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ATP and adenosine are purines that play dual roles in cell metabolism and neuronal signaling. Acting at the A_1 receptor (A_1R) subtype, adenosine acts directly on neurons to inhibit excitability and is a powerful endogenous neuroprotective and anticonvulsant molecule. Previous research showed an increase in ATP and other cell energy parameters when an animal is administered a ketogenic diet, an established metabolic therapy to reduce epileptic seizures, but the relationship among purines, neuronal excitability and the ketogenic diet was unclear. Recent work in vivo and in vitro tested the specific hypothesis that adenosine acting at A_1Rs is a key mechanism underlying the success of ketogenic diet therapy and yielded direct evidence linking A_1Rs to the antiepileptic effects of a ketogenic diet. Specifically, an in vitro mimic of a ketogenic diet revealed an A_1R-dependent metabolic autocrine hyperpolarization of hippocampal neurons. In parallel, applying the ketogenic diet in vivo to transgenic mouse models with spontaneous electrographic seizures revealed that intact A_1Rs are necessary for the seizure-suppressing effects of the diet. This is the first direct in vivo evidence linking A_1Rs to the antiepileptic effects of a ketogenic diet. Other predictions of the relationship between purines and the ketogenic diet will be discussed. Taken together, recent research on the role of purines may offer new opportunities for metabolic therapy and insight into its underlying mechanisms.

Keywords: adenosine; ATP; epilepsy; hemichannel; hippocampus; long-term potentiation; metabolism; seizure; transgenic mouse

1.0 Purines: regulation of adenosine and adenosine A_1 receptor activity

Purines are integral to many diverse cellular processes. The purine adenine is a nitrogenous base that likely played a role in prebiotic evolution (Oro and Kimball, 1961). Therefore, it is not surprising that it is both a building block of DNA and RNA, reflecting the metabolic activity of a cell, as well as a component of the adenine nucleotide adenosine triphosphate (ATP), the main cellular energy metabolite reflecting the metabolic capacity of a cell. Thus, ATP and its core molecule adenosine adapt cellular activity to energy homeostasis throughout the body. ATP and adenosine are evolutionarily ancient biochemical regulators but also participate directly in cell signaling by binding to their own families of membrane-bound cell surface receptors.

Adenosine's presence throughout the extracellular space exerts a tonic effect on central nervous system activity via G-protein coupled receptors, and - due to its interrelationship with adenine nucleotides, including ATP - adenosine is in a unique position to link metabolism and neuronal activity. Of the utmost relevance to epilepsy, increased activation of the adenosine A_1 receptor subtype (A_1R) profoundly inhibits neuronal activity. Due largely to these effects, adenosine is well known as a powerful seizure-reducing and neuroprotective molecule. The A_1R exerts both presynaptic and postsynaptic inhibitory actions: presynaptically it reduces transmitter release by closing Ca^{2+} channels (Ribeiro et al., 1979), and postsynaptically it hyperpolarizes neurons by opening K^+ channels (Haas and Greene, 1984).

Adenosine is released during cellular stress, including during hypoxia, ischemia, and seizures. Its neuroprotective role during conditions of high metabolic demand - where adenosine's powerful inhibitory effect can reduce excitability and reduce excitotoxicity – has earned adenosine a reputation as a retaliatory metabolite (Newby et al., 1985). However, adenosine has also been shown to be regulated by a number of non-pathological stimuli, such as decreased pH and increased temperature (Gabriel et al., 1998; Masino and Dunwiddie, 1999; Dulla et al., 2005; Gourine et al., 2005; Dulla et al., 2009), and a

main ongoing source of adenosine in the extracellular space is dephosphorylated ATP released from astrocytes (Pascual et al., 2005). Because of its prevalence throughout the brain, its connection to metabolism, and its dynamic and powerful actions, adenosine is a primary homeostatic bioenergetic network regulator (Boison et al., 2011).

2.0 Ketogenic diet – an established metabolic therapy

The ketogenic diet is a high-fat, low-carbohydrate diet which mimics fasting. It restricts glucose and forces metabolism of ketones (acetoacetate, acetone, β-hydroxybutyrate) to conversion into acetyl-CoA and thus to generate ATP. Metabolic treatment with a ketogenic diet reduces seizures, and has been noted recently for neuroprotective benefits. Overall, ketogenic diet therapy is as effective as drugs, and is particularly effective in pediatric and medically refractory epilepsy (Hemingway et al., 2001; Freeman et al., 2007; Neal et al., 2008). There are some side effects – typically minor with good medical management – but the strict protocol can be unpalatable and difficult to maintain. The ketogenic diet was established in 1921 (Wilder, 1921), and although it has been in continuous use ever since – the key mechanism(s) are not known. An incomplete understanding of the translation between metabolic changes and reduced seizures has hampered efforts to develop better diets or pharmaceutical strategies.

3.0 Purines and the Ketogenic Diet

In 2008 we published an opinion paper hypothesizing that increased adenosine acting at A_1Rs is critical to the therapeutic success of the ketogenic diet (Masino and Geiger, 2008). This concept was based initially on several parallel observations, including: (1) some of the stimuli thought to increase adenosine may be mobilized by the metabolic switch to ketolysis versus glycolysis (e.g. reduced glucose and/or pH), (2) the ketogenic diet increases ATP and mitochondrial biogenesis (thus providing additional

substrate for adenosine formation), (3) both adenosine and the ketogenic diet are known to be effective, even in drug-resistant epilepsy, and (4) initial reports of neuroprotective effects of the ketogenic diet, thus matching predications of the hypothesis that a ketogenic diet increases adenosine A_1R activation.

Extended predictions of this hypothesis are that the ketogenic diet should be therapeutic in clinical conditions where adenosine is known to be effective. For example, the ketogenic diet, like adenosine, should offer neuroprotection, influence sleep and reduce pain. Indeed, multiple reports in recent years note neuroprotective effects of the ketogenic diet (Noh et al., 2003; Prins et al., 2005; Van der Auwera et al., 2005; Zhao et al., 2006; Puchowicz et al., 2008; Cheng et al., 2009; Hu et al., 2009). Furthermore, fasting – which similarly forces use of ketone bodies for fuel – has paradoxically been shown to improve recovery after injury (Davis et al., 2008). Metabolic manipulations such as mitochondrial uncouplers (Sullivan et al., 2004; Pandya et al., 2007) and glycolytic inhibition (Massieu et al., 2003; Garriga-Canut et al., 2006) are thought to mimic at least some aspects of a ketogenic diet; these manipulations can offer seizure protection (Garriga-Canut et al., 2006) and have been shown to increase adenosine (Tekkök et al., 1999). More limited reports link the ketogenic diet to improved sleep in epileptic children (Panico et al., 2000; Hallböök et al., 2007), and improved circadian patterns and rest-activity cycles in chronically epileptic Kcna1-null mice (Fenoglio-Simeone et al., 2009). We showed that a ketogenic diet reduced pain and inflammation in juvenile and adult rats (Ruskin et al., 2009).

Here we describe recent work testing the hypothesis that the ketogenic diet increases activation of purine receptors, particularly A_1Rs, as a key mechanism underlying its therapeutic effects. We tested this hypothesis in vitro, using normal rat hippocampal slices, and in vivo, using several transgenic mouse models with chronic seizures due to altered adenosine signaling. In both cases we found evidence for increased A_1R activation (Kawamura et al., 2010; Masino et al., 2011). Also in accord with increased

adenosine, we found that a ketogenic diet reduces pain and inflammation in juvenile and adult rats (Ruskin et al., 2009).

