KETOGENIC DIET
AND METABOLIC THERAPIES
KETOGENIC DIET AND METABOLIC THERAPIES

Expanded Roles in Health and Disease

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OXFORD UNIVERSITY PRESS
## CONTENTS

<table>
<thead>
<tr>
<th>Preface</th>
<th>ix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contributors</td>
<td>xi</td>
</tr>
</tbody>
</table>

### SECTION I: Ketogenic Diet for Epilepsy in the Clinic

**ERIC H. KOSSOFF, MD, SECTION EDITOR**

1. Overview: Ketogenic Diets and Pediatric Epilepsy: An Update  
**ERIC H. KOSSOFF, MD**  
3  
2. “Alternative” Ketogenic Diets  
**ELIZABETH NEAL, RD, MSC, PHD**  
5  
3. Dietary Therapy in Adults: History, Demand, and Results  
**EMILY L. JOHNSON, MD AND MACKENZIE C. CERVENKA, MD**  
16  
4. How Do You Implement the Diet?  
**A. G. CHRISTINA BERGQVIST, MD**  
26  
5. Glut1 Deficiency and the Ketogenic Diets  
**JOERG KLEPPER, MD, PHD**  
35  
6. Ketogenic Diet in Established Epilepsy Indications  
**ANN M. BERGIN, MB, SCM, MRCP(UK)**  
40  
7. Ketogenic Diet for Other Epilepsies  
**DAVID T. HSIEH, MD AND ELIZABETH A. THIELE, MD, PHD**  
50  
8. The Ketogenic Diet and Related Therapies in “Novel” Situations: Idiopathic Generalized Epilepsy Syndromes  
**SUDHA KILARU KESSLER, MD, MSCE**  
56  
9. Ketogenic Diet in Status Epilepticus  
**RIMA NABBOUT, MD, PHD**  
60  
10. Preventing Side Effects and Diet Discontinuation  
**CHERIE L. HERREN, MD AND RANA R. SAID, MD**  
66

### SECTION II: Ketogenic Diet: Emerging Clinical Applications and Future Potential

**JONG M. RHO, MD, SECTION EDITOR**

11. Overview: Expanded Uses of Ketogenic Therapies  
**JONG M. RHO, MD**  
77  
12. Metabolism-Based Treatments to Counter Cancer: Scientific Rationale  
**THOMAS N. SEYFRIED, PHD AND LAURA M. SHELTON, PHD**  
79  
13. Ketogenic Diet as Adjunctive Therapy for Malignant Brain Cancer  
**ERIC C. WOOLE, PHD AND ADRIENNE C. SCHECK, PHD**  
88  
14. Metabolic Therapy for Autism Spectrum Disorder and Comorbidities  
**NING CHENG, PHD, SUSAN A. MASINO, PHD, AND JONG M. RHO, MD**  
101  
15. Glucose and Ketone Metabolism in the Aging Brain: Implications for Therapeutic Strategies to Delay the Progression of Alzheimer’s Disease  
**STEPHEN C. CUNNAANE, PHD, ALEXANDRE COURCHESNE-LOYER, MSC, VALERIE ST-PIERRE, BSC, CAMILLE VANDENBERGHE, BSC, ETIENNE CROTEAU, PHD, AND CHRISTIAN-ALEXANDRE CASTELLANO, PHD**  
113
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Authors</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>Ketogenic Diet and Ketones for the Treatment of Traumatic Brain</td>
<td>Femke Streijger, PhD, Ward T. Plunet, PhD, and Wolfram Tetzlaff, MD, Dr. Med, PhD</td>
<td>133</td>
</tr>
<tr>
<td></td>
<td>and Spinal Cord Injury</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Anti-Inflammatory Effects of a Ketogenic Diet: Implications for New</td>
<td>Nina Dupuis, PhD and Stéphane Auvin, MD, PhD</td>
<td>147</td>
</tr>
<tr>
<td></td>
<td>Indications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Dietary Therapy for Neurological Disorders: Focus on Amyotrophic</td>
<td>Carl E. Stafstrom, MD, PhD</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>Lateral Sclerosis, Parkinson's Disease, Mood Disorders, and Migraine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SECTION III: Ketogenic Diet in the Laboratory</td>
<td>Detlev Boison, PhD, Section Editor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Overview of Ketogenic Diet in the Laboratory: Progress on Models and Mechanisms</td>
<td>Detlev Boison, PhD</td>
<td>165</td>
</tr>
<tr>
<td>20</td>
<td>Ketogenic Diet and PPARgamma</td>
<td>Timothy A. Simeone, PhD</td>
<td>167</td>
</tr>
<tr>
<td>21</td>
<td>Ketogenic Diet in a Hippocampal Slice: Models and Mechanisms</td>
<td>Masahito Kawamura Jr., MD, PhD</td>
<td>186</td>
</tr>
<tr>
<td>22</td>
<td>Metabolic Therapy and Pain</td>
<td>David N. Ruskin, PhD</td>
<td>196</td>
</tr>
<tr>
<td>23</td>
<td>Ketogenic Diet, Adenosine, Epigenetics, and Antiepileptogenesis</td>
<td>Theresa A. Lusardi, PhD and Detlev Boison, PhD</td>
<td>209</td>
</tr>
<tr>
<td>24</td>
<td>Ketogenic Diet, Aging, and Neurodegeneration</td>
<td>Kui Xu, MD, PhD, Joseph C. Lamanna, PhD, and Michelle A. Puchowicz, PhD</td>
<td>216</td>
</tr>
<tr>
<td>25</td>
<td>Endocrine and Reproductive Effects of Ketogenic Diets</td>
<td>Jacob P. Harney, PhD, Kathryn Gudsnuk, MS, Ami Patel, MD, Anantha R. Vellipuram, MD, Sathyajit Bandaru, MS, and David Butler, PhD</td>
<td>227</td>
</tr>
<tr>
<td>26</td>
<td>Alzheimer's Disease: Causes and Treatment</td>
<td>Richard L. Veech, MD, PhD, DPhil and M. Todd King</td>
<td>241</td>
</tr>
<tr>
<td>27</td>
<td>Mitigation of Damage from Reactive Oxygen Species and Ionizing</td>
<td>William Curtis, Martin Kemper, PhD, Alexandra Miller, PhD, Robert Pawlosky, PhD, M. Todd King, and Richard L. Veech, MD, PhD</td>
<td>254</td>
</tr>
<tr>
<td></td>
<td>Radiation by Ketone Body Esters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>Metabolic Seizure Resistance via BAD and (K_{ATP}) Channels</td>
<td>Juan Ramón Martínez-François, PhD, Nika N. Danial, PhD, and Gary Yellen, PhD</td>
<td>271</td>
</tr>
<tr>
<td>29</td>
<td>Lactate Dehydrogenase: A Novel Metabolic Target</td>
<td>Nagisa Sada, PhD and Tsuyoshi Inoue, PhD</td>
<td>281</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SECTION IV: Ketone-Based Metabolism: General Health and Metabolic Alternatives</td>
<td>Dominic P. D’Agostino, PhD, Section Editor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Effects of the Ketogenic Diet on the Blood-Brain Barrier</td>
<td>Manoj Banjara, PhD and Damir Janigro, PhD</td>
<td>289</td>
</tr>
<tr>
<td>31</td>
<td>Overview of Ketone-Based Metabolism: General Health and Metabolic</td>
<td>Dominic P. D’Agostino, PhD</td>
<td>307</td>
</tr>
<tr>
<td></td>
<td>Alternatives</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>Ketone Supplementation for Health and Disease</td>
<td>Angela M. Poff, PhD, Shannon L. Kesler, PhD, and Dominic P. D’Agostino, PhD</td>
<td>310</td>
</tr>
<tr>
<td>33</td>
<td>Identifying the Molecular Mechanism of the Medium Chain Triglyceride</td>
<td>Matthew C. Walker, FRCp, PhD and Robin S.B. Williams, PhD</td>
<td>328</td>
</tr>
<tr>
<td></td>
<td>(Ketogenic) Diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>Triheptanoin in Epilepsy and Beyond</td>
<td>Karin Borges, PhD</td>
<td>336</td>
</tr>
<tr>
<td>35. Amino Acids in the Treatment of Neurological Disorders</td>
<td>36. 2-Deoxyglucose: Metabolic Control of Seizures through Inhibition of Glycolysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ADAM L. HARTMAN, MD</strong></td>
<td><strong>CARL E. STAFSTROM, MD, PHD AND THOMAS P. SUTULA, MD, PHD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>346</td>
<td>353</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36. Ketogenic Diets as Highly Effective Treatments for Diabetes Mellitus and Obesity</td>
<td>37. Advancing the Awareness and Application of Ketogenic Therapies Globally: The Charlie Foundation and Matthew’s Friends</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ERIC C. WESTMAN, MD, MHS, EMILY MAGUIRE, MSC, AND WILLIAM S. YANCY JR., MD, MHS</strong></td>
<td><strong>BETH ZUPEC-KANIA, RD, CD, JIM ABRAHAMS, EMMA WILLIAMS, MBE, AND SUSAN A. MASINO, PHD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>362</td>
<td>386</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PARKER HYDE, CSCS, CISSN, VINCENT J. MILLER, MS, AND JEFF S. VOLEK, PHD, RD</strong></td>
<td><strong>BETH ZUPEC-KANIA, RD, CD, JIM ABRAHAMS, EMMA WILLIAMS, MBE, AND SUSAN A. MASINO, PHD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>376</td>
<td>386</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Index 397
Metabolism is a fundamental cellular process, and metabolic dysfunction is associated with disease. The ketogenic diet is a metabolic therapy first published in 1921 as an effective treatment for seizures in both children and adults, and it has been prescribed to a subset of patients with epilepsy ever since. Today there are many drugs available to control epileptic seizures, yet this metabolic therapy can stop seizures even when all medications fail: for some patients a ketogenic diet is superior to all known drug treatments. The ketogenic diet was developed nearly 100 years ago because it had been observed—for centuries—that fasting would stop seizures. Adhering to a medically prescribed and carefully formulated high-fat ketogenic diet can maintain the ketone-based metabolism used during fasting.

Metabolic therapy targets the most fundamental aspect of cell function: cell energy. Targeting cell function or dysfunction metabolically is conceptually distinct from treating a disease specifically and pharmacologically. While a pharmacological approach has dominated drug development, and can be effective for some symptoms and conditions, it is also more likely to produce off-target side effects and less likely to produce lasting changes. In contrast, supporting cell energy and promoting metabolic homeostasis can improve overall health and may offer long-term benefits in preventing or modifying disease.

Recent basic and translational research has provided new insight into mechanisms as well as evidence that metabolic therapy with a ketogenic diet can treat diverse conditions beyond epilepsy. New research has also provided evidence that alternatives which can substitute for or complement the diet—and potentially augment its efficacy—may be close at hand. Evidence is also mounting that ketogenic diets can reverse chronic health conditions and provide general health benefits beyond treating any particular disease. Understanding key mechanisms underlying the success of metabolic therapy is of the highest biomedical significance: it is anticipated these mechanisms will apply to provide breakthroughs for multiple common, chronic, and poorly treated disorders. Similarly, a comprehensive understanding of the range and type of acute and chronic conditions that metabolic therapies can prevent, delay, or reverse is of urgent clinical importance.

Here we provide a fresh view on the promise of using the biochemistry of metabolism to treat disease and promote health by compiling the latest research and perspectives of leading experts on ketogenic diets and metabolic therapies. This volume is an up-to-date and comprehensive resource organized into four key subsections spearheaded by leaders in each area: the latest clinical research for treatment of epilepsy (Eric Kossoff, MD), emerging clinical applications (Jong Rho, MD), laboratory research into key mechanisms (Detlev Boison, PhD), and diverse metabolic therapies to treat disease and improve health (Dominic D’Agostino, PhD). The last chapter is devoted to two key organizations: the Charlie Foundation, established in 1994 in the United States, and Matthew’s Friends, established in 2004 in the United Kingdom. In the last two decades growth of research in the ketogenic diet field has been exponential, and the Charlie Foundation played an enormously important role in raising awareness and spearheading its resurgence in the clinic and the laboratory. Ongoing efforts of the Charlie Foundation have been furthered and multiplied by Matthew’s Friends, and together these foundations are devoted to research, education, outreach, and applications of ketogenic therapies throughout the world.
My personal path to a research program on the ketogenic diet was unusual: it arose organically from a basic science hypothesis on the regulation of adenosine. Adenosine is present throughout the body and the central nervous system and is a powerful neuromodulator and bioenergetic regulator of network homeostasis. Like the ketogenic diet, adenosine links metabolism and brain activity and has been proven to have powerful anti-seizure, neuroprotective, and disease-modifying benefits. Years of basic research on adenosine led me unexpectedly to the most important and exciting work of my career thus far and connected me with a motivated and collaborative global community of researchers, clinicians, patients, and advocates. The ketogenic diet has been proven to cure devastating cases of epilepsy, and we know that unlocking its key mechanisms—whatever they may be—will be a major biomedical breakthrough. Together we look forward to the 100th anniversary of the ketogenic diet in 2021 with optimism that metabolic therapies will offer new, safe, and effective options to promote health and cure disease.

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As it approaches its 100-year anniversary, the ketogenic diet (KD) is reaching an interest level not previously seen. Originally published in 1921 by Dr. Russell Wilder at the Mayo Clinic, its creation came at a time in which there were few other options for epilepsy (Wilder, 1921). The KD was widely used for the next several decades in both children and adults, with approximately 50% of patients reporting at least a 50% reduction in seizures in multiple studies. The advent of phenytoin and other modern pharmaceutical antiseizure drugs in the 1940s and afterward relegated the KD to “alternative” medicine and it was largely ignored by epilepsy specialists. For many decades it was used only as a last resort in children with intractable epilepsy; only very select institutions were still implementing it sporadically.

In 1993, one such refractory case prompted renewed interest in dietary therapies. Hollywood producer Jim Abrahams brought his 2-year old son Charlie to Johns Hopkins Hospital, where Charlie experienced rapid seizure control within days after starting the KD. Abraham created the Charlie Foundation in 1994, which revitalized research efforts, and produced First Do No Harm, a TV movie starring Meryl Streep, which promoted the KD. In 1998, the first multicenter prospective study of the KD in children with refractory epilepsy demonstrated that more than half of patients had a greater than 50% reduction in seizure frequency after 6 months (Vining et al., 1998).

In the now 20+ years since the formation of the Charlie Foundation, dietary therapies have experienced a rapid resurgence in research and use. The majority of countries have implemented KDs, and more than 100 research articles are published yearly (Kossoff & McGrogan, 2005). Several randomized controlled clinical trials, crossover studies, and prospective studies have confirmed a response rate of approximately 50% in children with refractory epilepsy. In 2009, Dr. Freeman and colleagues performed the first blinded study of the KD by having all participants consume the ketogenic diet plus a daily supplement of either saccharin (treatment group) or glucose (to prevent ketosis; control group) (Freeman et al., 2009). They found a trend toward improved seizure frequency in the saccharin group, though the effect did not reach statistical significance, possibly due to complex actions of the KD that were not prevented with ingestion of glucose once a day. Neal and colleagues randomized patients to no change in standard medical management or addition of the KD; they found that patients with refractory epilepsy who were randomized to receive the KD were more likely to have a 50% decrease in seizure frequency than the control group (Neal et al., 2008). Another study by Sharma et al. in 2013, using a similar study design to Dr. Neal’s 2008 trial, found the modified Atkins diet to be effective in a randomized controlled study as well (Sharma et al., 2013). In light of the accumulating evidence to support the efficacy of KDs, the International Ketogenic Diet Study Group, a panel of 26 neurologists and dietitians, recommended that dietary therapies be strongly considered in patients of any age who had failed two to three medications (Kossoff et al., 2009).

Beyond the formal prospective studies which have proven efficacy, perhaps an even more important factor that has led to the resurgence of dietary therapies has been a combination of flexibility in implementation and recognition of true indications for its use (Kossoff et al., 2009). Treating the appropriate patients (sooner rather than later) as well as considering alternative diets and methods of starting this treatment have led to widespread availability, willingness of patients and neurologists to consider it in their treatment algorithm, and better (and safer) outcomes. In this section,
“Ketogenic Diet for Epilepsy in the Clinic,” these factors are discussed in more detail.

First, Dr. Neal highlights that now there are not one but four types of KD now available, each with excellent reported efficacy: the classic ketogenic diet (KD), the medium chain triglyceride (MCT) diet, the low glycemic index treatment (LGIT), and the modified Atkins diet (MAD) (chapter 2). The latter two diets have certainly been responsible for the acceptance of dietary therapies by adults, which is discussed by Drs. Cervenka and Johnson in chapter 3 (see Cervenka et al., 2013). Flexibility during the initiation week of the classic KD has also revolutionized approaches to the diet by many epilepsy centers as outlined by Dr. Bergqvist (chapter 4; Bergqvist et al., 2005).

Second, pediatric epilepsy experts discuss the indications for dietary therapy in pediatric patients. Approximately 20 years ago, there was little to no ability to predict which child would be a KD responder. That has radically changed due to research and large cohort studies. The most famous indication, GLUT1 (glucose-1 transporter) deficiency syndrome, uses the KD as its primary, gold-standard therapy, and Dr. Klepper has been involved in much of the research on this condition and its response to the KD (chapter 5; Klepper, 2012). Drs. Bergin, Hsieh, and Thiele then discuss some of the other well-known epilepsy syndromes and genetic indications for dietary therapy such as infantile spasms, myoclonic-astatic epilepsy, Dravet syndrome, Rett syndrome, tuberous sclerosis complex, and more (Bergin, chapter 6; Hsieh and Thiele, chapter 7). In chapters 8 and 9 Drs. Kessler and Nabbout highlight the more recent, “novel” indications such as absence epilepsy, juvenile myoclonic epilepsy, status epilepticus, and others that have attracted investigators in the last few years (Nabbout et al., 2010).

Lastly, Drs. Herren and Said conclude this Section with a review of the latest research on how to identify and treat the adverse effects inherent in dietary therapy as well as how to eventually discontinue treatment when clinically indicated (chapter 10). This important chapter shows how clinical researchers are attempting to make the diet safer for those who require it, especially long-term. We hope you enjoy reading this Section and gain understanding of just how far the clinical use of dietary therapy has come in such a short time.

REFERENCES


“Alternative” Ketogenic Diets

ELIZABETH NEAL, RD, MSC, PHD

INTRODUCTION
As the classical ketogenic diet fast approaches a centennial anniversary, the wider ketogenic landscape has expanded considerably both in application and implementation. Although still extensively used today, this traditional dietary therapy has been the basis for development of alternative ketogenic protocols. One ketogenic diet incorporating medium chain fatty acids is used for many children and adolescents, who benefit from the generous carbohydrate allowance facilitated by the increased ketogenic potential of medium chain triglycerides. More recently, two less restrictive dietary approaches have been developed: the low glycemic index treatment and the modified Atkins diet. These are now being used worldwide as the advantages of a more liberal ketogenic diet are recognized, especially in adults and older children, supported by an increasing body of scientific data. This chapter explores the background and evidence for use of these alternative ketogenic diets.

THE MEDIUM CHAIN TRIGLYCERIDE KETOGENIC DIET
The predominant fatty acids in the human diet contain 12 or more carbon atoms and originate from animal and plant sources of long chain triglycerides (LCT), which can be saturated, mono-unsaturated, or polyunsaturated. The shorter chain length medium chain fatty acids (6 to 12 carbon atoms) originate from medium chain triglycerides (MCT), whose main constituents are octanoic (C8) and decanoic (C10) fatty acids. Dietary sources are limited: mainly coconut and palm kernel oils. Medium chain triglycerides have distinct physical and metabolic differences from LCT with a more efficient digestion, absorption, and mitochondrial transport process facilitating faster metabolism to acetyl CoA. Hepatic ketone body production is primarily determined by the rate of acetyl CoA generation, which led to suggestions by Dr. Huttenlocher and colleagues that a ketogenic diet (KD) replacing LCT with MCT would induce higher ketosis and allow inclusion of significantly more carbohydrate and protein, improving palatability and acceptance. After an initial trial of a KD providing 60% of total dietary energy from MCT in 12 children and adolescents with epilepsy (Huttenlocher et al., 1971), further results were reported from 18 patients aged 1.5–18 years, of whom 16 had over 50% seizure reduction (Huttenlocher 1976).

Interest in the MCT diet continued with further studies reported from the United States (Trauner et al., 1985), the United Kingdom (Sills et al., 1986), and Taiwan (Mak et al., 1999). A dietary modification with less MCT (30% energy) was also suggested in response to concerns about gastrointestinal side effects of MCT given in large doses (Schwartz et al., 1989). In 2008, researchers based at Great Ormond Street Hospital in London published a trial of classical and MCT KDs in intractable childhood epilepsy in which children aged 2–16 years were randomized to receive a diet either immediately or after a 3-month delay with no additional treatment changes (control group). After 3 months, seizure frequency was significantly lower in the 54 children in the diet group compared with the 49 controls (Neal et al., 2008a). Of the children who were randomized, 125 received dietary treatment at some stage (61 classical and 64 MCT diets). Comparing the two diet groups using an intention to treat analysis found no significant difference between the two diets; 29% of the MCT group had over 50% seizure reduction at 3 months (Neal et al., 2008a). Tolerability or withdrawals were also not significantly different at 3 and 6 months, with no evidence that the MCT diet caused more gastrointestinal problems; indeed a history of vomiting was significantly higher in the classical KD children at 12 months. In this trial, the MCT diet was initiated at a starting dose of 40%–50% energy from the MCT supplement, aiming to
provide the optimal balance between gastrointestinal tolerance and good ketosis. However many children and adolescents will need a higher dose to achieve optimal seizure control. Christiana Liu reports that in her extensive experience of using the MCT diet in Canada, MCT at 40% to greater than 70% energy can be well tolerated without side effects (Liu and Wang, 2013). Prospective follow-up of 48 children and adolescents aged 1–18 years on mostly (79%) the MCT diet has recently been reported from Holland. Responder rates were lower in this study, only 17% achieving over 50%

**Fig. 2.1** Ketogenic diet therapies: a comparison of dietary energy contribution from macronutrients.
seizure reduction after 3 months, increasing to 23% after 6 months (Lambrechts et al., 2015).

