

## POINT OF VIEW

# Metabolism-based therapies for epilepsy: new directions for future cures

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## Funding Information

The authors appreciate the editorial contributions of Alison F. Davis, Ph.D. Dr. Pascual was supported by US National Institutes of Health (grants NS094257 and NS102588). Dr. Rho was supported by Canadian Institutes of Health Research FRN-162177 and NS104513. Dr. Yellen was supported by US National Institutes of Health (NS102586 and GM124038).

Received: 14 May 2021; Accepted: 28 June 2021

*Annals of Clinical and Translational Neurology* 2021; 8(8): 1730–1737

doi: 10.1002/acn3.51423

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## Introduction

Dietary treatment of disease dates back to ancient Greek physicians' use of fasting as a strategy to alleviate seizures. Thousands of years later, although low-carbohydrate, high-fat ketogenic, and dietary therapies are now used to treat medically resistant epilepsy and other disorders, the precise role of altered metabolism in epilepsy remains elusive. To optimize efficacy, individualize treatment, and decrease adverse effects, understanding how metabolic

## Abstract

**Objective:** Thousands of years after dietary therapy was proposed to treat seizures, how alterations in metabolism relates to epilepsy remains unclear, and metabolism-based therapies are not always effective. **Methods:** We consider the state of the science in metabolism-based therapies for epilepsy across the research lifecycle from basic to translational to clinical studies. **Results:** This analysis creates a conceptual framework for creative, rigorous, and transparent research to benefit people with epilepsy through the understanding and modification of metabolism. **Interpretation:** Despite intensive past efforts to evaluate metabolism-based therapies for epilepsy, distinct ways of framing a problem offer the chance to engage different mindsets and new (or newly applied) technologies. A comprehensive, creative, and inclusive problem-directed research agenda is needed, with a renewed and stringent adherence to rigor and transparency across all levels of investigation.

perturbations affect neurological conditions such as epilepsy remains important. Ketogenic diets, the epitome of metabolic treatments for epilepsy, are not uniformly effective and may harbor health risks, but pharmacological therapy also has limitations. Many attempts to reverse-engineer the ketogenic diet to glean mechanistic insights across a wide array of basic, translational, and clinical models have yet to uncover a common therapeutic target or process—and in fact, they have suggested various distinct and overlapping causal or modulatory pathways.

This diversity of processes presents a challenge, particularly given our incomplete appreciation of the vast complexity of metabolism and how it differs based on the model or organism studied. Our field is at a crossroads.

Despite intensive past efforts to evaluate metabolism-based therapies for epilepsy, distinct ways of framing a problem offer the chance to engage different mindsets and new (or newly applied) technologies. In this perspective, we present a broad overview of what is known and what remains unclear about metabolism-based therapies for epilepsy. This article, not intended to be a comprehensive review, reflects an analysis by established investigators in this area of science.<sup>1</sup> Collectively, we call for a comprehensive, creative, and inclusive problem-directed research agenda with a renewed and stringent adherence to rigor and transparency across all levels of investigation. A deeper understanding of the interdependence of metabolic and neuronal excitability pathways and relevant regulatory processes could benefit from unbiased data mining methods and other types of secondary analyses of existing data sets, as well as the use of novel technologies that can capture more informative measurements of previously studied biological phenomena. Also likely to be valuable is a re-examination of clinical data from past trials that did not yield definitive conclusions or generated conflicting results, but that may harbor hidden clues that may be unearthed through novel study designs. These future investigations are essential for the benefit of the millions of individuals who experience unpredictable and often disabling seizures and may also illuminate the etiology and treatment of other conditions with known metabolic components such as cancer,<sup>2</sup> dementia,<sup>3</sup> and migraine.<sup>4</sup>

## What Do We Know about Metabolism-Based Therapies for Epilepsy?

The human brain remains a poorly understood organ that sustains life and enables cognition, sensation, emotion, and behavior despite numerous and changing internal and external demands. The brain modulates body functions via an interplay between metabolic signaling and neurochemical circuitry. Interactions between the brain and the rest of the body include well-characterized systems, such as endocrine organs, as well as through processes where our understanding is still emerging—including the gut–brain axis, plasticity of neural excitability in normal and disease states, and the epigenomic regulation of gene expression. All of these aspects, once properly elucidated, may constitute targets for disease modulation.