3.1 In vitro model of metabolic effects of a ketogenic diet

We mimicked a ketogenic diet in vitro by focusing on two key metabolic endpoints – (1) increased intracellular ATP and (2) low extracellular glucose. It has been reported that a ketogenic diet increases brain concentrations of ATP in rodents (DeVivo et al., 1978; Nakazawa et al., 1983; Nylen et al., 2009). Clinically, Pan et al. (1999) showed that in cerebral cortical gray matter a ketogenic diet increased the phosphocreatine/ATP ratio (a measure of positive bioenergetic status). In parallel, blood glucose levels are known to remain low during chronic treatment with the ketogenic diet (Huttenlocher, 1976; Bough et al., 2006) and mild hypoglycemia has been reported to control seizures in both animal models (Greene et al., 2001; Mantis et al., 2004) and clinical trials (Muzykewicz et al., 2009). Thus, we focused on these metabolic endpoints (increased ATP and reduced glucose) and developed a simple *in vitro* model of the ketogenic diet using whole-cell patch-clamp methods in hippocampal CA3 pyramidal cells, a cell type and region known to be involved in seizures.

While recording from individual CA3 neurons we simulated the effects of a ketogenic diet by varying the amount of ATP in the intracellular solution (above and below the standard ATP (2 mM)) and reducing extracellular glucose from 11 mM (higher than physiological levels, but standard for brain slice recordings) to 3 mM (still physiologically relevant for brain tissue). We found that with high or sufficient intracellular ATP concentrations (1-5 mM), reducing glucose caused CA3 hippocampal pyramidal neurons to hyperpolarize - measured as an outward current in voltage-clamp mode. The current induced by reduced extracellular glucose was dose-dependent upon intracellular ATP between 0.5 mM and 2 mM, suggesting an autocrine modulation of the recorded neuron. The reduced glucose-induced outward current was abolished by an A_1R antagonist and not observed in A_1R knock-out mice.

In contrast to the linear relationship between intracellular ATP and the membrane current observed for ATP concentrations between 0.5 mM and 2 mM, we found that the highest concentration of intracellular ATP - 5 mM – produced a lower amplitude of the reduced glucose-induced outward current, suggesting inhibition by high intracellular ATP. Furthermore, and in support of this observation, ATP-sensitive potassium channel blockers inhibited the outward currents. Gap-junction blockers and a peptide specific for blocking pannexin-1 channels also inhibited the outward current completely. Together, these results suggest that with high or sufficient intracellular ATP concentration and reduced extracellular glucose (a set of conditions present during ketogenic diet treatment), CA3 pyramidal neurons hyperpolarize themselves via direct ATP release through pannexin-1 channels with the subsequent activation of A_1Rs and opening of ATP-sensitive potassium channels (Fig 1; Kawamura et al., 2010).

The in vitro experiments described above demonstrate that relevant diet-induced metabolic shifts can produce an inhibitory purinergic autocrine/paracrine regulation. A combination of the synaptic inhibition and hyperpolarizing CA3 pyramidal neurons directly should confer a strong anticonvulsant effect, and this type of inhibition could be occurring in other brain regions; more work is needed to determine if this is observed in other brain regions and neuron subtypes. While this detailed mechanism has not been proven in vivo, a similar metabolic regulation of A_1R actions might be an important mechanism underlying the clinical success of a ketogenic diet (Rho, 2010).

3.2 In vivo ketogenic diet administration in animals with spontaneous seizures and altered adenosine signaling

To test directly the relationship between a ketogenic diet and A_1R activation, we tested adult wild-type (WT) and three types of transgenic mice that exhibit spontaneous hippocampal seizures and reduced A_1R signaling (Li et al., 2007). The mice used were engineered genetically to have a complete absence of

A_1Rs ($A_1R^{-/-}$), a 50% reduction in A_1Rs ($A_1R^{+/-}$) (Johansson et al., 2001), or an overexpression of adenosine kinase (Adk-tg) (Li et al., 2008b); adenosine kinase is an intracellular astrocyte-based enzyme (Studer et al., 2006) that catalyzes the metabolism of adenosine to 5'-AMP, and its overexpression is expected to decrease extracellular levels of adenosine resulting in increased susceptibility to neuronal injury (Pignataro et al., 2007) and spontaneous electrographic seizures (Li et al., 2008a). In comparison to the WT control mice, the $A_1R^{-/-}$, $A_1R^{-/+}$ and Adk-tg mice all exhibit spontaneous hippocampal seizures that are occasionally accompanied by behavioral arrest or staring episodes. The characterization of these adenosine-deficient mice is consistent with previously published results that spontaneous hippocampal seizures (Li et al., 2007; Li et al., 2008b) and enhanced vulnerability of brain to injury (Kochanek et al., 2006; Pignataro et al., 2007; Li et al., 2008b) are caused by deletion of A_1Rs or decreased adenosine levels resulting from elevated expression levels of adenosine kinase.

If anti-seizure effects of a ketogenic diet are mediated through adenosine signaling systems, and particularly A_1Rs, we predicted that the ketogenic diet *(i)* would suppress seizures in Adk-tg mice with their inherent deficiency in adenosine (but intact A_1Rs), *(ii)* would suppress seizures somewhat in mice with reduced levels of adenosine receptors ($A_1R^{+/-}$ mice), and *(iii)* would not exhibit anti-seizure effects in mice where A_1Rs are absent ($A_1R^{-/-}$ mice). Indeed, we found that when mice were fed the ketogenic diet for 3 weeks, that the incidence of spontaneous seizures was reduced by about 88% in Adk-tg mice, by about 53% in $A_1R^{+/-}$ mice, and by only 4% in $A_1R^{-/-}$ mice (Table 1; Masino et al., 2011). To confirm the metabolic specificity of the diet, we administered a single injection of glucose. In every case where seizure frequency was reduced by the ketogenic diet, it was increased within 30-90 minutes by acute glucose, confirming the metabolic specificity of the effect. Together, these data suggest strongly that A_1Rs are an important mechanism that underlies the anti-seizure effects of the ketogenic diet.

4.0 Testing predictions

Adenosine is a pervasive neuromodulator, and so influences a wide range of neural and behavioral processes. Thus, in addition to the direct evidence above supporting a link between the ketogenic diet and enhanced adenosine acting at A_1Rs, correlative evidence supports the idea of a ketogenic diet promoting adenosine. Thus, predictions of this hypothesis can be "tested" by looking at published literature as well as by conducting additional experiments.

4.1 Ketogenic Diet, Adenosine and Neuroprotection

Adenosine acting at A_1Rs is well-established as a neuroprotective mechanism, and reduces brain injury from mechanical, ischemic, and hypoglycemic insults (Evans et al., 1987; Goldberg et al., 1988; Varma et al., 2002). There is now abundant evidence from a number of laboratories that the ketogenic diet can similarly reduce brain injury from mechanical damage (Prins et al., 2005; Appelberg et al., 2009; Hu et al., 2009) and ischemic/hypoglycemic damage (Yamada et al., 2005; Puchowicz et al., 2008; Tai et al., 2008). Furthermore, treatment with ketones themselves is also effective against ischemic/hypoglycemic damage to neural tissue (Suzuki et al., 2001; Massieu et al., 2003; Samoilova et al., 2010). In addition to epilepsy, neuroprotection is an ideal target to test directly the relationship between ketogenic diet and adenosine.