The MCT diet is implemented using commercially available products of MCT oil or emulsion (Liquigen, Nutricia, 50% MCT; Betaquik, Vitafo, 20% MCT), which are supplied in some countries on medical prescription. The remaining energy is provided from carbohydrate, protein, and LCT. Calculation of this diet is not based on the ketogenic ratio but instead looks at the percentage of dietary energy provided by macronutrients (Box 2.1). Total energy intake is controlled as with the classical KD, although it will theoretically depend on the figure applied for energy content of MCT, which is lower than LCT (Ranhotra et al., 1995); this is not always reflected in the conversion factors listed on the products or used for dietary calculation. The MCT diet is strictly prescribed and all food weighed, often using food choice lists to develop meal plans. It is the most generous in carbohydrate of all ketogenic therapies (see Figure 2.1), and many children and adolescents benefit from the flexibility this offers. Medium chain triglycerides should be included in all meals and snacks, and compliance is improved by encouraging creative incorporation into recipes and ketogenic drinks. The dose of MCT should be slowly built up over the first week or two of treatment according to tolerance, ketosis, and seizure control (see Box 2.1).

Recent data indicate there may be additional specific efficacy benefits of medium chain fatty acids. Chang et al. (2013) found C10 significantly outperformed valproic acid in both in vitro and in vivo models of seizure control. Neuronal cell-line data from Hughes et al. (2014) suggested that C10 might increase mitochondrial number, mediated via activation of the PPARγ (peroxisome proliferator-activated) receptor and its target genes involved in mitochondrial biogenesis.

**THE MODIFIED ATKINS DIET**

In 2003 Dr. Kossoff and colleagues at Johns Hopkins Hospital in Baltimore published a brief communication to report their use of the Atkins’s diet in six patients with epilepsy aged between 7 and 52 years; three had over 90% seizure reduction, of whom two became seizure free (Kossoff et al., 2003). The Atkins diet, designed in the 1970s as a weight loss treatment, restricts carbohydrates and encourages fat in a similar way to the classical KD but allows free protein. It was suggested that this could be the basis of a less restrictive ketogenic therapy for epilepsy, the goal being seizure control rather than weight loss. The team at Johns Hopkins went on to trial this modified Atkins diet (MAD) in 20 children: 13 achieved over 50% seizure reduction after 6 months, including four who became seizure free (Kossoff et al., 2006). In a further study in 30 adults, seizures were reduced by over 50% in 10 patients after 6 months on the MAD (Kossoff et al., 2008a). The real advantage of this diet is that it allows free protein and calories, so can be easier to implement and comply with than the classical KD. Although approximating a ketogenic ratio of 1:1 (see Figure 2.1), the only macronutrient strictly controlled on the MAD is carbohydrate. A randomized crossover comparison of daily carbohydrate limits in children suggested a lower intake (10 g vs. 20 g) during the initial 3 months of the MAD was associated with significantly higher likelihood of over 50% seizure reduction at 3 months, after which time carbohydrate could be increased (Kossoff et al., 2007).

The MAD has led the way in a shift of approach to implementation of ketogenic therapy. The emphasis had been previously on absolute precision in calculation and accuracy in food weighing, albeit with very successful outcomes in many who followed the strict KD, but with compliance problems in others. As practitioners of the diet, we initially viewed what appeared to be such a liberal MAD protocol with caution. Now, over 10 years on, this is being increasingly adopted as an alternative ketogenic therapy, especially suited to adolescents, adults, and those unable to comply with the stricter classical KD. It is used with success worldwide in children and adults (see Table 2.1, studies included if five or more subjects), and has potential for use in resource-poor countries with more limited dietetic support (Kossoff et al., 2008b).

A review in 2012 combined data from published MAD studies to examine responder rates. After 3 months of treatment, 54% of 105 children (six studies, both retrospective and prospective) and 34% of 56 adults (three studies, all prospective) had greater than 50% seizure reduction. Prospective data was available on 82 children (four studies), of whom 52% had greater than 50% seizure reduction after 3 months (Auvin, 2012). The following year, Dr. Kossoff and colleagues comprehensively reviewed 10 years of the MAD with similar findings. Their review included all published primary studies in which the MAD was used as the first dietary treatment and also case reports of single patients. Of a combined total of 342 children, 53% had over 50% seizure reduction, with 15% achieving seizure freedom; in a combined total of 92 adults these figures were lower at 30% and 3%, respectively.
**Box 2.2**

**Modified Atkins Diet Protocol**

Carbohydrate for first month: 10 g daily for children, 10–15 g daily for adolescents and 20 g daily for adults (does not include fiber but does include sugar alcohols)

Encourage high-fat foods, eat with each meal/snack

Free protein

Free calories but need to avoid excess weight gain

Full vitamin and mineral supplementation

Carbohydrate-free medications

Ketocal formula can be used as daily supplement for first month

*Source: (Kossoff et al., 2011b)*

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**Table 2.1 Worldwide Use of the Modified Atkins Diet**

<table>
<thead>
<tr>
<th>Country</th>
<th>Study</th>
<th>Dietary Prescription—Daily Carbohydrate Allowance*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Children and Adolescents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Argentina</td>
<td>Vaccarezza et al., 2014 (Retrospective, n = 9)</td>
<td>10% energy</td>
</tr>
<tr>
<td>Denmark</td>
<td>Weber et al., 2009 (Prospective, n = 15)</td>
<td>10% energy, restricted further to 10 g after 1–2 weeks if poor seizure control (Weber)</td>
</tr>
<tr>
<td></td>
<td>Miranda et al., 2011 (Prospective, n = 33)</td>
<td>10 g for 1st 3 months (Miranda)</td>
</tr>
<tr>
<td>Egypt</td>
<td>El-Rashidy et al., 2013 (Prospective, n = 15)</td>
<td>10 g for 1st month then can increase by 5-g increments up to 10% energy</td>
</tr>
<tr>
<td>France</td>
<td>Porta et al., 2009 (Prospective, n = 10)</td>
<td>10 g for 1st month then can increase by 5-g increments up to 10% energy</td>
</tr>
<tr>
<td>India</td>
<td>Sharma et al., 2012 (Prospective, n = 15)</td>
<td>10 g</td>
</tr>
<tr>
<td></td>
<td>Sharma et al., 2013 (Randomized controlled trial, n = 50 in diet group)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sharma et al., 2015 (Retrospective, n = 25)</td>
<td></td>
</tr>
<tr>
<td>Iran</td>
<td>Tonekaboni et al., 2010 (Prospective, n = 51)</td>
<td>10 g</td>
</tr>
<tr>
<td></td>
<td>Ghazavi et al., 2014 (Retrospective, n = 20)</td>
<td></td>
</tr>
<tr>
<td>Japan</td>
<td>Ito et al., 2011 (Retrospective, n = 6)</td>
<td>10 g initially (one child needed 30 g–20 g step down start, Kumada et al.)</td>
</tr>
<tr>
<td>Korea</td>
<td>Kumada et al., 2012 (Prospective, n = 10)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kang et al., 2007 (Prospective, n = 14)</td>
<td>10 g for 1st month then can increase by 5-g increments up to 10% energy</td>
</tr>
<tr>
<td></td>
<td>Kim et al., 2012 (Retrospective, n = 20)</td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>Kossoff et al., 2003 (Retrospective, n = 6)</td>
<td>10 g for 1–2 months then can increase by 5-g increments up to 20 g</td>
</tr>
<tr>
<td></td>
<td>Kossoff et al., 2006 (Prospective, n = 20)</td>
<td>(20 g initially for five Sturge-Weber syndrome children in 2010 paper)</td>
</tr>
<tr>
<td></td>
<td>Kossoff et al., 2007 (Prospective, n = 20)</td>
<td></td>
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<tr>
<td></td>
<td>Kossoff et al., 2010a (Prospective, n = 5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kossoff et al., 2011a (Prospective, n = 30)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Groomes et al., 2011 (Retrospective, n = 13)</td>
<td></td>
</tr>
<tr>
<td><strong>Adults</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Belgium</td>
<td>Carrette et al., 2008 (Prospective, n = 8)</td>
<td>20 g</td>
</tr>
<tr>
<td>Canada</td>
<td>Smith et al., 2011 (Prospective, n = 18)</td>
<td>20 g</td>
</tr>
<tr>
<td>USA</td>
<td>Kossoff et al., 2008a (Prospective, n = 30)</td>
<td>15 g</td>
</tr>
<tr>
<td></td>
<td>Cervenka et al., 2012 (Prospective, n = 25)</td>
<td>20 g</td>
</tr>
<tr>
<td></td>
<td>Kossoff et al., 2013a (Retrospective, n = 8)</td>
<td>20 g</td>
</tr>
</tbody>
</table>

*All studies allowed free calories except Kim et al., 2012, who report using 75% of recommended daily intakes*
This review included results from Dr. Sharma and colleagues in India, who published the first randomized trial of the MAD in 102 children with intractable epilepsy aged 2–14 years. Using a delayed diet start control group in a design similar to that of Neal et al. (2008a), seizure frequency after 3 months was significantly lower in the 50 diet children compared with the 52 controls (Sharma et al., 2013). One study has looked at long-term outcomes of children on the MAD: over 50% seizure reduction was maintained in 55% of the 54 who continued treatment for over 6 months (Chen and Kossoff, 2012).

Most centers prescribe the diet as recommended by Dr. Kossoff and his team at Johns Hopkins (Box 2.2). A dietary variant adopted by some UK centers and described by Magrath et al. (2012) is frequently and more appropriately termed the modified ketogenic diet; this is distinguished from the MAD by a more generous initial carbohydrate allowance (up to 30 g with further reduction during fine-tuning depending on seizure control) and a prescribed fat intake using food choice lists. There is no published data on this type of modified diet.

**LOW GLYCEMIC INDEX TREATMENT**

The glycemic index (GI) is a measurement based on the blood glucose response to carbohydrate-containing foods (Jenkins et al., 1981); this will be influenced by their rate of digestion and absorption, slower absorbed foods having a lower GI rating. Glycemic index values are compared with a standard reference value of glucose. Other variables influencing the GI of a food include fiber content, cooking methods, processing, ripeness, and combination of different macronutrients within a meal. Whole grains and other high-fiber foods will lower GI, as will the addition of fat or protein. Most vegetables and many fruits are low in GI.

Blood glucose levels tend to be fairly stable while on the low-carbohydrate KD, and this observation led to the suggestion that a diet based on only low GI carbohydrate (<50) choices could maintain this glucose stability and facilitate a more liberal type of ketogenic regime (see Figure 2.1). This alternative low GI treatment (LGIT) (Box 2.3) was first tested by researchers from Boston: a preliminary retrospective review of 20 patients found half to have greater than 90% seizure reduction (Pfeifer & Thiele, 2005). Results were updated in 2009 with a larger review of 76 patients aged 1.5–22 years, half of whom had over 50% seizure reduction at 3 months, increasing to 66% by 12 months (Muzykewicz et al., 2009). Interestingly, carbohydrate intake ranged from 15 to 150 g daily (mean 53 g at 3 months); some individuals needed a more restricted amount for seizure control, whereas others were able to relax the carbohydrate without adverse effect. The same group has successfully used LGIT in 15 children with tuberous sclerosis (Larson et al., 2012) and a small group of six children with Angelman syndrome studied prospectively (Thibert et al., 2012). Again, reported carbohydrate intake was very varied, between 30 and 137 g at the 4-month follow-up.

The LGIT is now used in many centers worldwide. Results in children and adolescents have been reported from Italy (retrospective data on

<table>
<thead>
<tr>
<th>BOX 2.3</th>
<th>LOW GLYCEMIC INDEX TREATMENT DIETARY PROTOCOL</th>
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<tbody>
<tr>
<td><strong>Dietary goals set for carbohydrate, protein, and fat intake based on a target energy prescription</strong></td>
<td></td>
</tr>
<tr>
<td>Carbohydrate prescribed at 10% energy (approximately 40–60 g daily depending on baseline intake); this figure includes dietary fiber. Only carbohydrates with glycemic index &lt; 50 allowed</td>
<td></td>
</tr>
<tr>
<td>Approximately 30% energy protein</td>
<td></td>
</tr>
<tr>
<td>Approximately 60% energy LCT</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate should be evenly distributed over the day and always eaten with some fat and/or protein</td>
<td></td>
</tr>
<tr>
<td>Foods not weighed but based on household portion sizes</td>
<td></td>
</tr>
</tbody>
</table>

*Source: (Pfeifer, 2012)*
15 patients: over half had over 50% seizure reduction after a mean treatment duration of 14 months; Coppola et al., 2011) and Iran (prospective study of 42 patients: over 50% seizure reduction in 74% after 1 month; Karimzadeh et al., 2014). An interesting case study reports a 13-year-old Japanese girl who was able to maintain a 50-g carbohydrate daily LGIT choosing from specially designed menus including unpolished rice and Natto (fermented soybeans) (Kumada et al., 2013).

ARE “ALTERNATIVE” DIETS AS EFFECTIVE AS THE CLASSICAL KETOGENIC DIET?

With the range of ketogenic therapies available, the initial clinic assessment consultation will include consideration of which diet to choose for an individual patient. A key question for those embarking on ketogenic therapy is which diet could work best to treat the seizures. Will an alternative protocol with potential compliance and tolerance advantages still be as effective as the strict classical KD?

The scientific literature has also discussed this question and certainly review of the many MAD trials shows results comparable to those for the KD (Kossoff et al., 2013b), including longer-term follow-up data (Chen and Kossoff, 2012). Direct comparison trials of different ketogenic therapies are limited. In the trial of Neal et al. previously discussed, there were no significant differences in the mean percentage of baseline seizures or numbers, with over 50% or 90% seizure reduction between the classical and MCT diet groups after 3, 6, and 12 months (Neal et al., 2009). The authors concluded classical and MCT KDs were comparable in efficacy and tolerability and both had their place in the treatment of childhood epilepsy. In view of the similar design between this trial and the more recent MAD randomized trial already mentioned (Sharma et al., 2013), results of the two studies are provided together in Table 2.2. Although we cannot directly compare data, seizure improvement was lower in the KD trial, which may in part reflect differences in baseline patient characteristics, as a similar difference was seen in the control group.

A number of studies have tried to compare classical KD and the MAD. A retrospective review of children on classical KD or MAD found significantly more KD children had greater than 50% seizure reduction at 3 months, but not at 6 months (Porta et al., 2009). A prospective evaluation of children on the MAD compared with a previously treated KD group found an initial trend toward greater KD efficacy disappeared when data were age-adjusted (Miranda et al., 2011). Data from Iran on 40 children of whom half were prescribed a classical KD and the others a MAD showed no significant difference in numbers achieving 50% seizure reduction after 1–3 months (Ghazavi et al., 2014).

### TABLE 2.2 RESULTS OF RANDOMIZED CONTROLLED TRIALS ON THE KETOGENIC DIET AND THE MODIFIED ATKINS DIET

<table>
<thead>
<tr>
<th>Sharma et al., 2013 (MAD)</th>
<th>Neal et al., 2008a (classical and MCT KDs)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subjects</strong></td>
<td></td>
</tr>
<tr>
<td>Number enrolled in study (ages)</td>
<td>102 (2–14 years)</td>
</tr>
<tr>
<td>Inclusion criteria</td>
<td>Minimum 7 weekly seizures, tried at least 3 anticonvulsant medications, no compliance issues or medical contraindications</td>
</tr>
<tr>
<td><strong>Results</strong></td>
<td></td>
</tr>
<tr>
<td>Mean % baseline seizure frequency (CI)</td>
<td>59% diet group (44–75)</td>
</tr>
<tr>
<td></td>
<td>95% control group (82–109)</td>
</tr>
<tr>
<td>&gt;90% seizure reduction</td>
<td>30% diet</td>
</tr>
<tr>
<td></td>
<td>8% controls</td>
</tr>
<tr>
<td>&gt;50% seizure reduction</td>
<td>52% diet</td>
</tr>
<tr>
<td></td>
<td>12% controls</td>
</tr>
<tr>
<td>Most frequent side effect</td>
<td>Constipation (46%)</td>
</tr>
</tbody>
</table>
However other data suggest there may be benefits of a stricter diet. In a retrospectively analyzed multicenter group of 27 children switched from the MAD to classical KD, additional seizure reduction was reported in 10, of whom five with Doose syndrome (myoclonic astatic epilepsy) became seizure-free; the authors identified the KD as a “higher dose” of ketogenic therapy than the MAD (Kossoff et al., 2010b). In a small pilot study, researchers in Egypt randomly assigned 40 young children (aged 1–3 years) to either classical KD fed by a liquid formula, MAD, or no diet treatment. After 3 months the classical KD group showed significantly reduced seizure frequency and severity compared with the MAD group; at 6 months the reduction in frequency was still significant, but not that in severity (El-Rashidy et al., 2013).

A randomized trial comparing efficacy, safety, and tolerability of classical KD and MAD in 104 children aged 1–18 years (51 KD, 53 MAD) has recently been published. Mean percentage of baseline seizures was lower in the KD group at 3 months (38.6% KD, 47.9% MAD) and 6 months (33.8% KD, 44.6% MAD), although differences were not statistically significant. In infants aged 1–2 years the mean and median baseline seizure frequencies were much lower in the classical KD group with seizure freedom after 3 months achieved in 9 (53%) of 17 KD patients compared to 4 (20%) of 20 MAD patients. The MAD showed tolerability and side effect advantages. The authors concluded that while the MAD may be suitable for many children, the classical KD should always be first choice in those under 2 years (Kim et al., 2016).

Although one review of studies on dietary treatment in adults with refractory epilepsy concluded that both classical KD and MAD were equally effective and tolerated (Klein et al., 2014), this was challenged by a recent meta-analysis of 12 studies on ketogenic therapy in adults: 7 classical KD, 5 MAD, and one MCT. A total of 270 patients were evaluated with a combined efficacy rate of 52% for classical KD and 34% for MAD and an odds ratio for therapeutic success of classical KD relative to MAD of 2.04, a significant difference between the two types of diet (Ye et al., 2015). This analysis examined compliance, which was also significantly different between the two diets, at 38% classical KD and 56% MAD, suggesting that while the classical KD may be more effective in adults, it is not as well tolerated.

There is some evidence from the above studies that a stricter diet might be more efficacious. This could be particularly important at the outset of treatment, a hypothesis supported by two randomized trials examining the use of different prescriptions within a particular ketogenic therapy. A lower carbohydrate MAD initiation (10 g vs. 20 g) was associated with improved efficacy outcome (Kossoff et al., 2007), as was a higher classical KD ketogenic ratio (4:1 vs. 3:1) (Seo et al., 2007); in both these studies the benefits of a stricter diet at the outset were maintained even after increasing carbohydrate intake later in the course of treatment. Although it is now recognized that ketosis may not be directly linked to seizure control, any correlations between the two seem limited to the first 3 months of dietary treatment (Neal et al., 2009).

Further support is provided by results of a study of 30 children at Johns Hopkins who were given the 4:1 ratio classical KD supplement Ketocal daily during the first month of the MAD; this had benefit on seizure control when responder rate outcomes were compared to published results of the MAD alone (Kossoff et al., 2011a). This practice is now frequently implemented at their institution (see Box 2.2), where the importance of a strict initiation period during the first month has been recognized (Kossoff et al., 2013b). It could perhaps be argued that key questions to ask when embarking on ketogenic therapy are not only “which dietary protocol to choose?” but also “how we are going to manage the initiation period for optimal seizure outcomes?”

**SO WHICH DIET TO CHOOSE?**

The question of which diet to use for an individual will also take into account age, lifestyle, food preferences, and feeding method. As illustrated in Figure 2.1, all three of the alternative dietary protocols have less fat than the classical KD as a proportion of the overall dietary energy. The MCT diet is the most generous in carbohydrate, but consideration must be given to the need to incorporate the MCT supplement into all meals and snacks and the use of a strict prescription with food weighing. However this can work well for those who find it too difficult to follow a stricter carbohydrate restriction and need a more structured dietary prescription. Adolescents and adults usually prefer the flexibility of the MAD with no food weighing and free protein and calories, and to maintain ongoing compliance this option would routinely be recommended for these age groups. An alternative would be LGIT if unable to adhere to the stricter MAD carbohydrate restriction. The classical KD is recommended for all ages if using a feeding tube and also for infants under 2 years. In older children aged between 2 and 12 years it may
be sensible to start with a stricter KD in light of the

efficacy data previously discussed; this can then be
changed to the MAD at a later date if children and
parents are happy to switch (Kossoff et al., 2013b).
The classical KD will be preferable for children
who have poor appetites and need small meals, the
nutritionally at risk, and families who require close
supervision on meal planning and the control of
their child's energy intake. In other cases it is clear
that both the child and the family would not be
able to comply with the high-fat classical KD and
the MAD or MCT diet are better options. In view of
its reduced demands on time for training and
supervision, the MAD would be first choice in cen-
ters with fewer dietitians. This potential in devel-
op ing countries has been identified (Kossoff et al.,
2009b), including the possibility of e-mail-based
MAD management in adults (Cervenka et al.,
2012). However both the MAD and LGIT require
patients or families to design their own meals from
the food choices given; in some situations such as
a residential multicarer setting this may be more
difficult and require greater dietetic input.

The stricter classical KD has been recom-
mended in epilepsy syndromes where rapid
improvement is needed, such as infantile spasms
or status epilepticus (Auvin, 2012) and in Glut 1
deficiency syndrome and Doose syndrome
(Miranda et al., 2012), although the MAD has
been used successfully in both spasms (Sharma
et al., 2012) and Glut 1 deficiency syndrome (Ito
et al., 2011). Following observed benefits in Doose
syndrome children changed from the MAD to
classical KD, this switch should be considered in
this group if not seizure-free after 6–12 months on
the MAD (Kossoff et al., 2010b).

