The metabolic basis of epilepsy

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Abstract | The brain is a highly energy-demanding organ and requires bioenergetic adaptability to balance normal activity with pathophysiological fuelling of spontaneous recurrent seizures, the hallmark feature of the epilepsies. Recurrent or prolonged seizures have long been known to permanently alter neuronal circuitry and to cause excitotoxic injury and aberrant inflammation. Furthermore, pathological changes in bioenergetics and metabolism are considered downstream consequences of epileptic seizures that begin at the synaptic level. However, as we highlight in this Review, evidence is also emerging that primary derangements in cellular or mitochondrial metabolism can result in seizure genesis and lead to spontaneous recurrent seizures. Basic and translational research indicates that the relationships between brain metabolism and epileptic seizures are complex and bidirectional, producing a vicious cycle that compounds the deleterious consequences of seizures. Metabolism-based treatments such as the high-fat, antiseizure ketogenic diet have become mainstream, and metabolic substrates and enzymes have become attractive molecular targets for seizure prevention and recovery. Moreover, given that metabolism is crucial for epigenetic as well as inflammatory changes, the idea that epileptogenesis can be both negatively and positively influenced by metabolic changes is rapidly gaining ground. Here, we review evidence that supports both pathophysiological and therapeutic roles for brain metabolism in epilepsy.

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Epilepsy is a common neurological disorder that can occur at any age and has a worldwide prevalence of approximately 1% in the general population[1]. Spontaneous recurrent seizures (SRS), the defining hallmark of epilepsy, have been linked to increased neuronal excitability and hypersynchrony, and often result from genetic or other brain insults, the effects of which are modified by environmental factors[1]. Irrespective of the aetiology, epilepsy creates a high energy need in the brain to initiate seizure onset (ictogenesis), to sustain prolonged seizure activity, to promote recovery from seizures and to repair damage. Indeed, without sufficient ATP — the universal energy currency of life, mostly produced by mitochondria[2] — it would be impossible to regenerate the membrane potentials that are needed to enable epileptiform activity[3]. Conversely, metabolic treatments such as the ketogenic diet, which increases fuel to the brain in the form of ketone bodies, are uniquely suited to preventing seizures and to aiding recovery after a seizure.

SRS and prolonged seizures such as status epilepticus have long been known to induce secondary aberrations in cellular bioenergetics and metabolism[4,5]. These aberrations include glutamate-mediated and free radical-mediated injury, neuroinflammation[6–8] and widespread disruptions in brain homeostatic mechanisms. However, less well appreciated is the observation that inherent defects in brain energy metabolism, whether resulting from mutations in genes that encode mitochondrial proteins or metabolic substrates and enzymes constituting major biochemical pathways[5,9], or from redox instability[10] or epigenetic changes[11], can independently precipitate seizures. Irrespective of the 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 antiseizure medications (ASMs), most of which are designed to target cell membrane-bound ion channels and transporters to reduce excitation and/or increase inhibitory neurotransmission at the synaptic level[14]. Although ASMs have been shown to control SRS in most patients with epilepsy (but not without potentially serious adverse effects), the seizures remain medically intractable in approximately one-third of cases[1]. Despite decades of basic and translational epilepsy research and the approval of dozens of new drugs for clinical use, the relative proportion of this refractory population has remained unchanged[15], probably in part because drug development for epilepsy has traditionally been based on acutely provoked seizure models that do not faithfully recapitulate the

Key points

- Epileptic seizures induce widespread derangements in cellular and mitochondrial metabolism, as well as cerebral flood flow.
- Primary defects in genes that encode mitochondrial proteins and/or metabolic substrates and enzymes can increase neuronal and glial network excitability.
- Brain metabolic homeostasis and function can be viewed as an interplay among the cerebral circulation, glia and neurons, also known as the neurovascular unit.
- Epilepsy can be viewed as a metabolic disease, and primordial mechanisms evoked by compounds such as adenosine could be highly relevant to seizures and epileptogenesis.
- Metabolism-based treatments such as the high-fat ketogenic diet and its variants can help to restore metabolic homeostasis and enable seizure control.
- As dietary therapies can often control seizures in individuals with medically intractable epilepsy, experimental therapeutics based on metabolic targets should be explored.

pathophysiological changes seen in chronic epilepsy, 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 seem to have novel mechanisms of action, such as binding to synaptic vesicle protein 2A and inhibition of cholesterol 24-hydroxylase[17,18]. In addition, the use of chronic epilepsy models in the screening process for preclinical development of ASMs is steadily increasing[16]. Importantly, however, metabolic targets have only recently begun to attract attention in the field of epilepsy experimental therapeutics[18].

The century-old high-fat, low-carbohydrate ketogenic diet, the best-established metabolism-based treatment for epilepsy, provides the strongest evidence that targeting of brain bioenergetics and metabolism can mitigate seizure activity, most 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], the modified Atkins diet[23] and the low glycaemic index treatment (LGIT)[24], have provided further evidence-based validation of metabolic and dietary therapies for the spectrum of epilepsies encountered in clinical practice[25]. Although the fundamental mechanisms that underlie the antiseizure efficacy of such treatments remain unclear, evidence is accumulating that metabolic approaches derived from these diets can have neuroprotective — and perhaps even disease-modifying — effects[26]. In this Review, we highlight the growing recognition that disruptions in cellular metabolism can be both a cause and a consequence of epileptic seizures and discuss how this emerging science might be exploited to develop innovative therapeutic strategies.

Brain energy metabolism

The brain is a highly energy-dependent organ: at rest, it accounts for approximately 20% of oxygen and 25% of glucose consumption in the body even though it comprises only 2–3% of total body weight[27]. Neurons have an exceedingly high energy demand owing to their many cellular housekeeping functions, including 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 signalling, synaptic activity and plasticity change[2,28,29]. Despite its high demands, however, the brain lacks an adequate endogenous energy reserve, so it relies on a range of exogenous energy sources to maintain normal function, mostly via transport of substrates across the blood–brain barrier[30,31]. Astrocytes are known to store glycogen as a source of glucose-6-phosphate, a major substrate for glycolytic ATP production[32], but this resource is insufficient to meet the immediate energy needs of the brain. Therefore, brain glycogen might act more as an emergency energy reserve and may also serve unique signalling 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]. By contrast, neurons are much more dependent on the immediate availability of glucose, which is extracted from the capillaries via glucose transporters, in particular, GLUT3[33,38]. Although neurons can generate substantial quantities of ATP via oxidative metabolism to meet their high energy needs, these cells might maintain viability and long-term function by accessing metabolic fuel from astrocytes, for example, via an astrocyte–neuron lactate shuttle (ANLS)[39–41] (FIG. 1). Lactate cannot passively diffuse across the blood–brain barrier[30], and, as it cannot be directly utilized for energy production, it must be first transported into cells via monocarboxylic transporters[42] and be converted enzymatically to pyruvate by lactate dehydrogenase (LDH). This enzyme exists in multiple isoforms, with LDH1 and LDH5 being primarily expressed in neurons and astrocytes, respectively (FIG. 1). The ANLS hypothesis is not without controversy, however, as some evidence suggests that oxidative metabolism of lactate in neurons is not always 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 might rely more on their own aerobic glycolysis than on 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, which together comprise the neurovascular unit (FIG. 1). Mitochondria are the most important determinants of bioenergetic capacity and are essential for both normal and pathological function of all cell types in the brain[2,45]. Any compromise of mitochondrial function can induce dysregulation of intracellular calcium, increased oxidative stress and, ultimately, apoptotic cell death.

