Published in final edited form as:
Epilepsia. 2008 November ; 49(Suppl 8): 91–93. doi:10.1111/j.1528-1167.2008.01846.x.

Energy Metabolism as Part of the Anticonvuslant Mechanism of the Ketogenic Diet

Kristopher Bough, PhD
NIH – National Institute on Drug Abuse, Division of Pharmacotherapies and Medical Consequences, 6001 Executive Boulevard – Room 4122, Bethesda, MD 20852

Summary

The efficacy of the ketogenic diet (KD) develops gradually over a period of 1-3 weeks, suggesting that adaptive changes in gene expression are involved in its anticonvulsant effects. Previously, we employed microarrays to define patterns of gene expression in the hippocampus of rats maintained on either a KD or control diet for three weeks. The density of mitochondria in hippocampal tissue was assessed by electron microscopy. Levels of selected energy metabolites, enzyme activities, and the effect of low glucose on synaptic transmission were also investigated in hippocampal tissue taken from either KD- or control-fed animals. We found a coordinated upregulation of transcripts encoding energy metabolism enzymes and a dramatic 46% increase in the density of mitochondria observed in neuronal processes. These changes were accompanied by an increased phosphocreatine (PCr):creatine (Cr) energy-store ratio. Consistent with heightened energy reserves, hippocampal synaptic transmission in KD-fed animals was maintained ~50% longer compared to controls after exposure to a mild metabolic stressor. Taken together, several lines of evidence indicate that the KD enhances energy production in the brain. As a consequence, brain tissue appears to become more resistant to metabolic stress. We propose that the observed KD-induced enhancements in energy metabolism help to compensate for the metabolic deficits exhibited (interictally) within epileptic foci and transient failures of GABAergic inhibition, which would otherwise favor the initiation and propagation of seizure activity.

Keywords

Ketogenic Diet; Epilepsy; Microarray; Mitochondria; Hippocampus; Rat

The ketogenic diet (KD) has long been used as an alternative treatment for intractable, pediatric epilepsy and is remarkably effective against multiple seizure types. It is comprised of >90% fat by weight, is low in carbohydrates, adequate in proteins, vitamins and minerals, and typically calorie restricted by 10-25%. During diet treatment, the body synthesizes ketone bodies as an energy supplement to the brain, since dietary sources of glucose are dramatically reduced. Despite its successful use for nearly a century, we still do not know how the KD results in improved seizure control.

Both clinical and experimental data suggest that adaptations to the KD underlie its anticonvulsant effects. In rodents, maximal seizure control develops gradually over a period of 10-14 days (Appleton & DeVivo, 1974). Similarly, in most human patients, maximal seizure control is not achieved until after two weeks of dietary therapy (Freeman et al., 2000). Therefore, we hypothesized that the adaptive changes in gene expression are involved in the anticonvulsant mechanisms of the KD.

Disclosure: The author declares no conflicts of interest.

Microarrays were employed to identify coordinated patterns of gene expression in rats maintained on a calorie-restricted KD for three weeks (Bough et al., 2006). Three hundred eighty-four transcripts were upregulated after KD treatment, whereas 274 were downregulated. Seventy percent of these transcripts could be assigned to one of eight functional categories. These included metabolism (16%), signal transduction (15.3%), growth & development (11%), biosynthesis (10%), synaptic transport (8%), transport (8%), protein biosynthesis (6.5%), miscellaneous (6.5%), immune function (5%), transcriptional regulation (4.5%), cytoskeleton (3.8%), homeostasis (3.2%) and apoptosis (2.2%). The most prominent category of genes differentially expressed following chronic KD treatment was functionally attributed to energy metabolism. Of these, remarkably, 33 of the 34 transcripts were upregulated and 21 encoded genes involve oxidative phosphorylation.

We subsequently asked whether this coordinated increase in energy metabolism transcripts was accompanied by mitochondrial biogenesis. We visually scored electron micrographs of hippocampal tissue taken from animals maintained on either a control or ketogenic diet for at least three weeks. The inter-animal variability was consistent across treatment groups (i.e., coefficient of variation, CV = 3% for both KD and controls), indicating a striking 46% increase in the density of mitochondrial profiles in tissue taken from KD-fed animals compared to controls. Most mitochondria appeared to be located in neuronal processes (i.e., dendrites or axon terminals). In support of this result, we found that 39 of the 42 genes encoding mitochondrial proteins were upregulated after the KD.

In light of these findings, we next investigated whether the production of energy metabolites was heightened within hippocampal tissue. Although ATP, ADP and AMP levels did not change appreciably after the KD, there was an increase in the phosphocreatine (PCr)–creatine (Cr) energy-store ratio and tissue levels of beta-hydroxybutyrate (BHB). Somewhat surprisingly, concentrations of glutamate and glutamine were also elevated in hippocampal tissue from KD-fed animals versus controls. However, because most glutamate in the brain is used as an energy substrate rather than neurotransmitter, these results are consistent with notion that energy reserves in hippocampus are elevated after KD treatment.