These aspects are exemplified by the oldest and best-understood metabolic therapies: ketogenic diets. These diets mimic fasting by limiting glucose metabolism and increasing blood levels of ketones produced in the liver from dietary or adipose-derived fatty acids. Such metabolic switches affect brain excitability<sup>5</sup> and plasticity.<sup>6</sup> Yet, precise or quantitative relationships between metabolism and neuronal activity have been difficult to establish, in part, because ketogenic diets used by children and adults with epilepsy vary considerably in composition; some rely mainly on carbohydrate restriction, while others also manipulate the type of carbohydrate<sup>7</sup> or fatty acids.<sup>8</sup> Studies in model systems have contributed to our knowledge about the impact of dietary consumption of various ketogenic substances, which have also been extended to less well-known dietary constituents. For example, whey (which contains tryptophan as well as branched-chain amino acids) reduces hyperexcitable or seizure-like activity in fruit flies<sup>9</sup> work that could be extended to human studies given the commercial availability of this protein source. Further, the administration of isolated ketone esters and other metabolic alternatives has demonstrated anti-seizure effects in various models.<sup>10,11</sup> Past studies suggest a linkage between metabolism and neuronal excitability via interactions among energy sources,<sup>12</sup> micronutrients, and hormones. In addition, accumulating evidence supports an important role of the gut microbiome in brain function and dysfunction, particularly related to seizure development.<sup>13–15</sup>

These studies highlight the fact that our general understanding of the wide range of effects of metabolism on body systems continues to evolve through the appreciation of the complex interplay between diet, genetics, the microbiome, physical activity, and other factors such as disease states, which have been receiving renewed attention in only recent years. These complexities have prompted some to consider nutrition as a fundamental (albeit complex) biological variable.<sup>16</sup> Cell type-specific activities forge connections between metabolic activities (and responses) and the electrochemical environments of neurons and glia. Genetic defects in brain glucose metabolism are associated with focal excitation, despite lower overall cerebral electrical activity.<sup>17,18</sup> Although brain metabolism is primarily oxidative and yields adenosine triphosphate from glucose for the organ's high-energy needs, glial cell populations that co-occupy brain tissue are known to metabolize glucose via anaerobic glycolysis despite their high concentration of mitochondria. Upon stimulation by various neurotransmitters and other neural activities, glial cells produce lactate as a metabolic intermediate that may play a role in metabolic coupling to help regulate neuronal homeostasis and plasticity.<sup>19,20</sup>

## Gaps in knowledge about the interdependence of metabolic pathways and neuronal function

Our incomplete understanding of the nature of epilepsy is a primary challenge toward identifying new, effective treatments for the range of disorders characterized by increased seizure risk, especially when considering their occurrence in children and adults, since they may not stem from the same mechanisms. Disease mechanisms for epilepsies remain ill-defined, aside from known contributory factors such as genetics, infectious and inflammatory factors, focal brain injury, and other co-occurring neurological conditions. Single-gene defects characterize some epilepsy syndromes, such as Dravet Syndrome (caused by mutations in the *SCN1A* gene), glucose transporter type 1 deficiency syndrome (caused by impaired cerebral glucose transport<sup>21</sup>), pyruvate dehydrogenase deficiency,<sup>22</sup> and other metabolic disorders.<sup>23</sup> Other investigative paths have implicated various physiological processes in seizure development, including metabolic energy production (e.g., dynamic interactions between glucose and other aspects of intermediary metabolism), type of metabolic substrate utilization, oxidative stress, and extra-metabolic, far-reaching effects of metabolites. Past studies have also suggested an epileptogenic role for amino acid metabolism and signaling, as reflected by shifts in the balance of inhibitory/excitatory neurotransmitter concentration and action predominantly through gamma-aminobutyric (GABA) and glutamate.<sup>24</sup> Fasting, which has anti-seizure properties, has been associated with elevated GABA synaptic release and related effects on neuronal activity.<sup>25</sup> Nevertheless, the quantitative relationships and constraints that determine the impact of all of these processes at the neuronal network or whole-brain level remain poorly understood beyond studies in simplified experimental models.

Conclusions derived from clinical studies also remain perplexing: among individuals with epilepsy who respond favorably to a ketogenic diet, there is neither a consistent phenotype or set of clinical or experimental biomarkers or surrogate markers,<sup>26</sup> nor are there indicators of susceptibility to adverse effects. A better assessment of efficacy requires careful and quantitative analyses of the various ketogenic diet therapies in current use—which vary in proportions of fat, carbohydrates, and protein, and can include exogenous supplements such as medium-chain triglycerides, ketone salts, and ketone esters.<sup>27</sup> It should be emphasized, however, that not all clinically effective dietary treatments for epilepsy produce significant ketosis and whether the degree of ketosis correlates with seizure reduction remains under investigation.<sup>28</sup> Studies involving diet manipulation are challenging because they may be

complicated by low participant recruitment, varying definitions for and implementation of ketogenic diet therapies utilized (despite attempts to standardize protocols),<sup>29</sup> and lack of adherence to study diets. Innovative, information-maximizing study designs may help address these challenges.