Though often neglected in the epilepsy field, glia have major roles in both seizure prevention and seizure genesis by controlling the ionic balance between intracellular and extracellular compartments and modulating synaptic neurotransmission[37]. During normal brain activity, astrocytes help to prevent neuronal hyperexcitability and seizures by buffering potassium ions and regulating glutamate uptake[37]. Following disruption

during seizure activity, ionic and neurotransmitter homeostasis can be restored by astrocytes through various mechanisms, the most important of which are reuptake of glutamate from the synaptic cleft and buffering of extracellular potassium through glial end-feet, which link to the brain microvasculature[30,37]. Importantly, in an in vitro stem cell model, reuptake of glutamate into astrocytes triggered enhanced glycolysis, leading to increased generation of pyruvate, which was converted to lactate and subsequently transported to neurons via the ANLS[46]. Moreover, astrocytes are increasingly recognized to form large networks of highly interconnected cells, mostly through gap junction channels (connexins 30 and 43), which enable bidirectional exchange of ions, nutritional metabolites, second messengers, amino acids, peptides, nucleotides and even RNA[37]. Hence, astrocytes are uniquely poised to modulate network activity, both electrically and metabolically. Owing to their ability to use glycogen as fuel, astrocytes have a crucial role in maintaining not only energy metabolism but also excessive neuronal firing, as seen during epileptic seizures, and evidence is growing that these cells are involved in seizure genesis and epileptogenesis[37,47–49].

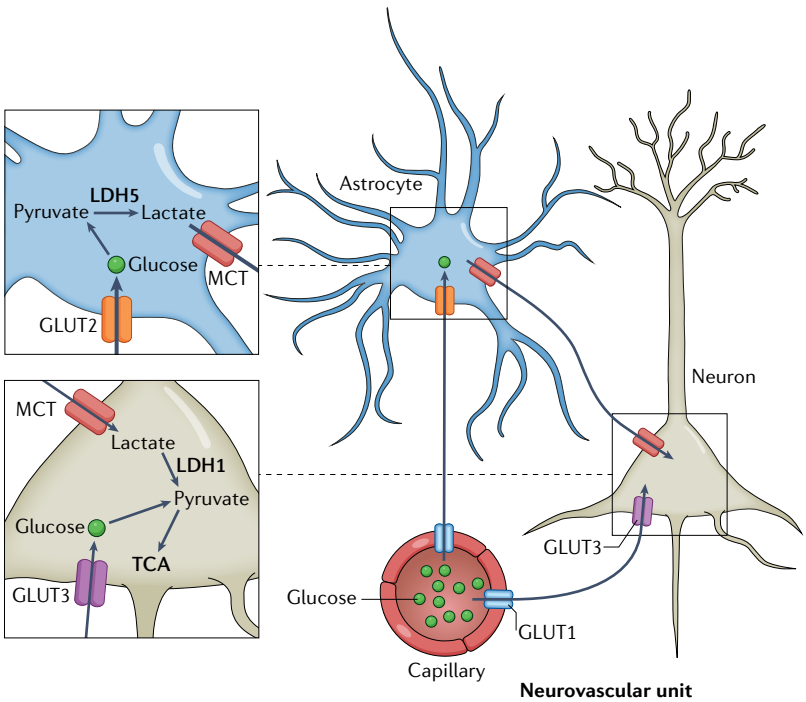

Fig. 1 | **The astrocyte–neuron lactate shuttle.** The astrocyte–neuron lactate shuttle hypothesis was formulated to explain how astrocytes control neurometabolic coupling. Glucose is taken up from the peripheral circulation by glucose transporters (GLUTs), which are differentially localized to different elements of the tripartite neurovascular unit. GLUT1 is the main astrocyte glucose transporter, whereas in neurons the principal isoforms are GLUT2 and GLUT3. Glucose is metabolized to pyruvate via the glycolytic pathway and is then converted to lactate by lactate dehydrogenase (LDH), which also exists as distinct isoforms, with LDH1 and LDH5 showing robust expression in neurons and astrocytes, respectively. Lactate cannot passively diffuse down its concentration gradient through the blood–brain-barrier and must be transported via monocarboxylic acid transporters (MCTs). Our current understanding is that the lactate generated in astrocytes is transported into neurons and is subsequently converted to pyruvate, which then enters the tricarboxylic acid (TCA) cycle to generate energy.

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 functions ordinarily ascribed to that area. These seizures can sometimes secondarily generalize to the entire cortex via adjacent spread or through commissural pathways. By contrast, primary generalized seizures manifest through disruption and enhancement of bilateral and reciprocal thalamocortical connections, which are modulated by subcortical structures via multiple ascending pathways[50]. Although this framework is often 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 stood the test of time.

The electroclinical phenomenology of epilepsy is well understood, and the role of adaptations in cerebral blood flow and metabolic coupling within the tripartite neurovascular unit in sustaining seizure activity is becoming increasingly clear. However, we know little about the mechanisms, including circadian and environmental factors, that initiate and terminate seizure activity[51]. For decades, the simple conceptual notion has been that an overall central excitatory–inhibitory imbalance precipitates seizure activity. Although this model makes intuitive sense, the mechanisms that induce network instability cannot all be reduced to increased excitation and/or reduced inhibition. For example, increased activity of GABAergic interneurons, which are usually inhibitory, has been shown to paradoxically induce network excitability and seizures[52–54], and ephaptic (non-synaptic) mechanisms[55–58] can either increase or decrease neuronal synchrony. Those bidirectional functions also depend on transmembrane ion gradients, which might change during the development and progression of epilepsy.

Pathophysiological mechanisms for epilepsy that have been elucidated over the past quarter century include ion channel dysfunction (primarily owing to genetic defects in various subunit proteins, resulting in either loss or gain of function)[59], structural brain pathologies such as focal cortical dysplasia or other malformations of cortical development[60,61], and changes secondary to postnatal brain insults such as hypoxic–ischaemic encephalopathy or traumatic brain injury, including cellular loss and synaptic reorganization, aberrant neurogenesis and/or blood–brain barrier dysfunction[30,31,62]. Molecular disruptions in cell signalling pathways such as mammalian target of rapamycin (mTOR)[63] have also been implicated, as have neuroinflammation (caused by intrinsic and extrinsic factors[6,7]), stress and hormones. In addition, developmental changes in neurotransmitter subunit expression and immaturity of homeostatic mechanisms affecting ionic gradients and transport have been linked to increased seizure propensity, especially in the developing brain[64].

In recent years, the gut microbiota has been implicated in both the pathogenesis of seizures and the therapeutic efficacy of the ketogenic diet[65], further strengthening the idea 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. Moreover, inborn errors of metabolism, particularly those that impair normal mitochondrial physiology and respiratory chain function, can manifest prominently with epileptic seizures[9].

The ever-expanding list of seizure-modifying factors indicates that virtually any pathophysiological mechanism involving the brain — in particular, those that induce stress — can elevate the risk of seizures[68,69]. An increasing number of studies have shown seizures or epilepsy arising from unexpected mechanisms or genes[59,70,71], and epilepsy is recognized as a common comorbidity of many neurological disorders. Though bewildering to comprehend in some respects, this mechanistic heterogeneity affords innovative opportunities to exploit advanced molecular approaches to develop more efficacious and better tolerated (and even precision) therapies, and ultimately to mitigate epileptogenesis[64,72] and identify relevant biomarkers[73].

Several lines of evidence suggest that physiological and metabolic changes are a cause and/or a consequence of seizure activity in humans. Functional and biochemical imaging techniques such as PET, functional MRI (fMRI) and magnetic resonance spectroscopy (MRS) have revealed alterations in cerebral blood flow and metabolism in the brains of individuals with epilepsy[74–76]. ^{18}F-Fluorodeoxyglucose (^{18}F-FDG) PET studies have shown notable increases in focal or regional blood glucose utilization during seizure activity[77]. Although such findings have traditionally been interpreted as evidence of a seizure focus, hypermetabolism per se might reflect other pathologies such as changes associated with brain plasticity[77]. Concomitant EEG monitoring during ^{18}F-FDG uptake can be used to distinguish between local seizure foci and more general plasticity changes. Interestingly, in children with Sturge–Weber syndrome, interictal hypermetabolism was observed in the cortex during the period of epileptogenesis[78]. A follow-up MRS-based study suggested that this hypermetabolism is related to increased glutamate release in the affected brain regions[79]. Conversely, ^{18}F-FDG PET studies have shown interictal hypometabolism in individuals with temporal lobe epilepsy (TLE)[80], which might indicate an energy crisis as a functional basis for impaired astrocytic potassium buffering and glutamate uptake and, hence, a lowering of seizure thresholds. Animal studies have consistently shown increased metabolic flux in the brain during epileptic seizures, and single-photon emission CT (SPECT) studies in humans have shown dynamic changes in cerebral perfusion during both interictal and ictal periods[76].

fMRI, which measures blood oxygenation levels as a surrogate for neuronal function and connectivity during cognitive tasks, has also proved useful for studying brain function in individuals with epilepsy[76]. Although fMRI has been used mostly to conduct language mapping for epilepsy surgery, its list of applications in the epilepsy field is growing, including broader assessments of neuronal functioning (information processing), brain network connectivity and activity, and even treatment outcomes. fMRI cannot directly interrogate brain metabolism, but when used in combination with MRS, PET, SPECT and EEG (non-invasive or invasive), it can provide a valuable tool to aid delineation of the functional and biochemical properties of the brain in people with epilepsy.

