

THE KETOGENIC DIET: STOKING THE POWERHOUSE OF THE CELL

Mitochondrial Biogenesis in the Anticonvulsant Mechanism of the Ketogenic Diet. Bough KJ, Wetherington J, Hassel B, Pare JF, Gawryluk JW, Greene JG, Shaw R, Smith Y, Geiger JD, Dingledine RJ. *Ann Neurol* 2006;60:223–235. **OBJECTIVE:** The full anticonvulsant effect of the ketogenic diet (KD) can require weeks to develop in rats, suggesting that altered gene expression is involved. The KD typically is used in pediatric epilepsies, but is effective also in adolescents and adults. Our goal was to use microarray and complementary technologies in adolescent rats to understand its anticonvulsant effect. **METHODS:** Microarrays were used to define patterns of gene expression in the hippocampus of rats fed a KD or control diet for 3 weeks. Hippocampi from control- and KD-fed rats were also compared for the number of mitochondrial profiles in electron micrographs, the levels of selected energy metabolites and enzyme activities, and the effect of low glucose on synaptic transmission. **RESULTS:** Most striking was a coordinated upregulation of all ($n = 34$) differentially regulated transcripts encoding energy metabolism enzymes and 39 of 42 transcripts encoding mitochondrial proteins, which was accompanied by an increased number of mitochondrial profiles, a higher phosphocreatine/creatine ratio, elevated glutamate levels, and decreased glycogen levels. Consistent with increased energy reserves, synaptic transmission in hippocampal slices from KD-fed animals was resistant to low glucose. **CONCLUSION:** These data show that a calorie-restricted KD enhances brain metabolism. We propose an anticonvulsant mechanism of the KD involving mitochondrial biogenesis leading to enhanced alternative energy stores.

COMMENTARY

One of the perplexing mysteries in the field of epilepsy research is how the ketogenic diet (KD) exerts broad therapeutic efficacy against multiple seizure types and epilepsy syndromes, often producing a cessation of seizures—even in patients who have failed to respond to antiepileptic drugs. The KD is a high-fat content diet in which carbohydrates are heavily restricted. In the metabolic state that results from chronic treatment with the KD, high rates of fatty-acid oxidation in mitochondria produce large amounts of acetyl-CoA, which leads to hepatic synthesis of ketone bodies, such as β -hydroxybutyrate and acetoacetate. Ketone bodies then enter the circulation and are used as substrates for fuel by the brain and, in developing mammals, as building blocks for membrane lipids.

Growing appreciation of the diet's efficacy has sparked the dramatic growth of clinical KD programs at epilepsy centers throughout the world. This development, in turn, has stimulated scientific interest in the question of how the KD works. Recently, clinicians have discovered that versions of the diet that have liberalized amounts of carbohydrate and protein still can provide seizure protection but are better tolerated than the classical KD, which consists of 80–90% fat and is often unpalatable and potentially unhealthy (1,2). An understanding of the underlying mechanisms of KD action eventually should enable optimization of dietary therapies for epilepsy and may permit the identification of clinically useful biomarkers that correlate with efficacy.

Multiple theories have been proposed to explain how the KD protects against seizures (2). The presence of high-serum ketone body levels raises the obvious possibility that these metabolites themselves could be endogenous antiepileptic substances. While acetoacetate and acetone (but not β -hydroxybutyrate) have been found to protect against seizures in a variety of animal models (3), a clear demonstration that brain levels of these ketone bodies correlate with treatment success or failure has not yet been forthcoming.

An alternate hypothesis focusing on bioenergetics was first proposed by DeVivo et al. (4), who noted an increased cerebral energy charge in chronically ketotic rats—reflected as an increased ATP/ADP ratio—and proposed that this finding may account for the “increased neuronal stability” required for seizure control. Indirect support for this bioenergetic theory has come from studies showing enhanced mitochondrial biogenesis in skeletal muscle, heart, and liver in response to fatty acids (5). Moreover, a ^{31}P spectroscopic imaging study of patients with intractable epilepsy treated with the KD has provided preliminary confirmation of increased brain energy stores (6); and a recent study of hippocampal gene expression, using cDNA microarrays in mice fed a KD, demonstrated increased expression of genes in mitochondrial metabolic pathways (7).