Because neuronal energy consumption is largely dependent upon action potential and post-synaptic depolarizing activity (Attwell & Laughlin, 2001), we then asked whether the observed genetic and biochemical changes could produce a functional change in synaptic transmission. We found that reducing the glucose concentration in the perfusion medium from 10 to 2 mM for 7-10 minutes reversibly depressed the slope of the field EPSP by 53±9% in control tissue; this was nearly twice that exhibited in slices made from KD-fed animals (27±8%). The latency to a −25% reduction of the fEPSP took nearly 50% longer in hippocampal KD slices than it did in controls.

How might these observed genetic, biochemical, and functional changes lead to improved seizure control? Human imaging studies have shown that interictally, epileptic foci are hypometabolic areas. More specifically, there is a concomitant loss of “metabolically-inexpensive” inwardly-rectifying potassium channels and an increase in Na^+/K^+-ATPase activity at the same time that there is a net reduction in glucose transport via GLUT-1, perhaps uncoupling metabolic supply and demand (Janigro, 1999). These data suggest that metabolic perturbations contribute significantly to synaptic instability, epileptic hyperexcitability, and the development of seizures. We propose that the ~50% increase in the density of neuronal mitochondria increases ATP production capacity, where the excess high-energy phosphates are stored as PCr. The enhanced ability of neurons to produce and sustain ATP levels during heightened levels of activity should allow neurons to more readily re-fuel the energy-requiring transporters (e.g., the Na^+/K^+-ATPase) and stabilize the membrane potential. This action would be expected to maintain ionic homeostasis and

normal synaptic function in a time of need. Indeed, our finding that hippocampal synaptic transmission in slices taken from KD-fed animals could be maintained for approximately 60% longer when exposed to a mild metabolic stressor compared to synaptic transmission in slices taken from controls is consistent with the notion that KD enhances energy production capacity and energy stores.

Another consideration is that the KD-induced enhancement in energy metabolism compensates for a metabolic deficiency that compromises, in particular, GABAergic inhibition. This may occur via three synergistic mechanisms. First, the KD may re-establish energy stores important for GABAergic output. In cases of human temporal lobe epilepsy, the PCr/ATP energy-store ratio has been inversely correlated with the recovery of the membrane potential following a stimulus train of neuronal bursting (Williamson et al., 2005); because the creatine kinase enzyme is predominantly localized within GABAergic interneurons (Boero et al., 2003), it has been proposed that PCr and energy stores are especially critical to the maintenance of GABAergic inhibitory output. Consistent with this possibility, we found that the KD induced an elevation in the PCr/Cr energy-store ratio in rats, and a similar finding was observed previously in humans (Pan et al., 1999). These results suggest that the KD may act to metabolically re-establish energy stores and, in so doing, help maintain network GABAergic inhibitory output that might have otherwise become compromised during non-accommodating bursts of activity.

Second, the KD-induced enhancement in energy production may help prevent rundown of post-synaptic $GABA_A$ receptors. Endogenous phosphorylation of the $GABA_A$ receptor prevents rundown of GABAergic inhibitory responses (Stelzer et al., 1988). Notably, the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) metabolic enzyme has been shown to be physically associated with the $GABA_A$ receptor and autophosphorylate the alpha-1 subunit using ATP (Pumain & Laschet, 2006). This energy-dependent mechanism of GABA receptor phosphorylation is significantly reduced in human epileptic tissue. We found that GAPDH was significantly upregulated after KD. Furthermore, in earlier studies, electrophysiological recordings performed in KD-fed animals *in vivo* demonstrated that paired-pulse inhibition was enhanced at the 30-ms time point compared to controls, a measure consistent with enhanced fast, $GABA_A$-mediated inhibition (Bough et al., 2003). These data are consistent with the notion that the postsynaptic $GABA_A$ receptor is better able to withstand functional rundown as it likely remains in a phosphorylated state.

Third, the KD-induced changes in energy metabolism also seem likely to modify the metabolism of brain amino acids (Yudkoff et al., 2007). Treatment with the KD may limit the availability of oxaloacetate to the aspartate aminotransferase reaction. As a result, glutamate becomes more accessible to the glutamate decarboxylase (GAD) reaction. This would be expected to increase the production of GABA, and, presumably, functional inhibition. Although we did not note an increase in hippocampal GABA concentration, the concentrations of both glutamate and glutamine – the essential precursors to GABA synthesis – were elevated following the KD. Diet therapy has been shown to increase the abundance of GAD in several brain regions (Cheng et al., 2004), and, in mice, KD treatment modified amino acid metabolism in a manner consistent with enhanced GABA production (Yudkoff et al., 2001).