Despite numerous scientific and technological advances over the past few decades, an important caveat must be considered when attempting to gain an accurate mechanistic understanding of the epileptic brain. It is often tempting to assign causality to any identifiable alteration in the brain that could theoretically enhance neuronal excitation towards seizure genesis. However, the key mediators and pathways that are directly responsible for ictogenesis and epileptogenesis are not always straightforward to identify. Seizures reflect a complex array of perturbations occurring at multiple hierarchical levels, from biochemistry to cellular structure and function, and the consequences of changes in neuronal and glial network activity are often unpredictable.

Seizure-induced impairment of metabolic homeostasis

To truly appreciate the crucial role of metabolism in epilepsy, we must acknowledge the existence of bidirectional and multidirectional molecular interactions that add layers of complexity to the many vicious cycles that have been implicated in epileptogenesis[62,64,81]. Seizures themselves cause impairments in metabolic homeostasis and, in turn, derailment of key metabolic and biochemical functions contributes to an increased likelihood of seizure generation. In this section, we discuss how epileptic seizures affect cellular metabolism.

During acute seizures, the brain uses glucose to fuel the heightened energy demand, resulting in preferential formation of lactate over acetyl-CoA[82]. Glycolytic flux increases during seizure activity but decreases during the interictal periods, which might help to explain the phenomena of ictal hypermetabolism and interictal hypometabolism, long considered to be metabolic hallmarks of human and experimental epilepsies[83,84]. Although glycolysis is a less efficient process for ATP production than 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 towards glycolysis under aerobic conditions during seizures is supported by reports of increased lactate production in the human hippocampus[85]. This type of aerobic glycolysis mirrors the Warburg effect that has been described in cancer cells, which exhibit metabolic derangements similar to those observed in the epileptic brain[86].

Increased ictal glucose metabolism might support plasticity processes in the brain, such as gliosis, neurogenesis and axonal sprouting[87]. 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 to be responsible for profound plasticity changes in the brain in response

to seizure activity. 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 versus mitochondrial oxidation), the impact of seizures on mitochondrial function is of paramount importance. Several enzymes involved in the mitochondrial TCA cycle, which converts pyruvate to carbon dioxide, are known to be compromised by acute seizures (for example, status epilepticus) or chronic seizure activity. Reductions in the activity of pyruvate dehydrogenase, α-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) that enable oxidative phosphorylation, such as complex I, are known to be impaired in TLE and rodent models of epilepsy[95–97]. The existence of mitochondrial dysfunction associated with these alterations is supported by findings from experimental status epilepticus-induced epileptogenesis models, in which oxygen consumption rates transiently increase within minutes of status epilepticus onset, return to baseline during the seizure-free latent period and decrease during the chronic epilepsy phase[98]. These experimental data are consistent with clinical findings from individuals with 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, a superoxide-generating enzyme that is found in mitochondria and the plasma membrane, has a major role in the generation of ROS in response to status epilepticus[92,100,101]. Seizure-induced increases in mitochondrial ROS production can be attributed to the following factors: increased substrate utilization and electron transfer to oxygen during interictal periods; to an overload of calcium; and to the inhibition of ETC complexes, all of which combine to transfer electrons to oxygen. Additional mechanisms that might contribute to seizure-induced oxidative stress include inactivation of the mitochondrial antioxidant superoxide dismutase 2 via decreased activity of sirtuin 3 or alterations in peroxide detoxification activity. ROS generation can result in inhibition of ETC complex I, leading to further ROS production, thus forming a pathologically regenerative cycle that drives increased oxidative stress. This process results in further free radical damage to cellular macromolecules — a major cause and consequence of extended seizure activity[102]. Seizure-induced neuronal death can be directly attributed to oxidative stress and the formation of reactive aldehydes, hydroxyl radicals and redox-active iron, which damage mitochondrial DNA, proteins and lipids[10,103]. Consistent with increased oxidative stress as a pathological hallmark of epilepsy, depletion of the endogenous antioxidant glutathione and formation of oxidized glutathione disulfide have been demonstrated in both human epilepsy and rodent models of acquired epilepsy[94,104–106]. In addition, at low concentrations, ROS are thought to possess signalling properties and to modulate GABAergic neurotransmission through both pre-synaptic and post-synaptic mechanisms[107].

Seizure-induced disruption of calcium homeostasis might also contribute to the detrimental effects of seizures. Excitotoxic neuronal death is primarily driven by excessive activation of NMDA receptors and subsequent intraneuronal accumulation of toxic levels of calcium. This calcium overload in turn promotes the generation of ROS or reactive nitrogen species, which compromise mitochondrial function, cause metabolic impairment and activate necrotic and apoptotic pathways. In a mouse model, calcium imaging during an ictal event revealed increased calcium influx in almost all neurons and astrocytes[108]. Mitochondrial calcium homeostasis depends on the mitochondrial calcium uniporter, rapid mitochondrial calcium uptake and mitochondrial ryanodine receptors[109]. Changes in calcium concentration in the mitochondrial matrix regulate the activity of the mitochondrial ETC, which is required for ATP production[110,111]. Through this mechanism, increases in seizure-induced calcium fluxes could directly compromise mitochondrial function.

Metabolic and epigenetic changes in epilepsy

The regulation of energy homeostasis has been crucial for the evolution of all living systems. Under conditions of excessive energy consumption or depletion of energy supplies, a rheostat-like system is needed to conserve energy. 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 a regulator of the *S*-adenosylmethionine-dependent transmethylation pathway[115,116], an energy crisis would also affect RNA synthesis by lowering ATP levels and DNA methylation by increasing adenosine levels. Both processes are expected to 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, conventional pharmacotherapy development starts with 'druggable' targets at the top of the pyramid of life (FIG. 2). For example, benzodiazepines were almost discovered by chance in 1957, leading to the subsequent characterization of the 'benzodiazepine receptor' in the CNS in 1977 (REF.[117]). The benzodiazepine binding site was only later revealed to be an integral part of the $GABA_A$ receptor complex[117]. Therefore, drug-driven therapy development led to a major focus

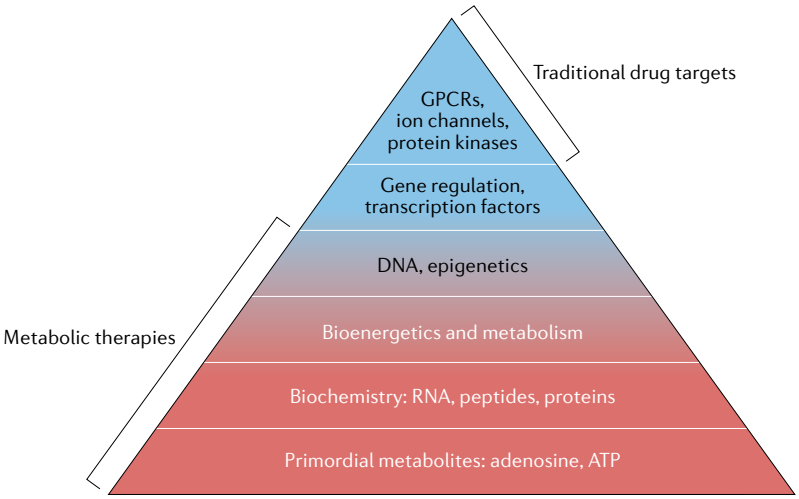

Fig. 2 | **Epilepsy treatment targets in the pyramid of life.** Traditional drug targets are found exclusively at the top of the pyramid, whereas metabolic therapies are uniquely positioned to reset fundamental self-regulatory mechanisms that form functional homeostatic systems. Primordial metabolites 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, and DNA and gene regulatory mechanisms, such as epigenetic modifications and use of non-coding RNAs, emerged later. Transcription factors provided further refinement to these mechanisms. Finally, regulatory systems based on G protein-coupled receptors (GPCRs), ion channels and protein kinases evolved. Disruption of fundamental mechanisms at the base of the pyramid is likely to result in disease such as epilepsy. Adapted from REF.[205], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

on G protein-coupled receptors, ion channels and protein kinases, which still form the mainstay of CNS therapeutics and do not target mechanisms at the base of the evolutionary pyramid. If the metabolic base of the pyramid depicted in FIG. 2 is disrupted in epilepsy, the limitations of the traditional pharmacological approach become obvious.