Many unanswered questions remain: Are existing or proposed mechanisms for metabolic impacts on neuronal activity feasible within defined physiological constraints (including the time scale of the observed effects), and do they fit within the current knowledge framework of what we know about both epilepsy and metabolism in humans? At present, we do not know whether multiple mechanisms synergize; whether there is a convergence of effects resulting from neurochemical, genomic, microbiomic, and other changes; or whether the established mechanisms operate independently of each other. What causes susceptibility and resilience to seizures at a given time and across different species? Is a seizure event a common final phenomenon, many types of phenomena arbitrarily simplified as one, or a secondary event? How does epilepsy respond and evolve in one individual, over time, as well as in response to metabolic shifts such as those invoked by ketogenic diets and non-adherence to them? Do metabolic therapies exert any synergy with pharmacological interventions? Parsing these distinct research questions and pairing them with suitable research designs and new tools is important to refine the search for safe and effective treatments for epilepsy unresponsive to pharmacological or surgical interventions.

## Ensuring rigor and transparency in studying the role of metabolism in neurological diseases

A heightened awareness of the vitality of rigor and reproducibility in all biomedical research has prompted the development of a range of new tools to help investigators design and conduct studies and analyze data in the field of metabolism-based therapies,<sup>30,31</sup> including the recent call for “rigor champions.”<sup>32</sup> Several other approaches have been introduced to prevent experimental bias, such as checklists for reporting data<sup>33</sup> and limited reliance on using p values to assign statistical significance.<sup>34</sup> In any line of biomedical inquiry from cells to humans, researchers must choose model systems that match specific research questions and that make the most efficient use of resources. Bench-to-bedside translation of metabolism-based therapy for epilepsy has been limited for many reasons, including distinct metabolic and behavioral differences and developmental timescales between rodents and humans, differing metabolic physiologies, and interspecies differences in the abundance and proportions of glial and

neuronal cells. Research with rodents, in particular, has been called into question given that these animals do not share morphological similarities with humans (e.g., they are agyric and have little white matter), and exhibit distinct behaviors (e.g., cognition, sleep, and rest-activity cycling). Additional species may serve as alternate models, such as domestic dogs, which experience diverse seizure types and share some human behaviors. Several randomized clinical trials of ketogenic diet therapies involving humans have been designed to evaluate efficacy in patients of all ages with drug-resistant epilepsy and specific epilepsy syndromes. These clinical investigations have been encouraging but not without limitations such as high nonadherence rates, variable protocols, recruitment difficulties,<sup>35,36</sup> and challenges with successfully blinding participants and researchers.<sup>37</sup> Some success, however, has been apparent.<sup>38</sup>

## **New Directions for the Study of Metabolism-Based Therapies in Epilepsy**

Various research areas and technologies seem poised to push this field toward a needed inflection point to deepen knowledge about metabolism and metabolic-synaptic coupling. Among others, these include cell and circuit mapping analyses, biomarker validation studies, data science and omics methods, and novel approaches to clinical science.

### **Cell type mapping and neuronal network analyses**

We do not yet know how system-wide treatments such as dietary therapies can modulate brain networks in patients with epilepsy. A better understanding of the drivers of network behavior beyond current anatomical constructs will help answer this question; however, brain-imaging evidence suggests that neuronal networks can become either erratic or too rigid by varying levels of fuels.<sup>39</sup> Converging evidence from various model systems will benefit from the use of new experimental methods such as optogenetics to align common pathways of metabolic activity and neuronal vulnerability, suggested by the existence of “seizure gates.”<sup>40</sup>

### **Identifying and validating biomarkers**

Exploratory avenues toward identifying epilepsy biomarkers include metabolic fate labeling, high-resolution respirometry, and other methods to quantify neuronal and glial metabolites. Fluorodeoxyglucose positron emission tomography (FDG-PET), fluorescence lifetime

imaging (FLIM), fluorescence resonance energy transfer (FRET) approaches, and other imaging methods, such as functional near-infrared spectroscopy, might be combined with electroencephalography to monitor and elucidate metabolic changes in brain activity from the ketogenic diet. The gut microbiome is highly variable between and within individuals and species, with recent advances demonstrating that thousands of previously uncharacterized metabolites arise either directly from diet or in response to host and microbiome digestion and metabolism.<sup>41</sup> Further studies in humans using rigorous protocols<sup>42</sup> could help clarify the role of ketone bodies, carbohydrate restriction, and amino acids in epilepsy and provide candidate biomarkers for future studies in both preclinical and clinical studies.