4.2 Ketogenic Diet, Adenosine and Pain

Adenosine agonists are well-known to reduce pain by acting at both central and peripheral receptors (Yarbrough and McGuffin-Clineschmidt, 1981; Karlsten et al., 1992), primarily via A_1Rs (Jarvis et al., 2000; Johansson et al., 2001; Schmidt et al., 2009), and are effective against pain induced by thermal, chemical, and mechanical stimuli. Antinociceptive actions of adenosine are also present clinically (Belfrage et al., 1995) and animal research suggests that adenosine may underlie the antinociceptive

effects of acupuncture (Goldman et al., 2010). Despite the common use of antiepileptic drugs to reduce pain (Vanotti et al., 2007), very little work has been done with the ketogenic diet and pain.

We tested the ketogenic diet for effects on pain using thermal stimulation (paw withdrawal from a hotplate) in rats, and found significant diet-related hypoalgesia at several temperatures (Ruskin et al., 2009). This effect was observed consistently in juvenile and adult rats (pooled data shown in Fig. 2, left). Intriguingly, hypoalgesia evolved gradually over many days after diet onset, with a similar time-course to the delayed appearance of the ketogenic diet's anticonvulsant effect, and the thermal hypoalgesia did not depend directly on reduced glucose or increased ketones (Ruskin et al., 2010). These results provide initial evidence that the diet can reduce pain sensation, consistent with a hypothesis linking a ketogenic diet to increased actions of adenosine.

4.3 Ketogenic Diet, Adenosine, and Inflammation

Adenosine can attenuate peripheral and central inflammation by acting locally in the periphery as well as by reducing peripheral inflammation indirectly through central effects (Akimitsu et al., 1995; Poon and Sawynok, 1999; Sorkin et al., 2003; Tsutsui et al., 2004). Both A_1Rs and A_{2A}Rs appear to be involved (Tsutsui et al., 2004; Sitkovsky and Ohta, 2005). In the study described above we also tested the ketogenic diet for anti-inflammatory effects by chemically-inducing paw inflammation in juvenile and adult rats after three weeks of control or ketogenic diet feeding (Ruskin et al., 2009). When we tested inflammatory parameters (two days after paw injection with complete Freund's adjuvant) we found significantly less tissue swelling in animals fed the ketogenic diet (Fig. 2, middle). In addition, fluid movement out of the local blood vessels (fluid extravasation, a measure of vascular permeability), measured by tracking movement of the dye Evans Blue, was significantly reduced by ketogenic diet feeding (Fig. 2, right). Similar to the effects of the ketogenic diet on thermal nociception, the reduced inflammation was found consistently in juvenile and adult rats. Other researchers are also beginning to

characterize the anti-inflammatory effects of the ketogenic diet, including in clinical settings (Tendler et al., 2007; Yang and Cheng, 2010).

4.4 Ketogenic Diet, Adenosine and Synaptic Plasticity

Adenosine interacts with synaptic plasticity, including long-term potentiation at hippocampal synapses. Typically, prior application of adenosine or receptor agonists has been shown to block induction of long term potentiation (Arai et al., 1990; Mitchell et al., 1993), including evidence in vivo (Dolphin, 1983), and there is mixed evidence that endogenous extracellular adenosine acts as a gate for synaptic plasticity (Moore et al., 2003; Kukley et al., 2005). Despite the clear effect of a ketogenic diet on seizures, and its use in children who are in a period of rapid growth and plasticity, there has been little research on the effects of a ketogenic diet on synaptic plasticity.

Using chronic recording in awake, behaving rats, we found baseline parameters of synaptic transmission at entorhinal cortex-to-dentate gyrus synapses (input/output relationship, paired-pulse plasticity) were not changed significantly by 3 weeks of ketogenic diet feeding. After a theta-burst stimulus was applied to induce long term potentiation, all animals showed significant synaptic potentiation in the dentate gyrus. However, a significantly reduced magnitude of potentiation was found in ketogenic diet-fed animals both short-term (1 – 15 minutes) and long-term (30 minutes +) after induction (Koranda et al., 2011). The amplitude of the long-term potentiation remained diminished significantly in the ketogenic diet group out to the longest recorded time, 48 hours. Non-significant effects of the ketogenic diet on long term potentiation in vivo in a prior electrophysiology study (Thio et al., 2010) might have been due to the use of general anesthesia, which could overwhelm the mild inhibition produced by a ketogenic diet. Overall, the effects of a ketogenic diet on long term potentiation observed so far are minor – it was diminished, not abolished - and in the expected direction based on a relationship among a ketogenic diet, adenosine and long term potentiation.

4.5 Emerging Evidence and Additional Mechanisms

Together, these findings suggest multiple commonalities in adenosine-based and ketogenic effects. Few studies have explored other implications of this relationship. For example, adenosine is well-known as a sleep-promoting agent, particularly in the basal forebrain (Porkka-Heiskanen, 1999; Huang et al., 2007); thus far there is only one published report (in children with epilepsy) linking a ketogenic diet to changes in sleep behavior and an overall increase in sleep quality (Hallböök et al., 2007). However, epilepsy is associated with sleep disruption, and chronically epileptic Kcna-1null mice with impaired circadian rhythms showed both decreased seizures and improved rest-activity cycles after treatment with a ketogenic diet (Fenoglio-Simeone et al., 2009). Decreased adenosine increases anxiety, and the regulation of the influence of adenosine by caffeine has been implicated in a number of disorders (Lara, 2010; Ribeiro and Sebastiao, 2010). There is ample evidence for adenosine/dopamine receptors forming functional heteromers (Fuxe et al., 2007), and thus additional predictions arise from this adenosine/dopamine interaction. Recently we found that a ketogenic diet delays weight loss and does not negatively affect locomotor activity in a mouse model of Huntington's Disease, suggesting some promise in aspects of this progressive genetic disorder (Ruskin et al., 2011). Further experiments with pharmacological and transgenic methods will be needed to test strictly the involvement of adenosine in these and other phenomena (Masino et al., 2009; Gomes et al., 2011).

Certainly the metabolic effects of a ketogenic diet are many, and its benefits may rely on a key set of mechanisms for a given clinical condition or even for a particular individual. Non-adenosine actions are likely to be involved in some of its therapeutic benefits: for instance, a ketone-based metabolism modulates mitochondrial energy production (Sato et al., 1995) to produce fewer free radicals (Noh et al., 2006) which may alleviate inflammation, and polyunsaturated fatty acids (elevated

by ketogenic diets) might reduce pain through activation of potassium channels in nociceptive circuitry (Lauritzen et al., 2000; Xu et al., 2008). More research is needed into these and other mechanisms.

5.0 Conclusions

Data is accumulating that purines play a key role in the anticonvulsant effects of a ketogenic diet, particularly recent in vivo work showing that the ketogenic diet can suppress seizures via A_1Rs in vivo (Masino et al., 2011). In general, a cohort of recent work on purines incorporates and comports with previous evidence outlining a connection between increased mitochondria and energy molecules with a ketogenic diet, and previous research linking purines to epilepsy in general – e.g. the relationship between ATP and seizures, and anticonvulsant effects of adenosine - even in pharmacoresistant epilepsy.