**PRACTICAL CONSIDERATIONS:**

**INITIATION AND FOLLOW-UP OF
“ALTERNATIVE” DIETS**

Medical contraindications to ketogenic therapy
are detailed in recommendations for clinical KD
management (Kossoff et al., 2009). These will
apply to both classical and alternative KDs and
should be excluded prior to initiation with any
necessary baseline biochemistry. Although some
centers do hospitalize children on initiation of
the MCT diet, as with the classical KD it is now
generally accepted that this can be started at home
without any prior fasting period. The daily intake
of MCT will be stepwise, increased as tolerated
(Box 2.1). The MAD and LGIT are started on an
out-patient basis. All dietary protocols require full
vitamin and mineral supplementation to ensure
requirements of micronutrients are met while
on a restricted diet. Adequate training from the
ketogenic team must be given prior to initiation of
any ketogenic therapy to ensure patients and car-
ers understand the dietary prescription and how
to manage the practicalities of ketogenic therapy
at home, including strategies for illness and acute
situations.

All ketogenic therapies must be carefully mon-
titored to ensure they can be implemented safely.
Home monitoring will include regular weight
checks; this is also important on the MAD and
LGIT, where the calorie content is not strictly pre-
scribed. Traditionally, ketones would be checked
up to twice daily at home while on the KD, as good
ketosis was thought to have been key to the success
treatment. This premise has been challenged
with alternative diets where ketones are often
much lower. Regular home testing of urine or
blood ketones is recommended on the MCT diet,
but levels are not as high as those seen on the clas-
sical KD (Neal et al., 2009). After the first month
of the MAD, with a relaxing of dietary restrictions,
ketones will usually be much lower than those seen
on the KD, especially in older children and adults.
Although Kossoff et al. (2011b) recommend only
a weekly check after this point, others suggest con-
sistently high ketones above 3 mmol/L (blood
β-hydroxybutyrate) in children on the MAD could
be important for maintaining efficacy (Kang et al.,
2007). Ketones on the LGIT will usually be very
low and undetectable in some cases; no correlation
was seen between ketosis and seizures on this diet
(Muzykewicz et al., 2009).

Clinic monitoring at regular follow-up visits
will include full laboratory studies, growth assess-
ment, review of seizures, tolerance, and other
benefits or adverse events. Side effects have been
reported with the MCT diet, primarily gastrointes-
tinal and usually managed with dietary manipu-
lation (Neal et al., 2009; Liu and Wang, 2013).
Growth faltering has been reported in children on
MCT diets, similar to that seen in children on a
classical KD despite the significantly higher pro-
tein intake on the MCT diet (Neal et al., 2008b).
Gastrointestinal symptoms including constipation
have also been reported as a side effect of the MAD
(Kang et al., 2007; Auvin, 2012; Chen & Kossoff,
2012; Sharma et al., 2013). Raised lipid levels on
the MAD have been reported in children (Kang
et al., 2007) and adults (Cervenka et al., 2014);
these were transient and normalized within a year
of treatment. No significant side effects have been
reported in LGIT studies, although increases in
blood urea nitrogen were detected in about a third of patients (Muzykewicz et al., 2009; Karimzadeh et al., 2014).

Fine-tuning of ketogenic therapy will aim to alleviate side effects where possible, and to optimize seizure outcomes. Adjustments to prescriptions and micronutrient supplementation will also be needed as a child grows older. The MCT dose may be increased or decreased on the MCT diet, as can carbohydrate intake, aiming to maximize benefit. As an addition to the MAD or modified ketogenic diet, MCT has also been used to give a boost to ketosis and seizure control and aid compliance by facilitating increased carbohydrate allowance. This practice is evidence of a more flexible approach to ketogenic therapy, designing an individualized treatment based primarily on specific dietary and lifestyle requirements rather than on a rigid diet protocol that is offered by a particular hospital center. This may primarily use one type of diet, but alternatively may use different aspects of some, or indeed all, of the ketogenic therapies. Well-defined dietary parameters are needed if conducting research studies on a specific diet, but anecdotal reports suggest more dietitians are tending toward this “patient-tailored” prescription of ketogenic therapy in clinical practice (Miranda et al., 2012).

CONCLUSION
As worldwide use of the KD continues to grow, it is clear that the alternative dietary protocols described in this chapter have an important place within the treatments we can offer to children and adults with intractable seizures. The MAD in particular has emerged as a therapy with great potential for treating not only children with epilepsy but also adults and those in countries with more limited resources. Further research will enable us to optimize protocols for clinical implementation to ensure the best possible outcome for those embarking on dietary treatment of epilepsy.

REFERENCES


**HISTORY**

Dietary therapy has been used for the treatment of epilepsy since antiquity. Hippocrates wrote of fasting “purifications” as a cure for seizures, and reported that some of his contemporaries believed certain foods such as eel and goat to exacerbate or cause seizures (Hippocrates, c. 400 BC). In the Roman era, drinking gladiators’ blood was thought to be a cure for epilepsy (Barborka, 1929).

In modern times, intermittent fasting has been studied for over 100 years. Drs. Marie and Guelpa described a cyclical fasting regimen of 4 days of fasting and purges followed by 4 days of a restricted vegetarian diet. Three-quarters of the 20 patients (adults and adolescents) with epilepsy that were studied could not adhere to the diet for more than one cycle. Of the remaining patients, those who followed the diet had significant benefit and in some cases had seizure remission; however, long-term compliance with the diet was limited (in more than one case, by friends of the patient who provided foods that were not permitted) and they concluded that their regimen was too difficult for most adults to follow (Marie and Guelpa, 1911).

Dr. Geyelin of New York Presbyterian observed a 10-year-old boy with 4 years of refractory epilepsy become cured after intermittent fasting (four fasts over 4 months) under the care of Dr. Conklin of Battle Creek. Dr. Geyelin then treated a 9-year-old boy with a 3-day fast; his multiple daily seizures stopped after the 2nd day. Dr. Geyelin went on to treat patients with intermittent fasting of lengthening duration (Geyelin, 1921) and expanded these treatments to adults as well as children. In 22/26 patients (ages 3–35 years), he observed seizure remission by the 10th day of fasting; 18/26 had marked improvement 1 year following fasting, and had no further seizures.

R.M. Wilder of the Mayo Clinic, analyzing Geyelin’s work, was the first to speculate that the benefit… may be dependent on the ketonemia which much result from such fasts, and that possibly equal good results could be obtained if a ketonemia were produced by some other means. The ketone bodies, acetoacetic acid and its derivatives, are formed from fat and protein whenever a disportion exists between the amount of fatty acid and the amount of sugar actually burning in the tissues…. it is possible to provoke ketosis by feeding diets which are very rich in fat and low in carbohydrate. It is proposed, therefore, to try the effect of such ketogenic diets on a series of epileptics. (Wilder, 1921)

The Mayo Clinic began treating adults with epilepsy with this “ketogenic diet” in 1924. C.J. Barborka wrote that “epileptic patients have an unusual ability to consume and utilize fat,” and hypothesized that the benefits of ketosis may be due to changes in nerve cells, and “decreased irritability of nerves.” General wisdom held that the acid-base balance contributed to seizures or seizure protection.

Barborka believed that dietary therapy offered a “ray of hope,” and recognized that while the diet was difficult, it was far better to try it than to “merely employ a sedative, and to wait.”

Barborka published several articles on the Mayo Clinic experience with the ketogenic diet. He emphasized the need for patient education, and required patients to spend 2–3 weeks under strict supervision while learning the diet (Barborka, 1928). The diet was designed to mimic the metabolism of a fasting person to produce mild ketosis, using a method originally developed for diabetics. The target maintenance diet was calculated to have sufficient calories to maintain a neutral weight in adults; carbohydrates were limited to develop and maintain ketosis. The original diet consisted of six
phases with varying amounts of carbohydrates and fat, with a stepwise decrease in the content of carbohydrates and increase in the amount of fat. Sample menus reveal an emphasis on heavy cream (100 cc of 40% cream with each meal), mayonnaise, and butter (Barborka, 1929). Patients were educated to test their urine for ketosis.

In 1930, Barborka published a series of 100 adolescent and adult patients (ages 16–51) remaining on diet from 3 months to 5 years. Twelve of the 100 patients achieved complete seizure remission on the ketogenic diet, and of those, two relaxed to a less strict diet without food weighing, and maintained seizure control. Seven patients had at least a 90% reduction in seizures, and 37 additional patients experienced significant benefit, giving a 56% response rate (Barborka, 1930). Of the 44 patients who had no improvement, 23 had not achieved ketosis (though some patients with substantial improvement lacked consistent ketosis as well).

In addition to seizure control, Barborka reported an improvement in patients’ cognition, the “appearance of intelligence, more normal attitude,” and decreased irritability.

Twelve of the 56 women had complete cessation of their menses; the seven women who restarted a standard diet had resumption of normal menstrual cycles within a few months. One woman with a history of menorrhagia had normalization of her menstrual cycle.

Across the Atlantic, Dr. C. Bastible studied 29 institutionalized women with epilepsy in Dublin. Their diet included low carbohydrate biscuits made with local Carrigeen moss, “an inexpensive seaweed found off the shores of Ireland . . . which gave excellent results.” Two of the 29 women became seizure-free. Six of the remaining patients had a 50%–90% decrease in seizures, and six had an increase in seizures. Bastible concluded that “there is a definite hope of improvement or cure” for adults with epilepsy (Bastible, 1931).

With the introduction ofphenytoin in 1938, which was more straightforward to initiate and to maintain, the ketogenic diet was used and studied less for the next 7 decades (Jóźwiak et al., 2011).

DEMAND

Children Transitioning to Adult Epilepsy Providers

The ketogenic diet for children is widely used and growing in popularity; in 2013, there were 148 diet centers for children in North America, of which half were started since 2000 (Jung et al., 2015). The ketogenic diet is used in over 40 countries worldwide (Kossoff and McGrogan, 2005). Altogether, it is estimated that there are thousands of children currently on the ketogenic diet. While many children do not continue dietary treatments into adulthood, there is a large and growing population of children who will transition to dietary therapy as adults.

Not all children who are treated with the ketogenic diet require transition to adult epilepsy care. Many children become seizure-free on the ketogenic diet and successfully wean off of the diet within 2 years; however, there is a risk of seizure recurrence with change to a less restrictive diet.

At the Johns Hopkins Ketogenic Diet Center, Martinez et al. reviewed 557 children who started the ketogenic diet between 1993 and 2007 (Martinez et al., 2007). Sixty-six children who were seizure-free discontinued the diet (after median 2.1 years, range 0.5–8 years). Of those, 20% (13 children) had seizure recurrence up to 5.5 years after discontinuing the diet (median: 2.4 years; minimum 0 years). Seven of those patients decided to restart the ketogenic diet. Risk factors for recurrence included an abnormal MRI and EEG with epileptiform abnormalities. Parents and patients with a higher risk of recurrence due to MRI and EEG findings may elect to continue dietary therapy into adulthood.

Children and adolescents with chronic diseases require thoughtful transition from pediatric to adult specialists, generally at age 18; however, discussions and planning for this transition must take place much earlier. Kossoff et al. identified 10 patients who started the ketogenic diet or the modified Atkins diet as children in the pediatric epilepsy center, and who remained on dietary therapy until at least age 18 (the mean age at initiation was 10.3, range 6–16). These patients remained on the diet from 4 to 32 years (mean: 15.5 years). All had good to complete seizure control (2 with 100% seizure control, 8 with 50%–99% reduction) while on dietary therapy. Four patients had previously attempted to reduce the ketogenic diet ratio or increase carbohydrates, with immediate seizure worsening. Eight patients transitioned to adult epilepsy clinics; the oldest patients did so at ages 26 and 43 years (after several years of self-management). Four patients switched from ketogenic diet to modified Atkins diet (MAD; 20 grams per day net carbohydrate limit) with no worsening of seizures. All remained on anticonvulsants. Six patients remained on dietary therapy, five at the Johns Hopkins Adult Epilepsy Diet Center
(AEDC), and maintain good seizure control. At the AEDC, most patients transition to adult providers by 21 years (Kossoff et al., 2013c).

Children and adolescents with specific genetic or mitochondrial conditions represent a population that requires adult dietary therapy, as they age past 18. The ketogenic diet is frequently helpful for mitochondrial disorders. While mitochondrial disorders with onset in infancy or early childhood may be fatal within a few years, those with onset in later childhood may benefit from the ketogenic diet and require transitioning to an adult epilepsy provider familiar with the ketogenic diet (Kossoff et al., 2014).

Glucose transporter type 1 (GLUT1) deficiency is a rare genetic condition, caused by impaired glucose transport into the brain and associated with an abnormality in the gene SLC2A1. The optimum treatment for GLUT1 deficiency is the ketogenic diet, which may be prescribed life-long. It is not known whether GLUT1 deficiency can successfully transition to less restrictive forms of dietary therapy such as the MAD (Kossoff et al., 2014). While more commonly diagnosed in childhood, GLUT1 is also diagnosed in adults. Ninety-one cases of adults with GLUT1 deficiency have been described in the literature, and the ketogenic diet remains a cornerstone of treatment (Leen et al., 2014).

Juvenile myoclonic epilepsy (JME) is highly treatment responsive, with 90% of patients achieving seizure freedom with appropriate antiepileptic drugs (AEDs). However, the remaining 10% have medically resistant seizures. Dietary therapy has been shown to be effective for JME in a small case series. Eight adolescents and adults (ages 15–44, mean 24.3) were started on the MAD for treatment of JME. After 1 month, 7 remained on the MAD; 6 patients (75%) had >50% seizure reduction; after 3 months, 5 had >50% reduction. Two patients became seizure-free (25%). The mean duration on diet at the time of publication was 13.2 months (range, 0.5–40 months). Three patients had increased seizures during brief periods of non-compliance, but returned to seizure control when they reinitiated the diet (Kossoff et al., 2013b). As JME is a diagnosis requiring lifelong treatment, patients with medically refractory JME are a large population of adolescents and adults who could benefit from dietary therapy.

Refractory Epilepsy

Worldwide, there are 65 million people with epilepsy; 30% are medically refractory (Moshe et al., 2015), leaving approximately 19.5 million people in the world with seizures uncontrolled by medications. Many of these patients are not surgical candidates, due to generalized epilepsy (of whom up to 26% may be refractory), multifocal nature, or nonresectable locations of ictal onset.

Patients with seizures resistant to two or more AEDs have a low chance of seizure freedom with additional drugs added. In a longitudinal study of 1,098 newly diagnosed epilepsy patients followed 2–26 years, 49% of patients were seizure-free on the first AED prescribed; an additional 13.2% became seizure-free with the second drug tried, 3.7% with the third AED, and 1% with the fourth; with successive AEDs added or attempted, the percent of patients achieving seizure freedom with each additional AED was less than 1% (Brodie et al., 2012).

In addition to medically refractory epilepsy, the desire to avoid additional AED side effects is a consideration leading patients to pursue the ketogenic diet. Adverse effects from medications (along with psychiatric comorbidities such as depressive symptoms) are the largest predictors of health-related quality of life in patients with epilepsy, much more strongly predictive of patients’ perceived quality of life than seizure frequency. In fact, in a study of 809 adult Italian patients with pharmaco-resistant epilepsy, seizure frequency and the presence of generalized tonic-clonic seizures did not significantly affect quality of life, whereas quality of life declined with increased medication side effects (Luoni et al., 2011). In patients without comorbid depression, adverse medication effects are the main drivers of health-related quality of life (Luoni et al., 2011). The ketogenic diet has the benefit of freedom from many of the adverse effects that can accompany additional medications, particularly cognitive side effects.

Super-Refractory Status Epilepticus

Patients with status epilepticus (prolonged seizure lasting more than 5 minutes, or recurrent seizures without return to baseline) are generally treated with benzodiazepines or other medications; if seizure activity continues despite treatment with intravenous antiepileptic drugs, the condition is termed refractory status epilepticus, and the patient may be placed in a medically induced coma. If status epilepticus continues after at least 24 hours of general anesthetic medications, it is deemed super-refractory status epilepticus (SRSE), which is associated with high morbidity and mortality, with up to 61% mortality reported (Brophy et al., 2012).
The ketogenic diet has been used in children for SRSE since 1999 (Baumeister et al., 2004), and is now used for refractory status of different etiologies in children (O’Connor et al., 2014).

In 2008, the first report of ketogenic diet for SRSE in an adult was published in France (Bodenant et al., 2008). At the University of Pennsylvania, two adults in super-refractory status were then successfully treated with the ketogenic diet, after 20 days and 101 days of seizures, with successful medication weaning at 6 and 11 days following diet initiation, respectively (Wusthoff et al., 2010). Thakur et al. published the largest series of adults, a series at four medical centers of 10 adult patients (median age, 33 years) treated with the ketogenic diet for SRSE, of whom 70% had encephalitis. The diet started after a median of 21.5 days (range, 2–60) and a median of seven antiepileptic drugs were tried (range, 5–13). The SE ceased in all nine patients who achieved ketosis, in a median of 3 days (Thakur et al., 2014).

Dietary therapy is now used as an adjunct strategy for SRSE in both children and adults, and in proposed treatment strategies the recommendation has been made that the ketogenic diet “should probably be tried in all severe cases of super-refractory status epilepticus” (Shorvon & Ferlisi, 2011).

**RESULTS**

**Feasibility, Tolerability, and Adherence**

While the ketogenic diet has been widely used in children in modern times, concerns over adults’ ability to tolerate the diet and maintain ketosis has slowed adoption for use in adults (Swink et al., 1997; Barborka, 1930). Modern studies report a wide range of adherence to the ketogenic diet in adults, 22%–75% at 3 months (Mosek et al., 2009; Klein et al., 2010), with significant variation. The MAD, first published in 2003 as a less restrictive alternative to the classic ketogenic diet (Kossoff et al., 2003), has had published adherence rates of 56%–100% at 3 months and 22%–77.8% at 1 year (Kossoff et al., 2003; Cervenka et al., 2012; Smith et al., 2011). These retention rates are somewhat lower than those seen in add-on drug trials for new AEDs (75%–80% retention after 12–18 weeks; Elger et al., 2007; Ben-Menachem et al., 2007).

The initial decision to begin dietary treatment is not undertaken lightly. In some studies, up to two-thirds of eligible patients screened decline to participate, due to concerns about restrictiveness or complexity of the diet (Mosek et al., 2009, 18/27 declined; Klein et al., 2010, 23/35 declined). However, many people choose to continue dietary treatment beyond the initial study periods requested (Carrette et al., 2008; Klein et al., 2010; Cervenka et al., 2012), and some patients have remained on dietary therapy as long as 32 years (Kossoff et al., 2013c).

A meta-analysis in 2015 comparing six classic ketogenic diet studies and five MAD studies concluded that adherence rates are higher in the MAD (combined compliance rate 56%) than the classic ketogenic diet (38% adherence) (Ye et al., 2015).

Not surprisingly, when dietary treatment is effective, patients are motivated to continue treatment. When patients decide to stop dietary treatment, the most common reason cited is lack of efficacy, followed by restrictiveness of the diet (Kossoff et al., 2008; Lambrechts et al., 2012; Schoeler et al., 2014). Financial reasons have been cited in a few patients due to higher cost of meats compared to processed carbohydrates (Smith et al., 2011).

**Efficacy**

Adults with pharmacoresistant epilepsy have response rates (defined as a ≥50% decrease in seizures) of 33%–54% to the newer antiepileptic drugs (Mbizvo et al., 2012; Elger et al., 2007; Ben-Menachem et al., 2007). Rates of seizure freedom with additional agents are much lower, with each additional add-on agent after the second providing a less than 5% chance of seizure freedom (Brodie et al., 2012). Dietary therapy compares favorably with these rates in most published studies (Payne et al., 2011), especially as the patients starting dietary treatment are typically the most refractory patients, with mean prior AEDs tried ranging from 5.4 to 10.6 (Sirven et al., 1999; Kossoff et al., 2003).

The classic ketogenic diet reduces seizures by ≥50% in 22%–55% of patients, using intent-to-treat analysis (Sirven et al., 1999; Mosek et al., 2009; Klein et al., 2014; Figure 3.1 and Table 3.1). Many patients have even higher response rates, with 8%–27% of patients seeing >90% decrease in seizures (Sirven et al., 1999; Schoeler et al., 2014) and seizure freedom in up to 8% (Klein et al., 2010). In a comparison of seizure-free months, Klein et al. found an improvement from 20% of months seizure-free at baseline to 56.2% of months seizure-free on the ketogenic diet (Klein et al., 2010).

The MAD has wider variability in published response rates, ranging from 12% to 67% with ≥50% seizure reduction (Smith et al., 2011; Kossoff et al., 2008; Kossoff et al., 2013b; Kossoff et al., 2013c) and up to 33% of patients with
>90% reduction (Cervenka et al., 2012; Kossoff et al., 2013b; Kossoff et al., 2013c; Figure 3.1 and Table 3.1).

Other dietary therapies such as the medium chain triglyceride (MCT) diet and the low glycemic index treatment (Pfeifer and Thiele, 2005) have not been widely studied in adults. In a series of 11 patients on the MCT diet (and four on the classic ketogenic diet or a combination of MCT/ketogenic diet during the study) Lambrechts found that 5/12 patients continued the diet at 1 year, and of those, two had a 50%–90% reduction in seizures, while the remaining three patients had a <50% reduction (Lambrechts et al., 2012). The mean AEDs used decreased slightly, from 2.7 at baseline to 2.2 at the end of the diet.

Disproving the initial speculations that adults could not maintain ketosis, the majority of adults on ketogenic diets have been successful at achieving and maintaining urinary and/or serum ketosis (range of published rates, 58.3%–87.5%; Sirven et al., 1999; Klein et al., 2010; Mosek et al., 2009); levels of ketosis have not been predictive of seizure improvement (Mosek et al., 2009; Klein et al., 2010; Nei et al., 2014).

Kossoff et al. found a trend toward patients with more frequent seizures at baseline having a larger proportion of ≥50% response rates (Kossoff et al., 2008), though this has not been detected in other studies (Mady et al., 2003, Mosek et al., 2009).