### **Data science and omics methods**

Machine learning approaches offer an opportunity for the systematic study of the biochemical spectrum of dietary components<sup>43</sup> toward the ability to manipulate host and microbiome biology to treat or prevent seizures or other diseases. Wearable devices offer the opportunity to capture dietary data dynamically and in a less biased manner compared to self-reporting or periodic measurement of metabolites. Other potential strategies include the collection and high-throughput analysis of metabolomic data from spinal or other fluids of affected individuals or research organisms and liquid biopsies containing various subcellular components such as mRNA, exosomes, mitochondria, and mitochondrial DNA. In addition to various applications of bio- and clinical informatics, disease-guided, bench-to-bedside approaches resulting from observant clinicians and patient perspectives will remain critical.

### **Clinical research innovation**

The use of rigorous diet intervention trials (for instance, feeding studies<sup>44</sup>) may ultimately improve adherence and reproducibility by strictly monitoring food intake and defined biomarkers. Currently, as few as half of the participants adhere to ketogenic diet therapies despite multiple modifications to create less strict diet protocols.<sup>45</sup> Additional tools, such as continuous glucose monitoring or other wearables could help monitor adherence and collect data on micronutrients. Novel trial designs used in pharmacologic studies can enhance the inclusion of additional research participants and allow for direct comparison between different treatment modalities. For example, master protocols (basket, umbrella, and platform) consist of a single trial design and multiple sub-studies that can share a control arm.<sup>46</sup> These approaches offer flexibility

in the ability to have different objectives, evaluate one or more drugs, and include one or more therapies. For example, basket trials can include participants with different underlying diseases (or in the case of epilepsy, syndromes or epilepsy types<sup>47</sup>) in a single trial. Additional strategies might compare one dietary therapy between two treatment arms (e.g., Dravet syndrome and focal epilepsy) that share a control group to maximize recruitment. Finally, an impactful clinical trial evidence base benefits from the perspective of individuals with epilepsy and their caregivers,<sup>48</sup> toward helping to shape clinical outcomes that are meaningful to patients (e.g., cognition,<sup>49</sup> independence, and quality of life) and improve the yield of actionable knowledge.

### All Hands on Deck: a Vision for Future Metabolism-Guided Treatment of Disease

Despite decades of research, the current body of knowledge describing features and proposed mechanisms for epilepsy has been insufficient to drive the development of effective prevention and therapeutic strategies for the majority of epilepsies. New approaches and novel collaborations are desperately needed to explore the interdependence of metabolic pathways that generate normal neuronal function or seizures—while adhering to the highest standards of rigor and transparency (see Table 1 for challenges and opportunities).

What else is missing? Who can be recruited to the field to propose new ways of looking at existing problems in different ways, and /or using new technologies? Widening the community of investigators in this field may answer many research questions we continue to grapple with and also deepen understanding of numerous other conditions with both neurological and metabolic contributions and manifestations. Epilepsy research is ripe for a diverse set of new minds and skill sets including engineers, data analysts, dynamic systems modelers, genomicists, and medical informaticists – to name a few – to propose hypotheses based on observations and decipher undetected patterns and connections amid the wealth of data already generated and that still are to be generated. These new lenses can augment ongoing research approaches to clarify proposed mechanisms toward the very practical and urgent goal of bringing needed relief to patients and families managing such difficult and life-altering medical conditions.

Given the complexity of neurological and metabolic conditions, as summarized here and detailed more comprehensively elsewhere, team science models that coalesce investigative groups from various disciplines that do not typically interact may be productive. The National Institute

**Table 1.** Toward a future state of metabolism-based treatment for epilepsy

Challenges and opportunities	
Challenge	Opportunity
Insufficient rigor in pre-clinical and clinical studies	<ul style="list-style-type: none"> <li>Adherence to principles of rigor, reproducibility, sex as a biological variable, and including appropriate representation in both preclinical and clinical studies</li> <li>Reporting adherence to published rigor guidelines</li> </ul>
Animal models do not mirror the human condition	<ul style="list-style-type: none"> <li>Studying species that more closely mimic human pathophysiology</li> <li>In silico modeling human physiology</li> <li>Basic science studies in humans</li> </ul>
Incomplete understanding of network properties of metabolism	Build on existing knowledge, tools, and approaches (e.g., by the BRAIN Initiative) to investigate metabolic circuits
Missing or incomplete data sets	Adherence to data sharing guidelines
Clinical trial recruitment	<ul style="list-style-type: none"> <li>Optimizing remote participation</li> <li>Use of monitoring devices</li> <li>Novel clinical trial designs (using appropriate statistics)</li> </ul>
Poor relevance of measured clinical trial outcomes to patient wellbeing	Inclusion of advocacy organizations
Neglect of individual biological differences in clinical trials	Data-intensive sub-analysis methods to capitalize on potentially important biological information
Excessive proportion of negative trial results	Rigorous failure analysis
No validated biomarkers	Development of biomarkers (at various stages of investigation) within the BEST framework for future clinical studies

of Neurological Disorders and Stroke (NINDS) introduced the Center Without Walls team science approach starting in 2015 with multidisciplinary teams focused on identifying genes involved in epilepsies. NINDS has supported teams focused on investigating sudden unexpected death in epilepsy (SUDEP), anti-epileptogenesis, and post-traumatic epilepsy—and two groups are studying the consequences of gene variants in ion-channel genes and other genes associated with epilepsies.