An in vitro mimic of a ketogenic diet revealed new cellular mechanisms and a metabolic autocrine hyperpolarization of neurons via A_1Rs linked to K_{ATP} channels (Kawamura et al., 2010). Previous work linked direct application of two ketones – β-hydroxybyturate and acetoacetate – to hyperpolarization and reduced firing rate via K_{ATP} channels (Ma et al., 2007), so metabolic or pharmaceutical enhancement of adenosine itself, A_1R activation, or direct activation of neuronal post-synaptic K_{ATP} channels to hyperpolarize neuron membrane potential would be key targets. Recent work has found ketone bodies reduce the activity of vesicular glutamate transporters (Juge et al., 2010). The role of these mechanisms in the clinical effects of the ketogenic diet is unknown, but the consequences of such a global diet-induced change in metabolism are diverse and multiple targets could offer anticonvulsant potential.

Many questions remain regarding the relationship between purines and a ketogenic diet: Does a ketogenic diet *in vivo* open pannexins, reduce adenosine kinase, and/or increase the number of A_1Rs? Are there changes in the metabolic profile of all purines, and are there differential effects across brain

regions and cell types? Is there a key combination of diet-mobilized mechanisms and effects for optimal clinical benefits? Based on initial evidence, the adenosine-enhancing effects have the potential to be strongest exactly where more adenosine is needed – regions of gliosis and high adenosine kinase activity. More mechanistic details are needed on the effects of purines in vivo.

Understanding the metabolic control of central nervous system adenosine offers opens many powerful clinical opportunities beyond epilepsy. If a key role for purines in ketogenic diet therapy - and specifically adenosine acting at the A_1R - is borne out through additional research, this lends new insight into metabolic therapy and new targets for drug development. In addition, diet-based approaches are cost-effective across cultures (Kossoff et al., 2009). Ultimately, and for the first time, the relationship between a ketogenic diet and adenosine may provide insight into a global regulation of network activity – increased adenosine can enhance the signal-to-noise ratio, and prevent inappropriate hyperexcitability, regardless of the underlying cause(s). This emerging work that the ketogenic diet enhances the actions of a homeostatic bioenergetic network regulator such as adenosine (Boison et al., 2011), is an exciting development in ketogenic diet research and may yield insight into other neurological disorders.

References

Akimitsu, T., White, J.A., Carden, D.L., Gute, D.C., Korthius, R.J., 1995. Fructose-1,6-diphosphate or adenosine attenuate leukocyte adherence in postischemic skeletal muscle. Am. J. Physiol. Heart Circ. Physiol. 269**,** H1743-H1751.

Appelberg, K.S., Hovda, D.A., Prins, M.L., 2009. The effects of a ketogenic diet on behavioral outcome after controlled cortical impact injury in the juvenile and adult rat. J. Neurotrauma 26**,** 497-506.

Arai, A., Kessler, M., Lynch, G.S., 1990. The effects of adenosine on the development of long-term potentiation. Neurosci Lett 119**,** 41-44.

Belfrage, M., Sollevi, A., Segerdahl, M., Sjölund, K.F., Hansson, P., 1995. Systemic adenosine infusion alleviates spontaneous and stimulus evoked pain in patients with peripheral neuropathic pain. Anesth. Analg. 81**,** 713-717.

Boison, D., Masino, S.A., Geiger, J.D., 2011. Homeostatic bioenergetic network regulation: a novel concept to avoid pharmacoresistance in epilepsy. Expert Opin. Drug Discov. in press.

Bough, K.J., Wetherington, J., Hassel, B., Pare, J.F., Gawryluk, J.W., Greene, J.G., Shaw, R., Smith, Y., Geiger, J.D., Dingledine, R.J., 2006. Mitochondrial biogenesis in the anticonvulsant mechanism of the ketogenic diet. Ann. Neurol. 60**,** 223-235.

Cheng, B., Yang, X., An, L., Gao, B., Liu, X., Liu, S., 2009. Ketogenic diet protects dopaminergic neurons against 6-OHDA neurotoxicity via up-regulating glutathione in a rat model of Parkinson's disease. Brain Res. 1286**,** 25-31.

Davis, L.M., Pauly, J.R., Readnower, R.D., Rho, J.M., Sullivan, P.G., 2008. Fasting is neuroprotective following traumatic brain injury. J. Neurosci. Res. 86**,** 1812-1822.

DeVivo, D.C., Leckie, M.P., Ferrendelli, J.S., McDougal, D.B., Jr., 1978. Chronic ketosis and cerebral metabolism. Ann. Neurol. 3**,** 331-337.

Dolphin, A.C., 1983. The adenosine agonist 2-chloroadenosine inhibits the induction of long-term potentiation of the perforant path. Neurosci. Lett. 39**,** 83-89.

Dulla, C.G., Dobelis, P., Pearson, T., Frenguelli, B.G., Staley, K.J., Masino, S.A., 2005. Adenosine and ATP link P_{CO2} to cortical excitability via pH. Neuron 48**,** 1011-1023.

Dulla, C.G., Frenguelli, B.G., Staley, K.J., Masino, S.A., 2009. Intracellular acidification causes adenosine release during states of hyperexcitability in the hippocampus. J. Neurophysiol. 102**,** 1984-1993.

Evans, M.C., Swan, J.H., Meldrum, B.S., 1987. An adenosine analogue, 2-chloroadenosine, protects against long term development of ischaemic cell loss in the rat hippocampus. Neurosci. Lett. 83**,** 287-292.

Fenoglio-Simeone, K.A., Wilke, J.C., Milligan, H.L., Allen, C.N., Rho, J.M., Maganti, R.K., 2009. Ketogenic diet treatment abolishes seizure periodicity and improves diurnal rhythmicity in epileptic *Kcna1*-null mice. Epilepsia 50**,** 2027-2034.

Freeman, J.M., Kossoff, E.H., Hartman, A.L., 2007. The ketogenic diet: one decade later. Pediatrics 119**,** 535-543.

Fuxe, K., Ferré, S., Genedani, S., Franco, F., Agnati, L.F., 2007. Adenosine receptor–dopamine receptor interactions in the basal ganglia and their relevance for brain function. Physiol. Behav. 92**,** 210-217.

Gabriel, A., Klussmann, F.W., Igelmund, P., 1998. Rapid temperature changes induce adenosine-mediated depression of synaptic transmission in hippocampal slices from rats (non-hibernators) but not in slices from golden hamsters (hibernators). Neuroscience 86**,** 67-77.

Garriga-Canut, M., Schoenike, B., Qazi, R., Bergendahl, K., Daley, T.J., Pfender, R.M., Morrison, J.F., Ockuly, J., Stafstrom, C., Sutula, T., Roopra, A., 2006. 2-Deoxy-D-glucose reduces epilepsy progression by NRSF-CtBP-dependent metabolic regulation of chromatin structure. Nat. Neurosci. 9**,** 1382-1387.

Goldberg, M.P., Monyer, H., Weiss, J.H., Choi, D.W., 1988. Adenosine reduces cortical neuronal injury induced by oxygen or glucose deprivation in vitro. Neurosci. Lett. 89**,** 323-327.

Goldman, N., Chen, M., Fujita, T., Xu, Q., Peng, W., Liu, W., Jensen, T.K., Pei, Y., Wang, F., Han, X., Chen, J.-F., Schnermann, J., Takano, T., Bekar, L., Tieu, K., Nedergaard, M., 2010. Adenosine A1 receptors mediate local anti-nociceptive effects of acupuncture. Nat. Neurosci. 13**,** 883-888.