Metabolic, biochemical and epigenetic alterations clearly have major roles in the pathophysiology of acquired epilepsies[118]. Among these alterations, maladaptive changes in adenosine metabolism, which link metabolism with neuronal excitability and gene expression, constitute a central mechanism. 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} and A_3 (REFS[119–121]). Together, these receptors control neuronal excitability, neuroprotection, synaptic plasticity and inflammatory responses. Adenosine is under the metabolic control of adenosine kinase (ADK), which exists in a cytoplasmic form, ADK-S, that regulates tissue levels of adenosine, and a nuclear form, ADK-L, that controls adenosine metabolism in the cell nucleus[122]. ADK-L drives the flux of methyl groups through the transmethylation pathway, and increased ADK-L activity drives global hypermethylation of DNA (FIG. 3). Increasing adenosine levels through various methods leads to reduced global 5-methylcytosine levels — an effect that is independent of adenosine receptors. Importantly, cultured cell lines that are engineered to overexpress ADK-L have increased global 5-methylcytosine levels compared with ADK-S-expressing or ADK-deficient cells[116]. These findings identify ADK-L as a therapeutic target to modify maladaptive DNA methylation changes during epileptogenesis. Given the pleiotropic roles of adenosine metabolism and receptor modulation described above, it becomes obvious that purinergic neurotransmission is a key factor in the epileptic brain.

Metabolic and mitochondrial epilepsies

The discovery of a metabolic basis for acquired epilepsy has led to renewed interest in epilepsies caused by metabolic and mitochondrial disorders. Metabolic epilepsy in humans can usually be linked to inborn errors of metabolism, which typically present during infancy or childhood. These genetically defined aberrations in metabolism provide valuable insights into the aetiology 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, which is the active form of vitamin B_6 and functions as a coenzyme for the synthesis and metabolism of amino acids. Pyridoxal 5′-phosphate deficiency leads to decreased GABA concentrations in the brain, which is thought to be one of the underlying ictogenic mechanisms[123].

Of note, too much GABA can also trigger seizures, as exemplified by mutations that lead to deficiency of succinic semialdehyde dehydrogenase — an enzyme in the GABA degradation pathway. Succinic semialdehyde dehydrogenase deficiency causes developmental delay, autism, epilepsy, hypotonia and extrapyramidal movement disorders[124]. In addition, deficiency of this enzyme disrupts lysine catabolism, which results in the accumulation of toxic intermediary substrates such as α-aminoadipic semialdehyde, thereby increasing oxidative stress.

Pyridoxine-dependent epilepsy can also be caused by mutations in the *PLPBP* gene, which encodes a pyridoxal 5′-phosphate binding protein[125]. In a related but distinct condition affecting pyridoxine metabolism, pyridoxamine 5′-phosphate oxidase deficiency affects the key enzyme that is responsible for the conversion of pyridoxine to its active metabolite pyridoxal phosphate[126]. Clinically, this deficiency results in neonatal seizures, which are unresponsive to pyridoxine and are associated with hypoglycaemia, lactic acidosis and encephalopathy[127].

Cerebral folate deficiency, another condition that can present with seizures, has multiple aetiologies and is characterized by low levels of 5-methyltetrahydrofolate (the active metabolite of folate) in the brain but normal folate metabolism in peripheral organs[128]. Conversion of homocysteine to methionine requires the enzyme methionine synthetase, methyl tetrahydrofolate as a methyl donor and folate as a cofactor. Folate deficiency leads to disruption of methylation reactions and reduced methionine synthesis, resulting in DNA

hypomethylation — an epigenetic mechanism that modifies gene expression[129].

Non-ketotic hyperglycinaemia results in glycine encephalopathy, a rare inborn defect in the glycine cleavage enzyme complex that is characterized by accumulation of glycine in the cerebrospinal fluid[130]. Glycine acts as obligatory co-agonist of the NMDA receptor by binding to its strychnine-insensitive glycine binding site[131]. Because this recognition site is normally not saturated[132], 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 type deficiency syndrome is characterized by impaired glucose transport into the brain[133], which results in infantile-onset refractory seizures, developmental delay, intellectual impairment and movement disorders[133]. Interestingly, in individuals with this syndrome, seizure frequency increases before meals, and provision of 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 illustrated by 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 of pancreatic β-cells[134–136]. Those channels normally open under conditions of low glucose or low ATP to ADP ratios and, through potassium efflux, keep the post-synaptic neuron hyperpolarized. Mutations in this channel directly impair the brain's ability to shut down its activity in response to a metabolic challenge, and they result in diabetes and seizures.

Metabolic epilepsies can also arise from mutations that cause urea cycle disorders, which lead to the accumulation of toxic compounds such ammonia. Hyperammonaemia can cause irreversible neurological damage[137] and increases neuronal excitability, probably through excessive NMDA receptor activation[138].

Mitochondrial dysfunction is known to precipitate a wide range of neurological manifestations[139], one of which is mitochondrial epilepsy — a distinct subset of epilepsy that is often refractory to treatment and tends to carry a poor prognosis[140]. Patients with mitochondrial epilepsy exhibit distinct neuropathological changes, including reduced expression of mitochondrial complex I and complex IV subunits in neurons and reactive astrocytes[141,142]. On the basis of these observations, a brain slice model of mitochondrial epilepsy was developed that relies on the pharmacological inhibition of mitochondrial complexes I and IV, as well as specific inhibition of the astrocytic TCA cycle enzyme

