THE KETOGENIC DIET IN EPILEPSY - MOLECULAR BASIS OF ANTI-SEIZURE EFFICACY

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ABSTRACT

Discovered 85 years ago, the ketogenic diet (KD) continues to serve as an effective therapy for treating patients suffering from intractable epilepsy. KD is rich in fat and has low protein and carbohydrate content. That such a strict dietary regimen involving the consumption of bare minimum amounts of carbohydrates is useful for treating epilepsy suggests that energy metabolism can modulate seizure frequency, intensity and disease progression. KD produces metabolites with anticonvulsant and neuroprotective properties, but how it confers protection from seizures remains elusive. In this article, we discuss a potential mechanism that has surfaced recently involving the neuron-restrictive silencing factor (NRSF) which attempts to explain how carbohydrate metabolism is linked to epileptogenesis.

Since the time of Hippocrates it has been known that fasting is an effective way to treat epilepsy. The ketogenic diet (KD) was first introduced in the 1920s and still continues to be in use.[1] It gained popularity during a period when phenobarbital was the only anti-epileptic drug that was available but which caused severe side-effects. Prior to the introduction of KD, patients, who were primarily children, had to fast or go on a water-only diet for prolonged periods. Since such a treatment was considered extremely challenging for children and their parents, KD was introduced as a substitute and found to work just as well. In an article published in 1922, Hugh Conklin, a strong proponent of the epilepsy curing effects of KD, reported that his isocaloric dietary regimen was capable of curing or significantly reducing seizure frequency in a large fraction of children with epilepsy.[2] A preliminary study carried out in 1924 showed that KD had a complete cure rate of 60% and could reduce seizure frequency in 35% of the patients by one-half.[3]

FACTS AND HISTORY

KD is composed of high fat, and low amounts of proteins and carbohydrates. Typically the ratio of fat to protein and carbohydrate is 4:1 but lower ratios have been found to be equally effective. Changes to the diet are usually considered if seizure frequency remains constant or if the child begins to lose weight; in situations where the person is gaining weight but KD is effective, the diet is left untouched. Since its introduction, KD has seen many changes, including a liquefied form,[4] and several new diets have been introduced such as a modified Atkin's diet in which the amount of carbohydrates is kept low but that of protein increased substantially.[5,6] Like KD, the modified Atkin's diet also induces production of the ketone bodies acetoacetate, β-hydroxybutyrate, and acetone, and has equivalent efficacy. Adverse effects of KD include hypoglycemia, acidosis, and dehydration but these are easily reversed after the diet is discontinued; prolonged consumption of KD, however, results in high cholesterol levels, growth retardation, and formation of kidney stones.[7]

KD and its various derivatives continued to gain popularity until the anti-convulsant medication phenytoin was introduced in the late 1930s. Subsequently, more attention was devoted to finding additional anticonvulsants and studying their modes of action. Introduction of small-molecule drugs therefore ushered in a new era of epilepsy treatment which surpassed KD, which had proved both expensive as well as rather difficult to adapt to. This trend continued for almost 60 years and left behind a small number of pediatricians and dieticians who were familiar with the diet; an overwhelming majority of physicians who were not knowledgeable about the original KD either never

prescribed it or modified it in ways that rendered it ineffective. Such failures generated bad publicity for KD and as a result its use in the clinical setting was almost totally abandoned.

BORN AGAIN

Despite widespread use of progressively more sophisticated anticonvulsants, a handful of trained pediatric neurologists continued to gather data on the usefulness of KD on children with epilepsy. It is due to their efforts and collective teamwork that KD has seen a revival in the past decade and is now being used worldwide.8 During this period, several studies were carried out. Most notable among these is a 1998 study in which 150 children (mean age 5.2 years), each experiencing about 410 seizures per month and having taken on average six anticonvulsant medications, were placed on KD. Results showed that after 3 months 83% remained on the diet, of which a remarkable 34% had their seizure frequency reduced by 90%; of the 55% who continued the diet for one year, about 50% were either completely cured or on average experienced about one seizure per day.[9] Interestingly, these numbers are similar to those reported 74 years earlier.[3]