Gomes, C.V., Kaster, M.P., Tomé, A.R., Agostinho, P.M., Cunha, R.A., 2011. Adenosine receptors and brain diseases: neuroprotection and neurodegeneration. Biochim Biophys Acta 1808**,** 1380-1399.

Gourine, A.V., Llaudet, E., Dale, N., Spyer, K.M., 2005. ATP is a mediator of chemosensory transduction in the central nervous system. Nature 436**,** 108-11.

Greene, A.E., Todorova, M.T., McGowan, R., Seyfried, T.N., 2001. Caloric restriction inhibits seizure susceptibility in epileptic EL mice by reducing blood glucose. Epilepsia 42**,** 1371-1378.

Haas, H.L., Greene, R.W., 1984. Adenosine enhances afterhyperpolarization and accommodation in hippocampal pyramidal cells. Pflugers Archiv 402**,** 244-247.

Hallböök, T., Lundgren, J., Rosén, I., 2007. Ketogenic diet improves sleep quality in children with therapy-resistant epilepsy. Epilepsia 48**,** 59-65.

Hemingway, C., Freeman, J.M., Pillas, D.J., Pyzik, P.L., 2001. The ketogenic diet: a 3- to 6-year follow-up of 150 children enrolled prospectively. Pediatrics 108**,** 898-905.

Hu, Z.G., Wang, H.D., Jin, W., Yin, H.X., 2009. Ketogenic diet reduces cytochrome c release and cellular apoptosis following traumatic brain injury in juvenile rats. Ann. Clin. Lab. Sci. 39**,** 76-83.

Huang, Z.L., Urade, Y., Hayaishi, O., 2007. Prostaglandins and adenosine in the regulation of sleep and wakefulness. Curr. Opin. Pharmacol. 7**,** 33-38.

Huttenlocher, P.R., 1976. Ketonemia and seizures: metabolic and anticonvulsant effects of two ketogenic diets in childhood epilepsy. Pediatr. Res. 10**,** 536-540.

Jarvis, M.F., Yu, H., Kohlhass, K., Alexander, K., Lee, C.-H., Jiang, M., Bhagwat, S.S., Williams, M., Kowaluk, E.A., 2000. ABT-702 (4-Amino-5-(3-bromophenyl)-7-(6-morpholinopyridin-3-yl)pyrido[2,3-*d*]pyrimidine), a novel orally effective adenosine kinase inhibitor with analgesic and anti-inflammatory properties: I. In vitro characterization and acute antinociceptive effects in the mouse. J. Pharmacol. Exp. Ther. 295**,** 1156-1164.

Johansson, B., Halldner, L., Dunwiddie, T.V., Masino, S.A., Poelchen, W., Giménez-Llort, L., Escorihuela, L.M., Fernández-Teruel, A., Wiesenfeld-Hallin, Z., Xu, X.-J., Hårdemark, A., Betscholtz, C., Herlenius, E., Fredholm, B.B., 2001. Hyperalgesia, anxiety, and decreased hypoxic neuroprotection in mice lacking the adenosine A_1 receptor. Proc. Natl. Acad. Sci. USA 98**,** 9407-9412.

Juge, N., Gray, J.A., Omote, H., Miyaji, T., Inoue, T., Hara, C., Uneyama, H., Edwards, R.H., Nicoll, R.A., Moriyama, Y., 2010. Metabolic control of vesicular glutamate transport and release. Neuron 68**,** 99-112.

Karlsten, R., Gordh, T., Post, C., 1992. Local antinociceptive and hyperalgesic effects in the formalin test after peripheral administration of adenosine analogues in mice. Pharmacol. Toxicol. 70**,** 434-438.

Kawamura, M., Jr., Ruskin, D.N., Masino, S.A., 2010. Metabolic autocrine regulation of neurons involves cooperation among pannexin hemichannels, adenosine receptors and K_{ATP} channels. J. Neurosci. 30**,** 3886-3895.

Kochanek, P.M., Vagni, V.A., Janesko, K.L., Washington, C.B., Crumrine, P.K., Garman, R.H., Jenkins, L.W., Clark, R.S.B., Homanics, G.E., Dixon, C.E., Schnermann, J., Jackson, E.K., 2006. Adenosine A1 receptor knockout mice develop lethal status epilepticus after experimental traumatic brain injury. J. Cereb. Blood Flow Metab. 26**,** 565-575.

Koranda, J.L., Ruskin, D.N., Masino, S.A., Blaise, J.H., 2011. A ketogenic diet reduces long-term potentiation in the dentate gyrus of freely-behaving rats. J. Neurophysiol. accepted with revisions.

Kossoff, E.H., Zupec-Kania, B.A., Rho, J.M., 2009. Ketogenic diets: an update for child neurologists. J. Child Neurol. 24**,** 979-988.

Kukley, M., Schwan, M., Fredholm, B.B., Dietrich, D., 2005. The role of extracellular adenosine in regulating mossy fiber synaptic plasticity. J Neurosci 25**,** 2832-2837.

Lara, D.R., 2010. Caffeine, mental health, and psychiatric disorders. J. Alzheimers Dis. 20 (Suppl. 1)**,** S239-S248.

Lauritzen, I., Blondeau, N., Heurteaux, C., Widmann, C., Romey, G., Lazdunski, M., 2000. Polyunsaturated fatty acids are potent neuroprotectors. EMBO J. 19**,** 1784-1793.

Li, T., Lan, J.Q., Boison, D., 2008a. Uncoupling of astrogliosis from epileptogenesis in adenosine kinase (ADK) transgenic mice. Neuron Glia Biol. 4**,** 91-99.

Li, T., Lan, J.Q., Fredholm, B.B., Simon, R.P., Boison, D., 2007. Adenosine dysfunction in astrogliosis: cause for seizure generation? Neuron Glia Biol. 3**,** 353-366.

Li, T., Ren, G., Lusardi, T., Wilz, A., Lan, J.Q., Iwasato, T., Itohara, S., Simon, R.P., Boison, D., 2008b. Adenosine kinase is a target for the prediction and prevention of epileptogenesis in mice. J. Clin. Invest. 118**,** 571-582.

Ma, W., Berg, J., Yellen, G., 2007. Ketogenic diet metabolites reduce firing in central neurons by opening K_{ATP} channels. J. Neurosci. 27**,** 3618-3625.

Mantis, J.G., Centeno, N.A., Todorova, M.T., McGowan, R., Seyfried, T.N., 2004. Management of multifactorial idiopathic epilepsy in EL mice with caloric restriction and the ketogenic diet: role of glucose and ketone bodies. Nutr. Metab. 1**,** 11.

Masino, S.A., Dunwiddie, T.V., 1999. Temperature-dependent modulation of excitatory transmission in hippocampal slices is mediated by extracellular adenosine. J. Neurosci. 19**,** 1932-1939.

Masino, S.A., Geiger, J.D., 2008. Are purines mediators of the anticonvulsant/neuroprotective effects of ketogenic diets? Trends Neurosci. 31**,** 273-278.

Masino, S.A., Kawamura, M., Jr., Wasser, C.D., Pomeroy, L.T., Ruskin, D.N., 2009. Adenosine, ketogenic diet and epilepsy: the emerging therapeutic relationship between metabolism and brain activity. Curr. Neuropharmacol. 7**,** 257-268.

