intermediate[87]. Abbreviations: NAD^+/NADH, oxidized/reduced forms of nicotinamide adenine dinucleotide; CytC, cytochrome C; G6P. glucose 6-phosphate; mPTP, mitochondrial permeability transition pore; TCA, tricarboxylic acid cycle; UCP, mitochondrial uncoupling protein; Gln, glutamine; Glu, glutamate; GABA, γ-aminobutyric acid;

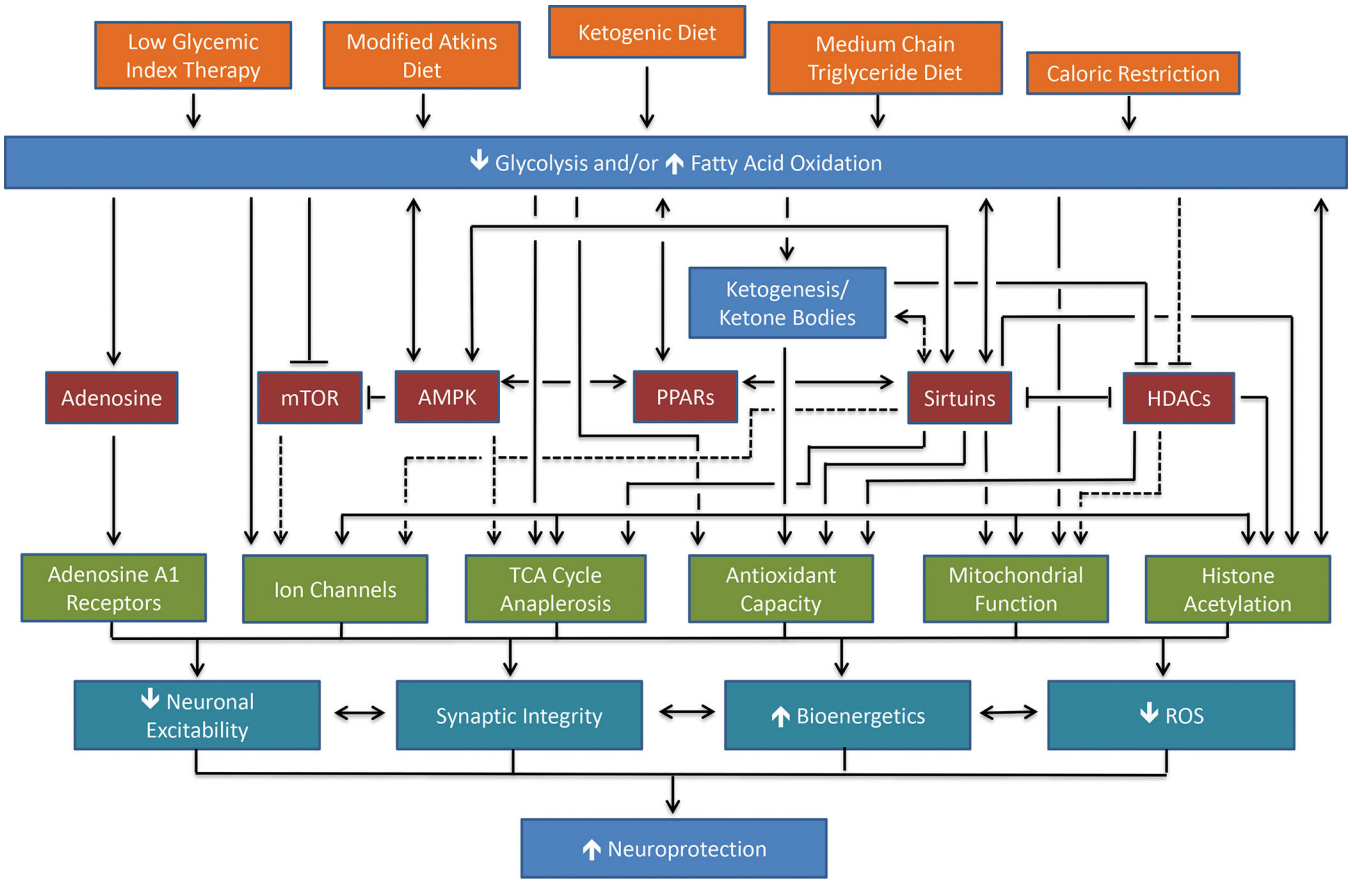

Figure 7: Mechanisms Underlying the Neuroprotective Effects of Ketogenic and Related Diets

Proposed mechanisms for the neuroprotective effects of the ketogenic diet and its variants. The major dietary interventions are shown in orange, including caloric restriction which mirrors many but not all the biochemical or physiological effects of the exogenous diets. The metabolic effects of the various diets are shown in blue; the energy-sensing pathways that may mediate the effects of the dietary alterations are shown in red; the cellular effects resulting from the diets and/or the energy-sensing pathways are shown in green; and the broad protective effects of the diets and the resulting cellular effects are in shown cyan. Solid black lines indicate linkages detailed in the literature; dashed black lines represent possible, but as yet unproven links. Abbreviations: mTOR, mammalian target of rapamycin; AMPK, adenosine monophosphate-activated kinase; PPARs, peroxisome proliferator-activated receptors; HDACs, histone deacetylases; TCA, tricarboxylic acid cycle; ROS, reactive oxygen species. Modified with permission from Gano et al., Ketogenic Diets, Mitochondria and Neurological Diseases, Journal of Lipid Research, 2014. 55: 2211–2228.