Revival of KD is attributable to several factors. First, it is more effective on children as well as adults of both sexes with intractable epilepsy who do not respond to anticonvulsants. Second, side effects from KD are mild and readily reversible as compared with available drugs. Third, KD has been made more palatable. It is noteworthy that patients without intractable epilepsy have an excellent chance (>60% success rate) of being cured completely by KD.[5]

MECHANISMS OF ACTION

Even though its modus operandi has thus far remained elusive, there is reliable data from animal models suggesting that metabolism of KD leads to production of compounds with anticonvulsant and seizure-protective activities. It is well known that KD promotes production of ketone bodies, among which acetone has well established anticonvulsant properties.[10] KD neither influences glutamate levels,[11] nor the levels of its transporters EAAC1, GLT-1 and GLAST, but it preferentially increases levels of γ-aminobutyric acid (GABA).[12] Intriguingly, mitochondrial biogenesis in hippocampal neurons is promoted by KD.[13] Since cell death has been implicated in epileptogenesis, there is sound experimental evidence to support the notion that KD is neuroprotective.[14,15] The diet's low carbohydrate content also guards integrity of neurons by reducing the amount of mitochondrially produced reactive oxygen species (ROS) and also by upregulating expression of glutathione peroxidase, an enzyme which protects the plasma membrane from damage by lipid peroxidation.[16] Taken together, these findings indicate that KD increases energy production in neurons, tips the balance towards inhibition, and is neuroprotective.

The fact that KD is low in carbohydrates indicates that reduced glycolysis is beneficial for epilepsy patients. Supporting this deduction, a recent report has shown that the glycolytic inhibitor 2-deoxy-D-glucose (2-DG) has anti-epileptic properties.[17] 2-DG traps hexokinase upon binding and does not enter glycolysis. Experiments have shown that rats administered 2-DG require significantly more evoked electrically stimulated after-discharges before they can experience spontaneous class III, IV and V seizures in the kindling model of epilepsy.[17] As a glycolysis inhibitor, 2-DG is a prototypical drug that has shown promise in animal models and has now entered clinical trials.

NEURON-RESTRICTIVE SILENCING FACTOR - THE LINK BETWEEN ENERGY METABOLISM AND EPILEPTOGENESIS

The molecule that has been found to bridge energy metabolism with epilepsy is the neuron-restrictive silencing factor (NRSF), a zinc finger harboring

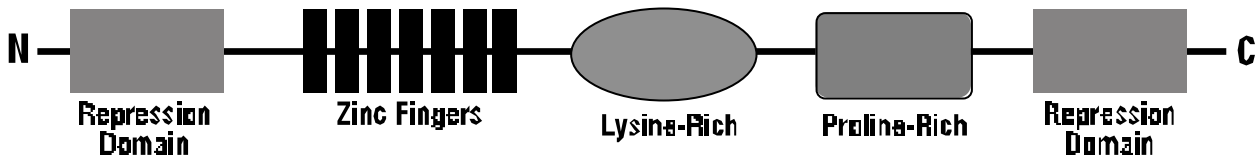

Figure 1: Modular structure of the neuron-restrictive silencing factor (NRSF). Shown are the respective locations of N-terminal and C-terminal repression domains, as well as the zinc finger region, and lysine and proline-rich domains.