With regard to seizure type and response to diet therapies, Nei et al. detected a trend toward greater seizure reduction in patients with symptomatic generalized epilepsy, with 64% of symptomatic generalized patients having ≥50% reduction versus 28% of focal epilepsies (Nei et al., 2014). In Mady et al.’s study of 45 adolescents, those with multiple seizure types had a greater improvement than those with complex partial or generalized seizure types alone (Mady et al., 2003). As discussed previously, high response rates and seizure freedom were seen in a small series of adolescents and adults with JME, with 4/6 adults showing >50% decrease in seizures, 2/6 >90% decrease, and 1/6 seizure-free (Kossoff et al., 2013b).

In patients with GLUT1 deficiency, up to 90% of patients were seizure-free on the ketogenic or MAD, including three adults. One adult had resolution of generalized convulsive seizures, but had persistence of likely nonepileptic events; the other two adults were seizure-free (Ramm-Petersen et al., 2013).

Beyond a reduction in the number of seizures, the severity or duration of seizures reportedly
<table>
<thead>
<tr>
<th>Study first author</th>
<th>Year</th>
<th>Journal</th>
<th>Diet</th>
<th>Number of patients</th>
<th>&gt;=50% improvement</th>
<th>&gt;=90% improvement</th>
<th>Retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sirven</td>
<td>1999</td>
<td>Epilepsia</td>
<td>Ketogenic</td>
<td>11</td>
<td>55% (6/11)</td>
<td>27% (3/11)</td>
<td>7/11 (63.6%) at 8 months</td>
</tr>
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<td>Coppola</td>
<td>2002</td>
<td>Epilepsy Res</td>
<td>Ketogenic</td>
<td>5 (18–23)</td>
<td>No age-specific information</td>
<td>No age-specific information</td>
<td></td>
</tr>
<tr>
<td>Mady</td>
<td>2003</td>
<td>Epilepsia</td>
<td>Ketogenic</td>
<td>45 adolescents (12–19)</td>
<td>13/28 (46%); no age-specific information</td>
<td>8/28 (28%); no age-specific information</td>
<td>28/45 (62%) at 6 months</td>
</tr>
<tr>
<td>Groesbeck</td>
<td>2006</td>
<td>Dev Med Child Neurol</td>
<td>Ketogenic</td>
<td>28</td>
<td>No age-specific information</td>
<td>24/28</td>
<td>No age-specific information</td>
</tr>
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<td>Mosek</td>
<td>2009</td>
<td>Seizure</td>
<td>Ketogenic</td>
<td>9</td>
<td>2/9 (22%)</td>
<td>No age-specific information</td>
<td>2/9 (22%) at 3 months</td>
</tr>
<tr>
<td>Klein</td>
<td>2010</td>
<td>Epilepsy Behav</td>
<td>Ketogenic</td>
<td>12</td>
<td>5/12 (42%)</td>
<td>2/12 (17%)</td>
<td>9/12 (75%) at 4 months</td>
</tr>
<tr>
<td>Lambrechts</td>
<td>2012</td>
<td>Epilepsy Behav</td>
<td>KD or MCT</td>
<td>15</td>
<td>2/15 (13%)</td>
<td>No age-specific information</td>
<td>5/15 (33%) at 12 months</td>
</tr>
<tr>
<td>Nei</td>
<td>2014</td>
<td>Seizure</td>
<td>Ketogenic</td>
<td>29 (11–51)</td>
<td>13 (45%)</td>
<td>1/29 (3%)</td>
<td>Mean 9 months</td>
</tr>
<tr>
<td>Schoeler</td>
<td>2014</td>
<td>Epilepsy Behav</td>
<td>Ketogenic</td>
<td>23</td>
<td>9/23 (39%)</td>
<td>2/23 (8%)</td>
<td>9/23 (29%) at 12 months</td>
</tr>
<tr>
<td>Kossoff</td>
<td>2003</td>
<td>Neurology</td>
<td>MAD</td>
<td>3 (18–52)</td>
<td>1/3 (33%)</td>
<td>1/3 (33%)</td>
<td>3/3 (100%) at 3 months</td>
</tr>
<tr>
<td>Carrette</td>
<td>2008</td>
<td>Clin Neurol</td>
<td>MAD</td>
<td>8</td>
<td>1 (12%)</td>
<td>No age-specific information</td>
<td>3/8 (37.5%) at 6 months</td>
</tr>
<tr>
<td>Kossoff</td>
<td>2008</td>
<td>Epilepsia</td>
<td>MAD</td>
<td>30</td>
<td>14/30 (47%)</td>
<td>1/30 (3%)</td>
<td>20/30 (67%) at 3 months</td>
</tr>
<tr>
<td>Smith</td>
<td>2011</td>
<td>Epilepsia</td>
<td>MAD</td>
<td>18</td>
<td>4/18 (22%)</td>
<td>No age-specific information</td>
<td>14/18 (78%) at 6 months</td>
</tr>
<tr>
<td>Coppola</td>
<td>2011</td>
<td>Seizure</td>
<td>LGID</td>
<td>3 adults</td>
<td>3/3 (100%)</td>
<td>No age-specific information</td>
<td>3/3 (100%) at 2 months</td>
</tr>
<tr>
<td>Cervenka</td>
<td>2012</td>
<td>Epilepsia</td>
<td>MAD</td>
<td>22</td>
<td>6 (27%)</td>
<td>4/22 (18%)</td>
<td>14/22 (64%) at 3 months</td>
</tr>
<tr>
<td>Kossoff</td>
<td>2013</td>
<td>Epilepsy Behav</td>
<td>MAD</td>
<td>6 adults</td>
<td>4/6 (67%)</td>
<td>2/6 (33%)</td>
<td>5/6 at (83%) 2 months</td>
</tr>
<tr>
<td>Ramm-Pettersen</td>
<td>2013</td>
<td>Dev Med Child Neurol</td>
<td>MAD</td>
<td>3 adults</td>
<td>2/3 (67%)</td>
<td>2/3 (67%)*</td>
<td>1 year (at least)</td>
</tr>
</tbody>
</table>

* suspicion of PNES in remaining patient not seizure-free
decreased, or the amount of time to recover from a seizure shortened in a subset of patients (Schoeler et al., 2014, Smith et al., 2011).

Some studies have shown that weight loss predicts diet efficacy, with 67% of patients with greater than 0.9 kg/m² decrease in BMI having >50% seizure reduction, compared with 27% of patients with <0.9 kg/m² decrease in BMI, with \( p = .03 \) (Kossoff et al., 2008). However, this is not a consistent finding in all studies (Smith et al., 2011), and patients with weight gain can also respond to the diet (Kossoff et al., 2008).

**Beneficial Effects**

**Cognition and Mood**

Dietary treatment often has positive cognitive and mood effects in studies of adults with epilepsy (Table 3.2). Many patients report an improvement in cognition and mood, as well as (or even despite the lack of) improved seizure control; in fact, some patients with no or <50% improvement in seizure frequency opt to continue dietary treatment for the cognitive benefits alone (Sirven et al., 1999; Coppola et al., 2002). The majority (7/11) of patients in one study of the ketogenic diet saw an improvement in mood and cognition, though 2/11 also reported impaired concentration (Sirven et al., 1999). Increased alertness and energy are common findings, seen in 33%–65% of adults and adolescents on the ketogenic diet (Mady et al., 2003; Mosek et al., 2009; Lambrechts et al., 2012; Schoeler et al., 2014). One study of the MAD that administered detailed cognitive and depression questionnaires found reduction in depression scores and improved concentration in 6/7 patients on diet for 1 month, and in all three patients completing 6 months of the study (Carrette et al., 2008). Quality of life scores tend to rise rather than decrease on both the MAD and the ketogenic diet, though not significantly (Carrette et al., 2008, Klein et al., 2010; Lambrechts et al., 2012). Anxiety, tension, and fatigue may all be improved as well (Lambrechts et al., 2012).

**Weight Loss**

Weight loss is often a desirable effect of ketogenic diet therapies, and many patients are able to lose significant amounts of weight, and may successfully move from a clinically “obese” body-mass index (BMI) to a “normal” or “overweight” BMI. Weight loss is of particular importance in the adult population as it is estimated that over one-third of adults in the United States are obese (BMI ≥ 30 kg/m²; Ogden et al., 2014). Obesity can lead to type II diabetes, obstructive sleep apnea, and metabolic syndrome, all of which can be combated with weight loss. In a series of studies investigating weight loss with the MAD, mean weight loss was 7 kg over 3 months (Kossoff et al., 2008) and 10 kg over 6 months (Carrette et al., 2008), including 4 of the 11 patients who were obese when they started the diet who were no longer obese at the conclusion (Kossoff et al., 2008). In a separate study of the ketogenic diet, mean BMI improved 18%, from 33.8 (obese) to 27.5 (overweight) in 12 adults over 4 months, and the majority of overweight or obese subjects had at least a 10% reduction in BMI (Klein et al., 2010).

When patients are of average weight or underweight when starting diet therapy, total calories can be adjusted to prevent or reverse weight loss.

**Adverse Effects**

**Gastrointestinal**

Gastrointestinal side effects are common, with half to all patients reporting some degree of nausea, constipation, bloating, or vomiting at some point on diet therapy; these generally resolve after the
first few days or weeks of treatment with the ketogenic diet (Sirven et al., 1999; Coppola et al., 2002; Klein et al., 2010). Rarely, patients are unable to continue dietary treatment due to intractable nausea or vomiting.

Lipids
Lipids may increase on ketogenic diets and should be monitored. Sirven et al. found a significant increase in total fasting cholesterol at 3 and at 6 months on diet, with an increase of the mean cholesterol from 208 mg/dL (range, 120–304) to 291 mg/dL (220–395). Triglycerides also increased at 3 months from mean 190 mg/dL (41–542) to 203 mg/dL (68–417), then plateaued (Sirven et al., 1999). The extension of this study continued to show a significant increase in total cholesterol and in the cholesterol/HDL ratio at the time of diet discontinuation after up to 35 months on diet (Nei et al., 2014). If extreme, lipid changes may prompt discontinuation of dietary therapy (Mosek et al., 2009). However, elevated lipids are not present in all patients or in all studies, and triglycerides and LDL may not change (Klein et al., 2010).

Lipids increase on the MAD as well (Carrette et al., 2008), though end lipid levels in some studies remained within average cardiovascular risk ranges (Kossoff et al., 2008, Smith et al., 2011). One study of the MAD found a decrease in triglycerides with dietary treatment over 12 months (Smith et al., 2011).

Lipids may increase during the initial phase of the diet, then return to baseline: one study of 37 adults on the MAD for at least 3 months found that while total cholesterol and LDL had increased at 3 months, there was no difference from baseline after 1 year ($p = 0.2$ and $p = 0.5$, respectively) (Cervenka et al., 2014).

If cholesterol elevation is present, this may be manageable without stopping dietary therapy; one patient whose LDL doubled after 3 months continued the MAD, and with carnitine supplementation and the substitution of saturated fats for polyunsaturated fats saw his cholesterol and LDL return to normal (Cervenka et al., 2012). Carnitine supplementation successfully decreased elevated triglycerides in three patients as well (Nei et al., 2014).

Effects on the Menstrual Cycle
Menstrual irregularities and cessation of menstruation are common in the starvation state. Given that the ketogenic diet is designed to mimic starvation, it is not surprising that it can also cause menstrual irregularity. Barborka reported that 12/56 women had cessation of their menses during ketogenic diet treatment; however, in the seven that stopped the diet, normal menstruation resumed (Barborka, 1930). In Sirven’s 1999 study, all nine women developed menstrual irregularities (irregular cycles or cessation of menses), which resolved on diet discontinuation (Sirven et al., 1999). Menstrual irregularities were also frequent in Mady et al.’s 2003 study of the ketogenic diet (45% of women). Menstrual irregularities seem to be much less common with the MAD: there were no menstrual irregularities in any of the 19 women in one study (Kossoff et al., 2003) and none reported in nine women in a second study (Smith et al., 2011), and they were present in only 1 out of 17 women in a third (Cervenka et al., 2012). Lambrechts found none in two women on the ketogenic diet and two women on the MCT diet (Lambrechts et al., 2012).

Other Side Effects
Long-term effects in patients on the ketogenic diet for 6 years or more (in patients ages 7–23 years) included decrease in growth rate: at diet initiation, 14/28 (50%) were at or below the 10th percentile for weight, which increased to 23/28 (82%) at last follow-up (Grosbeck et al., 2006). Growth restriction was not related to degree of ketosis and is less of a concern in patients who begin ketogenic diets as adults. In one study, one-quarter of patients on ketogenic diets developed kidney stones, with a median of 2 years after diet onset (Grosbeck et al., 2006). Subsequent studies have shown that urine alkalization with potassium citrate reduces the risk of kidney stones (Sampath et al., 2007). Six patients in the long-term study (21%) had skeletal fractures, occurring a median of 18 months after diet initiation (Grosbeck et al., 2006).

Kidney stones have not been reported in other studies of adults on dietary treatment. One patient had a jaw fracture related to a seizure and stopped the diet (Mosek et al., 2009), but other skeletal fractures have not been reported.

CONCLUSIONS
Dietary treatment is feasible in adults and often highly effective, with seizure reduction rates in medically refractory populations of 33%–67%, comparable with response rates in children. A significant proportion of patients may become seizure-free. In addition to seizure reduction, patients may also benefit from improved mood
and cognition, as well as intentional weight loss. Lipids should be monitored, but in most cases persistent lipid elevations can be managed with diet adjustments. Diet adherence remains a major challenge for adults.

REFERENCES
Chapter 3: Dietary Therapy in Adults


How Do You Implement the Diet?

A. G. CHRISTINA BERGQVIST, MD

INTRODUCTION
The ketogenic diet (KD) has survived and thrived for almost 100 years as an effective treatment of intractable epilepsy. For many years the implementation of the diet remained in large parts unchanged from its initial conception by Wilder (Livingston, 1951; Wilder, 1921). Management was based on our clinical practice, strongly tied to its history of “fasting, calorie and fluid restrictions.” As the use of the ketogenic diet increased locally in the United States and spread across the world, and side effects became better understood, many of the standard practices were questioned and some were changed or discontinued (Kossoff and McGrogan, 2005). As a result, alternative implementation of the classic KD and creation of newer diets have emerged. Most of the data analyzed consist of retrospective chart reviews but there is some support from randomized trials. In 2008 a statement regarding the management of the KD was written by 28 authors from nine countries. This served as a basis from which future improvements to dietary therapies were made (Kossoff et al., 2009). This document, the International Consensus Statement for Ketogenic Diet, is referred to as the ICSKD in this chapter. There are multiple books detailing site-specific implementation practices of the KD (Freeman et al., 2007; Kossoff et al., 2011b; Snyder, 2006; Stafstrom et al., 2008). It is not within the scope of this chapter to provide the same detail; instead I discuss the implementation changes that have occurred in the 100-year history of the KD.

NEED FOR A FAMILY-CENTERED, TEAM-BASED APPROACH TO DIETARY THERAPIES
The KD is a prescribed medical diet therapy requiring supervision from a healthcare team. It should never be confused with weight loss diets that individuals can manage safely themselves. Dietary therapies for epilepsy require periodic physical examination and laboratory testing to remain safe, promote general health, and prevent side effects. This is particularly true in children, whose nutritional needs are continuously changing during childhood and adolescence. Technology has changed much of the interaction between the care provider and the KD team, and allows for long-distance communication. Communication with the team via phone, e-mail, and electronic medical records (such as EPIC) is necessary when managing the ketogenic diet. However, it is not a replacement for a follow-up visit with the KD team.

The ICSKD recommends that patients be evaluated at least four times in the first year of treatment and twice a year thereafter. Younger children, particularly infants, need more frequent (even monthly) evaluations to keep up with their nutritional needs.

COMPOSITION OF THE KETOGENIC DIET TEAM
The ketogenic diet is best supervised by a team of experienced healthcare professionals. For a small program, this comprises, at a minimum, an epileptologist and a dietitian. For truly comprehensive care and for a larger program, this team is expanded to also include a nurse and social worker. Each team member contributes unique knowledge to optimize the care for these children and will grow as the number of patients in the dietary treatment program increases. The latest addition to our team at the Children's Hospital of Philadelphia is a chef to assist with creation of recipes in our in-patient KD kitchen (Fenton et al., 2014; Groveman et al., 2014). Any institution that supports dietary therapies must also provide pharmacy services to find medications with the lowest carbohydrate content, a very important task, as the content in medication is not routinely reported by industry and is subject to frequent change.
Access to other subspecialties such as gastroenterology and nutrition, nephrology, urology, endocrinology, a feeding team, and bone health experts is also essential to provide truly comprehensive care. The children started on these diets today rarely have just “treatment resistant epilepsy” but carry many additional diagnoses: cerebral palsy, developmental disability, intellectual disability, feeding difficulties some with tube dependencies, behavioral difficulties, autism spectrum disorder, and genetic conditions. Adding the KD to their treatment regimen makes their care truly complex. Parents are important partners, and essential to the success of the diet and a keto program. They can be effective coaches of other parents, assisting them with nonmedical information, and also become trained educators as the keto-community grows to include school, nursing agencies, and so forth (Chee et al., 2014; MacCracken and Scalisi, 1999). Creating a keto community makes it possible to provide comprehensive care.

PREDIET EVALUATION(S), EDUCATION AND COUNSELING

For families considering the KD, education about dietary therapies is provided in various forms: reading materials; referrals to Internet sites; foundations that advocate for the KD such as the Charlie Foundation for ketogenic therapies (www.charliefoundation.org), Matthews Friends (www.matthewsfriends.org), and the Epilepsy Foundation (www.efa.org); DVDs, and so on. Some dietary treatment programs offer separate education classes to assist the families in their decision to try dietary therapies (e.g., the Children’s Hospital of Philadelphia [CHOP], www.chop.edu/treatment/ketogenicdiet)

The KD requires the body to switch from using carbohydrates as the primary energy source to using lipids. There are some disorders for which the use of the KD or fasting can lead to significant morbidity, even mortality. These include inborn errors of metabolism related to carnitine (mitochondrial transport), beta oxidation defects, pyruvate carboxylase deficiencies, and porphyria (which requires a high-carbohydrate diet). As a group, mitochondrial cytopathies are in their own category, as some may benefit from KD therapies, such as pyruvate dehydrogenase deficiency (Di Pisa et al., 2012; Weber et al., 2001; Wijburg et al., 1992), while for other cytopathies the KD would worsen the condition (Bergqvist, 2004; Horvath et al., 2008; Kang et al., 2007). Assistance from metabolic or neuromuscular specialists before attempting the KD in these disorders is recommended. A metabolic screen is recommended for any child for whom the KD is considered. The ICSKD suggests that this include acylcarnitine esters, urine organic acids, serum amino acids, lactate, and pyruvate. For screening labs that are followed every 3–6 months while on the KD treatment, see the ICSKD.

INITIATION OF KETOGENIC DIET

Initiating the KD can be intimidating to the family, caretaker and KD Team alike. A well prepared admission and a well educated parent is the best assurance for success. In the following section I will review the various types of initiation methods that have been used and discuss both their benefits and drawbacks.

In-Patient versus Out-Patient Initiation

The classic ketogenic diet created by Wilder, and advocated by Johns Hopkins University (Freeman et al., 2007) is started in the hospital so that the patient can be closely observed, monitored, and treated if needed. Most centers in the United States will use an in-patient setting to start the KD, as this makes it possible to advance the KD relatively quickly and achieve “ketosis” within a few days (Kossoff et al., 2009). The child is closely observed by nursing staff and physicians, and interventions for hypoglycemia, acidosis, dehydration, vomiting, weight loss, or feeding intolerances can be instituted in a timely fashion to minimize any complications. Although seizures more often improve during the admission they can worsen from the stress of switching metabolic substrate. In this situation the in-patient setting allows for immediate adjustments in medication and ICU care, should it be needed. While the family is in the hospital, many hours of direct teaching—“hands-on” education—is provided by the KD team. Many centers admit one child at a time to start the KD. The KD team is on 24/7 permanent call to initiate the KD. Organization of the admissions to a monthly basis, using a “small group setting” for teaching, improves efficiency and frees up the team’s time to assist with out-patient management. The Children’s Hospital of Philadelphia now also provides cooking classes in its keto-kitchen as part of starting the KD (Grovenman et al., 2014). These classes provide hands-on experience with the KD food before leaving the hospital, and improve chances of acceptance and success with the KD at home. Finally, although inborn errors in fatty
acid oxidation are rare, screening is not infallible. We have picked up a handful of children with beta-oxidation defects during admission in the past 20 years. It is more likely that these children’s defects would have been missed, and resulted in significant morbidity had the diet been started as an out-patient.

Out-patient initiation of the KD can be successful, and in some countries it is the standard of care (Neal et al., 2008; Rizzutti et al., 2007; Vaisleib et al., 2004). The out-patient advancement of the KD is in general slower, often over several weeks before a “full KD is achieved.” The centers that use out-patient initiation of the KD must have a flexible, available staff, and the ability to schedule these patients for frequent out-patient visits to make sure the diet is proceeding as expected and that the child is safe. Transition into ketosis is not directly observed. Any issues after hours must be directed to the emergency department. The amount of education provided is limited and it requires that the family live near the epilepsy center to minimize time traveling. Benefits with an out-patient initiation include starting the KD in the comfort of the child’s own home and a cheaper overall cost. However, centers that use out-patient initiation often have a higher dropout rate before the 3-month mark when effectiveness is typically determined, perhaps due to the above factors (Levy et al., 2012).

**THE CLASSIC KETOGENIC DIET**

The classic ketogenic diet begins with fasting, and only fluids excluding carbohydrates are consumed. The duration of fasting varies. In the initial protocols from the 1920 to the 1930s fasting was commonly extended until 10% of body weight was lost (Livingston, 1951; Wilder, 1921). The actual time centers fast their patients has decreased, but 12–72 hours is often implemented, or “until ketones are large.” The KD is then started at the full 90% fat composition and at a third of the calories, advanced daily, until the full calorie meal is tolerated.