Masino, S.A., Li, T., Theofilas, P., Ruskin, D.N., Fredholm, B.B., Geiger, J.D., Aronica, E., Boison, D., 2011. A ketogenic diet suppresses seizures in mice through adenosine A_1 receptors. J. Clin. Invest. in press.

Massieu, L., Haces, M.L., Montiel, T., Hernández-Fonseca, K., 2003. Acetoacetate protects hippocampal neurons against glutamate-mediated neuronal damage during glycolysis inhibition. Neuroscience 120**,** 365-378.

Mitchell, J.B., Miller, K., Dunwiddie, T.V., 1993. Adenosine-induced suppression of synaptic responses and the initiation and expression of long-term potentiation in the CA1 region of the hippocampus. Hippocampus 3**,** 77-86.

Moore, K.A., Nicoll, R.A., Schmitz, D., 2003. Adenosine gates synaptic plasticity at hippocampal mossy fiber synapses. Proc Natl Acad Sci USA 100**,** 14397-14402.

Muzykewicz, D.A., Lyczkowski, D.A., Memon, N., Conant, K.D., Pfeifer, H.H., Thiele, E.A., 2009. Efficacy, safety, and tolerability of the low glycemic index treatment in pediatric epilepsy. Epilepsia 50**,** 1118-1126.

Nakazawa, M., Kodama, S., Matsuo, T., 1983. Effects of ketogenic diet on electroconvulsive threshold and brain contents of adenosine nucleotides. Brain Dev. 5**,** 375-380.

Neal, E.G., Chaffe, H., Schwartz, R.H., Lawson, M.S., Edwards, N., Fitzsimmons, G., Whitney, A., Cross, J.H., 2008. The ketogenic diet for the treatment of childhood epilepsy: a randomised controlled trial. Lancet Neurol. 7**,** 500-506.

Newby, A.C., Worku, Y., Holmquist, C.A., 1985. Adenosine formation: Evidence for a direct biochemical link with energy metabolism. Adv. Myocardiol. 6**,** 273-284.

Noh, H.S., Hah, Y.-S., Nilufar, R., Han, J., Bong, J.-H., Kang, S.S., Cho, G.J., Choi, W.S., 2006. Acetoacetate protects neuronal cells from oxidative glutamate toxicity. J. Neurosci. Res. 83**,** 702-709.

Noh, H.S., Kim, Y.S., Lee, H.P., Chung, K.M., Kim, D.W., Kang, S.S., Cho, G.J., Choi, W.S., 2003. The protective effect of a ketogenic diet on kainic acid-induced hippocampal cell death in the male ICR mice. Epilepsy Res. 53**,** 119-128.

Nylen, K., Velazquez, J.L.P., Sayed, V., Gibson, K.M., Burnham, W.M., Snead, O.C., III, 2009. The effects of a ketogenic diet on ATP concentrations and the number of hippocampal mitochondria in *Aldh5a1*$^{-/-}$ mice. Biochim. Biophys. Acta 1790**,** 208-212.

Oro, J., Kimball, A.P., 1961. Synthesis of purines under possible primitive earth conditions. I. Adenine from hydrogen cyanide. Arch. Biochem. Biophys. 94**,** 217-227.

Pan, J.W., Bebin, E.M., Chu, W.J., Hetherington, H.P., 1999. Ketosis and epilepsy: ^{31}P spectroscopic imaging at 4.1 T. Epilepsia 40**,** 703-707.

Pandya, J.D., Pauly, J.R., Nukala, V.N., Sebastian, A.H., Day, K.M., Korde, A.S., Maragos, W.F., Hall, E.D., Sullivan, P.G., 2007. Post-injury administration of mitochondrial uncouplers increases tissue sparing and improves behavioral outcome following traumatic brain injury in rodents. J Neurotrauma 24**,** 798-811.

Panico, L.R., Ríos, V.G., Demartini, M.G., Carniello, M.A., 2000. [The electroencephalographic evolution of a group of patients on a ketonic diet]. Rev. Neurol. 30**,** 8-15.

Pascual, O., Casper, K.B., Kubera, C., Zhang, J., Revilla-Sanchez, R., Sul, J.Y., Takano, H., Moss, S.J., McCarthy, K., Haydon, P.G., 2005. Astrocytic purinergic signaling coordinates synaptic networks. Science 310**,** 113-116.

Pignataro, G., Simon, R.P., Boison, D., 2007. Transgenic overexpression of adenosine kinase aggravates cell death in ischemia. J. Cereb. Blood Flow Metab. 27**,** 1-5.

Poon, A., Sawynok, J., 1999. Antinociceptive and anti-inflammatory properties of an adenosine kinase inhibitor and an adenosine deaminase inhibitor. Eur. J. Pharmacol. 1999**,** 123-138.

Porkka-Heiskanen, T., 1999. Adenosine in sleep and wakefulness. Ann. Med. 31**,** 125-129.

Prins, M.L., Fujima, L.S., Hovda, D.A., 2005. Age-dependent reduction of cortical contusion volume by ketones after traumatic brain injury. J. Neurosci. Res. 82**,** 413-420.

Puchowicz, M.A., Zechel, J.L., Valerio, J., Emancipator, D.S., Xu, K., Pundik, S., LaManna, J.C., Lust, W.D., 2008. Neuroprotection in diet-induced ketotic rat brain after focal ischemia. J. Cereb. Blood Flow Metab. 28**,** 1907-1916.

Rho, J.M., 2010. How does altered metabolism lead to seizure control? Partially filling the knowledge gap. Epilepsy Curr. 10**,** 159-161.

Ribeiro, J.A., Sá-Almeida, A.M., Namorado, J.M., 1979. Adenosine and adenosine triphosphate decrease ^{45}Ca uptake by synaptosomes stimulated by potassium. Biochem. Pharmacol. 28**,** 1297-1300.

Ribeiro, J.A., Sebastião, A.M., 2010. Caffeine and adenosine. J. Alzheimers Dis. 20 (Suppl. 1)**,** S3-S15.

Ruskin, D.N., Kawamura, M., Jr., Masino, S.A., 2009. Reduced pain and inflammation in juvenile and adult rats fed a ketogenic diet. PLoS One 4**,** e8349.

Ruskin, D.N., Ross, J.L., Kawamura, M., Jr., Ruiz, T.L., Geiger, J.D., Masino, S.A., 2011. A ketogenic diet delays weight loss and does not impair working memory or motor function in the R6/2 1J mouse model of Huntington's disease. Physiol. Behav. 103**,** 501-507.

Ruskin, D.N., Suter, T.A.C.S., Masino, S.A., 2010. Ketogenic diet-induced thermal hypoalgesia follows a delayed time course in comparison to ketosis in juvenile rats. Soc. Neurosci. Abstr. 375.4.

Samoilova, M., Weisspapir, M., Abdelmalik, P., Velumian, A.A., Carlen, P.L., 2010. Chronic *in vitro* ketosis is neuroprotective but not anticonvulsant. J. Neurochem. 113**,** 826-835.

Sato, K., Kashiwaya, Y., Keon, C.A., Tsuchiya, N., King, M.T., Radda, G.K., Chance, B., Clarke, K., Veech, R.L., 1995. Insulin, ketone bodies, and mitochondrial energy transduction. FASEB J. 9**,** 651-658.