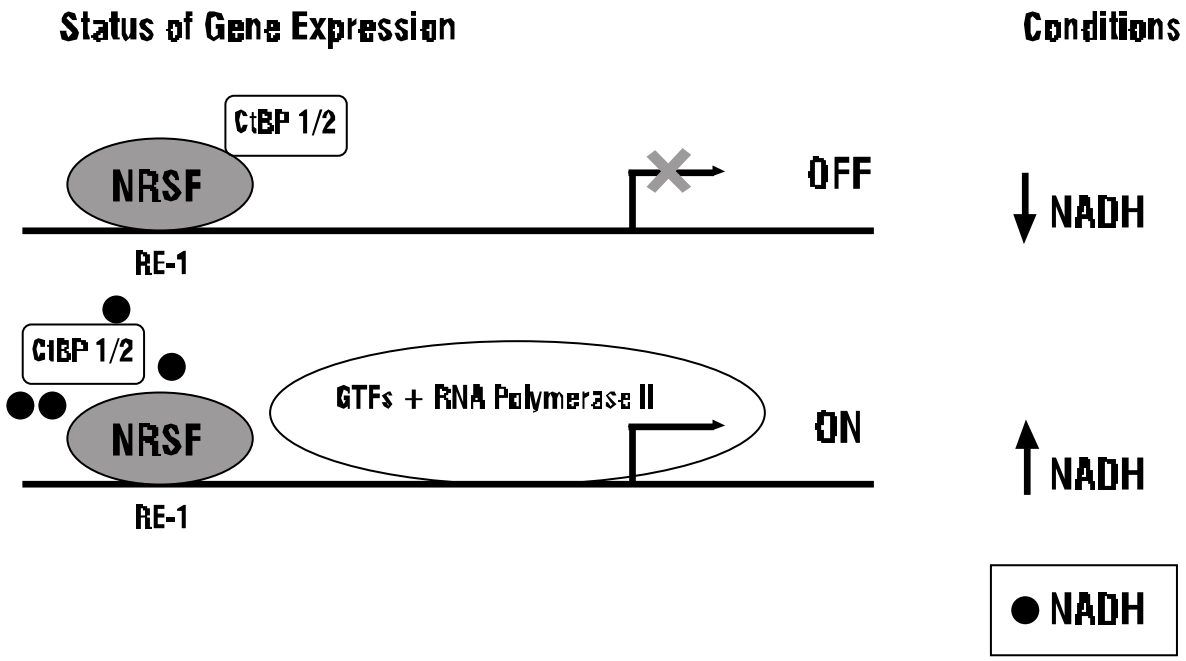

Figure 2: NRSF links energy metabolism to epilepsy. In conditions when carbohydrates are abundant, high levels of NADH would be generated which would in turn bind to CtBP1/2 and destabilize its interaction with NRSF. In presence of the glycolysis inhibitor 2-DG or during periods of KD consumption, NADH production falls well below physiological levels (~ 100 nM); under these conditions CtBP1/2 associates with NRSF as co-repressor, and down regulates expression of NRSF target genes such as trkB and BDNF.

transcription factor which binds sequence-specifically to the 21 base-pair restrictive elements (RE1) found in promoter regions of well over 1800 different genes, including those encoding the neurotrophin brain-derived neuronal factor (BDNF) and its receptor TrkB.[18,19] Interestingly, a conditional knock-out of the trkB gene in mice has been found to stop epilepsy progression, suggesting that down-regulating trkB expression could potentially be advantageous to an individual with epilepsy.[20] The main role of NRSF is to suppress the expression of neuronal genes in non-neuronal cells, but it also regulates gene expression in neurons.[21] Full-length NRSF contains two transcription repression domains at its N- and C-termini; eight zinc finger motifs through which it binds sequence-specifically to RE1 sites; and two separate regions rich in lysine and proline, the functions of which remain unknown (Figure-1).[22,23] NRSF mRNA is alternatively spliced to produce different smaller and, most likely, biochemically distinct isoforms.