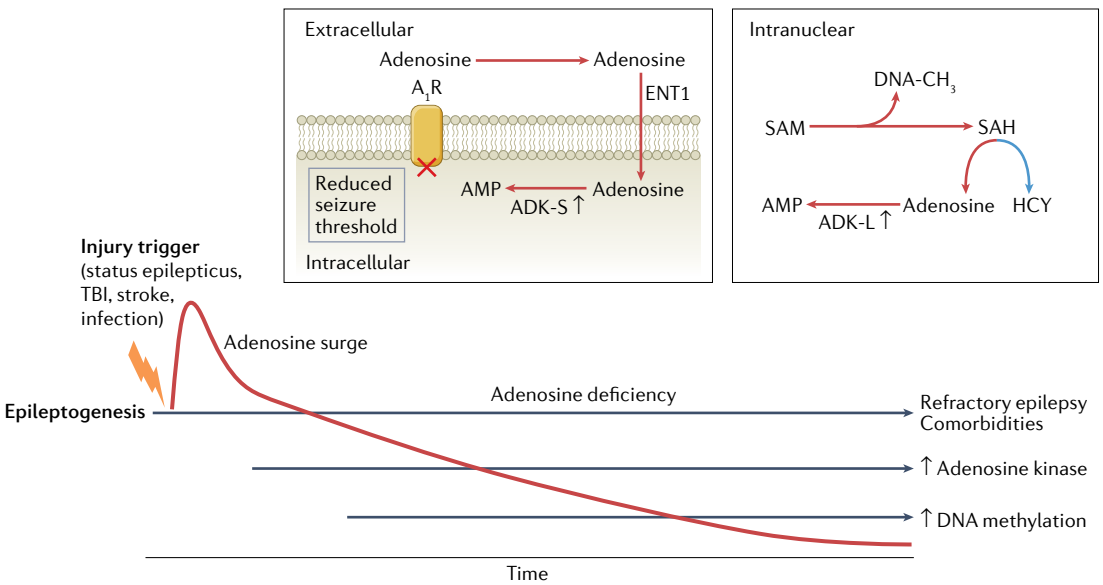

Fig. 3 | **Adenosine metabolism and epileptogenesis.** The epileptogenic process leading to acquired epilepsy can be initiated through a variety of injurious triggers, including status epilepticus, traumatic brain injury (TBI), stroke or brain infection. Such precipitating events lead to a biphasic response of the adenosine system: an acute neuroprotective adenosine surge followed by progressive adenosine deficiency during epileptogenesis. Chronic adenosine deficiency is driven by maladaptive overexpression of the key adenosine metabolizing enzyme adenosine kinase (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 ADK (ADK-S), which drives the uptake of adenosine through equilibrative nucleoside transporter 1 (ENT1). Metabolism-driven cellular uptake of adenosine leads to reduced binding of this molecule to its A_1 receptor (A_1R), which could explain 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 regulates DNA methylation (DNA-CH_3). The reaction product, S-adenosylhomocysteine (SAH), is hydrolysed 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. Consequently, increased ADK-L activity drives increased DNA methylation. The acute adenosine surge takes place during the first 24 h 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 (not shown to scale in the figure).

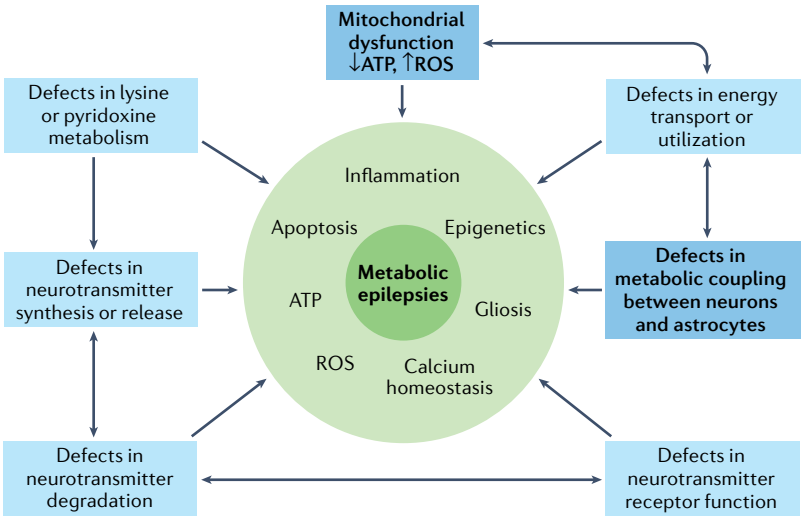

Fig. 4 | **Principal mechanisms involved in the pathogenesis of metabolic epilepsies.** Metabolic epilepsies are complex syndromes characterized by seizures, comorbidities and developmental impairment. The complex pathology of metabolic epilepsies can be explained by congenital defects that lead to an imbalance of neurotransmitter function at various levels, including synthesis, degradation, transport and receptor binding. Congenital or acquired defects in mitochondrial function and in metabolic coupling between neurons and astrocytes combine to trigger major defects in energy transport or utilization, and could represent a preventable — for example, through metabolic therapies — cause of metabolic epilepsies. ROS, reactive oxygen species.

aconitase[141]. In a study involving this model, astrocytes were shown to have a key role in the generation of epileptiform discharges; specifically, the astrocytic GABA–glutamate–glutamine cycle was affected, thereby compromising the regulation of GABA-mediated inhibition[141]. Findings from this study suggest that glutamine is a crucial mediator of the interactions between astrocytic and neuronal compartments that control the epileptiform network. In the clinical context, mutations that perturb mitochondrial TCA cycle function, such as nonsense and missense mutations in the citrate transporter gene *SLC13A5*, have been found to 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 that are altered in inborn errors of metabolism and can result in synaptic dysfunction are depicted in FIG. 4.

Metabolism-based treatments

The signature metabolic therapy for medically intractable epilepsy is the ketogenic diet. This diet was formulated a century ago to recreate the key biochemical changes associated with fasting, which had been anecdotally observed to control seizures without creating substantial 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, this diet — like fasting — induces prominent ketonaemia through enhanced fatty acid oxidation, which elevates blood levels of the principal ketone bodies β-hydroxybutyrate and acetoacetate[19,34,35]. In clinical practice, patients are treated with dietary formulations adhering to ketogenic to antiketogenic (fat to carbohydrate plus protein) ratios of 3:1 to 4:1 by weight, which results in serum β-hydroxybutyrate levels in the very low millimolar range (typically 2–5 mM, but can be as low as 0.4 mM or as high as 10 mM)[19,143].

Over the years, clinicians have used variations of the ketogenic diet to address common adverse effects and theoretically increase ketosis (the medium-chain triglyceride diet)[22] or to enhance palatability (the modified Atkins diet)[23], or have employed diets that capitalize on another key biochemical change induced by the ketogenic diet, that is, relative hypoglycaemia (the LGIT)[144]. Clinicians have reported that these variations of the ketogenic diet can all improve seizure control in patients (in particular, very young infants) with treatment-resistant epilepsy, with more than 50% seizure reduction being observed in up to 60% of individuals[20,25,145,146]. Evidence is also accumulating that these diets can be successfully implemented in adults[147]. The traditional ketogenic diet and the medium-chain triglyceride diet seem to be comparable in terms of clinical effectiveness[21], but whether the modified Atkins diet and LGIT show similar response rates is unclear, as no rigorously controlled, prospective head-to-head comparisons are yet available[25,148,149].

Given the remarkable clinical efficacy of the ketogenic diet and its variants, scientific interest in elucidating the mechanisms of these metabolism-based treatments is growing[14,150]. At present, despite the multiplicity of potential mechanisms that have been advanced in the literature, and the surprising finding that specific metabolic substrates and enzymes can act like ASMs or modulate neuronal excitability in unexpected ways (FIG. 5), how the diets generate their clinical effects remains unclear. Dietary therapies are often hampered by compliance and tolerability issues, and insights into the basic mechanisms of ketogenic diet action, gleaned from basic and translational studies, could uncover novel therapeutic targets or paradigms. Indeed, the 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[151,152].

The proposed mechanisms of metabolic therapies can be grouped into several broad categories (FIG. 6): first, restoration of impaired bioenergetics and mitochondrial function, notably modulation of TCA cycle and respiratory chain activity[153–155], as well as improved redox regulation and antioxidant capacity[102,103,106,156]; second, regulation of both excitatory and inhibitory neurotransmission via metabolic substrates and enzymes[88] at pre-synaptic and post-synaptic levels[157,158], involving $GABA_A$, glutamate and adenosine receptors[158] as well as K_{ATP} channels[159]; third, glycolytic restriction with 2-deoxy-D-glucose[160] and regulation of glycolysis by apoptotic factors[161], or augmentation of the pentose phosphate pathway by fructose-1,6-bisphosphate (a glycolytic intermediate)[162]; fourth, signalling pathways involved in cellular metabolism, such as the mTOR pathway[163,164];

fifth, direct and indirect anti-inflammatory actions on neurons and blood–brain barrier permeability[31,165–167]; and last, epigenetic effects on DNA[168] and histones[165], which form the basis for disease-modifying and anti-epileptogenic effects of the diet. A summary of proposed mechanisms underlying the neuroprotective effects of various dietary treatments is provided in FIG. 7. An exhaustive discussion of these mechanisms is beyond the scope of this Review, and the reader is referred to several other references for additional details[14,26,150,169,170]. However, we should emphasize that, analogous to the majority of ASMs, the mechanisms underlying the clinical effects of the ketogenic diet are multiple and potentially synergistic[14,18].