Schmidt, A.P., Böhmer, A.E., Antunes, C., Schallenberger, C., Porciúncula, L.O., Elisabetsky, E., Lara, D.R., Souza, D.O., 2009. Anti-nociceptive properties of the xanthine oxidase inhibitor allopurinol in mice: role of A_1 adenosine receptors. Br. J. Pharmacol. 156**,** 163-172.

Sitkovsky, M.V., Ohta, A., 2005. The 'danger' sensors that STOP the immune response: the A2 adenosine receptors? Trends Immunol. 26**,** 299-304.

Sorkin, L.S., Moore, J., Boyle, D.L., Yang, L., Firestein, G.S., 2003. Regulation of peripheral inflammation by spinal adenosine: role of somatic afferent fibers. Exp. Neurol. 184**,** 162-168.

Studer, F.E., Fedele, D.E., Marowsky, A., Schwerdel, C., Wernli, K., Vogt, K., Fritschy, J.-M., Boison, D., 2006. Shift of adenosine kinase expression from neurons to astrocytes during postnatal development suggests dual functionality of the enzyme. Neuroscience 142**,** 125-137.

Sullivan, P.G., Rippy, N.A., Dorenbos, K., Concepcion, R.C., Agarwal, A.K., Rho, J.M., 2004. The ketogenic diet increases mitochondrial uncoupling protein levels and activity. Ann. Neurol. 55**,** 576-580.

Suzuki, M., Suzuki, M., Sato, K., Dohi, S., Sato, T., Matsuura, A., Hiraide, A., 2001. Effect of β-hydroxybutyrate, a cerebral function improving agent, on cerebral hypoxia, anoxia and ischemia in mice and rats. Jpn. J. Pharmacol. 87**,** 143-150.

Tai, K.-K., Nguyen, N., Pham, L., Truong, D.D., 2008. Ketogenic diet prevents cardiac arrest-induced cerebral ischemic neurodegeneration. J. Neural Transm. 115**,** 1011-1017.

Tekkök, S., Medina, I., Krnjević, K., 1999. Intraneuronal [Ca^{2+}] changes induced by 2-deoxy-D-glucose in rat hippocampal slices. J. Neurophysiol. 81**,** 174-183.

Tendler, D., Lin, S., Yancy, W.S., Jr., Mavropoulos, J., Sylvestre, P., Rockey, D.C., Westman, E.C., 2007. The effect of a low-carbohydrate, ketogenic diet on nonalcoholic fatty liver disease: a pilot study. Dig. Dis. Sci. 52**,** 589-593.

Thio, L.L., Rensing, N., Maloney, S., Wozniak, D.F., Xiong, C., Yamada, K.A., 2010. A ketogenic diet does not impair rat behavior or long-term potentiation. Epilepsia 51**,** 1619-1623.

Tsutsui, S., Schnermann, J., Noorbakhsh, F., Henry, S., Yong, V.W., Winston, B.W., Warren, K., Power, C., 2004. A1 adenosine receptor upregulation and activation attenuates neuroinflammation and demyelination in a model of multiple sclerosis. J. Neurosci. 24**,** 1521-1529.

Van der Auwera, I., Wera, S., Van Leuven, F., Henderson, S.T., 2005. A ketogenic diet reduces amyloid beta 40 and 42 in a mouse model of Alzheimer's disease. Nutr. Metab. 2**,** 28.

Vanotti, A., Osio, M., Mailland, E., Nascimbene, C., Capiluppi, E., Mariani, C., 2007. Overview of pathophysiology and newer approaches to treatment of peripheral neuropathies. CNS Drugs 21 (Suppl 1)**,** 3-12.

Varma, M.R., Dixon, C.E., Jackson, E.K., Peters, G.W., Melick, J.A., Griffith, R.P., Vagni, V.A., Clark, R.S.B., Jenkins, L.W., Kochanek, P.M., 2002. Administration of adenosine receptor agonists or antagonists after controlled cortical impact in mice: effects on function and histopathology. Brain Res. 951**,** 191-201.

Wilder, R.M., 1921. The effects of ketonemia on the course of epilepsy. Mayo Clin. Bull. 2**,** 307-308.

Xu, X.-p., Erichsen, D., Börjesson, S.I., Dahlin, M., Åmark, P., Elinder, F., 2008. Polyunsaturated fatty acids and cerebrospinal fluid from children on the ketogenic diet open a voltage-gated K channel: A putative mechanism of antiseizure action. Epilepsy Res. 80**,** 57-66.

Yamada, K.A., Rensing, N., Thio, L.L., 2005. Ketogenic diet reduces hypoglycemia-induced neuronal death in young rats. Neurosci. Lett. 385**,** 210-214.

Yang, X., Cheng, B., 2010. Neuroprotective and anti-inflammatory activities of ketogenic diet on MPTP-induced neurotoxicity. J. Mol. Neurosci. 42**,** 145-153.

Yarbrough, G.G., McGuffin-Clineschmidt, J.C., 1981. In vivo behavioral assessment of central nervous system purinergic receptors. Eur. J. Pharmacol. 76**,** 137-144.

Zhao, Z., Lange, D.J., Voustianiouk, A., MacGrogan, D., Ho, L., Suh, J., Humala, N., Thiyagarajan, M., Wang, J., Pasinetti, G.M., 2006. A ketogenic diet as a potential novel therapeutic intervention in amyotrophic lateral sclerosis. BMC Neurosci. 7**,** 29.

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Table 1. Predicted and observed effects of the ketogenic diet in mice.

Mouse Model	*A_1R Expression*	*Predicted change in seizure frequency*	*Observed change in seizure frequency*	*Glucose-induced change in seizure frequency*
Wild-type: (C57/BL6)	unaltered	n/a (no seizures)	n/a	no change
Transgenic: Adk-tg	Unaltered	Robust suppression	88% decrease ($p<0.001$)	reversed to 85% of baseline ($p<0.001$)
Transgenic: $A_1R^{+/-}$	50% normal	Partial suppression	53% decrease ($p<0.001$)	reversed to 89% of baseline ($p<0.001$)
Transgenic: $A_1R^{-/-}$	no receptors	No suppression	4% decrease (n.s.)	no change (n.s.)

Legend: Predicted and observed effects of a ketogenic diet in a complement of wild type and transgenic mice. All transgenic mice demonstrated adenosine-based recurrent electrographic seizures. Based on the level of A_1R expression in each mouse strain we predicted the correlated effects of a ketogenic diet in reducing seizure frequency. The A_1R receptor gene was not manipulated in the Adk-tg mice; thus, we predicted seizures would be reduced by a ketogenic diet. We observed a significant (88%) reduction in seizure frequency. Functional A_1Rs are expressed partially (50%) in the $A_1R^{+/-}$ mice, and thus we predicted partial seizure reduction. We observed that seizures decreased significantly but to a lesser extent than in the Adk-tg mice (53%). Finally, $A_1R^{-/-}$ mice have a complete loss of functional A_1Rs, we predicted no effects of the ketogenic diet on spontaneous seizures, and we observed no significant change (4%) in seizure frequency. The ketogenic diet-induced decrease in seizure frequency was reversed significantly by glucose injection in Adk-tg and $A_1R^{+/-}$ mice, and was unaffected in wild-type and $A_1R^{-/-}$ mice (adapted from Masino et al., 2011)