Gene-expression profiling also was used in the study by Bough et al., highlighted here, to confirm that the KD produces a coordinated upregulation of transcripts for genes encoding proteins involved in energy metabolism in rat hippocampus, including those specific to mitochondria. In addition, using electron microscopy, the authors directly demonstrated an increased number of hippocampal mitochondria, which appeared to be localized primarily to dendrites and axon terminals. The enhanced energy reserves following KD treatment were found to preserve normal synaptic transmission in brain slices for longer

periods of time under conditions of controlled metabolic challenge than in brain slices from rats fed a control diet.

The major implication of this study is that the KD results in adaptive changes in energy metabolism, including a striking enhancement in mitochondrial biogenesis that leads to an increase in energy stores. This effect on cellular bioenergetics presumably enables neurons to better withstand metabolic challenges in the face of increased energy demand. Whether the change in bioenergetics accounts for the seizure protection conferred by the KD remains unclear. There is little evidence that the triggering of seizures under ordinary conditions is related to energy failure, although it is well recognized that there is hypometabolism interictally in epileptic foci. Nevertheless, it is possible that a greater reserve pool of bioenergetic substrates could enhance the ability of neurons to maintain the activity of energy-requiring membrane transporters, most notably the $\text{Na}^+ - \text{K}^+ - \text{ATPase}$, thus biasing against aberrant epileptic depolarization. Along similar lines, there is increasing evidence that the KD may have beneficial neuroprotective and disease-modifying actions in a variety of neurological conditions believed to be associated with metabolic stress (3,8), and it is plausible that the effect of the KD on bioenergetics could contribute to these types of actions.

One way in which cellular energy stores could be linked to neuronal excitability is through ATP-sensitive potassium (K_{ATP}) channels, which represent a type of inwardly rectifying potassium channel (Kir6) that is activated when intracellular ATP levels fall. However, the ATP sensitivity of these channels is opposite to that required for dampening neuronal excitability, since enhanced ATP levels result in closure of these channels, which would tend to cause depolarization and destabilize neurons. Thus, a requirement for seizure control would be that the relevant K_{ATP} channels be present in inhibitory neurons. At least some inhibitory GABAergic neurons (e.g., in the substantia nigra) do contain K_{ATP} channels, and the activity of these neurons can be affected by ketone bodies (9).

Although the prevailing view is that the KD enhances brain energy stores, it is possible that some dietary manipulations may influence neuronal excitability through negative effects on cellular energetics. It has long been recognized that the KD is associated with modestly reduced glucose levels. Moreover, calorie restriction alone, even without high-fat ingestion, confers seizure protection (10). The reduced ATP levels that might occur under such situations could lead to less robust activity of membrane transporters, which in turn would limit the ability of neurons to reach (and maintain) high levels of activity necessary for seizures. Of course, low glucose and ATP also may activate K_{ATP} channels, which also could reduce excitability. Although carbohydrate restriction might be therapeutically useful, from a practical standpoint, such a diet is difficult to maintain, given the ubiquity of carbohydrates in contemporary

diets. Therefore, it is of interest that Stafstrom et al. (11) found that 2-deoxyglucose (2-DG), which blocks glucose metabolism by inhibiting phosphoglucose isomerase, inhibits epileptiform bursting in a brain slice model and also elevates the seizure threshold in perforant-path kindled rats. However, it is unclear if 2-DG mimics the KD in animal seizure models (12), and it is unlikely to represent a true replacement for the diet. Given the findings of Bough et al., it will be interesting to determine whether carbohydrate restriction or 2-DG can induce mitochondrial biogenesis.

