After nearly a century of clinical use, the mechanisms underlying the anti-seizure effects of the ketogenic diet (KD) remain unclear. Notwithstanding this dearth of knowledge, the KD’s clinical utility appears to be expanding, as there is increasing evidence of its broad neuroprotective properties (1, 2). For both anti-seizure and neuroprotective actions, there have emerged a broad array of proposed mechanisms, and more recently, biochemical and cellular effects that would not necessarily be predicted for bioenergetic substrates and enzymes (3). However, it is uncertain which putative mechanisms are relevant in the clinical context. Clearly, as perturbations in cellular metabolism are increasingly linked to the pathogenesis of neurologic disorders (4), a better mechanistic understanding of the KD (and indeed its variants, such as the modified Atkins diet and the low-glycemic index therapy), would potentially lead to more effective dietary/metabolic treatments, and even perhaps a “KD in a pill,” although the evidence thus far suggests that modulation of a single target mechanism is unlikely to recapitulate the entire clinical profile of the KD (5).

Not surprising, some investigators have taken a simple reductionist approach to studying KD mechanisms and asked whether the principal by-products of fatty acid oxidation (i.e., ketone bodies such as β-hydroxybutyrate [BHB], acetacetate [ACA], and acetone) might exert direct effects on brain network excitability, beyond their well-established role as alternative fuels for bodily tissues under conditions of decreased bioavailability of glucose. Thus far, the preclinical data support the notion that ACA and acetone might exert direct effects on brain network excitability, beyond their well-established role as alternative fuels for bodily tissues under conditions of decreased bioavailability of glucose. Therefore, the evidence thus far suggests that modulation of a single target mechanism is unlikely to recapitulate the entire clinical profile of the KD (5).

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the clinical data have not been helpful in resolving the issue of whether ketone bodies are important mediators or epiphenomena (6).

Against this backdrop, there have emerged two intriguing studies that have provided new insights into the age-old question of how the KD works. The first relates to ketone-induced epigenetic regulation through histone modifications, which may play a pivotal role in ictogenesis and epileptogenesis (7). Histones are important proteins that regulate chromatic structure in eukaryotic cells and are heavily post translationally modified. H3 is one of five main histone proteins whose sequence variants and variable modification states critically modulate long-term regulation of genes. Acetylation of lysine residues on histones is mediated by acetyltransferases, which enable unbound DNA to undergo transcription, whereas removal of acetyl groups on histone deacetylases (HDACs) results in tight binding of histones to DNA and transcriptional repression. HDAC inhibitors are increasingly becoming recognized as potentially important anticancer and anti-inflammatory agents (8). With respect to epilepsy, the most notable example of an HDAC inhibitor is the broad-spectrum anticonvulsant valproic acid (VPA), which inhibits both class I and II HDACs (9) and is cytotoxic to many different cancer types (8).

Shimazu and colleagues noted that BHB is structurally related to butyrate, a product of anaerobic fermentation in bacteria, and which is known to be a small-molecule inhibitor of class I and class II HDACs. Based on this observation, these investigators asked whether the major ketone body BHB might exhibit similar activity against HDACs. They exposed HEK293 (human embryonic kidney) cells in vitro to BHB for 8 hours and used antibodies against acetylated H3 (both lysine-9 and lysine-14 isoforms) to show that BHB increased histone acetylation in a dose-dependent manner, and importantly, over a clinically relevant concentration range (i.e., 1–2 mM) (6, 10).

To determine the selectivity of this effect, Shimazu and co-investigators purified recombinant human HDACs, incubated them with labeled acetylated histone peptides, and then measured their deacetylase activity. They found that BHB inhibited HDAC1, HDAC3, and HDAC4 (but not HDAC6) in a dose-dependent manner (IC50s of 2.4–5.3 mM). Of interest, they also found that “supratherapeutic” concentrations of acetoacetate also inhibited class I and class IIa HDACs in vitro and in HEK293 cells. Further evidence was provided by depleting cells of BHB dehydrogenase with a small interfering RNA (siRNA), which suppressed histone acetylation by BHB at concentrations up to 3 mM. Finally, to establish an in vivo effect of BHB, they treated mice with BHB for 10 hours. The importance of the HDAC receptor was further provided by demonstration of tissue protection when nicotinic acid (another ligand of the HDAC receptor) was administered 10 minutes prior to stroke induction in wild-type, but not knock-out, mice. In a more clinically relevant model, nicotinic acid also protected against stroke-mediated damage when given after stroke induction, although the magnitude of the effect was somewhat decreased. Of importance, stroke-induced behavioral abnormalities also were decreased in wild-type mice treated with BHB or nicotinic acid. Collectively, these studies indicate that HDAC receptors are necessary for ketone body– and nicotinic acid–mediated prevention of tissue destruction after stroke.