Detailed basic and translational studies of dietary therapies have not only strengthened the view that epilepsy can, in many respects, be considered a metabolic disease, but have also presented novel approaches that might be exploited for clinical development. One example is 2-deoxy-D-glucose, which inhibits

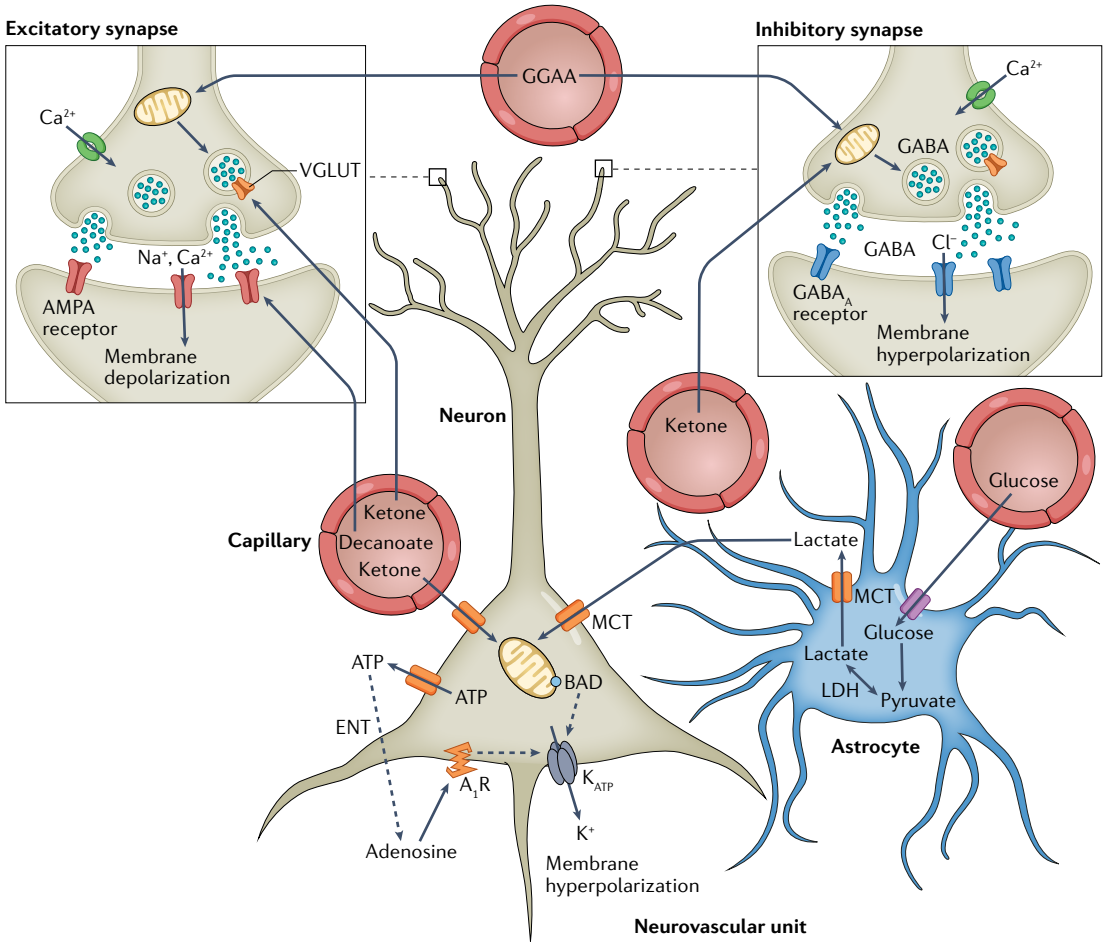

Fig. 5 | **Metabolic substrates and enzymes that regulate synaptic neurotransmission and neuronal excitability.** Schematic of the neurovascular unit (neuron, astrocyte and microvasculature) and expanded views of prototypical excitatory and inhibitory central synapses. Ketone bodies can directly compete with the chloride anion regulatory site of vesicular glutamate transporters (VGLUTs) to prevent presynaptic release of glutamate. Decanoate, a C10 medium-chain triglyceride, can inhibit post-synaptic AMPA receptors in a similar way to the antiseizure medication perampanel. Ketones can also serve as a substrate for glutamate production in inhibitory presynaptic terminals, which increases the synthesis of GABA via glutamic acid decarboxylase, thereby enhancing inhibitory neurotransmission. In addition, 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). In turn, adenosine can bind to inhibitory adenosine type 1 receptors (A_1R) in an autocrine fashion to limit membrane excitability. Probably through this mechanism, ketones can indirectly activate ATP-sensitive potassium (K_{ATP}) channels, which induce membrane hyperpolarization. Moreover, increased membrane activity might deplete ATP levels in proximity or subjacent to K_{ATP} channels, which would decrease the ATP to ADP ratio and activate these inhibitory channels. K_{ATP} channels can also be indirectly regulated by Bcl2-associated agonist of cell death (BAD), a protein that serves a dual function as a pro-apoptotic factor and a modulator of glycolysis. Glucose transported into astrocytes is broken down to produce pyruvate, which can be converted to lactate via lactate dehydrogenase (LDH); inhibition of LDH (for example, by stiripentol) produces antiseizure effects through mechanisms that are as yet unclear. The astrocyte–neuron lactate shuttle is shown in detail in FIG. 1. Ketogenic diet-induced alterations in the gut microbiome have been shown to decrease blood levels of γ-glutamyl amino acids (GGAA), resulting in changes in glutamate and GABA production, specifically, an increase in the GABA to glutamate ratio. MCT, monocarboxylic acid transporter.