As current investigations proceed and new efforts begin, we should continue to revisit basic questions and

assumptions. Will continued study of ketogenic diets reveal underlying mechanisms that can be more safely and easily mimicked with medications? It seems clear that the future treatment of medication-resistant epilepsy will continue to consist of a combination of dietary components, pharmacologic interventions (potentially at lower, safer doses), and perhaps device-mediated neuronal or cerebral network control. At this juncture, many doors have been cracked open by technology advances and knowledge gained in other fields of investigation that can be applied to the investigation of feasibility, safety, efficacy, and mechanisms of action of metabolism-based therapies. There has never been a better time to set and implement a comprehensive and rigorous research agenda to understand metabolism-based therapies for epilepsy for the benefit of the millions of individuals who experience unpredictable and often disabling seizures.

## Acknowledgments

The authors gratefully acknowledge the active participation of the workshop panelists and participants. The authors appreciate the editorial contributions of Alison F. Davis, Ph.D. Dr. Pascual was supported by US National Institutes of Health (grants NS094257 and NS102588). Dr. Rho was supported by Canadian Institutes of Health Research FRN-162177 and NS104513. Dr. Yellen was supported by US National Institutes of Health (NS102586 and GM124038). This report does not represent the official view of the National Institute of Neurological Disorders and Stroke (NINDS), the National Institutes of Health (NIH), or any part of the US Federal Government. No official support or endorsement of this article by the NINDS or NIH is intended or should be inferred.

## Conflict of Interest

Drs Pascual, Yellen, Thiele, Whittemore, and Hartman report no conflicts of interest. Dr Rho serves as a consultant for Aquestive Therapeutics, Cerecin Ltd., Biocodex, and Cypralis Ltd. Dr. Cervenka has received grants from Vitaflo International Ltd, honoraria from Nutricia and Vitaflo International Ltd, royalties from Demos Health/Springer Publishing Company, and consulting fees for Nutricia.

## Author Contributions

Drs Cervenka and Pascual participated in the overall concept development and drafting of the manuscript, critically reviewed it for content, and approved the final version for submission.

Drs Rho, Thiele, Yellen, and Whittemore critically reviewed the manuscript for content, and approved the final version for submission.

Dr Hartman led the concept review, participated in drafting the manuscript, critically reviewed it for content, and approved the final version for submission.

## References

1. This article reflects presentations and discussion during and after the November 9–10, 2020 Metabolism-based Therapies for Epilepsy Workshop hosted by the National Institutes of Health in Bethesda, Maryland. The contents of the workshop are available at <https://www.ninds.nih.gov/News-Events/Events-Proceedings/Events/Metabolism-based-TherapiesEpilepsy-Virtual-Workshop>.
2. Martin-McGill KJ, Marson AG, Tudur Smith C, et al. Ketogenic diets as an adjuvant therapy for glioblastoma (KEATING): a randomized, mixed methods, feasibility study. *J Neurooncol* 2020;147:213–227. <https://doi.org/10.1007/s11060-020-03417-8>. Epub 2020 Feb 8. PMID: 32036576; PMCID: PMC7076054.
3. Davis JJ, Fournakis N, Ellison J. Ketogenic diet for the treatment and prevention of dementia: a review. *J Geriatr Psychiatry Neurol* 2021;34:3–10. <https://doi.org/10.1177/0891988720901785>. Epub 2020 Jan 30 PMID: 31996078.
4. Di Lorenzo C, Pinto A, Ienca R, et al. A randomized double-blind, cross-over trial of very low-calorie diet in overweight migraine patients: a possible role for ketones? *Nutrients* 2019;11:1742. <https://doi.org/10.3390/nu11081742>. PMID: 31357685; PMCID: PMC6722531.
5. Lutas A, Yellen G. The ketogenic diet: metabolic influences on brain excitability and epilepsy. *Trends Neurosci* 2013;36:32–40. <https://doi.org/10.1016/j.tins.2012.11.005>. Epub 2012 Dec 8. PMID: 23228828; PMCID: PMC3534786.
6. Mattson MP, Moehl K, Ghena N, et al. Intermittent metabolic switching, neuroplasticity and brain health. *Nat Rev Neurosci* 2018;19:63–80. <https://doi.org/10.1038/nrn.2017.156>. Epub 2018 Jan 11. Erratum in: *Nat Rev Neurosci*. 2020 Aug;21(8):445. PMID: 29321682; PMCID: PMC5913738.
7. Pfeifer HH, Thiele EA. Low-glycemic-index treatment: a liberalized ketogenic diet for treatment of intractable epilepsy. *Neurology* 2005;65:1810–1812. <https://doi.org/10.1212/01.wnl.0000187071.24292.9e>. PMID: 16344529.
8. Huttenlocher PR. Ketonemia and seizures: metabolic and anticonvulsant effects of two ketogenic diets in childhood epilepsy. *Pediatr Res* 1976;10:536–540. <https://doi.org/10.1203/00006450-197605000-00006>. PMID: 934725.
9. Kasuya J, Iyengar A, Chen HL, et al. Milk-whey diet substantially suppresses seizure-like phenotypes of *paraShu*, a *Drosophila* voltage-gated sodium channel mutant. *J Neurogenet* 2019;33:164–178. <https://doi.org/10.1007/s10048-019-00000-0>.