**Fasting**

The KD has a strong historical tie to fasting. The father of medicine, Hippocrates prescribed fasting for his epilepsy patients. It is described in the Bible (Mark 9:29), and was used in the early 1900s in a cyclic fashion (for several weeks at a time) as treatments for patients with epilepsy (Wheless, 2008). The KD was created to mimic the metabolic changes that occur when we fast: lowering of the blood glucose and insulin levels, utilization of the body’s glycogen stores, slowing of the flux through the glycolytic pathway, and finally utilization of our fat stores via beta oxidation (Cahill, 1970; Cahill and Owen, 1970). In the process of breaking down fat, ketone bodies are produced and transported into our central nervous system for direct use in energy production or indirectly affecting a myriad of metabolic pathways leading to the “miracle of seizure reduction” (Lutas and Yellen, 2013). Ketone bodies are acidic, therefore, while fasting or while on the KD, the overall “acid load” is increased. Ketone bodies also suppress our appetite and it is often difficult to get a lethargic, acidic, dehydrated child to eat a 90% fat meal without vomiting. For children, particularly young ones, this could become an issue that prolongs admissions and worsens morbidity, and it frankly deters some families from trying the KD. Many centers have used a “kinder, gentler, gradual” advancement without fast initiation. Wirrell (Wirrell et al., 2002), first described their success with this approach in a retrospective case series of 14 children. The Korean group (Kim et al., 2004) stopped fasting all of their patients. They instead used the gradual caloric advancement approach of the 4:1 ratio and reported similar success in seizure reduction at 3 months in 41 patients compared with 81 historical fasting controls. Finally, a randomized prospective trial compared the classical fasting KD with a gradual initiation approach (Bergqvist et al., 2005). In 48 patients using equivalence testing, the gradual approach (1:1, 2:1, 3:1, 4:1, daily advancement, full calorie KD) was equally effective at reducing seizures at 3 months, compared with the classical KD protocol. Both protocols achieved strong ketosis by the 5th day discharge, the gradual protocol about 1 day later than the fasting. Side effects were reduced by about two-thirds, and interventions were significantly fewer in the gradual protocol, with less weight loss, mild and severe hypoglycemia, dehydration, acidosis, need for bicarbonate and intravenous fluid administration. Vomiting was not quantified but rather reported as present or not, and occurred in both groups. Additional days needed in the hospital were also reduced in the gradual group compared with the fasting group, with a dropout rate of 4% compared with 8% in the fasting group (very low, overall) (Bergqvist et al., 2005). With this data in hand many centers stopped the fasting process for their routine admission and use it only when speed of achieving ketosis is of the essence, as with a child in status epilepticus (Cobo et al., 2015). The gradual initiation protocol has been modified,
individualized to fit the child better. The CHOP protocol has eliminated the 1:1 ratio, instead using the (2:1, 3:1, 4:1) approach. Many centers do not advance to a full 4:1 ratio, depending on the severity of the child’s epilepsy, frequency of seizures, and the response to lower ratios (Seo et al., 2007). The ICSKD considers fasting as an option, but no longer necessary (Kossoff et al., 2009).

**Liquid/Formula versus Food**

Ketogenic diet formulas have been created for children who have gastrostomy tubes, and for infants. In an attempt to shorten the admission time, to quicken the acceptance of the high-fat KD, some centers use a formula or liquid eggnog to initiate the KD with similar success. Some centers have reported higher overall seizure reduction with the formula-fed children, perhaps due to lower chance of noncompliance (Kossoff et al., 2004). The problem with not modeling a home situation during the admission is that families are discharged having had no experience or assistance cooking 90% fat meals that are palatable and have to attempt this by themselves, be it with the support of websites, and other keto-coaches once home.

**Caloric Restriction**

**Initiation**

Caloric restriction was traditionally part of the KD initiation, with the calories gradually advanced during the initiation after the fast (1/3, 2/3, full calorie 90% fat diet as tolerated). Bansal et al. found better effectiveness at 3 months in 30 children started on full calorie 4:1 KD without fast, compared with 30 historical controls, but no difference in side effects or interventions, perhaps related to the lack of gradual adjustment to the 90% fat diet in their protocol (Bansal et al., 2014).

**Maintenance**

Calories are important to a growing child, whose needs are continuously changing during infancy and childhood. Calories are controlled on the KD until the dietitian changes the prescription. Clinical practice from KD centers tells us if a child is given excessive calories and gains weight too quickly, it is associated with less reduction in seizures and lower ketosis, until ideal body weight is achieved (Freeman et al., 2007). This has not been tested in any trial. The calories are traditionally determined by a weighed 3-day dietary record provided by parents before the KD is started. Comparison of this caloric estimate to age and gender RDA, estimated or measured resting energy expenditure (REE) and an activity factor. Children with epilepsy are less active than healthy children and those who have additional motor disabilities may need even fewer calories (Wong and Wirrell, 2006). All these factors makes it difficult to estimate calories for a KD plan.

Two studies have actually measured REE (an estimate of the basal metabolic rate) in children treated with the KD. In a short-term 6-month study the KD did not change REE in 18 children, but change in the respiratory quotient (RQ) correlated with seizure reduction (Tagliaabue et al., 2012). In a longer, 15-month, prospective trial of 24 children treated with the KD compared with 75 age-matched controls, linear growth status declined while weight status and REE were unchanged; REE remained reduced in children with CP (Groleau et al., 2014). Further, although the children gained weight as calories were adjusted, this weight gain came in the form of a change in body composition and relative increase in fat mass. That is, “you become what you eat.” The increased calories did not prevent the height deceleration seen in these children, a long-term side effect now well established in several prospective studies of the KD (Bergqvist et al., 2008; Nation et al., 2014; Spulber et al., 2009; Vining et al., 2002; Williams et al., 2002; see chapter on side effects). In summary, caloric restriction is not needed for initiation or maintenance of the KD. Normal growth during the treatment period is encouraged, but the KD appears to alter body composition and caloric adjustments do not prevent reduction in height velocity (growth failure), which maybe IGF-1 mediated.

**WHEN DO WE DETERMINE EFFECTIVENESS OF THE KD?**

Almost all efficacy trials related to dietary therapies determine initial effectiveness at 3 months. This is likely due to comparisons with anticonvulsant drug trials, where a 3-month design is standard practice (Sachdeo, 2007; Schmidt et al., 2014). Do we really need to stay on the diet that long to determine whether it works? Can we stop sooner, or perhaps should we stay on the diet longer? To answer these questions, Johns Hopkins University and Children's Memorial Hospital in a combined study looked at when seizures began to reduce on the ketogenic diet (Kossoff et al., 2008) in a retrospective analysis of 99 children started on a 4:1 fasting KD or 3–4:1 gradual KD protocol. They found that the median time to first improvement was 5 days, with a range of 1–65 days. Reduction in seizures occurred sooner in the children who were fasted compared with a gradual protocol, but
long-term effectiveness was not different between the two initiation protocols, confirming prior studies. Five subjects did not began to have any change in their seizures until day 60 or more. Then 4/5 had >50%–90% reduction in seizures and one became seizure-free. They concluded that if no change is seen in seizures frequency by 2 months, the KD could be discontinued and that there was no need for the full 3-month trial period. However, the data can also be interpreted to confirm the use of a 3-month minimal trial period of the KD. A significant improvement including seizure freedom in a 5% treatment resistant epilepsy cohort is not insignificant!

SEIZURE FREEDOM

Seizure freedom is the goal for majority of patients trying the KD. In the short-term randomized trials it has been reported in 15%–55% (Martin et al, 2016). For longer-term studies the data presented is not always a continuous variable, instead often obtained just prior to the time of interest, thereby artificially inflating the percentage reported as seizure-free. This makes it difficult to interpret the data and counsel our families appropriately. Taub et al. tried to answer this question in a retrospective chart review of 275 patients started on the KD, of whom 65 children (24%) achieved seizure freedom (defined as no seizure for 28 days) (Taub et al., 2014). All children had daily seizures before starting the KD. The median time to becoming seizure-free was 1.5 months, 72% became seizure-free before 3 months, (considered early seizure-free), while 28% became seizure-free after 3 months, (considered late seizure-free). The longest time to becoming seizure-free was 18 months in one subject. Seizure recurrence (defined as any seizure after having been seizure-free for 28 days) occurred in 84%, and median time to recurrence was 3 months. However, 60% of those who recurred still had less than one seizure per month. Timing of discontinuation of the AED did not affect return of seizures. In general, for those who became seizure-free and recurred, their seizures did not return to their prediet frequency. The chance of remaining seizure-free at 18 months was only 3%. Families should be counseled that seizure freedom may occur at some point during KD treatment, most often in the first few months. If it happens, recurrence is more common rather than complete remission. In general, seizures remain greatly improved and do not return to the frequency before the KD was started.

RATIOS: DO THEY MATTER?

As the KD practices were questioned and clinical protocols changed, less restrictive diets emerged: the Modified Atkins diet (MAD) (Kossoff et al., 2003), or unlimited protein diet, as it is called in Europe, and the low glycemic index diet (Pfeifer and Thiele, 2005). These diets have become popular alternatives to the KD because of their less strict nature, less need for resources, possibly milder side-effects and claims of “similar” effectiveness to the KD. Some have even suggested that the modified diets should replace the formal KD for the majority of our treatment-resistant patients (Auvin, 2012; Miranda et al., 2012). What data do we have to support this claim? Both diets are lower ratio KD, MAD at most a 2:1 if tightly controlled and LGIT a 1–1.5:1 ratio.

Animal data to date suggest that higher ratios are more effective in controlling seizures, although the ratios used in rodent models (4–6:3:1) are often much higher than what we would use in humans (Bough et al., 1999; Bough et al., 2000; Nylen et al., 2005). Some clinical data to compare ratios have been randomized, but not blinded. Seo et al. compared a 3:1 with a 4:1 KD in 76 randomized patients, and seizure reduction was measured at 3 months. They found that the 4:1 ratio KD reduced seizures more effectively and that there were significantly more seizure-free patients in this group than the 3:1 group (Seo et al., 2007). At 3 months, they crossed the two protocols for an additional 3-month extension. Children who were seizure-free at the 3-month mark on the 4:1 maintained their seizure freedom on the lower ratio, indicating that perhaps a lower ratio can be used later in the KD treatment without sacrificing effectiveness. El-Rashidy et al. studied 40 patients with symptomatic treatment-resistant epilepsy randomized to MAD, 4:1 KD formula, and standard diet continued medical treatment. They found that the 4:1 formula at 3 and 6 months resulted in better seizure reduction than the MAD and regular diet (El-Rashidy et al., 2013). Raju et al. randomized 38 children to a 4:1 versus 2.5:1 KD and measured effectiveness at 3 months. There was no significant difference in responders or seizure-freedom rate at 3 months (58% vs. 63% and 26 vs. 21%) (Raju et al., 2011).

Kossoff et al. looked at MAD versus MAD with liquid 4:1 supplement in the first month and found that the liquid supplement (i.e., higher ratio) had a better reduction in seizures at 3 months (Kossoff et al., 2011a). In a multicenter study, 27 patients from several countries were identified who had
not responded completely to the MAD and who were switched to the KD. Ten had further improvement, 11 did not. Children with myoclonic atonic epilepsy appeared to particularly improve with the formal KD (Kossoff et al., 2010). In summary, current data animal and human-based studies, some prospective and randomized but none blinded, indicate that higher ratios and the formal KD are more effective at reducing seizures than the modified diets, at least in the short term. It is premature to routinely discard the KD in favor for the modified diets. There are situations where the modified diets are more appropriate (see chapter on modified diets). When choosing the MAD, families should be counseled that these less restrictive diets might come at the expense of less reduction in seizures. A blinded randomized trial with long-term follow-up to assess retention of seizure control and seizure-freedom rate is needed to definitively answer this question.

**PREDICTOR OF RESPONSE**

The KD appears to work particularly well in some electroclinical epilepsy syndromes. However many patients do to fit into one of these categories, therefore a predictor of response to the dietary therapies would be very helpful when trying to guide parents about staying on the KD or not. In the multiple observational studies published there were no demographic parameters that predicted response (such as age, gender, seizure type).

The electroencephalogram however, appears to be a helpful tool. Kessler et al. in a prospective blinded study of 37 patients on the fast or gradual KD initiation protocol assessed the routine EEG at baseline, 1 and 3 months into KD treatment. The EEGs were analyzed for background slowing, interictal epileptiform discharges (IED), power spectrum analysis, and manual spike index. They found that 70% of the patients became responders, with 27% seizure-free. Over time the EEG background slowing improved and there was an overall increase in beta, but this change did not predict responder status. Intertial epileptiform discharges were reduced by 65% by the 1-month EEG. The 1-month reduction in IED strongly predicted response status to KD with odds ratio of 4.8 (95% CI 1.1–21.3, \( p = .03 \)). They concluded that the routine EEG is an excellent predictor of response and can assist neurologists when counseling and encouraging parents about staying with the KD in the first 3 months of therapy (Kessler et al., 2011). Ebus et al. confirmed these findings via 24-hour video-EEG in 34 patients on at baseline 6 weeks and 3 months into the KD. They had a low response rate overall, only 26%, but did notice a proportional reduction in IED of 30% during sleep strongly predicted reduction in seizure or response status (Ebus et al., 2014).

**DURATION OF TREATMENT**

The KD was initially created for a 2-year treatment period and a 6- to 12-month wean. However, there is no definitive treatment period, and it can be individualized based on the child’s underlying condition and response. There are some electroclinical epilepsy syndromes, such as infantile spasms, where if the patient responds and becomes seizure-free, the diet treatment period may be shortened to 6–12 months, particularly if the EEG has normalized (Kossoff et al., 2002). For others, the KD has resulted in such improvement in the child’s overall quality of life, not only reduction in seizures but also removal of all or most medications, that the family chooses to stay on the KD longer (Hallbook et al., 2007). Most centers have a few patients who have been on the KD for >15 years (Kossoff et al., 2007). Often the ratios in the KD in this group of patients have been weaned to the lowest ratio that successfully maintains seizure control, but attempts to come off the KD have resulted in return of seizures. Continued diet use of dietary therapies has to be weighed against the risk and complications and long-term side effects of a high-fat diet (Zupec-Kania and Zupanc, 2008; see chapter on side effects). Patients who are on the KD for life, due to their underlying inborn error of metabolism such as Glut1 syndrome and pyruvate dehydrogenase disorder require particular care in management of their diets (see chapter about Glut1).

**REFERENCES**


Chapter 4: How Do You Implement the Diet?


Glut1 Deficiency and the Ketogenic Diets

JOERG KLEPPER, MD, PHD

GLUT1 DEFICIENCY

In the fed state the human brain relies entirely on glucose for energy metabolism. Glucose entry into the brain is exclusively mediated by the facilitated glucose transporter protein Glut1. Impaired glucose transport into the brain resulting from Glut1 deficiency (Glut1D, OMIM 606777) (De Vivo et al., 1991) will cause a cerebral “energy crisis,” particularly as the developing brain requires 3–4 times more energy than the adult brain. Clinical features are global developmental delay, early-onset epilepsy, and a complex movement order (Klepper and Leiendecker, 2007). As such, the disease mechanism of Glut1D is intriguingly straightforward:

1. The Glut1 defect results in low CSF glucose concentrations, termed hypoglycorrhachia.
2. Hypoglycorrhachia results in an epileptic encephalopathy and movement disorder.
3. Glut1D is treatable by means of a ketogenic diet providing ketones as an alternative fuel for brain energy metabolism (Figure 5.1).

DIAGNOSIS

The diagnosis of Glut1D rests on (1) low glucose concentrations in CSF, termed hypoglycorrhachia, determined via fasting lumbar puncture, and (2) mutations in the SLC2A1 gene (De Giorgis and Veggiotti, 2013). The lumbar puncture should be performed in a metabolic steady-state, for example, following a 4- to 6-hour fast. In Glut1D, CSF glucose concentrations usually are below 40 mg/dL. A CSF/plasma glucose ratio below 0.45 is helpful to determine hypoglycorrhachia, and CSF lactate should always be low to normal (for age-dependent reference values of CSF glucose and lactate, see Leen et al., 2013b). Patients with milder phenotypes do have CSF glucose values above 41 mg/dL but never normal (De Vivo and Wang, 2008). Mild or borderline hypoglycorrhachia associated with missense mutations are often present in extended phenotypes and indicate that a SLC2A1 analysis should be performed along with a diagnostic lumbar puncture (Weber et al., 2008). In approximately 85%–90% of cases the condition can be confirmed by heterozygous mutations in the SLC2A1 gene. Of note, the absence of SLC2A1 mutations does not exclude the diagnosis (Klepper, 2013). Inheritance is predominantly autosomal dominant and mutations are mostly de novo, but individual cases of autosomal recessive transmission have been described (Brockmann et al., 2001; Klepper et al., 2009; Klepper et al., 2001). Prenatal testing is available if the disease-causing mutation has been identified. Neuroimaging is generally uninformative, but PET scanning has been reported to show a distinctive metabolic footprint in brain (Pascual et al., 2002). Further diagnostic tools such as glucose uptake assay into erythrocytes are available on a research basis only (Klepper et al., 1999).

CLINICAL PRESENTATION

Classic Glut1D presents with unspecific developmental delay associated with early-onset epilepsy, a complex movement disorder, and paroxysmal nonepileptic events. Any combinations of these features are possible (Figure 5.2) (Pearson et al., 2013). Severe cases develop secondary microcephaly, reflecting the impairment of the developing brain. Seizure type may vary from cyanotic attacks and staring spells in early infancy to childhood absence epilepsy and myoclonic/generalized grand-mal seizures in later childhood. Certain epilepsy syndromes such as early-onset absence epilepsy (onset < age 4 years) and myoclonic astatic epilepsy (Doose syndrome) are associated with Glut1D (Mullen et al., 2010; Suls et al., 2008). The complex movement disorder in Glut1D features ataxic-spastic and gait abnormalities, action limb dystonia, mild chorea, cerebellar action tremor, myoclonus, and dyspraxia (Pons
et al., 2010). In adults, exertion-induced dystonia, and stomatin-associated cryohydrocytosis (Flatt et al., 2011; Weber et al., 2008), a rare form of hemolytic anemia, have been identified as allelic diseases associated with SLC2A1-mutations.

**FIGURE 5.1** The metabolic concept of Glut1 Deficiency.

➊: the defect of the Glut1 transporter at the blood-brain barrier results in cerebral energy failure

➋: Ketones derived from either body fat (fasting) or nutritional fat (KDT) serve as an alternative fuel to restore cerebral energy.

**KETOGENIC DIET THERAPIES IN GLUT1D**

Currently ketogenic diet therapy (KDT) is the only treatment for this disorder of brain energy metabolism. The metabolic concept is to mimic

**FIGURE 5.2** The complex phenotype of Glut1 deficiency (with permission from Pearson et al., 2013).
the metabolic state of fasting and thus “refuel” the brain using ketones from the KDT (Figure 5.1). Anticonvulsant mechanisms described for KDT in intractable childhood epilepsy (Masino and Rho, 2012) also apply in Glut1D and contribute to seizure control. In addition, KDTs provide unspecific positive effects such as improved physical endurance, improved attention span, and alertness (Lambrechts et al., 2012; Rampp-Pettersen et al., 2014) and have a substantial positive impact on the associated movement disorder (Leen et al., 2013a).

However, KDTs for Glut1D differ from KDTs for intractable childhood epilepsy in the following aspects:

- Ketones in Glut1D provide an alternative fuel for the brain, restoring the cerebral energy deficit. The higher the ketosis, the more energy is provided for the developing brain. Within this concept the KDT providing the highest ketosis is best for the patient.
- Introduction of KDT should be as early as possible, even in infancy, to meet the energy demands of the developing brain.
- KDT in Glut1D should be continued throughout childhood into adolescence to provide ketones as an alternative fuel for adequate brain development. Discontinuation of KDT after approximately 2 years, as discussed in intractable childhood epilepsy, is not recommended in Glut1D patients.

The practical approach to KDT in Glut1D does not differ substantially from KDT for intractable childhood epilepsy (Klepper, 2012). Clinical management such as initiation, supplementation, and maintenance of KDT follow established guidelines (Kossoff et al., 2009). In theory, any KDT providing measurable ketosis may be used in Glut1D according to age, clinical presentation, and compliance. In practice, long-chain and medium-chain KDT and the MAD are established therapies for Glut1D, whereas the low glycemic index treatment is not.

Certain aspects are special to KDT in Glut1D. In contrast to the efficacy of KDT in the treatment of childhood epilepsy the vast majority of patients with Glut1D show an immediate and continuing response. This is even considered a diagnostic tool: if KDT is ineffective, reconsider the diagnosis. Long-term adverse effects of KDT such as growth retardation, prolonged QT interval in ECG, selenium deficiency, renal stones, elevated serum lipids, and atherosclerosis are serious concerns in Glut1D—long-term data is only just emerging to assess these questions. Emerging tools for follow-up screening are dexta-scans for bone density (Bergqvist et al., 2008) and ultrasound of the carotid arteries for atherosclerosis (Coppola et al., 2014; Kapetanakis et al., 2014). On follow-up into puberty, recommendations to maintain KDT in Glut1D throughout childhood and adolescence often are particularly difficult. Achieving compliance after years on KDT often is the most important practical problem in this age group.

Novel compounds such as ketone-esters are currently discussed to supplement or even replace KDT in childhood epilepsy and metabolic disease. Triheptanoin-based metabolic therapy and the concept of anaplerosis are discussed elsewhere in this book (see ; Borges, chapter 34). Clinical trials and experimental data in Glut1-deficient mice may offer novel approaches toward Glut1D therapy.

**OPEN QUESTIONS**

Clinical symptoms of Glut1D appear to be age-dependent. Epilepsy is the prominent clinical feature in infants and children, but it often stabilizes whereas movement disorders and paroxysmal events increase with age and seem to be the main clinical problem in adolescence and adulthood (Alter et al., 2015). Here the impact of KDT is less clear, although response to classic KDT and MAD have been described for Glut1D. In a small subgroup of patients with confirmed Glut1D symptoms do not respond adequately to KDT. For reasons yet unclear, epilepsy remains uncontrolled and movement disorders, in particular paroxysmal events, increase despite KDT.