Rahman and colleagues sought to determine which cells were responsible for mediating this protective effect. They used a genetically modified reporter mouse in which monomeric red fluorescent protein (mRFP) expression is directed by the HCA2 locus (Hca2mRFP). At baseline, mRFP was noted only in resident microglia, but after stroke induction, mRFP-positive cells were noted in the periphery of the ischemic zone. This observation could be explained by two distinct possibilities: either resident microglia were reactive or there was infiltration of monocytes/macrophages from peripheral blood. The authors implemented a clever strategy to distinguish between these possibilities using chimeric mice, with wild-type bone marrow transplanted to Hca2mRFP mice and Hca2mRFP marrow transplanted to wild-type mice, as well as flow cytometry of tissue and peripheral blood. This series of experiments demonstrated that both resident microglia were activated, and furthermore, bone marrow–derived monocytes/macrophages infiltrated the ischemic periphery.

The more surprising finding was that in blood, the number of “inflammatory” types of monocytes/macrophages decreased after stroke, which raised the possibility that “inflammatory” monocytes/macrophages differentiated into the “resident” (neuroprotective) subtype in tissue after infiltration. Experiments with different chimeric mice (using bone-marrow transplants of wild-type and Hca2 knock-out mice) showed that HCA2 in bone marrow–derived cells were responsible for nicotinic acid–mediated prevention of tissue destruction.
Because other cell types express HCA₃ (including neutrophils, which may play a role in the immunologic response to stroke), the authors used mice expressing the human diphtheria toxin receptor under the control of the monocyte/macrophage-specific CD11b promoter to demonstrate that nicotinic acid-mediated protection against stroke requires the presence of monocytes/macrophages.

One of the major problems with using nicotinic acid clinically is a cutaneous flushing response mediated via prostaglandin D2 (PGD₂) (15), which is synthesized in response to activation of HCA₂ receptors after nicotinic acid binding. PGD was shown previously to have neuroprotective effects in a neonatal hypoxia–ischemia model (16). Consistent with the hypothesis that the protective effect of HCA₂ receptor–mediated neuroprotection is mediated via PGD₂, Rahman and colleagues found that PGD₂ levels are elevated in both plasma and brain after nicotinic acid treatment. Further, the post stroke effect of nicotinic acid was eliminated in mice lacking one of the major enzymes responsible for PGD₂ synthesis in macrophages, COX1. Administration of a small molecule inhibitor of the other major PGD synthetic enzyme (hematopoietic PGD synthase) also partially reversed the neuroprotective effect of nicotinic acid. To summarize, pharmacologic activation of one BHB receptor, HCA₂, led to neuroprotection in a stroke model, and this effect was mediated via PGD₂ synthesis, primarily in cells of the monocyte/macrophage lineage that infiltrate margins of the ischemic zone from peripheral blood.

While the studies by Shimazu and colleagues and Rahman and co-investigators are indeed elegant in design and innovative in scope, there remain a number of unanswered questions. Do either or both of these mechanisms (i.e., modulation of histone acetylation and the HCA₂ receptor) play a role in ictogenesis or epileptogenesis? Neither study provides direct evidence in this regard, but based on emerging lines of research (1, 2, 7), both strongly indicate that BHB (and by extension, the KD) could influence the epileptic state. In support of this, the KD has recently been shown to affect epigenetic changes in epileptic brain (17) and is also proving useful in patients with neuroinflammation-related epilepsy (18). With respect to neuroprotection, there are indeed many other KD-related mechanisms that likely play a role (3), such as ketone-mediated inhibition of vesicular release (19). And as recognized by Rahman and colleagues, another potential mechanism may be the PGD₂ metabolite (15d-PGJ₂), an agonist of neuroprotective peroxisome proliferator alpha receptor gamma (PPARγ). Taken together, the recent studies by Shimazu and coinvestigators and Rahman and colleagues have revealed molecular mechanisms that may be relevant for KD-induced neuroprotection, and possibly, epileptogenesis. Although much more work needs to be done, the mystery of how the KD may induce lasting protective changes in the brain is now starting to be unraveled.

by Adam L. Hartman, MD, and Jong M. Rho, MD

References


American Epilepsy Society

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Disclosure of Potential Conflicts of Interest

Instructions
The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in four parts.

1. Identifying information.
   Enter your full name. If you are NOT the main contributing author, please check the box “no” and enter the name of the main contributing author in the space that appears. Provide the requested manuscript information.

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   This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking “No” means that you did the work without receiving any financial support from any third party – that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check “Yes”. Then complete the appropriate boxes to indicate the type of support and whether the payment went to you, or to your institution, or both.

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3. Are you the Main Assigned Author? ☒ Yes ☐ No
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4. Manuscript/Article Title: The New Ketone Alphabet Soup: BHB, HCA and HDAC

5. Journal Issue you are submitting for: 14.6

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<td>13. Other (err on the side of full disclosure)</td>
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