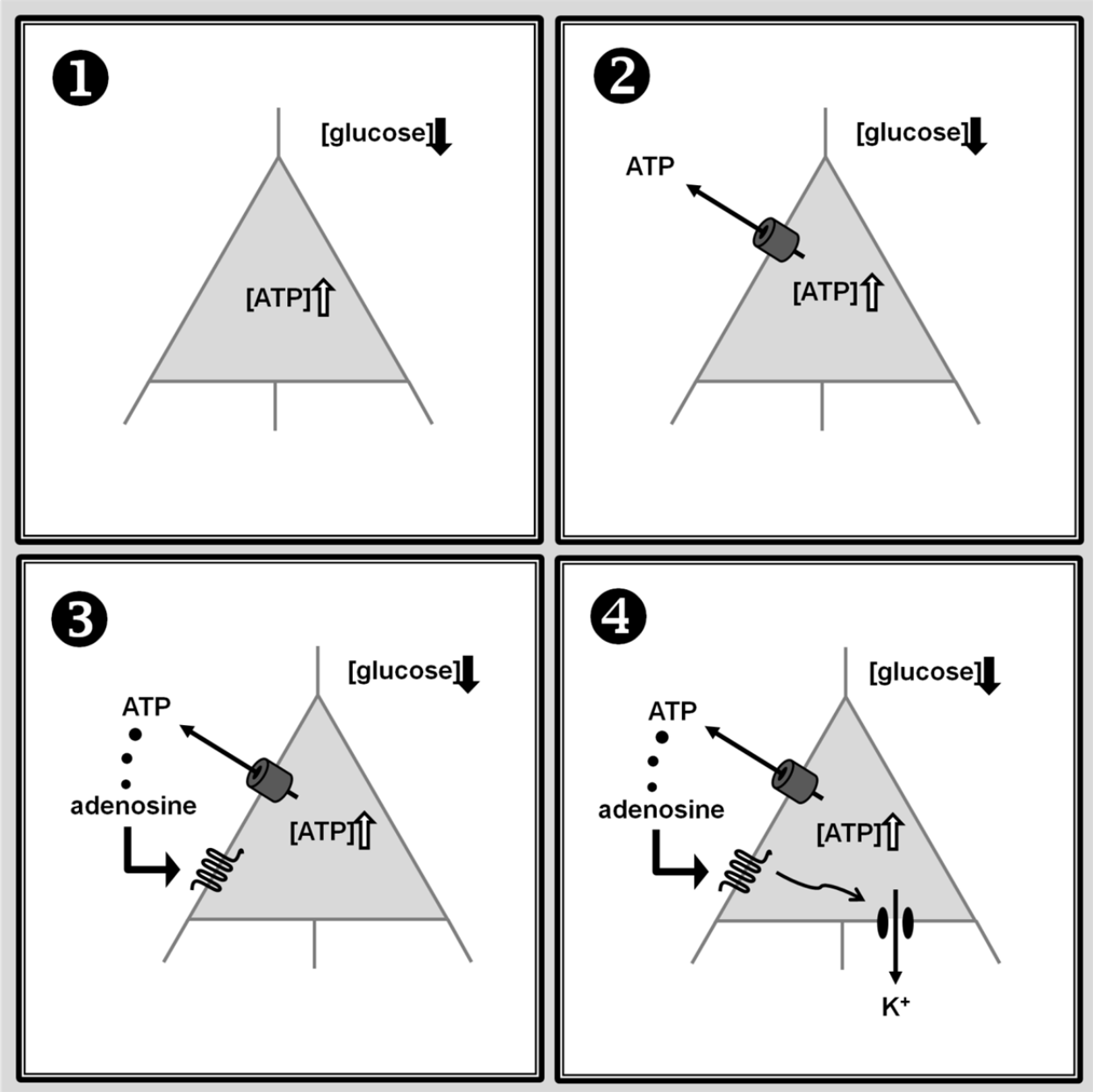

Figure 1. A schematic of purinergic autocrine regulation of CA3 pyramidal cell excitability. (1): With abundant intracellular ATP and moderately reduced extracellular glucose - a scenario a ketogenic diet is thought to produce: (2) ATP is released directly via pannexin hemichannels, and (3) released ATP is dephosphorylated subsequently to adenosine which activates adenosine A_1Rs. This in turn opens K_{ATP} channels which hyperpolarizes the membrane, and decreases excitability (4). In addition to these autocrine postsynaptic effects, the elevated adenosine can function in a paracrine manner to reduce

neurotransmitter release from afferent axon terminals. Adapted and modified from Kawamura et al., 2010.

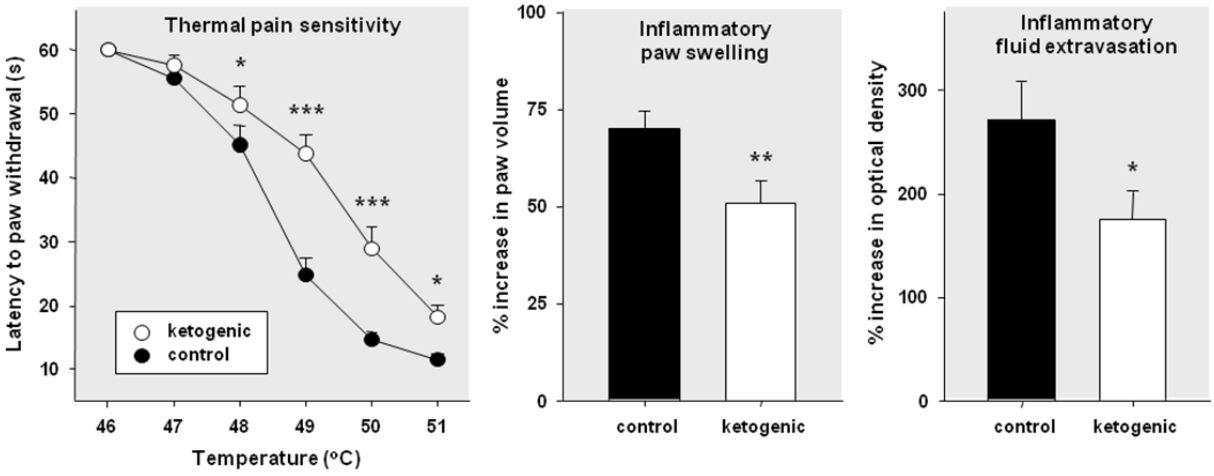

Figure 2. Effects of a ketogenic diet on nociception and inflammation. Left: Latency to nocifensive hindpaw response across a range of hotplate temperatures. Ketogenic diet feeding significantly lengthened latencies at a majority of tested temperatures, suggesting that the diet reduced sensitivity to thermal pain. Middle: Hindpaw swelling after experimentally-induced inflammation. Ketogenic diet reduced inflammatory swelling, expressed as percent increase in paw volume compared to pretreatment paw volume. Right: Movement of fluid out of blood vessels (extravasation) after experimentally-induced inflammation. Ketogenic diet feeding reduced this aspect of inflammation, expressed as percent increase in treated paw compared to contralateral untreated paw. For clarity, all panels show combined data from adolescent and adult rats; dietary effects were significant at both ages (Ruskin et al., 2009). Number of subjects = 26-28 (pain, left panel), 15-19 (swelling and extravasation, middle and right panels). *p<0.05, **p<0.01, ***p<.001, compared to control diet. Modified from Ruskin et al., 2009.