Taken together, several lines of evidence indicate that the KD dramatically enhances energy production in the brain. This effect appears to result from adaptive changes to chronic dietary treatment which produces a coordinated upregulation of several energy metabolism genes, mitochondrial biogenesis, and an increase in energy reserves. As a consequence, brain tissue appears to become more resistant to metabolic stress. It is noteworthy that the KD only increases seizure threshold; it does not appear to terminate breakthrough seizure

activity, and may actually provide a greater energy supply that exacerbates the spread of ictal activity once it has been initiated (Bough et al, 2000). We propose that KD-induced enhancements in metabolism compensate for metabolic deficits exhibited (interictally) within epileptic foci and transient failures of GABAergic inhibition (e.g., $GABA_A$ receptor rundown), which would otherwise favor the initiation and propagation of seizure activity.

Although many questions remain, the fact that a dietary regimen can have such profound therapeutic effects on neurological disease and cellular metabolism underscores the importance of elucidating the mechanistic underpinnings of the KD. Future studies will undoubtedly lead to a better understanding of the roles of cellular metabolism in normal neurological function, as well as disease, and should pave the way for the development of potent new treatment strategies for the intractable epilepsies.

Acknowledgments

This work was supported in part by the Charlie Foundation (Santa Monica, California). The author acknowledges Dr. David White for helpful comments in the preparation of this article.

References

Appleton DB, DeVivo DC. An animal model for the ketogenic diet. Epilepsia 1974;15:211–227. [PubMed: 4525180]

Attwell D, Laughlin SB. An energy budget for signaling in the grey matter of the brain. J Cereb Blood Flow Metab 2001;21:1133–1145. [PubMed: 11598490]

Boero J, Qin W, Cheng J, Woolsey TA, Strauss AW, Khuchua Z. Restricted neuronal expression of ubiquitous mitochondrial creatine kinase: changing patterns in development and with increased activity. Mol Cell Biochem 2003;244:69–76. [PubMed: 12701812]

Bough KJ, Matthews PJ, Eagles DA. A ketogenic diet has different effects upon seizures induced by maximal electroshock and by pentylenetetrazole infusion. Epilepsy Res 2000;38:105–114. [PubMed: 10642038]

Bough KJ, Schwartzkroin PA, Rho JM. Calorie restriction and ketogenic diet diminish neuronal excitability in rat dentate gyrus in vivo. Epilepsia 2003;44:752–760. [PubMed: 12790887]

Bough KJ, Wetherington J, Hassel B, Pare JF, Gawryluk JW, Greene JG, Shaw R, Smith Y, Geiger JD, Dingledine RJ. Mitochondrial biogenesis in the anticonvulsant mechanism of the ketogenic diet. Ann Neurol 2006;60:223–235. [PubMed: 16807920]

Cheng CM, Hicks K, Wang J, Eagles DA, Bondy CA. Caloric restriction augments brain glutamic acid decarboxylase-65 and -67 expression. J Neurosci Res 2004;77:270–276. [PubMed: 15211593]

Freeman, JM.; Freeman, JB.; Kelly, MT. The Ketogenic Diet: A Treatment for Epilepsy. 3rd. Demos Publishing; New York: 2000.

Janigro D. Blood-brain barrier, ion homeostasis and epilepsy: possible implications towards the understanding of ketogenic diet mechanisms. Epilepsy Res 1999;37:223–232. [PubMed: 10584972]

Pan JW, Bebin EM, Chu WJ, Hetherington HP. Ketosis and epilepsy: ^{31}P spectroscopic imaging at 4.1T. Epilepsia 1999;40:703–707. [PubMed: 10368066]

Pumain R, Laschet J. A key glycolytic enzyme plays a dual role in GABAergic neurotransmission and in human epilepsy. Crit Rev Neurobiol 2006;18:197–203. [PubMed: 17725522]

Stelzer A, Kay AR, Wong RK. GABAA-receptor function in hippocampal cells is maintained by phosphorylation factors. Science 1988;241:339–341. [PubMed: 2455347]

Williamson A, Patrylo PR, Pan J, Spencer DD, Hetherington H. Correlations between granule cell physiology and bioenergetics in human temporal lobe epilepsy. Brain 2005;128:1199–1208. [PubMed: 15728655]

Yudkoff M, Daikhin Y, Nissim I, Lazarow A, Nissim I. Ketogenic diet, amino acid metabolism, and seizure control. J Neurosci Res 2001;66:931–940. [PubMed: 11746421]

Yudkoff M, Daikhin Y, Melo TM, Nissim I, Sonnewald U, Nissim I. The ketogenic diet and brain metabolism of amino acids: relationship to the anticonvulsant effect. 2007;27:415–430.