In conclusion, the study of Bough et al. provides compelling evidence that the KD induces profound changes in brain energy metabolism. Indeed, the mitochondrion, the powerhouse of the cell, certainly appears to be “stoked” by the KD. A major issue that remains is whether the epileptic brain would respond in the same manner to the KD as the normal brain studied by Bough et al. Mitochondria from epileptic brain may be functionally impaired (13). Would these “epileptic mitochondria” also have an impaired bioenergetic response compared with those in normal brain, or would there perhaps be an even more robust response? The answer is presently unknown. In any case, the effects of the KD on energy metabolism may certainly contribute to the neuroprotective and disease-modifying effects of the diet (3,8). Whether such effects confer seizure protection, as do antiepileptic drugs, remains to be determined. Plasma membrane ion channels and transporters conventionally have been considered the major targets for antiepileptic drugs. Now, cellular elements implicated in bioenergetics—including enzymes and substrates involved in energy metabolism, mitochondrial proteins, and diverse regulatory factors—also must be considered potential antiepileptic targets. Perhaps, bioenergetic modulators will one day come to represent a new class of pharmacological agents useful to treat seizures resistant to conventional antiepileptic drugs.

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References

1. Kossoff EH, McGrogan JR, Bluml RM, Pillas DJ, Rubenstein JE, Vining EP. A modified atkins diet is effective for the treatment of intractable pediatric epilepsy. *Epilepsia* 2006;47:421–424.
2. Pfeifer HH, Thiele EA. Low-glycemic-index treatment: a liberalized ketogenic diet for treatment of intractable epilepsy. *Neurology* 2005;65:1810–1812.
3. Bough KJ, Rho JM. Anticonvulsant mechanisms of the ketogenic diet. *Epilepsia* 2007;48:43–58.
4. DeVivo DC, Leckie MP, Ferrendelli JS, McDougal DB, Jr. Chronic ketosis and cerebral metabolism. *Ann Neurol* 1978;3:331–337.
5. Totland GK, Madsen L, Klementsens B, Vaagenes H, Kryvi H, Froyland L, Hexeberg S, Berge RK. Proliferation of mitochondria and gene expression of carnitine palmitoyltransferase and fatty

- acyl-CoA oxidase in rat skeletal muscle, heart and liver by hypolipidemia fatty acids. *Biol Cell* 2000;92:317–329.
6. Pan JW, Bebin EM, Chu WJ, Hetherington HP. Ketosis and epilepsy: ^{31}P spectroscopic imaging at 4.1 T. *Epilepsia* 1999;40:703–707.
 7. Noh HS, Lee HP, Kim DW, Kang SS, Cho GJ, Rho JM, Choi WS. A cDNA microarray analysis of gene expression profiles in rat hippocampus following a ketogenic diet. *Brain Res Mol Brain Res* 2004;129:80–87.
 8. Gasior M, Rogawski MA, Hartman AL. Neuroprotective and disease-modifying effects of the ketogenic diet. *Behav Pharmacol* 2006;17:431–439.
 9. Yellen G. Ketone bodies and neuronal excitability. *Epilepsia* 2004;45(suppl. 7):2.
 10. Greene AE, Todorova MT, McGowan R, Seyfried TN. Caloric restriction inhibits seizure susceptibility in epileptic mice by reducing blood glucose. *Epilepsia* 2001;42:1371–1378.
 11. Stafstrom CE, Kriegler SM, Valley MT, Ockuly JC, Roopra AS, Sutula TP. 2-deoxyglucose exerts anticonvulsant and antiepileptic actions in experimental epilepsy models. *Epilepsia* 2005;46:268–269.
 12. Yankura J, French A, Rogawski MA, Hartman A, Gasior M. Assessment of the role of glucose deprivation in the ketogenic diet with 2-deoxyglucose. *Epilepsia* 2006;47(suppl. 4):338–339.
 13. Kunz WS. The role of mitochondria in epileptogenesis. *Curr Opin Neurol* 2002;15:179–184.