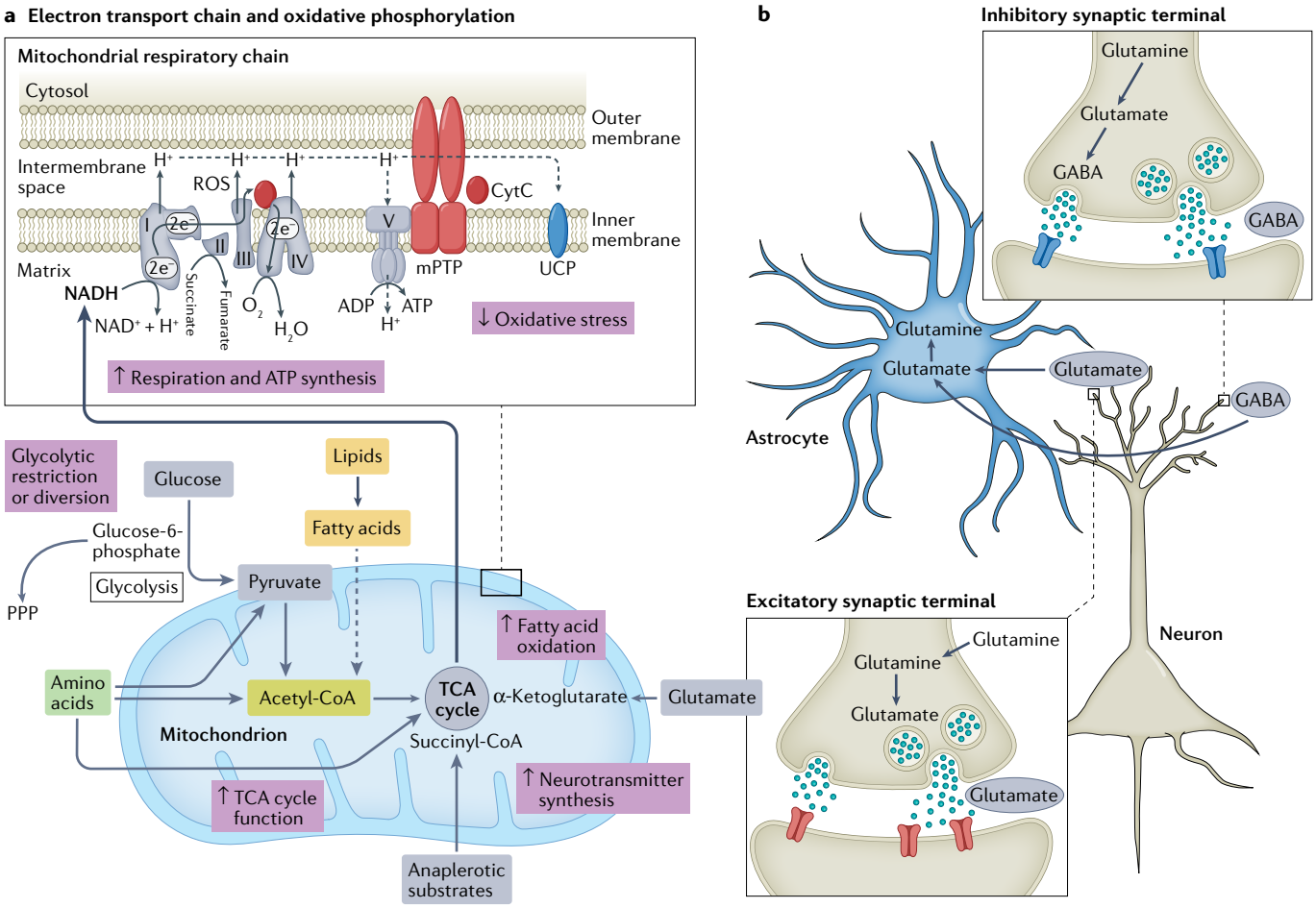

Fig. 6 | **Biochemical pathways and mitochondria. a** | Various experimental models have demonstrated a link between the biochemical effects of metabolic antiseizure treatments (pink boxes) and the control of seizures or epilepsy. Inhibition of glycolysis with 2-deoxy-D-glucose has been shown to control seizures in multiple animal models. In addition, the glycolytic intermediate fructose-1,6-bisphosphate exerts broad antiseizure effects, possibly by augmenting the pentose phosphate pathway (PPP). 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 can be converted to acetyl-CoA; and histidine and glutamate can be converted to α-ketoglutarate, which is part of the tricarboxylic acid (TCA) cycle. Fatty acids derived from lipids can undergo oxidation within the mitochondrial matrix to generate acetyl-CoA, which can be condensed into acetoacetyl-CoA — a precursor for the synthesis of acetoacetate and β-hydroxybutyrate. Ketones are not only utilized for ATP production, but 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 production. Triglycerides, such as triheptanoin, provide anaplerotic substrates that can produce antiseizure effects when metabolized to succinyl-CoA in mitochondria. In addition, enhancement of mitochondrial respiratory chain function, for example, through the ketogenic diet or oxidative metabolism, results in increased oxygen consumption and ATP production and a reduction in oxidative stress. **b** | The glutamine–glutamate cycle between neurons and astrocytes is crucial for presynaptic GABA biosynthesis, as well as for providing glutamate that is converted by glutamate dehydrogenase to α-ketoglutarate which enters the TCA cycle as an intermediate[87]. CytC, cytochrome *c*; I–V, respiratory chain complexes I–V; mPTP, mitochondrial permeability transition pore.

phosphoglucose isomerase, a key glycolytic enzyme, and has been shown to possess relatively broad antiseizure properties, to retard kindling (a model of epileptogenesis)[171] and to mitigate post-traumatic epilepsy[172]. However, the protective effects of 2-deoxy-D-glucose might not be attributable solely to inhibition of glycolysis, and other mechanisms have been implicated[160]. A medium-chain triglyceride product has also been shown to be effective in paediatric patients with drug-resistant epilepsy[173].

The anti-epileptogenic potential of transient focal adenosine delivery was tested in a rat model of systemic kainic acid-induced epilepsy[116]. Young male rats were treated with the excitotoxin kainic acid to trigger epilepsy, and adenosine-releasing silk was implanted into the lateral ventricles of the brain 9 weeks later. Strikingly, in the adenosine-treated group, epilepsy progression was completely suppressed and a more than 70% reduction in seizure frequency was maintained for at least 12 weeks — long after adenosine release would be expected to have expired. Progression of mossy fibre sprouting was also attenuated in the adenosine-treated rats. Moreover, the transient delivery of adenosine restored normal 5-methylcytosine levels in the hippocampus in the long term. A DNA methylation array analysis identified genes in which the methylation status

changed during epilepsy development and reverted to normal after adenosine therapy. The identified genes encoded molecules such as DNA polymerase PolD and various DNA-interacting proteins, including zinc finger proteins[116]. Taken together, these findings suggest that transient therapeutic adenosine augmentation can restore normal DNA methylation patterns, thereby preventing epilepsy progression (FIG. 3). Importantly, adenosine has been strongly implicated in the mechanism of ketogenic diet action, and the ketogenic diet has been shown to reverse DNA methylation changes in a chronic animal model of epilepsy[168].

Subsequently, transient and systemic use of the ADK inhibitor 5-iodotubercidin to increase adenosine levels was shown to be anti-epileptogenic in the intrahippocampal kainic acid mouse model of TLE[174]. Three days after induction of status epilepticus with kainic acid, 5-iodotubercidin or vehicle was injected twice daily for 5 days. Six weeks after status epilepticus induction, 94% of mice in the vehicle-injected mice had developed robust seizures, whereas 59% of the 5-iodotubercidin-treated mice showed virtually no seizure activity. These findings are consistent with the anti-epileptogenic activity of adenosine, mediated through an epigenetic mechanism[116]. Importantly, histopathological analysis demonstrated characteristic granule cell dispersion in vehicle-injected animals, which was prevented by 5-iodotubercidin[174]. Thus, transient inhibition of ADK holds promise as a therapeutic strategy for epilepsy prevention.

The nuclear and epigenetically active ADK isoform, ADK-L, is a unique molecular and therapeutic target that links metabolism with developmental and epigenetic functions. A ketogenic diet was shown to lead to a reduction in ADK-L expression[175], which could help to explain the epigenetic and disease-modifying properties of metabolic treatments[168,176]. Efforts are currently underway to develop novel small-molecule compounds to target ADK-L as potential therapeutic agents for epilepsy prevention[177].

Additional strategies based on network pharmacology and repurposing of FDA-approved drugs are currently being pursued for the prevention of epilepsy in humans[178,179]. These approaches include the antibiotic ceftriaxone[179], which increases astroglial glutamate uptake via the glutamate transporter GLT1. Interestingly, through its interaction with the adenosine A_{2A} receptor, adenosine has been shown to regulate GLT1 expression and function[180,181], representing a possible mechanism through which maladaptive changes in adenosine metabolism during epileptogenesis could influence a