- 1080/01677063.2019.1597082. Epub 2019 May 16. PMID: 31096839; PMCID: PMC6641994.
10. Poff AM, Rho JM, D'Agostino DP. Ketone administration for seizure disorders: history and rationale for ketone esters and metabolic alternatives. *Front Neurosci* 2019;15:1041. <https://doi.org/10.3389/fnins.2019.01041>. PMID: 31680801; PMCID: PMC6803688.
  11. Chang P, Augustin K, Boddum K, et al. Seizure control by decanoic acid through direct AMPA receptor inhibition. *Brain* 2016;139:431–443. <https://doi.org/10.1093/brain/awv325>. Epub 2015 Nov 25. PMID: 26608744; PMCID: PMC4805082.
  12. Marin-Valencia I, Good LB, Ma Q, et al. Heptanoate as a neural fuel: energetic and neurotransmitter precursors in normal and glucose transporter I-deficient (G1D) brain. *J Cereb Blood Flow Metab* 2013;33:175–182. <https://doi.org/10.1038/jcbfm.2012.151>. Epub 2012 Oct 17. PMID: 23072752; PMCID: PMC3564188.
  13. Olson CA, Vuong HE, Yano JM, et al. The gut microbiota mediates the anti-seizure effects of the ketogenic diet. *Cell* 2018;173:1728–1741.e13. <https://doi.org/10.1016/j.cell.2018.04.027>. Epub 2018 May 24. Erratum in: *Cell*. 2018 Jul 12;174(2):497. PMID: 29804833; PMCID: PMC6003870.
  14. Dinan TG, Cryan JF. The microbiome-gut-brain axis in health and disease. *Gastroenterol Clin North Am* 2017;46:77–89. <https://doi.org/10.1016/j.gtc.2016.09.007>. Epub 2017 Jan 4 PMID: 28164854.
  15. Lum GR, Olson CA, Hsiao EY. Emerging roles for the intestinal microbiome in epilepsy. *Neurobiol Dis* 2020;135:104576. <https://doi.org/10.1016/j.nbd.2019.104576>. Epub 2019 Aug 21 PMID: 31445165.
  16. Rodgers GP, Collins FS. Precision nutrition—the answer to "What to Eat to Stay Healthy". *JAMA* 2020;324:735–736. <https://doi.org/10.1001/jama.2020.13601>. PMID: 32766768.
  17. Jakkamsetti V, Marin-Valencia I, Ma Q, et al. Brain metabolism modulates neuronal excitability in a mouse model of pyruvate dehydrogenase deficiency. *Sci Transl Med* 2019;11:eaan0457. <https://doi.org/10.1126/scitranslmed.aan0457>. PMID: 30787166; PMCID: PMC6637765.
  18. Marin-Valencia I, Good LB, Ma Q, et al. Glut1 deficiency (G1D): epilepsy and metabolic dysfunction in a mouse model of the most common human phenotype. *Neurobiol Dis* 2012;48:92–101. <https://doi.org/10.1016/j.nbd.2012.04.011>. Epub 2012 Apr 23. PMID: 22683290; PMCID: PMC3495165.
  19. Magistretti PJ, Allaman I. Lactate in the brain: from metabolic end-product to signalling molecule. *Nat Rev Neurosci* 2018;19:235–249. <https://doi.org/10.1038/nrn.2018.19>. Epub 2018 Mar 8 PMID: 29515192.
  20. Bak LK, Walls AB. CrossTalk opposing view: lack of evidence supporting an astrocyte-to-neuron lactate shuttle coupling neuronal activity to glucose utilisation in the brain. *J Physiol* 2018;596:351–353. <https://doi.org/10.1113/JP274945>. Epub 2018 Jan 2. PMID: 29292507; PMCID: PMC5792606.