To negatively regulate its targets genes, NRSF employs the co-repressors mSin3A/B, CoREST and G9a.[24-28] CtBP1/2 is another NRSF-interacting protein with co-repressor function. Chromatin immunoprecipitation (ChIP) experiments have shown that CtBP1/2 promotes methylation of H3K9 (histone H3 lysine-9); this modification is known to compact chromatin in a way that the transcription machinery comprised of RNA polymerase II and general transcription factors (GTFs) is unable to access the condensed region. A unique feature of CtBP1/2 which distinguishes it from other co-repressors is that its interaction with NRSF is sensitive to NADH making CtBP1/2 a redox sensor.[29]

The elegant work of Garringa-Canut et al demonstrates how NADH levels influence trkB and BDNF expression.[17] Under carbohydrate-rich conditions, high concentration of NADH cause CtBP1/2 to dissociate from NRSF, thus allowing trkB and BDNF expression to occur. In contrast, inhibition of glycolysis through 2-DG (or with KD) reduces NADH from its physiological levels (approximately 100 nM) to a concentration at which it is no longer able to impact the integrity of the NRSF:CtBP1/2 complex; under such hypoglycemic conditions CtBP1/2 remains bound to NRSF and represses expression of trkB, BDNF and other target genes (Figure 2). The key to stopping epilepsy progression is therefore to maintain expression levels of trkB and BDNF to a minimum, and both KD as well as 2-DG help to achieve that goal.

CONCLUSIONS

KD serves as a useful therapy for treating individuals with intractable epilepsy regardless of seizure type, age and gender. In terms of efficacy, KD is comparable to the available epilepsy medications. Besides epilepsy, there is evidence to suggest the diet may be effective for autism, depression, and Parkinson's disease. KD has also shown promise in animal models of Alzheimer's disease, amyotrophic lateral sclerosis and cancer. Although mechanisms underlying the anti-epileptic efficacy of KD are poorly understood, it seems clear that changes in global gene expression patterns (induced by KD or glycolysis inhibitors) collectively act to stop epilepsy progression. NRSF has now been found to link energy metabolism to epilepsy via CtBP1/2; however, it remains to be determined if reduced expression of trkB and BDNF alone is sufficient to produce an anti-epileptic effect, or whether other NRSF target genes, co-repressors, and master transcriptional regulators are also involved.