The use of KDT in adult Glut1D seems reasonable, but is by no means an established recommendation—patients find it very difficult, symptoms such as epilepsy seem to stabilize in adults, and there is simply not enough data on the benefit and long-term adverse effects of KDT in this age group (Leen et al., 2014). The use of KDT in Glut1D allelic diseases such as paroxysmal exertion-induced dystonia, early-onset absence epilepsy, or stomatin-deficient cryohydrotaxis also remains unclear—originally considered an exclusively pediatric disorder, Glut1D has now moved into the focus of adult neurology.

**ABBREVIATIONS**

<table>
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<tr>
<th>Abbreviation</th>
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<tr>
<td>Glut1D</td>
<td>Glut1 Deficiency</td>
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<td>PED</td>
<td>Paroxysmal exercise-induced dystonia</td>
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CONFLICTS OF INTEREST
The author has received speaker honoraria and travel funds from Nutricia GmbH, SHS, Heilbronn, Germany, and has received project support from the parents support group Glut1-Förderverein, Bremen, Germany.

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Chapter 5: Glut1 Deficiency and the Ketogenic Diets


Ketogenic Diet in Established Epilepsy Indications

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INTRODUCTION
Arising from biblical anecdote and early 20th-century explorations into the effect of fasting for seizure management, the ketogenic diet (KD) developed alongside medical knowledge of the biochemistry of fasting/starvation and ketosis (Whelless, 2014). Falling into relative disuse with the arrival of phenytoin and subsequent anticonvulsant medications, it continued to be used mainly in pediatrics, and primarily under the auspices of Dr. John Freeman and colleagues at Johns Hopkins University. After the development of waves of new anticonvulsant medications that were associated with reduction in toxicity but not with greater efficacy in patients with refractory epilepsy, interest in the KD reemerged. Over the years, multiple case series, describing cases achieving remarkable and sustained efficacy in a substantial proportion of children with the most intractable epilepsies (>400 seizures/month) had been published from the Johns Hopkins group (Hemingway et al., 2001). With the renewed interest, a multicenter group showed that other centers using the same protocol could achieve similar results (Vining et al., 1998), and finally a randomized controlled study was conducted in London, again supporting the efficacy of this treatment in children with refractory epilepsies (Neal et al., 2008). This chapter reviews diet treatment in epilepsies where its use is well established—refractory nonsurgical epilepsies and epileptic encephalopathies: Lennox Gastaut syndrome, infantile spasms, myoclonic astatic epilepsy (Doose syndrome), and severe myoclonic epilepsy of infancy (Dravet syndrome).

REFRACTORY NONSURGICAL EPILEPSY
This broad group, defined by nonsuitability for a potentially curative surgery, includes lesional and nonlesional, focal, multifocal, and secondary generalized epilepsies. Etiologies include remote symptomatic injuries (pre-, peri- and postnatal brain injuries of hypoxic, traumatic, infectious, hemorrhagic origin), developmental brain malformations, genetic/metabolic disorders causing epilepsy as their primary problem, or resulting in symptomatic epilepsy, primary and secondary epileptic encephalopathies and progressive/degenerative epilepsies. In this era of molecular specificity, it seems counterintuitive to dwell on such a heterogeneous group. And indeed, increasing surgical prowess and advancing ability to define specific genetic etiologies and offer individualized molecularly based therapies will continue to “chip away” at this broad group. However it is in precisely such a heterogeneous group that the ketogenic diet’s efficacy was first described. And, in epilepsy practice, patients presenting with refractory epilepsy constitute exactly this mixed group of patients.

In treatment of epilepsy, serially trialed antiepileptic drugs (AEDs), after initial failure, are known to have diminishing likelihood of achieving seizure freedom, with only 14% achieving seizure freedom with a second or third agent and only 3% seizure-free with a combination of two drugs (Kwan and Brodie, 2000). Alternative approaches, such as surgery for patients with amenable lesions, and neurostimulation and/or diet manipulation for others, are available in this setting. "Drug resistant epilepsy may be defined as failure of adequate trials of two tolerated and appropriately chosen and used AED schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom" (Kwan et al., 2010). Once epilepsy is identified as refractory, nonpharmacologic approaches are appropriately considered. Among the options, resective epilepsy surgery offers the possibility of “cure” of epilepsy, in certain cases the best chance of seizure-free, and even medication-free survival. Consideration of surgical candidacy is therefore important at this point. Reasons for ineligibility for epilepsy surgery can be manifold—inability to lateralize or localize seizure focus, presence of multiple foci, high risk
of injury to eloquent cortex, patient/family preference. In this setting, as well as in those for whom epilepsy surgery has already failed, dietary manipulation is an important treatment option.

For many years, evidence of diet efficacy was in the realm of “expert opinion,” within a narrow community of experts familiar with the diet. This took the form of retrospective reviews (Kinsman, 1992; Prasad et al., 1996; Hemingway, 2001) describing diet experience in 58 of the most severely affected patients over an 18- to 24-month follow-up period. Studies of this type included etiologies and seizures of all kinds in children of a broad range of ages, often with very high seizure frequency. Reported efficacy was surprisingly good, comparable to industry standards for efficacy of new drugs today. Retrospective nature and lack of blinding and control groups limited the interpretability of these studies. Following media exposure in 1994 in a Dateline NBC review of the diet and in 1997 after release of the film First Do No Harm, there was a tidal wave of interest in the diet among parents of children with refractory epilepsy, pressuring their providers to provide, or refer them for a trial of, diet treatment. In this setting the first prospective studies emerged—a multicenter study (Vining et al., 1998) and a single institution report (Freeman et al., 1998), again describing diet efficacy in patients with an average monthly seizure frequency of 230/month and 410/month respectively. The multicenter study in particular assured the community of pediatric epileptologists that the efficacy of the Johns Hopkins protocols could be reproduced in other hands. At this point, in 2000, a review of 11 reports on diet efficacy, including the two cited prospective studies, cosponsored by the BlueCross and Blue Shield association, stated, “it is unlikely that this degree of benefit can result from a placebo response and/or spontaneous remission” and therefore concluded, “the evidence is sufficient to determine that the ketogenic diet is efficacious in reducing seizure frequency in children with refractory epilepsy” (Lefevre and Aronson, 2000). Finally, in 2008, a randomized, controlled but unblinded study of diet in a mixed group of children with refractory epilepsy revealed a statistically significant improvement in seizure control in a group of children treated with ketogenic diet now, compared with a matched group treated with ketogenic diet 3 months later, improving the quality of evidence in support of diet efficacy (Neal et al., 2008).

These studies have the great benefit of “generalizability” to the real world of refractory epilepsy. As might therefore be expected, subsequent studies from around the world in similar case series have reported roughly similar efficacy, which can be broadly stated as ~50% of refractory epilepsy patients starting the diet can expect to be responders (i.e. have >50% seizure reduction in seizures), 25%–30% will experience >90% seizure reduction, of whom approximately half will be seizure-free (Henderson et al., 2006; Nathan et al., 2009; Hallbook et al., 2015; Kossoff and McGrogan, 2005; Kossoff et al., 2012).

Diet therapy in epilepsy is rigorous, requiring precision in preparation and careful attention in administration by caregivers. Formula-fed infants and tube-fed infants are easiest to initiate and maintain on the diet, and usually use the classic ketogenic diet at a ratio sufficient to induce ketosis with plasma betahydroxybutyrate ≥ 4 mmol/L or seizure freedom, whichever comes first. These children are not troubled by dietary restriction and poor palatability as older, orally fed children are, who are accustomed to making dietary choices and may refuse unpalatable and preferred foods and drink. Reduced GI motility may worsen esophageal reflux and constipation in all, resulting in discomfort. Diet therapy is not without a variety of systemic adverse effects occurring at low frequency (Kang et al., 2004; Kossoff et al., 2009) but at least comparable in range and significance to those associated with anticonvulsant medications, although a significant advantage is the lack of sedating and/or behavioral adverse effects.

It would be extremely helpful to be able to predict which patients are most likely to respond to diet therapy in advance, instead of having to anticipate ~50% failure to achieve a useful seizure reduction. Unfortunately, though many studies and reports attempt to identify predictors, small study size, lack of control groups, heterogeneous diagnostic groups, and rarity of specific epilepsy syndromes have all limited the ability to reliably identify these factors. Schoeler et al. recently published an extensive review of the articles providing data on effectiveness and putative predictive factors (Schoeler et al., 2013). Twenty-one factors were examined. For each factor, the predominance of evidence in favor of an effect, the relative strength of evidence against an effect of that factor, and the number of patients in the cohorts “for” and “against” that factor were considered. No factor had strong evidence for a positive or negative response to diet. Strong evidence for absence of effect is also lacking, except in the case of gender and intellectual ability, which appear to have no effect on diet response. There were “mixed” findings (effect on response is reported in approximately half of
reported cases) for epilepsy cause/syndrome, seizure type, and biochemical markers other than ketosis and plasma glucose. “Weak evidence” for an effect on diet response (effect reported in less than half of reported cases) exists for age of seizure onset, age at diet initiation, time from seizure onset to diet initiation, seizure frequency, diet type, ratio, levels of ketosis (betahydroxybutyrate, acetone), EEG parameters, AEDs (individual or number/combinations), and BMI. Presence or absence of an effect of blood glucose, genetics, and imaging findings could not be assessed because of a limited number of studies reporting and patients reported. Assessment of predictive factors in this way is complicated by interactions between factors that cannot be resolved in small, uncontrolled, retrospective studies. Hopefully the future will include the development of consortia of expert centers devoted to analysis of greater numbers of patients managed prospectively and in a standard manner, which will allow dissection of factors predictive of successful treatment. Better understanding may also illuminate potential mechanisms for further study and manipulation.

Patients started on KD may have a seizure response within 14 days, but most will continue a trial of diet for at least 3 months, assuming no exacerbation of seizures occurs beyond the initial initiation period (Kossoff et al., 2009). Discontinuation after successful treatment is usually attempted after 2 years, though there is no data determining this to be the optimal time. Rarely, diet initiation is associated with persistent exacerbation of seizures beyond the initiation itself. Diet withdrawal is appropriate in this setting. Early diet discontinuation (<3 months) is usually due to lack of efficacy. Growing intolerance of dietary restriction can be problematic in young patients able to make diet choices.

Barriers to provision of ketogenic diets continue to include access to clinical expertise in diet treatment, cost of higher grade protein and high-fat foods in some communities, individual feeding/dietary preferences, co-morbid medical complexity, and systemic fragility raising concern for ability to tolerate the stress of dietary conversion.

In summary, for patients falling into the heterogeneous category of “nonsurgical epilepsy,” dietary therapy with a ketogenic diet should be considered once drug resistance and ineligibility for surgery has been established. In general, patients can expect a better chance of efficacy than with trial of another AED (Kwan and Brodie, 2000).

Many conditions have been recently established for which the ketogenic diet can be particularly beneficial. Several are epilepsy syndromes with refractory generalized seizures. This chapter covers the most common four conditions, for which there are sufficient data to recommend the ketogenic diet as potentially very helpful.

**INFANTILE SPASMS**

Infantile spasms (IS) are an age-dependent seizure type, typically occurring between 6 and 18 months of age, which are clinically characterized by serial body jerks occurring in clusters, often at the interfaces of waking and sleep. They represent the most common type of epileptic spasm. In infancy, they are often, but not always, associated with a characteristic EEG pattern known as hypsarrhythmia, a chaotic waking pattern of asynchronous high-voltage irregular slowing with superimposed multifocal spikes, which are more synchronous and discontinuous in sleep. When spasms are associated with developmental regression and hypsarrhythmia this triad constitutes West syndrome. Infantile spasms can be idiopathic or associated with genetic disorders, brain malformations, or pre-existing brain injuries. ACTH and vigabatrin are first-line treatments for this epileptic encephalopathy, which is refractory to initial therapies in 30%–40% overall (Pellock et al., 2010; Lux et al., 2005). Refractory IS are associated with serious delay in development in most survivors. Early and effective treatment of this seizure type is considered the best chance for normal developmental outcome. With first-line treatment, ACTH in most cases, elimination of clinical spasms by 14 days of treatment is the marker of successful treatment. Resolution of hypsarrhythmia is also considered a marker of successful treatment of IS and is usually assessed first at 14 days, with evaluation for maintenance of improvement in a further 28 days.

In comparison with other epilepsies, assessment of efficacy of any treatment for infantile spasms should consider the seizure-free rate to be the first desirable seizure outcome, with EEG resolution of underlying hypsarrhythmia of near equal importance. Complicating the assessment of efficacy of any treatment of infantile spasms is the known occurrence of spontaneous remission of infantile spasms in untreated cases, which can occur as early as 1 month after onset, and cumulatively in 10%–15% at 6 months and up to 25% of patients at 1 year (Hrachovy et al., 1991). Notably, in this retrospective cohort of untreated
infants ~90% suffered moderate to severe developmental impairment at follow-up, an average of 80 months later. There is also a significant rate of relapse of spasms during treatment with first-line agents, which therefore should also be considered in assessing dietary treatments for this condition.

The ketogenic diet is among the treatment options considered after failure of the first-line treatments, or if their use is contraindicated for any reason. In a study of ketogenic diet in infants by Nordli, 17 of the 32 infants with refractory epilepsy had infantile spasms. Of the 32 infants, 6 achieved seizure freedom all of whom had infantile spasms, (Nordli et al., 2001). Another 6 patients with spasms had “worthwhile improvement,” and as a group, patients with infantile spasms were more responsive to ketogenic diet than infants with other seizure types in this study. Following on this finding, 23 children with IS were studied, of whom 18 remained on diet at 6 months, 13 of whom had ≥50% reduction in seizures (3 seizure-free), and 13/13 remaining on diet at 12 months had ≥50% reduction (3 seizure-free) (Kossoff et al., 2002). In 2010, 104 infants with IS at the same institution, treated with KD after exposure to a mean of 3.6 AEDs, including steroids or vigabatrin in >70% were reported (Hong et al., 2010). Overall, 64% had a ≥50% reduction in seizures, and 38 achieved at least 6 months spasm free after a median of 2.4 months on the diet. Of these, 30 (79%) maintained spasm freedom. In a prospective case study of 20 patients with epileptic spasms, among 70% and 72% achieving a >50% reduction in seizures at 3 and 6 months respectively, 3 infants achieved and maintained seizure freedom for at least 6 months (Kayyali et al., 2014). Improvement in spasms was noted within 1 month of starting diet treatment. EEG improved in 12 (60% of patients) when assessed at 3–6 months, and in five of six infants with hypsarrhythmia the pattern resolved and did not recur up to 12 months after diet initiation. Pires et al. reported 6/17 (35%) seizure-free at 1 month after failing vigabatrin and hydrocortisone (Pires et al., 2013). Eleven of these 17 patients (65%) were seizure-free at 3 months, one after the addition of felbamate. The addition of felbamate to their regimen brought five more into the responder (>50% reduction) group. Dressler, in a retrospective, cross-sectional study comparing early (<1.5 yrs) with late diet initiation, included 59 infants with IS in their population of 115 infants. Nineteen (32%) were seizure-free at 3 months (Dressler et al., 2015a). Numis et al. had 9/26 (32%) seizure-free at 1–3 months. They noted usefully an association of spasm response and “other seizure” response in these patients (p = .02) (Numis et al., 2011).

Factors favoring diet therapy in IS include easily available ketogenic formula and lack of independent choice of diet intake in infants. Nonetheless, three of 20 caregivers discontinued the diet; of these one indicated difficulty maintaining the protocol (Kayyali et al., 2014). In an Asian population, 9 of 43 patients discontinued the diet due to “unacceptable” complications, and 7 due to intolerability. An additional 9 (21%) discontinued due to insufficient efficacy (Eun et al., 2006). In contrast, 6/26 (23%) had side effects (Numis et al., 2011), none serious enough to discontinue the diet. In this group, 5/26 discontinued the diet by ~1 year due to either lack of efficacy or difficulty maintaining the diet.

In these young patients, concerns regarding (Hong et al., 2010), weight gain and appropriate growth were amenable to adjustment downward in ratio for increased protein intake. Numis et al. described no significant differences between responders and nonresponders in terms of weight-for-height z scores, although the best responders (>90% seizure reduction) had lower weight-for-height z scores at all points.

Among the 104 patients described by Hong et al., 10/18 in whom diet was used as the first-line treatment became seizure-free, had normal EEG within 2 months, and were maintained on the diet for 6 months, after which the diet was withdrawn without relapse (Hong et al., 2010). The same group reported a retrospective case-controlled study of diet versus ACTH as initial therapy for new-onset spasms (Kossoff et al., 2008). Eight of 13 (62%) patients on diet therapy became seizure-free at a median of 6.5 days of treatment, compared with 18/20 (90%) treated with high dose ACTH who were spasm free at a median of 4 days (p = .06). One of the 8 relapsed (12.5%), versus 6 (33%) of the 18 ACTH responders (p = .23). Also, EEG normalization was more likely in the ACTH group (1/11 vs. 9/17, p = .02) at 1 month, though the EEG normalized in all eight responders by 5 months of diet treatment. Adverse effects were significantly less likely in the diet therapy group (p = .006). There was no difference in developmental outcome between the treatment groups at their last examination at a median of 12 months.

Predicting efficacy of diet has been difficult. Eun et al. associated underlying etiology with efficacy, cryptogenic cases responding at a higher rate than symptomatic cases, but no other predictor
emerged from their series (Eun et al., 2006). No significant predictive factor of diet efficacy was identified in the small diet group in the case control comparison with ACTH. Numis et al. reported 11/18 males and 1/8 females were responders \( (p = 0.04) \) but cited low sample size and skewed distribution of etiologies as potential biases (Numis et al., 2011). This has not been reported in other studies.

A final area of interest is the possibility of synergistic effects of combination of KD with other treatments for IS. A recent study of epileptic encephalopathies (EE) (IS, Lennox Gastaut syndrome/other generalized epilepsies, CSWS) examined the efficacy of KD added to steroid therapy when not controlled by the initial steroid treatment (Ville et al., 2015). Patients were on a mean of 3.5 mg/kg/day of hydrocortisone (1–10 mg/kg/day) when starting the diet. Ketosis was achieved in all but one case with status epilepticus (SE), and no diet adjustment was made to maintain ketonuria. Plasma ketones were not measured. There were 23 IS cases in the total of 42 with EE. Twenty were steroid-resistant, three steroid-dependent. Overall, 5/27 (18.5%) steroid-resistant cases and 9/15 (60%) steroid-dependent cases were considered responders. The data presented do not allow specific analysis with respect to IS.

In summary, studies of modest quality indicate that KD is a moderately effective therapy for refractory IS, and is generally safe and tolerable in this young population. As a first-line treatment, it has better efficacy for seizure freedom over time, but is not as quick as effective as traditional first-line treatments, in particular ACTH. Patients experiencing improvement do so within 1 month, and definitely by 3 months. There may be reason to examine the KD in combination with first-line agents rather than serially, with the goal of maximizing the chance of seizure freedom. High-quality studies would be best, and given the low frequency of infantile spasms would require a multicenter approach.

**LENNOX GASTAUT SYNDROME**

This is an epilepsy syndrome characterized by peak onset between 3 and 5 years of age. It is associated with a very refractory pleomorphic epilepsy, tonic seizures being most characteristic, and also including atypical absences and atonic and myoclonic seizures (Arzimanoglou et al., 2009). Focal seizures and complex partial seizures may also occur. Seizures may be very frequent, occurring multiple times per day. Episodes of SE, often prolonged, are common. Lennox Gastaut syndrome (LGS) may occur de novo or in about 20% develops following West syndrome, and might also follow other remote symptomatic brain injuries (e.g., hypoxic ischemic encephalopathy). If there is no preexisting developmental delay, cognitive regression occurs and at least moderate intellectual disability is expected, with virtually all individuals being dependent in adulthood. The EEG is characterized by slow (1.5–2 Hz) spike and wave activity, often multifocal spikes, and bursts of 10-Hz fast activity during sleep. Slow spike and wave activity may wane over the years. Treatment with broad-spectrum anticonvulsant drugs is the mainstay of treatment but is rarely effective in achieving seizure control. Side effects of lethargy and drowsiness are particularly damaging, as these states are associated with increased seizures. Nonpharmacologic treatments including surgical approaches (corpus callosotomy, vagus nerve stimulation) and diet therapies offer non-sedating adjunctive therapies that, if successful, may allow reduction of medication burden and a resultant improvement in seizures and quality of life.

The early reports of efficacy in the most refractory epilepsies typically included patients with this disorder, and this subgroup of patients were found to respond to diet with a >50% reduction in seizures within 5 days of diet initiation with 36 hours of fasting and the development of ketosis (Freeman and Vining, 1999). A blinded crossover study of 20 LGS patients, initiated on diet and subsequently, in a blinded manner, given either 60 gm glucose with saccharin to abort diet, or saccharin only (allowing continued ketosis) failed to show a significant difference in outcome, but this was likely due to the failure to eliminate ketosis in the control group (Freeman et al., 2009). In 2012, Lemmon et al. reported that in the literature to that date 88/189 (47%) LGS patients had responded to KD (Lemmon et al 2012). They retrospectively reviewed 71 patients from their institution, John Hopkins Hospital, of whom 36 (51%) achieved a >50% reduction in seizures, 16 (23%) a >90% reduction, and one was seizure-free, using an intention to treat analysis. The results were similar at 12 months. Caraballo et al. reported similar results in a smaller prospectively studied group of 20 LGS patients started on diet and followed for a minimum of 16 months with retention of 75% of patients on diet, and 2/20 (15%) seizure-free and 25% with 50%–99% reduction in seizures (Caraballo et al., 2014).
discontinued diet in this study, three because of lack of efficacy within 3 months, and two because of persistent vomiting, one of whom was also hypoglycemic. Patients with LGS have also been reported to respond with similar efficacy to the modified Atkins diet (Sharma et al., 2015), 12/25 were responders and 2 (8%) were seizure-free.