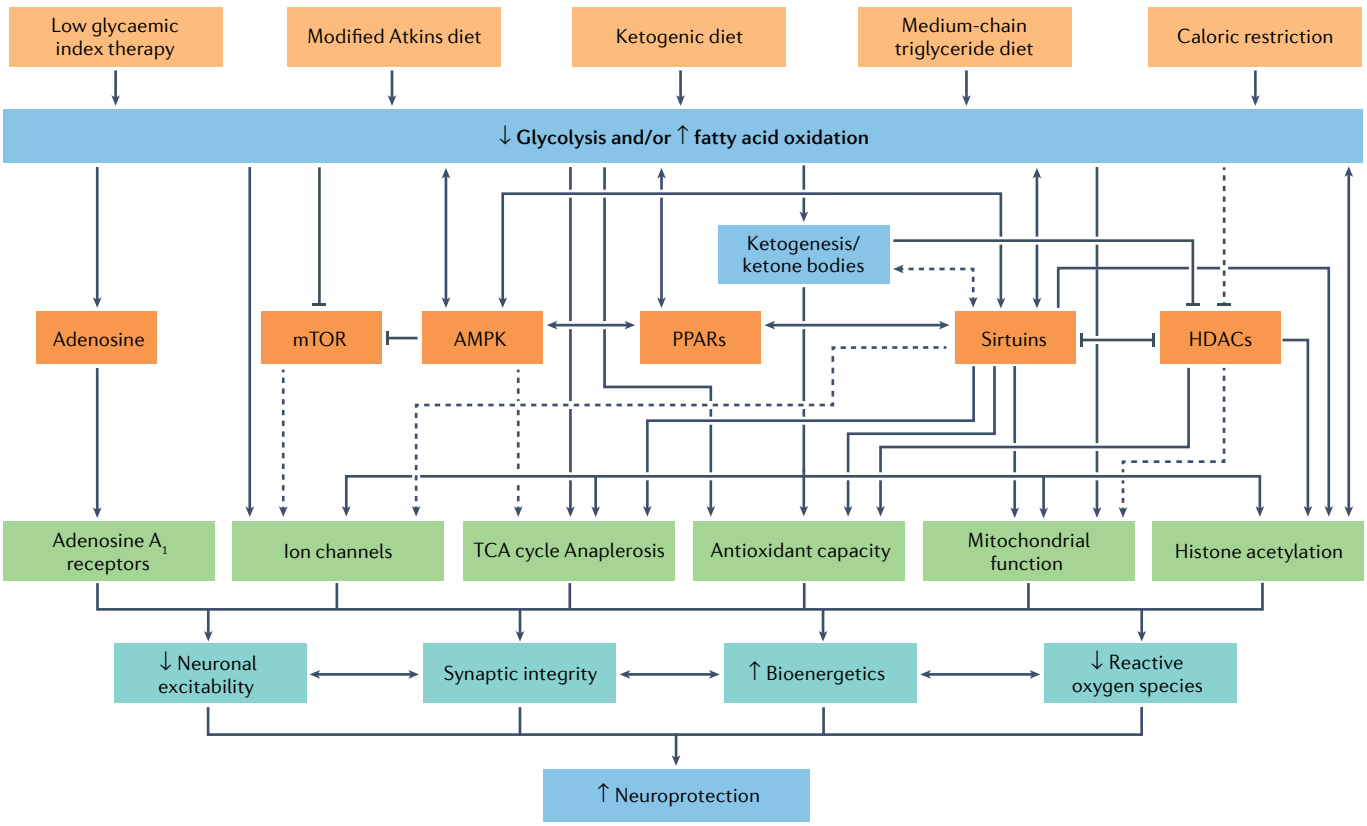

Fig. 7 | **Mechanisms underlying the neuroprotective effects of ketogenic and related diets.** The main dietary interventions, including caloric restriction, which mirrors many but not all the biochemical or physiological effects of the exogenous diets, are shown in light orange. The principal metabolic effects of the diets are shown in blue; the energy-sensing pathways that might mediate the effects of the dietary alterations are shown in dark orange; 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 in cyan. Solid black lines indicate linkages detailed in the literature; dashed black lines represent possible but as yet unproven links. AMPK, adenosine monophosphate-activated kinase; HDACs, histone deacetylases; mTOR, mammalian target of rapamycin; PPARs, peroxisome proliferator-activated receptors; TCA, tricarboxylic acid cycle. Adapted from REF.[26], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

downstream target that is amenable to correction by an existing FDA-approved drug.

Since the 1930s, investigators have been interested in examining whether ketone bodies exert direct antiseizure effects[182,183]. Despite numerous studies in preclinical models, however, whether ketones are directly responsible for the antiseizure effects of the ketogenic diet remains unclear, as peripheral ketone levels do not correlate well with seizure control in patients with epilepsy[182,184]. Nevertheless, the growing evidence that β-hydroxybutyrate can indirectly modulate K_{ATP} channels, act as an endogenous ligand for free fatty acid receptors[185], inhibit histone deacetylases[165], regulate the mitochondrial permeability transition pore[186] and prevent NLRP3 inflammasome assembly[166], among many other actions[187,188], provides a compelling scientific rationale for exploring ways to induce ketosis without dietary manipulation, such as administration of ketone esters[151].

Fatty acids have also attracted considerable attention in view of their roles as key constituents of ketogenic diets. Polyunsaturated fatty acids have long been known to directly inhibit voltage-gated sodium and calcium channels[189], but perhaps more importantly they can activate the transcription factor peroxisome proliferator-activated receptor-α (PPARα), which regulates genes involved in the β-oxidation of fatty acids and, more generally, energy homeostasis[190]. Moreover, the ketogenic diet has been shown to increase the expression of PPARγ, which exerts anti-inflammatory and antioxidant actions[191]. However, despite strong preclinical data, whether polyunsaturated fatty acid ingestion produces antiseizure effects remain unclear[192,193].

Medium-chain triglycerides, most notably decanoic acid, have been shown to inhibit post-synaptic AMPA receptors[194] much like the ASM perampanel[195], and also to increase mitochondrial content in neurons and inhibit mTORC1 activity independently of glucose and insulin signalling[164]. These triglycerides might, therefore, represent a novel metabolic treatment approach for epilepsy[196]. 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[173]. Furthermore, triheptanoin (the triglyceride of heptanoic acid) promotes anaplerosis (replenishment of substrates involved in the TCA cycle) and thereby induces antiseizure effects in both animal models[197] and patients[198] with epilepsy. Finally, ketogenic diet therapy led to an increase in adenosine[175,176], which not only reduced seizure thresholds[199] but also exerted potent disease-modifying and anti-epileptogenic effects[116,174].

The efficacy of ketone preparations, adenosine-laden delivery vehicles and fatty acid formulations for epilepsy therapeutics remains to be determined. In the meantime, another intriguing question that has emerged from the epilepsy and metabolism literature is whether any ASMs currently used in clinical practice affect metabolic pathways, and whether these actions might in part explain their efficacy against epileptic seizures. In general, metabolic targets are not believed to be relevant to ASM mechanisms, with one notable exception. Stiripentol, an ASM that is thought primarily to allosterically modulate $GABA_A$ receptors, seems to exert antiseizure effects via inhibition of LDH[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 these two areas for therapeutic innovation. A related idea is that ASMs could help to reduce the energy needs of the epileptic brain, such that the spared energy can be redirected for normal neuronal signalling. Given that synaptic activity creates a high energy demand, the direct inhibitory effects of ASMs on neurotransmission might yield an important secondary benefit.

In recent years, evidence has emerged that the ketogenic diet provides antiseizure effects by modulating the gut microbiome[65]. In a mouse model, the diet was shown to induce specific changes in the abundance and composition of bacterial species, which secondarily affected the blood–brain metabolome (for example, by decreasing γ-glutamyl amino acid levels) and influenced brain levels of key neurotransmitters such as GABA and glutamate. The gut microbiome is increasingly being linked to neurological and psychiatric disorders, and these potentially paradigm-shifting findings suggest that it could function as a powerful integrator of metabolism and inflammation through the gut–brain axis.

Conclusions and future perspectives

Cellular metabolism is emerging as an important biological framework within which epileptic seizures arise. Both normal and abnormal neuronal and glial activity rely on the fundamental substrates, enzymes and biochemical pathways that collectively mediate metabolic homeostasis and disruption in the brain. Consequently, epilepsy could be considered a metabolic disease or, more specifically, the heterogeneous epilepsies might represent electroclinical manifestations of complex perturbations in physiological networks fuelled by basic biochemical processes. In recent years, this notion has been amplified by basic, translational and clinical studies that have revealed 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 empirical and highly reasoned approaches towards expanding therapeutic options. In addition, drug discovery paradigms have begun to explore the utility of metabolic readouts in various model systems[141,199–202], and technical advances, including molecular biosensors and molecular imaging techniques, are providing new insights into phenomena such as cell membrane physiology, epigenetic changes and inflammation[3,203]. For decades, drug discovery for epilepsy has been hampered by a lack of understanding of the mechanisms responsible for drug refractoriness[204] 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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J.M.R has been a paid consultant to Aquestive Pharmaceuticals, Danone Nutricia, Mallinckrodt, Eisai Pharma and Zogenix, and has served on the Scientific Advisory Board of The Charlie Foundation for Ketogenic Therapies (Santa Monica, CA, USA). D.B. is a co-founder of PrevEp and J.M.R. is the Chief Medical Officer for Path Therapeutics.

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