REFERENCES

1. Freeman J, Veggiotti P, Lanzi G, Tagliabue A, Perucca E. The ketogenic diet: from molecular mechanisms to clinical effects. *Epilepsy Res* 2006; **68**:145-80.
2. Conklin JW. Cause and treatment of epilepsy. *J Amer Osteopath Assoc* 1922; **26**:11-14.
3. Peterman MG. The ketogenic diet in the treatment of epilepsy: a preliminary report. *Amer J Dis Child* 1924; **28**:28-33.
4. Kossoff EH, McGrogan JR, Freeman JM. Benefits of an all-liquid ketogenic diet. *Epilepsia* 2004; **45**:1163.
5. Kossoff EH, Krauss GL, McGrogan JR, Freeman JM. Efficacy of the Atkins diet as therapy for intractable epilepsy. *Neurology* 2003; **61**:1789-91.
6. 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**:421-4.
7. Groesbeck DK, Bluml RM, Kossoff EH. Long-term use of the ketogenic diet in the treatment of epilepsy. *Dev Med Child Neurol* 2006; **48**:978-81.
8. Kossoff EH, McGrogan JR. Worldwide use of the ketogenic diet. *Epilepsia* 2005; **46**:280-9.
9. Freeman JM, Vining EP, Pillas DJ, Pyzik PL, Casey JC, Kelly LM. The efficacy of the ketogenic diet-1998: a prospective evaluation of intervention in 150 children. *Pediatrics* 1998; **102**:1358-63.
10. Likhodii SS, Serbanescu I, Cortez MA, Murphy P, Snead OC, 3rd, Burnham WM. Anticonvulsant properties of acetone, a brain ketone elevated by the ketogenic diet. *Ann Neurol* 2003; **54**:219-26.
11. Dahlin M, Elfving A, Ungerstedt U, Amark P. The ketogenic diet influences the levels of excitatory and inhibitory amino acids in the CSF in children with refractory epilepsy. *Epilepsy Res* 2005; **64**:115-25.
12. Bough KJ, Paquet M, Pare JF, et al. Evidence against enhanced glutamate transport in the anticonvulsant mechanism of the ketogenic diet. *Epilepsy Res* 2007; **74**:232-6.
13. Bough KJ, Wetherington J, Hassel B, et al. Mitochondrial biogenesis in the anticonvulsant mechanism of the ketogenic diet. *Ann Neurol* 2006; **60**:223-35.
14. Noh HS, Kim YS, Lee HP, et al. The protective effect of a ketogenic diet on kainic acid-induced hippocampal cell death in the male ICR mice. *Epilepsy Res* 2003; **53**:119-28.
15. Noh HS, Kim YS, Kim YH, et al. Ketogenic diet protects the hippocampus from kainic acid toxicity by inhibiting the dissociation of bad from 14-3-3. *J Neurosci Res* 2006; **84**:1829-36.
16. Ziegler DR, Ribeiro LC, Hagenn M, et al. Ketogenic diet increases glutathione peroxidase activity in rat hippocampus. *Neurochem Res* 2003; **28**:1793-7.
17. Garriga-Canut M, Schoenike B, Qazi R, et al. 2-Deoxy-D-glucose reduces epilepsy progression by NRSF-CtBP-dependent metabolic regulation of chromatin structure. *Nat Neurosci* 2006; **9**:1382-7.
18. Chong JA, Tapia-Ramirez J, Kim S, et al. REST: a mammalian silencer protein that restricts sodium channel gene expression to neurons. *Cell* 1995; **80**:949-57.
19. Schoenherr CJ, Anderson DJ. The neuron-restrictive silencer factor (NRSF): a coordinate repressor of multiple neuron-specific genes. *Science* 1995; **267**:1360-3.
20. He XP, Kotloski R, Nef S, Luikart BW, Parada LF, McNamara JO. Conditional deletion of TrkB but not BDNF prevents epileptogenesis in the kindling model. *Neuron* 2004; **43**:31-42.
21. Roopra A, Huang Y, Dingledine R. Neurological disease: listening to gene silencers. *Mol Interv* 2001; **1**:219-28.
22. Tapia-Ramirez J, Eggen BJ, Peral-Rubio MJ, Toledo-Aral JJ, Mandel G. A single zinc finger motif in the silencing factor REST represses the neural-specific type II sodium channel promoter. *Proc Natl Acad Sci U S A* 1997; **94**:1177-82.
23. Thiel G, Lietz M, Cramer M. Biological activity and modular structure of RE-1-silencing transcription factor (REST), a repressor of neuronal genes. *J Biol Chem* 1998; **273**:26891-9.
24. Roopra A, Qazi R, Schoenike B, Daley TJ, Morrison

JF. Localized domains of G9a-mediated histone methylation are required for silencing of neuronal genes. *Mol Cell* 2004; **14**:727-38.
25. Roopra A, Sharling L, Wood IC, et al. Transcriptional repression by neuron-restrictive silencer factor is mediated via the Sin3-histone deacetylase complex. *Mol Cell Biol* 2000; **20**:2147-57.
26. Grimes JA, Nielsen SJ, Battaglioli E, et al. The co-repressor mSin3A is a functional component of the REST-CoREST repressor complex. *J Biol Chem* 2000; **275**:9461-7.
27. Naruse Y, Aoki T, Kojima T, Mori N. Neural restrictive silencer factor recruits mSin3 and histone deacetylase complex to repress neuron-specific target genes. *Proc Natl Acad Sci U S A* 1999; **96**:13691-6.
28. Huang Y, Myers SJ, Dingledine R. Transcriptional repression by REST: recruitment of Sin3A and histone deacetylase to neuronal genes. *Nat Neurosci* 1999; **2**:867-72.
29. Zhang Q, Piston DW, Goodman RH. Regulation of corepressor function by nuclear NADH. *Science* 2002; **295**:1895-7.