In summary, LGS patients, some of the most refractory pediatric epilepsy patients, respond to KD therapies. As a result, medication reduction or withdrawal provides special benefit to these patients, possibly reducing medications even further and improving quality of life. Diet therapy should be considered early once diagnosis is clear and refractoriness established.

**DRAVET SYNDROME (SEVERE MYOCLONIC EPILEPSY OF INFANCY)**

Dravet syndrome (severe myoclonic epilepsy of infancy; DS SMEI) is a clinical syndrome characterized by initial febrile seizure, often febrile SE, and/or hemiclonic febrile seizures in the first year of life, in the setting of initially normal development. It occurs in 1:20,000–1:40,000 individuals. Febrile and afebrile seizures are quickly recurrent and associated with subsequent developmental stagnation or regression. The epilepsy is pleomorphic (multiple seizure types—myoclonic, focal, generalized absence, and generalized motor seizures) and pharmacoresistant. Fever/hyperthermia continues to provoke seizures. Subsequent to seizure onset, ataxia, pyramidal signs and interictal myoclonus are seen. Early EEGs are normal but later, generalized spike and polyspike waves and multifocal spikes are seen. Historically, many children thought to have pertussis vaccine-related encephalopathy and long-term cognitive sequelae, were found to have underlying DS (Berkovic et al., 2006). Up to 80% are associated with a heterozygous mutation in the sodium channel gene SCN1A, although not all SCN1A mutations result in DS. Other genetic and/or environmental factors play an as yet unclear role in evolution of the syndrome. Development generally stabilizes, but at least a moderate degree of intellectual disability is the rule. The temporal relationship between seizure onset and developmental impairment suggests but does not prove a causal relationship between seizures, SE, and developmental regression. Nonetheless, vigorous efforts to control and prevent seizures are appropriate. Problems with this approach include medication side effects. The gold standard is combination treatment with stiripentol (STP), valproic acid (VPA), and clobazam (CLB). There is a higher mortality in children with this syndrome (Genton et al., 2011), due to a variety of factors (sudden unexpected death in epilepsy, SE, accident) further motivating efforts to control convulsive seizures. An Scn1A mutant mouse model of DS showed decreased latency to seizure onset after an epileptogenic challenge compared with wild-type littermates. Feeding the mutant mouse a ketogenic diet increased latency to seizures to levels that were not significantly different from the wild-type littermates (Dutton et al., 2011).

In 2011, Caraballo and colleagues reported their experience in 59 DS patients, of whom 24 were treated with a 4:1 ketogenic diet and followed for at least 2 years (Caraballo 2011). Sixteen remained on the diet at 2 years, of whom 2 (16%) were seizure-free, 10 (62.5%) had 75%–99% reduction in seizures and another 4 (25%) had 50%–74% reduction in seizures. All five with SE responded to diet and had no additional SE during follow-up. Medication reduction, even in those without dramatic seizure efficacy, resulted in improvement in quality of life. Ten of 15 patients followed by Nabbout et al. achieved a >75% reduction in seizures (Nabbout et al., 2011). All of these patients were already receiving triple combination therapy (VPA, STP, CLB). However, efficacy was lost in ~50% by 1 year. Behavioral improvements were noted in all responders.

Laux et al. followed a subgroup of 20/48 DS patients on KD at their own center, all of whom remained on diet at least 6 months, 17 continuing for at least 18 months. They saw 65% achieve a greater than 50% reduction in seizures and 30% a >90% reduction. They noted that the KD appeared to reduce the frequency of all seizure types, though quantification of myoclonic and absence seizures was not robust (Laux et al., 2013).

In a retrospective cross-sectional study, 10 of 32 mutation positive patients (31 SCN1A and 1 GABRG mutations) with DS were treated with KD (Dressler et al., 2015b) for at least 3 months. Seven of 10 patients were responders at 3 months, 6 at 6 months and 12 months. One child became seizure-free on KD. A striking finding in this group was the lack of SE in the eight patients who had previously suffered this seizure type. No child on KD, responder or not, had SE while on the diet. Given that excess mortality in this syndrome is partly related to SE, this is a notable finding that would bear further study. Early withdrawal from the KD treatment was due to failure of efficacy; later withdrawals were due to difficulty...
maintaining compliance. Responder status was not associated with mutation type in this group.

An interesting aspect of the Dressler study is the comparison with other treatments (Dressler et al., 2015b). There was no significant difference in responder rates between the gold standard treatment, a combination of STP, VPA, and CLB (89%); bromide (78%); KD (70%); VPA monotherapy (48%); TPM (topiramate) (35%); and VNS (vagus nerve stimulation) (37%). Ketogenic diet was more effective than LEV (levetiracetam). The conclusions are limited by small numbers but appear worthy of further study.

With respect to positive interaction with other treatments, 7 of 16 diet responders were on topiramate in Caraballo’s updated group, and 3 of 7 of Dressler’s responders were also.

In summary, treatment with KD in DS is associated with a reduction in seizures, and possibly in SE. In this highly pharmacoresistant epilepsy, polypharmacy is often the rule, with the attendant burden of medication side effects. The goal of optimizing seizure control while minimizing medication adverse effects may be facilitated by introduction of the ketogenic diet, often allowing reduction in medication burden and therefore in cognitive and behavioral side effects. An as yet unanswerable question is whether early use of the ketogenic diet, prior to seizure onset, with its lack of cognitive side effects and prominent effect on status epilepticus (SE), might ameliorate the developmental stagnation/regression associated with seizure onset in this disorder.

DOOSE SYNDROME
(MYOCLONUC ASTATIC EPILEPSY)

Doose syndrome (myoclonic astatic epilepsy; MAE) is a genetic generalized epilepsy, initially described by Doose in 1970 and distinguished from other causes of myoclonic epilepsy in childhood (SMEI, LGS), although the underlying genetic abnormality is not yet known. It occurs in previously normal children, with onset, sometimes explosively, possibly as early as the latter half of the first year of life, but more typically between 2 and 5 years of age. There is a high rate of preceding febrile seizures, and of a family history of epilepsy. It is characterized by frequent myoclonic and myoclonic astatic/atonic seizures; absences, with or without myoclonia; and generalized tonic-clonic, clonic, and/or tonic seizures. The occurrence of nocturnal tonic seizures is thought by some to indicate a poor outcome. With extremely frequent seizures, often absences interspersed with myoclonias, and nonconvulsive SE an epileptic encephalopathy can develop. Seizure-related falls are a major component of associated morbidity, and these children are at risk of injury. Many need to wear helmets for protection. The epilepsy is often highly pharmacoresistant, and medication adverse effects add to the impact of the disorder on cognitive performance and behavior regulation. Often EEG is associated with generalized spike and polyspike wave activity, ranging from 2 to 5 Hz, which also underlies the myoclonic seizures. Prominent theta activity is a feature of the interictal EEG. This disorder, among other refractory childhood epilepsies, is very responsive to the ketogenic diets. This has led to exploration of the relationship of this disorder to GLUT1 deficiency, a hypothesis that would provide an explanation for its particular responsiveness to ketosis. However, only 0%-5% of cases have been shown to be associated with this genetic disorder (Larsen et al., 2015; Mullen et al., 2011). Cognitive outcome is normal in about 60% of patients in the long term. Continued active seizures are correlated with varying degrees of intellectual disability, ranging from mild to moderate severity.

Using ILAE criteria for diagnosis, Oguni et al. reported treatment outcome in a group of 81 patients with MAE (Oguni et al., 2002). Seizure freedom for >2 years was achieved in 55/81 (65%) by 50 months (+/- 16 mo). Later recurrence of GTCs occurred in 14% up to 18 years later but were then easily controlled. Eighteen percent continued to have refractory epilepsy. Among the treatments used in this group, KD was used for 26 patients, of whom 15 (58%) became free of myoclonic (MS) and atonic (AS) seizures and 9 had >50% reduction in these seizures. This efficacy was better than that of ACTH (n = 22, 36% MS/AS free, 23% >50% decrease) and ethosuximide (n = 32, 32% MS/AS free, 32% >50% decrease), the best of the conventional AEDs used, and than valproic acid (n = 57, 12% MS/AS free, 28% >50% decrease). Generalized motor seizures gradually decreased thereafter in these patients.

Caraballo et al. reported 30 MAE patients of whom 11 were treated with diet (Caraballo et al., 2006). Six of these remained on the diet at 18 months of whom two (18.2%) were seizure-free, and two each had 75%-99% reduction and 50%-74% reduction. The EEG was improved in all six, and was normal in the seizure-free patients at 18 months. Five patients discontinued the diet within the first 3 months, four for lack of efficacy and one for persistent vomiting. Those remaining on the diet tolerated it without significant side effects. The authors remarked the good response
to the diet and considered that for this disorder, it should be considered earlier in the course.

Kilaru et al. reported the order of exposure to various treatments and their efficacy in 23 MAE patients (Kilaru et al., 2007). Ketogenic diet was tried last in 10 patients, and was the most effective treatment in achieving seizure remission. It was initiated an average of 17 months (range 2–58 months) after onset, and after an average of five AED trials. Five (50%) became seizure-free, three within 1 month, one by 7 months, and the last by 19 months after diet initiation. Three of these patients remained seizure-free for more than 6 months. Ethosuximide (25%) and topiramate (23%) were the next most effective in achieving remission of seizures. In this group, steroids (prednisone or ACTH) were ineffective. The authors report the possibility of spontaneous remission in 3 of 14 patients who became seizure-free in this group. When this is a factor, late-used treatments may appear more effective than those used early in the disorder, when spontaneous remission is less likely. Only 43% of patients in this group were cognitively normal at last follow-up, the rest having mostly mild disability. Cognitive outcome was not related to time to seizure freedom.

Perhaps because this epilepsy seems especially responsive to the ketogenic diet, use of a lower ratio, as in the modified Atkins diet has been considered as an option for treatment—with the potential of seeing efficacy with a less rigorous, more palatable diet. There is little information available about efficacy in comparison to the classic ketogenic diet. Simard-Tremblay used the modified Atkins diet in six and KD in three of nine patients after a median of four AEDs (Simard-Tremblay et al., 2015). They reported seven of nine patients achieved seizure remission, with one patient on KD and one on the modified Atkins diet failing to respond. One patient advanced from the modified Atkins diet to 4:1 KD and achieved seizure freedom. When 27 patients were switched from the modified Atkins diet to KD there was improvement (additional 10% decrease in seizures) in 10 (37%) (Kossoff et al., 2010). There were nine MAE patients in this group, of whom seven improved with the change to KD, five of them achieving seizure freedom.

In summary, MAE appears to be more responsive to ketogenic diets than other epilepsies, with approximately 50% achieving seizure freedom, allowing medication withdrawal in many and ultimately diet withdrawal also. Assessment of efficacy is complicated by a possible but poorly defined spontaneous remission rate. Diet therapy is being offered as an earlier option more frequently in light of the above reports. It remains to be seen if efficacy remains high in this setting.

**CONCLUSION**

In conclusion, there is general agreement, based on the literature described in this chapter, that patients with these four epilepsies (infantile spasms, Lennox-Gastaut syndrome, Dravet syndrome, and myoclonic-astatic epilepsy) may benefit from a trial of diet therapy once their epilepsy has become refractory to medication. The quality of the data for individual epilepsy syndromes is modest, at best. These diagnoses are rare, so that only multicenter studies are likely to produce more robust data, comparing diet therapy to other treatments in a controlled manner, and/or establishing the best timing of diet trials. There is also very little information on the efficacy of diet treatment as first-line therapy. The difficulty of administration of diet therapy is likely to limit its acceptance in this position unless it becomes possible to induce ketosis without diet restrictions, that is, via a supplement or pill. Future, multicenter collaborative studies will be necessary to examine these possibilities.

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Ketogenic Diet for Other Epilepsies

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INTRODUCTION
As discussed in previous chapters, the ketogenic diet (KD) is the treatment of choice for epilepsy in certain disorders of brain metabolism, in particular glucose transporter protein 1 (Glut1) deficiency and pyruvate dehydrogenase deficiency (PDHD). An understanding of the underlying metabolic pathophysiology of these genetic disorders has been coupled with successful clinical efficacy of the KD in patients with Glut1 deficiency or PDHD. However, there are also several other genetic disorders that manifest with epilepsy for which dietary therapies appear to be especially effective, but for which the possible underlying metabolic mechanisms are not as clearly delineated. The International Ketogenic Diet Study Group has listed several conditions for which the KD has been reported as being particularly beneficial (Box 7.1) and could be offered earlier. This list includes Doose syndrome, tuberous sclerosis complex (TSC), Rett syndrome (RTT), Dravet syndrome, and infantile spasms (Kossoff et al., 2009b). Whether efficacy in these conditions is due in part to the broad-spectrum efficacy of the KD or to specific mechanisms specific to these conditions is still under investigation. Furthermore, patient populations who are often fed with gastrostomy tubes, as in RTT, may be especially good candidates for the KD in liquid form, due to the relative ease of administration. In this chapter, we discuss the use of dietary therapies for the treatment of epilepsy in certain genetic disorders, including RTT and TSC, as listed by the International Ketogenic Diet Study Group, and additionally discuss the use of epilepsy dietary therapies in patients with Angelman syndrome (AS) and Sturge-Weber syndrome (SWS).

RETT SYNDROME
Rett syndrome is an X-linked dominant neurogenetic disorder characterized clinically by developmental regression around 12–18 months of age, specifically with regression of purposeful hand use and spoken language, development of a gait abnormality, and hand stereotypies such as hand wringing (Neul et al., 2010). Rett syndrome is associated with mutations in the methyl-CpG-binding protein 2 (MECP2) gene at Xq28, which are detected in 95%–97% of typical RTT patients (Neul et al., 2010). Epilepsy occurs in about 70% of patients with RTT, with a median onset of 3–4 years (Nissenkorn et al., 2010; Nissenkorn et al., 2015). Seizure semiology includes all types, with generalized tonic-clonic occurring more frequently than complex partial and secondarily generalized seizures (Cardoza et al., 2011). The treatment of epilepsy in RTT usually involves the use of standard anticonvulsant medications, but about a third of patients remain medication-refractory (Pintaudi et al., 2010). Patients with RTT are usually not typical candidates for epilepsy surgery, thus RTT patients with medication-refractory epilepsy are often considered for nonmedication treatments, such as dietary therapy.

Rett syndrome is listed by the International Ketogenic Diet Study Group as a condition for which the KD has been reported as having probable benefit, defined as having at least two publications in the literature. In a published case series of seven girls with clinical RTT and medication-refractory epilepsy, the KD was initiated in and tolerated by five of the patients (Haas et al., 1986). Of these five patients, four of them had a 50% or better decrease in seizures, including one with a 75% decrease and one with a 100% decrease. All five of the patients who were able to tolerate the diet also had reported slight behavioral improvements. In another published case of an 8-year-old girl with genetically confirmed RTT with medication-refractory seizures, a 70% reduction in seizures was achieved on the KD (Liebhaber et al., 2003). This patient was
able to tolerate the diet for over 4 years, and also had improvement reported in her behavior on the KD. Additionally, in a 10-year-old girl with genetically confirmed RTT and medication-refractory seizures, the KD significantly decreased seizure frequency, and this patient was also reported to concurrently became “more communicative” with her family members (Giampietro et al., 2006).

It is uncertain whether there is a specific underlying metabolic process in patients with RTT that would provide a physiological benefit to treatment with the KD. Some researchers have noted that many patients with RTT have serum markers suggestive of defects in energy metabolism, including serum lactate or pyruvate elevations (Haas et al., 1995) or cerebrospinal fluid lactate elevations (Matsuishi et al., 2011). It has been hypothesized that perhaps the KD alters energy metabolism in a favorable direction in patients with RTT (Haas et al., 1986; Mantis et al., 2009), but further research is needed to better understand the implications of the KD in patients with RTT.

**TUBEROUS SCLEROSIS COMPLEX**

Tuberous sclerosis complex is an autosomal dominant neurocutaneous disorder characterized by hamartomatous growths in multiple organ systems, including the skin, central nervous system, eyes, kidneys, heart, and lungs. Mutations in the TSC1 and TSC2 genes, encoding for hamartin and tuberin, are present in over 85% of cases, resulting in hamartomatous growths due to unregulated activation of the mammalian target of rapamycin (mTOR) pathway (Napolioni et al., 2008). The most common lesions found in the brain include cortical tubers, subependymal nodules, subependymal giant cell astrocytomas, and radial migration lines. Epilepsy occurs in up to 85% of TSC patients, and is a significant factor in neurological morbidity. Seizures begin within the first year of life in 63% and prior to 3 years of age in 83% (Chu-Shore et al., 2010a). The majority of patients have multiple seizure types, with the most common being complex partial seizures, followed by infantile spasms, generalized tonic-clonic seizures, and atypical absence (Chu-Shore et al., 2010a). The treatment of epilepsy in TSC usually starts with the use of standard anti-convulsant medications, but TSC is medication-refractory in 30%–60% of patients (Chu-Shore et al., 2010a; Vignoli et al., 2013). The TSC patients with medication-refractory epilepsy are often considered early for epilepsy surgery candidacy, with cortical tubers and abnormal surrounding cortex usually the targets of interest. When surgical candidates are chosen carefully, good outcomes with seizure freedom can be achieved in about 60% (Zhang et al., 2013). For those patients who are not surgical candidates, or for whom the family does not wish to pursue surgical management, alternative epilepsy treatment options such as dietary therapy should be considered.

Tuberous sclerosis complex is listed by the International Ketogenic Diet Study Group as a condition for which the KD has been reported as having probable benefit, defined as having at least two publications in the literature. In a published case series of 12 children with TSC between the ages of 8 months and 18 years treated with the KD, at 6 months, 92% had at least a 50% reduction in seizures, with 67% having at least a 90% reduction (Kossoff et al., 2005). In this series, five children had at least a 5-month seizure-free response. The authors recommended the KD in...
patients with TSC for whom medications fail and no clear epileptogenic tuber is identified. In another case series of three children with TSC and medication-refractory seizures who were followed for 12 months on the KD, two of the patients became seizure-free, and the third patient had his drop seizures decreased (Coppola et al., 2006). Within a larger series of 71 patients with TSC, six were started on the KD, and two of the six had a 50% decrease in seizures (Overwater et al., 2015).

When the KD is effective, the possibility of weaning off the KD can be considered after 2 years. Unfortunately, a diagnosis of TSC is a risk factor for seizure recurrence in patients weaned off of the KD after 2 or more years of seizure freedom. In one published series, all three patients with TSC who were weaned off of the KD after becoming seizure-free had a recurrence of seizures, and only one regained full control with reinitiation of the KD (Martinez et al., 2007). The authors concluded that patients with TSC may need to be treated for longer than 2 years, or possibly indefinitely if seizure control is achieved.

There is also evidence that the low glycemic index treatment (LGIT) diet can be effective in reducing seizures, and is better tolerated, in patients with TSC. In a case series of 15 patients with TSC who were weaned off of the KD after becoming seizure-free had a recurrence of seizures, and only one regained full control with reintroduction of the KD (Martinez et al., 2007). The authors concluded that patients with TSC may need to be treated for longer than 2 years, or possibly indefinitely if seizure control is achieved.

It has now been well characterized that the manifestations in TSC are largely due to dysfunctional regulation of the mTOR pathway. It is unclear, however, whether there is a similar underlying mechanism to explain why patients with TSC respond well to dietary therapies. There is some bench evidence that markers of the mTOR pathway, including pS6 and pAkt, are reduced in the hippocampus and liver of rats fed the KD (McDaniel et al., 2011). However, in a series of five patients with TSC who were treated with KD, three of the five patients had progression of known tumors (renal angiomylipomas or subependymal giant cell astrocytomas) or had new tumors develop while being treated with the KD (Chu-Shore et al., 2010b). Thus, if there is some mTOR inhibition provided by the KD, it does not appear, although only in a small sample, to provide the same level of tumor regression effects in TSC as better-studied mTOR inhibitors such as sirolimus and everolimus.

**Angelman Syndrome**

Angelman syndrome is a neurogenetic disorder characterized by cognitive and language impairment, epilepsy, ataxia and tremorous movements, and sleep disturbances, with patients also tending to have an apparent happy demeanor. Angelman syndrome is maternally inherited, with four possible known genetic mechanisms resulting in reduced expression of the ubiquitin-protein ligase E3A (UBE3A) gene, most commonly from a deletion in the 15q11.2-13.1 region (70%) (Jiang et al., 1999). Epilepsy is present in over 85% of patients with AS (Laan et al., 1997; Thibert et al., 2009), with the onset of epilepsy in 50% by 1 year of age, and over 75% by 3 years of age (Valente et al., 2006). The underlying etiology of epilepsy in AS is not clear, but there are known differences in epilepsy genotype-phenotypes correlations: AS patients with maternal deletions have higher rates of epilepsy (90%) than those without (55%–75%) (Thibert et al., 2009), have an earlier onset, and have a more severe epilepsy phenotype (Valente et al., 2005; Minassian et al., 1998). It has been hypothesized that there might be other factors related in patients with maternal deletions specifically associated within their 15q11-13 deletions, such as the genes coding for subunits of the γ-aminobutyric acid (GABA), a receptor complex within this region (Minassian et al., 1998). Epilepsy in AS typically manifests with multiple seizure types, including myoclonic, atypical absence, generalized tonic-clonic, and atonic seizures, and AS patients commonly have frequent or prolonged seizures (Thibert et al., 2009). Most patients are medication-refractory, with up to 77% of patients requiring combination medication therapy (Laan et al., 1997; Thibert et al., 2009). Patients with AS and epilepsy are unlikely to be candidates for epilepsy surgery, thus alternative therapies such as dietary therapies are often considered.