  21. Schwantje M, Verhagen LM, van Hasselt PM, Fuchs SA. Glucose transporter type 1 deficiency syndrome and the ketogenic diet. *J Inher Metab Dis* 2020;43:216–222. <https://doi.org/10.1002/jimd.12175>. Epub 2019 Nov 13. PMID: 31605543; PMCID: PMC7078900.
  22. Sears SM, Hewett SJ. Influence of glutamate and GABA transport on brain excitatory/inhibitory balance. *Exp Biol Med* (Maywood). 2021;246:1069–1083. <https://doi.org/10.1177/1535370221989263>. Epub ahead of print. PMID: 33554649.
  23. Pascual JM, Campistol J, Gil-Nagel A. Epilepsy in inherited metabolic disorders. *Neurologist* 2008;14(6 Suppl 1):S2–S14. <https://doi.org/10.1097/01.nrl.00000340787.30542.41>. PMID: 19225367.
  24. Yudkoff M, Daikhin Y, Horyn O, et al. Ketosis and brain handling of glutamate, glutamine, and GABA. *Epilepsia* 2008;49(Suppl 8):73–75. <https://doi.org/10.1111/j.1528-1167.2008.01841.x>. PMID: 19049594; PMCID: PMC2722878.
  25. Dolce A, Santos P, Chen W, et al. Different ketogenesis strategies lead to disparate seizure outcomes. *Epilepsy Res* 2018;143:90–97. <https://doi.org/10.1016/j.eplepsyres.2018.04.011>. Epub 2018 Apr 27 PMID: 29723773.
  26. Schoeler NE, Cross JH, Sander JW, Sisodiya SM. Can we predict a favourable response to Ketogenic Diet Therapies for drug-resistant epilepsy? *Epilepsy Res* 2013;106:1–16. <https://doi.org/10.1016/j.eplepsyres.2013.06.002>. Epub 2013 Jun 30 PMID: 23820448.
  27. Kossoff EH, Zupec-Kania BA, Auvin S, et al.; Charlie Foundation; Matthew's Friends; Practice Committee of the Child Neurology Society. Optimal clinical management of children receiving dietary therapies for epilepsy: updated recommendations of the International Ketogenic Diet Study Group. *Epilepsia Open* 2018;3:175–192. <https://doi.org/10.1002/epi4.12225>. PMID: 29881797; PMCID: PMC5983110.
  28. Buchhalter JR, D'Alfonso S, Connolly M, et al. The relationship between d-beta-hydroxybutyrate blood concentrations and seizure control in children treated with the ketogenic diet for medically intractable epilepsy. *Epilepsia Open* 2017;2:317–321. <https://doi.org/10.1002/epi4.12058>. PMID: 29588960; PMCID: PMC5862113.
  29. Martin-McGill KJ, Lambert B, Whiteley VJ, et al.; Ketogenic Dietitians Research Network (KDRN). Understanding the core principles of a 'modified ketogenic diet': a UK and Ireland perspective. *J Hum Nutr Diet* 2019;32:385–390. <https://doi.org/10.1111/jhn.12637>. Epub 2019 Mar 11. Erratum in: *J Hum Nutr Diet*. 2020 Oct;33(5):738. PMID: 30859652.
  30. Kroeger CM, Garza C, Lynch CJ, et al. Scientific rigor and credibility in the nutrition research landscape. *Am J Clin*

- Nutr 2018;107:484–494. <https://doi.org/10.1093/ajcn/nqx067>. PMID: 29566196; PMCID: PMC6248649.
31. Brito JJ, Li J, Moore JH, et al. Recommendations to enhance rigor and reproducibility in biomedical research. *Gigascience* 2020;9:giaa056. <https://doi.org/10.1093/gigascience/giaa056>. Erratum in: *Gigascience*. 2020 Sep 17;9(9): PMID: 32479592; PMCID: PMC7263079.
  32. Koroshetz WJ, Behrman S, Brame CJ, et al. Framework for advancing rigorous research. *Elife* 2020;4:e55915. <https://doi.org/10.7554/eLife.55915>. PMID: 32127131; PMCID: PMC7056268.
  33. Did a change in Nature journals' editorial policy for life sciences research improve reporting? *BMJ Open Sci* 2019;3:e000035. <https://doi.org/10.1136/bmjopen-2017-000035>
  34. Wasserstein RL, Schirm AL, Lazar NA. Moving to a world beyond " $p < 0.05$ ". *Am Stat* 2019;73:1–19.
  35. Titre-Johnson S, Schoeler N, Eltze C, et al. Ketogenic diet in the treatment of epilepsy in children under the age of 2 years: study protocol for a randomised controlled trial. *Trials* 2017;18:195. <https://doi.org/10.1186/s13063-017-1918-3>. PMID: 28446244; PMCID: PMC5406967.
  36. Kverneland M, Molteberg E, Iversen PO, et al. Effect of modified Atkins diet in adults with drug-resistant focal epilepsy: a randomized clinical trial. *Epilepsia* 2018;59:1567–1576. <https://doi.org/10.1111/epi.14457>. Epub 2018 Jun 14 PMID: 29901816.
  37. Martin-McGill KJ, Bresnahan R, Levy RG, Cooper PN. Ketogenic diets for drug-resistant epilepsy. *Cochrane Database Syst Rev* 2020;6:CD001903. <https://doi.org/10.1002/14651858.CD001903>. pub5. PMID: 32588435; PMCID: PMC7387249.
  38. Freeman JM, Vining EP, Kossoff EH, et al. A blinded, crossover study of the efficacy of the ketogenic diet. *Epilepsia* 2009;50:322–325. <https://doi.org/10.1111/j.1528-1167.2008.01740.x>. Epub 2008 Aug 19 PMID: 18717710.
  39. Mujica-Parodi LR, Amgalan A, Sultan SF, et al. Diet modulates brain network stability, a biomarker for brain aging, in young adults. *Proc Natl Acad Sci USA* 2020;117:6170–6177. <https://doi.org/10.1073/pnas.1913042117>. Epub 2020 Mar 3. PMID: 32127481; PMCID: PMC7084077.
  40. Krook-Magnuson E, Armstrong C, Bui A, et al. In vivo evaluation of the dentate gate theory in epilepsy. *J Physiol* 2015;593:2379–2388. <https://doi.org/10.1113/JP270056>. Epub 2015 Mar 31. PMID: 25752305; PMCID: PMC4457198.
  41. Chen H, Nwe PK, Yang Y, et al. A forward chemical genetic screen reveals gut microbiota metabolites that modulate host physiology. *Cell* 2019;177:1217–1231.e18. <https://doi.org/10.1016/j.cell.2019.03.036>. Epub 2019 Apr 18. PMID: 31006530; PMCID: PMC6536006.
  42. Çavuş I, Romanyshyn JC, Kennard JT, et al. Elevated basal glutamate and unchanged glutamine and GABA in refractory epilepsy: microdialysis study of 79 patients at the yale epilepsy surgery program. *Ann Neurol* 2016;80:35–45. <https://doi.org/10.1002/ana.24673>. Epub 2016 Jun 13 PMID: 27129611.
  43. Barabási AL, Menichetti G, Loscalzo L. The unmapped chemical complexity of our diet. *Nat Food* 2020;1:33–37.
  44. Burén J, Ericsson M, Damasceno NRT, Sjödin A. A ketogenic low-carbohydrate high-fat diet increases LDL cholesterol in healthy, young, normal-weight women: a randomized controlled feeding trial. *Nutrients* 2021;13:814. <https://doi.org/10.3390/nu13030814>. PMID: 33801247; PMCID: PMC8001988.
  45. Ye F, Li XJ, Jiang WL, et al. Efficacy of and patient compliance with a ketogenic diet in adults with intractable epilepsy: a meta-analysis. *J Clin Neurol* 2015;11:26–31. <https://doi.org/10.3988/jcn.2015.11.1.26>. Epub 2015 Jan 2. PMID: 25628734; PMCID: PMC4302176.
  46. Woodcock J, LaVange LM. Master protocols to study multiple therapies, multiple diseases, or both. *N Engl J Med* 2017;377:62–70.
  47. A phase 2, multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy, safety, and tolerability of TAK-935 (OV935) as an adjunctive therapy in pediatric participants with developmental and/or epileptic encephalopathies (ELEKTRA), ClinicalTrials.gov. Available at: <https://clinicaltrials.gov/ct2/show/NCT03650452>. Accessed March 22, 2021.
  48. CDER Patient-Focused Drug Development, FDA. Available at: <https://www.fda.gov/drugs/development-approval-process-drugs/cder-patient-focused-drug-development>. Accessed March 22, 2021.
  49. Diet treatment glucose transporter type 1 deficiency (G1D), ClinicalTrials.gov. Available at: <https://clinicaltrials.gov/ct2/show/NCT03181399>. Accessed March 22, 2021.