Several examples of successful treatment of epilepsy in AS with the KD have been published. In one series of 19 patients with genetically confirmed AS and medication-refractory epilepsy, the KD was described as “effective” for all four patients it was initiated in (Valente et al., 2006). In a case report of an infant with AS, the KD produced a rapid and significant reduction in seizures from hundreds a day to near complete control, and was accompanied by increased alertness and smiling (Stein et al., 2010). In another report, a 5-year-old with AS became seizure-free within 2 months on the KD and was able to wean off of two of her three anticonvulsant medications (Evangelion et al.,
2010). This patient also had an improvement in her sleep pattern and reduced hyperactivity. In a large electronic survey sent through the Angelman Syndrome Foundation to 461 family members of patients with AS, 11% reported trying dietary therapy, to include the KD in 8% and LGIT in 2%. Of those trying the KD, 36% reported that the KD was the best overall treatment, although only 19% reported still being on the KD (Thibert et al., 2009). Finally in a series of six children with AS followed prospectively on the LGIT for 4 months, five of six of the patients had at least an 80% reduction in seizures, with five remaining on the LGIT for over a year (Thibert et al., 2012).

It is also uncertain whether there are underlying mechanisms explaining the efficacy of dietary therapies in patients with AS. The anticonvulsant effects of the ketogenic diet are probably multiple (Bough et al., 2007). It has been reported that children on the KD have an increase in GABA, taurine, serine, and glycine in their cerebrospinal fluid (Dahlin et al., 2005), thus a possible specific action partly explaining the efficacy of dietary therapies in patients could be the increase of GABA synthesis in the brain. Specifically for AS, as mentioned, there are genes encoding for the GABAA receptor within the region of the maternal 15q deletions associated with AS (Minassian et al., 1998), thus it has been hypothesized that increasing GABA levels through the KD may be an important component in its effectiveness in patients with AS.

**Sturge-Weber Syndrome**

Sturge-Weber syndrome is a sporadic neurocutaneous disorder characterized by capillary malformations in the distribution of the trigeminal nerve, glaucoma, and cerebral venous malformations. Sturge-Weber syndrome is associated with a somatic mutation in the GNAQ gene, which plays a key role in cell proliferation (Shirley et al., 2013). Epilepsy is an especially common manifestation, occurring in over 85% of patients (Pascal-Castroviejo et al., 2008), with onset by 1 year of age in up to 75% (Sujansky et al., 1995; Kossoff et al., 2009a). Most seizures are focal, but generalized seizures can also occur (Ewen et al., 2007), with seizures commonly occurring in a pattern of clustering and intense seizures followed by prolonged seizure-free periods (Kossoff et al., 2009a). Seizure control in SWS is particularly important because of radiographic evidence of hypoperfusion that may occur ictally in some patients, which may contribute to progressive cortical atrophy (Aylett et al., 1999). The treatment of epilepsy in SWS usually starts with the use of standard anticonvulsant medications. The SWS patients with medication-refractory epilepsy are often considered for an epilepsy surgical evaluation to consider options of either a lesionectomy or hemispherectomy (Arzimanoglou et al., 2000; Kossoff et al., 2002). Hemispherectomy can result in seizure freedom in 81% (Kossoff et al., 2002), without significant worsening in postoperative hemiparesis or cognitive functioning (Arzimanoglou et al., 2000).

The successful implementation of the KD has been reported in a 4-year-old patient with SWS, although a recurrence of seizures occurred 4 months into treatment (Petit et al., 2008). There is a theoretical risk in SWS with the KD due to the fasting and fluid restrictions implemented during the initiation of the KD, which could possibly provoke stroke-like events that SWS patients are prone to (Kossoff et al., 2010). Thus, the use of less restrictive dietary therapies has been proposed for patients with SWS. In 2010, Kossoff et al. published a case series of the modified Atkins diet (MAD) in five children with SWS: three of the patients had at least a 50% reduction in seizures, including one having a 90% reduction, and one becoming seizure-free (Kossoff et al., 2010). Only one patient had a stroke-like event on the MAD, although this patient had a prior history of stroke-like events prior to initiation of the MAD.

**Conclusions**

In conclusion, dietary therapies are effective for a wide spectrum of epilepsy types, and we present four examples of genetic epilepsy syndromes that have been reported to respond well to dietary therapies. Whether efficacy in these conditions is due in part to the broad-spectrum efficacy of the KD or to specific mechanisms specific to these conditions is still under investigation. If specific pathways affected in particular genetic syndromes are better characterized, this may help us further understand the underlying pathophysiologic mechanisms contributing to the efficacy of dietary therapies in the treatment of epilepsy.

**Disclosures**

The view(s) expressed herein are those of the author(s) and do not reflect the official policy or position of Brooke Army Medical Center, the US Army Medical Department, the US Army Office of the Surgeon General, the United States Air Force, the Department of the Army and Department of Defense, or the US Government.
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INTRODUCTION

The ketogenic diet (KD) and related dietary therapies, which are characterized by carbohydrate restriction, are now widely accepted as a treatment option for epilepsies that are resistant to medications. Though many studies have demonstrated the efficacy of the KD in symptomatic generalized or mixed epilepsies including infantile spasms, Dravet syndrome, Doose syndrome, and Lennox Gastaut syndrome, the KD is perhaps less often considered for idiopathic generalized epilepsies, which include childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME), and generalized epilepsy with generalized tonic-clonic seizures (GE-GTC). This chapter covers these “novel” epilepsy uses.

CHILDHOOD ABSENCE EPILEPSY

Childhood absence epilepsy (CAE) is the most common pediatric epilepsy syndrome, accounting for more than 10% of all cases of epilepsy in children (Berg et al., 2000). Onset occurs between 4 and 8 years of age in an otherwise neurologically normal child. Typical seizures are staring spells, lasting only seconds, occurring many times daily, and characterized on EEG by 3-Hz generalized spike and wave activity with a normal background. Childhood absence epilepsy has classically been considered a benign epilepsy syndrome that responds to medication, remits with time, and does not affect neurodevelopmental outcome. Now it appears that treatment response and remission rates are not as high as once assumed, and long-term psychosocial difficulties are common (Masur et al., 2013; Nickels; Wirrell et al., 1997). Juvenile myoclonic epilepsy presents later in childhood, typically between 11 and 17 years of age, and carries a higher risk of treatment resistance and lower rate of eventual remission. The occurrence of generalized tonic-clonic (GTC) seizures is more common in JAE than in CAE, but in both syndromes, GTC seizures portend a poorer prognosis for remission.

Ethosuximide is the first-line treatment for CAE, and valproic acid is also efficacious but has a greater impact on tests of attention than does ETX (Glauser et al., 2010). Lamotrigine is efficacious in a smaller proportion of patients. Second-line therapies are broad-spectrum AEDs such as topiramate, zonisamide, and clobazam, or conventional benzodiazepines such as clonazepam. Antiepileptic drugs for focal mechanisms of action in epilepsy, including the sodium channel blockers, are avoided because they might exacerbate seizures with generalized mechanisms of action. Thus, treatment choices are limited when first-line drugs fail.

Unfortunately the published literature on ketogenic diet related therapies for absence epilepsy is quite limited. One study from Johns Hopkins specifically evaluated the efficacy of the KD and the modified Atkins diet (MAD) in children with treatment-resistant absence epilepsy, and also included a review of studies in the literature that included absence epilepsy patients (Groomes et al., 2011). In the Johns Hopkins cohort, 8 patients with absence epilepsy were treated with the KD and 13 patients with absence epilepsy were treated with the MAD. Of the combined 21 patients, only 2 had a history of GTC seizures in addition to absence, but 8 had early-onset absence with seizures beginning prior to 4 years of age. Diet initiation occurred 1 to 12 years after seizure onset, and the median number of antiepileptic medications used was 4 (range 2–10). After 3 months of dietary therapy, 18 children (82%) had a >50%
seizure reduction, and 4 (19%) were seizure-free. Too few patients (only three) had EEG data before and during dietary therapy to determine the effect on spike wave discharges.

The same paper also included a summary of 17 previously published KD studies dating from 1922 to 2008, which included patients with CAE or JAE. Of the 133 children with absence epilepsy and documented outcomes (with responses recorded at 3 days to 3 months), more than two-thirds reported a greater than 50% seizure reduction and one-third experienced periods of seizure freedom. What is not known from this historical review is the proportion of patients presenting with a CAE-like syndrome due to glucose transporter type 1 deficiency, which is expected to respond well to the ketogenic diet.

A recent case report describes a 7-year-old girl with absence epilepsy who became seizure-free with the paleolithic ketogenic diet consisting entirely of meat, offal, fish, egg, and animal fats without calorie restriction, which she apparently tolerated well (Clemens et al., 2013). Of note, dietary therapy was used as first-line antiepileptic treatment because of parental concerns about the potential for medication side effects. Also of note, the child had other neurologic abnormalities (developmental delays, social withdrawal, and a history of febrile seizures)—and thus was atypical for classic CAE. Whether the child underwent diagnostic testing for glucose transporter type 1 deficiency is not reported. After an immediate and gradual decline in seizure frequency, the patient became seizure-free at 6 weeks of therapy. At 3 months, carbohydrates were reintroduced, in the form of low glycemic index vegetables. The patient had no evidence of epileptiform discharges on long-term video-EEG monitoring, and continued to be seizure-free 20 months after diet initiation.

Interestingly several large, single-center series of KD effectiveness include no patients with absence epilepsy (Dressler et al., 2010; Hallböök et al., 2015; Sharma et al., 2009; Wibisono et al., 2015). In a recent large, single-center series from Australia that included 61 patients initiating the KD, the 29 patients who were responders at 3 months included two with treatment-resistant CAE, both of whom continued to be responders at 12 months (Thammongkol et al., 2012).

**JUVENILE MYOCLOTONIC EPILEPSY**

Another genetic generalized epilepsy syndrome infrequently appearing in ketogenic diet series, JME is a common and highly recognizable epilepsy syndrome with onset in adolescence or young adulthood (Dreifuss, 1989). The hallmarks of the syndrome are myoclonic seizures (brief bilateral jerks with persevered awareness), often clustering in the morning and sometimes triggered by photic stimulation, and 4-Hz or faster generalized spike and wave or polyspike and wave discharges on EEG. Many patients initially come to medical attention not when the myoclonic jerks begin, but when the first GTC seizure occurs. Absence seizures occur in a subset of patients, and may be a marker of worse long-term seizure prognosis (Senf et al., 2013).

Because valproic acid (VPA) is efficacious in most patients with JME, it has long been considered the treatment of choice (Dreifuss, 1989). However, growing concerns about the long-term side effects and teratogenic potential of VPA have made it a less desirable choice, particularly in young women (Tomson et al., 2015; Wheless et al., 2005). In addition, treatment resistance, even to VPA, does occur in JME. Thus, dietary therapy may be considered in this population as well.

Very little published literature has addressed the use of KD-related therapies in JME. In 2013, Dr. Kossoff and colleagues described the use of the MAD in eight JME patients ages 15 to 44 years, six of whom were female (Kossoff et al., 2013). Except for one patient who was medication naïve, all others were treatment-resistant to four or more AEDs, and all but three had tried valproate. Of note, the mean age of onset was 10.5 years, which is atypically young for JME. Seven of the eight remained on the diet at 1 month and achieved at least moderate urinary ketosis. After 3 months, five (63%) reported a greater than 50% drop in seizure frequency, and two became seizure-free. At the time the paper was published, patients had remained on the diet 0.5 to 40 months; two patients were able to reduce medications, but none had stopped all AEDs.

Just as for CAE, many of the larger, single-center, long-term follow-up studies of the KD included no patients with JME. The case series from Australia discussed previously also included a single patient with JME, who was a responder at 3 months (no 12-month follow-up reported). A very recent study from Norway prospectively evaluated 13 adults with idiopathic generalized epilepsy (IGE), ages 16 to 57 years, initiating the MAD (Kverneland et al., 2015). The majority, nine, had JME, while two had CAE, one had Jeavon syndrome (absence epilepsy with eyelid myoclonia), and one had an IGE not otherwise specified. Nine
patients continued the MAD for at least 4 weeks, and only six reached the 12-week time point. Four patients achieved a 50% or greater reduction in seizure frequency—in one, frequent GTC seizures stopped. Lack of motivation or lack of adherence to the diet were the reasons most frequently cited for dropping out of the study.

While the reason for referral for KD treatment for most IGE patients may be treatment-resistant seizures, the KD has other potential advantages over AEDs besides efficacy. Though specific cognitive and behavioral challenges have been noted (inattention in CAE, impulsivity and other frontal lobe dysfunction in JME, for example), patients with IGE syndromes typically have normal intelligence at baseline—thus, cognitive adverse effects of AEDs may have a substantial impact on function, particularly for those on polytherapy. Though rigorous controlled studies are still lacking, the potentially beneficial effects of the KD on cognition have been repeatedly noted. Therefore, for IGE patients who are experiencing cognitive side effects from one or more AEDs, the KD may be a desirable alternative, or a strategy for reducing the number of AEDs.

A key challenge for dietary epilepsy therapy in patients with IGE is the feasibility and tolerability of the diet at a time when food choices are typically made more autonomously than in early childhood. In addition, maintaining the minimum protein requirements in an adolescent or adult in the context of a protein and carbohydrate restricted, but normal caloric, KD can be difficult. The MAD, which is high-fat and low carbohydrate, but less stringent in protein restriction, might be more feasible and tolerable than a classic KD in these patients. However, as shown in the study by Kverneland and colleagues, even adult patients may find the MAD difficult to carry out consistently (Kverneland et al., 2015). There are no reports of the use of two other KD-related therapies, the low glycemic index treatment (LGIT) and the MCT oil diet, in IGE syndromes specifically. The published series on LGIT include patients with generalized seizure types, but specific syndrome information is not reported (Muzykewicz et al., 2009; Pfeifer and Thiele, 2005).

In conclusion, the KD or MAD are viable treatment options for patients with medication-resistant IGE syndromes, and may be preferred over AEDs causing cognitive adverse effects. However, feasibility and tolerability continue to be a major barrier. Further investigations of the long-term outcomes of IGE treated with dietary treatments are needed.

REFERENCES


INTRODUCTION
Status epilepticus (SE) is defined by continuous epileptic status with alteration of consciousness. Status epilepticus might be a single prolonged seizure or recurrent seizures repeated frequently without recovery of consciousness between the seizures. The incidence of SE is 3–41/100,000 individuals per year (Chin et al., 2006, Raspall-Chaure, 2007, Dham, 2014) and it is the second most common neurologic emergency in adults and the first in children. The duration of SE was long debated, and an operational definition is proposed for tonic clonic status defining T1 as the time that emergency treatment should be started and T2 as the time at which long-term consequences on the brain might be expected (Trinka et al., 2015). For instance, T1 is from 5 minutes and above and T2 is over 30 minutes of duration (Trinka et al., 2015). 12%–43% of patients with SE fail to respond to first- and second-line therapy, and they enter refractory SE (after 2 hours of the onset of SE). Super-refractory SE (SRSE) is defined by the persistence of SE over 24 hours (Ferlisi and Shorvon, 2012). Overall, ~15% of SE cases admitted to the hospital become super-refractory (DeLorenzo, 1995; Krumholz et al., 1999; Ferlisi and Shorvon, 2012).

In adults, SRSE presents a high rate of mortality (>60%) (Ferlisi and Shorvon, 2012). Although the risk of death is low in the pediatric population, the risk of subsequent neurological morbidity and cognitive problems is high (Scott, 2009). The therapeutic intervention aims to reduce SRSE duration, mortality, and short- and long-term comorbidities.

Status epilepticus can be tonic clonic, tonic or myoclonic. It can be associated with acute or chronic brain disease or can occur in patients with known epilepsy. It might occur at the onset or in the course of the disease (Trinka et al., 2015). For instance, inaugural convulsive SE without previous known epilepsy occurs in CNS infections (encephalitis, meningitis), autoimmune epilepsies, epilepsies with presumed immune etiology such as FIRES and NORSE, metabolic disorders, and stroke. SE might occur during the course of epilepsy syndromes; tonic clonic or clonic in Dravet syndrome, tonic in Lennox Gastaut syndrome. In myoclonic atonic epilepsy (Doose syndrome), myoclonic status might occur at onset or during the first months. Myoclonic nonconvulsive status is frequent in some genetic syndromes such as Angelman syndrome, and in mitochondrial diseases (Trinka et al., 2015).

Identifying a possible underlying cause and treating it is an important step in the treatment of SE. An etiology-targeted therapy should be instituted as soon as the etiology is identified (antibiotics for CNS infections, antiviral agents for encephalitis, immune therapy for auto-immune encephalitis).

Benzodiazepines are the first-line therapy in SE, with different molecules used and different routes of administration; buccal, intra rectal or intra muscular. If benzodiazepines fail to stop the SE, phenytoin is usually the second-line drug. The use of other AEDS with IV formulations is the usual next step after the failure of phenytoin (Shorvon and Ferlisi, 2011). Barbiturate anesthesia is the most common third-line drug and is used in adults more often than in children. Propofol and ketamine can also be used (Ferlisi et al., 2015). The failure rate of anesthetic barbiturate and the frequent recurrence after withdrawal with associated high mortality and morbidity make it essential to develop other therapies for medication-resistant SE.

The KD has reported efficacy in SE (Kossoff and Nabbout, 2014). The multiple mechanisms of action of KD make it a good choice for refractory SE. The inherent combination of these mechanisms can mimic AED polytherapy, an approach that is suggested to be a good choice for RSE (Löscher, 2015, Gama et al., 2015, Lusardi et al., 2015).
The first report of efficacy of KD in a series of patients with highly recurrent seizures was in 2009 by Villeneuve et al. (Villeneuve et al., 2009). The authors reported KD response in a retrospective pediatric series of patients with focal pharmacoresistant epilepsies. The efficacy of KD was higher in patients who presented with SE or recent worsening of seizure frequency (Villeneuve et al., 2009). In this first series, efficacy was reported in different etiologies such as Sturge-Weber syndrome, FIRES, and cryptogenic focal SE (Villeneuve et al., 2009). A second paper in 2010 reported an international series of nine patients with SRSE due to FIRES with 7/9 responders to KD (Nabbout et al., 2010). The KD was well tolerated via nasogastric tube in the pediatric ICU setting. Ketosis was achieved mostly within 24 hours and SE stopped during the first days of the diet and in all responders within the first week (Nabbout et al., 2010).

Other reports of SE due to FIRES or similar immune entities were further reported in the literature, including pediatric (Vaccarezza et al., 2012, Nam et al., 2011, Sort et al., 2013, O'Connor et al., 2014) and adult patients (Cervenka et al., 2011, Thakur et al., 2014). The pathophysiology of FIRES, based on activation of an inflammatory cascade (Nabbout et al., 2011), makes this syndrome a possible specific target for KD. The majority of patients reported had RSE due to inflammatory etiologies (Table 9.1). This trend is confirmed by the largest pediatric series with FIRES (Nabbout et al., 2010) and in adult series (Thakur et al., 2014). In this last series of 10 patients, 4 patients presented with NORSE, 2 with anti-NMDA encephalitis, and 1 with LGI1 encephalitis.

Other etiologies, possibly involving inflammation cascades, were reported as a possible indication for KD in SE; the over representation of these inflammatory etiologies might be due to their frequency in SRSE. Patients with Rasmussen syndrome, an encephalitis involving the activation of the T cell pathway were reported as responders in a few pediatric (Villeneuve et al., 2009) and adult patients (Wusthoff et al., 2010).

SE in mitochondrial diseases as in Alpers disease with POLG1 mutations (Martikainen et al., 2012) and with stroke-like accidents as in MELAS syndrome (Steriade et al., 2014) are good candidates for KD to be introduced early at onset (Desguerres et al., 2014). Other small series of patients with focal SE due to structural lesions were also reported as responders to KD in pediatric (Villeneuve et al., 2009, Caraballo et al., 2014, Lin et al., 2015) or adult series (Bodenant et al, 2008).

**KETOGENIC DIET IN NONCONVULSIVE STATUS EPILEPTICUS**

The efficacy of KD is not restricted to convulsive SE. The efficacy of KD was reported in myoclonic etiologies (Table 9.1). This trend is confirmed by the largest pediatric series with FIRES (Nabbout et al., 2010) and in adult series (Thakur et al., 2014). In this last series of 10 patients, 4 patients presented with NORSE, 2 with anti-NMDA encephalitis, and 1 with LGI1 encephalitis.

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### TABLE 9.1 REPORTS OF EFFICACY OF KETOGENIC DIET (KD) IN SE IN CHILDREN AND ADULTS. *KD WAS INTRAVENOUS (IV)*

<table>
<thead>
<tr>
<th>Author</th>
<th>Population</th>
<th>Diet</th>
<th>Etiology</th>
<th>Time to response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bodenant (2008)</td>
<td>1 adult</td>
<td>KD</td>
<td>Partial</td>
<td>7 days</td>
</tr>
<tr>
<td>Villeneuve (2009)</td>
<td>5 children</td>
<td>KD</td>
<td>SWS, encephalitis…</td>
<td>1–10 days</td>
</tr>
<tr>
<td>Kumada (2009)</td>
<td>2 children</td>
<td>MAD</td>
<td>Frontal lobe, heterotopia</td>
<td>5–10 days</td>
</tr>
<tr>
<td>Wusthoff (2010)</td>
<td>2 adults</td>
<td>KD</td>
<td>Rasmussen, head trauma</td>
<td>8–10 days</td>
</tr>
<tr>
<td>Nabbout (2010)</td>
<td>9 children</td>
<td>KD</td>
<td>FIRES</td>
<td>4–6 days</td>
</tr>
<tr>
<td>Cervenka (2011)</td>
<td>1 adult</td>
<td>KD</td>
<td>Idiopathic, autoimmune</td>
<td>7 days</td>
</tr>
<tr>
<td>Ismail (2012)</td>
<td>1 child</td>
<td>KD</td>
<td>FIRES</td>
<td>10 days</td>
</tr>
<tr>
<td>Nam (2012)</td>
<td>4 children, 1 adult</td>
<td>KD</td>
<td>Encephalitis</td>
<td>1–19 days</td>
</tr>
<tr>
<td>Vaccarezza (2012)</td>
<td>5 children</td>
<td>KD</td>
<td>FIRES, partial status</td>
<td>?</td>
</tr>
<tr>
<td>Sort (2013)</td>
<td>3 children</td>
<td>KD</td>
<td>FIRES, HHE, mito</td>
<td>1–6 days</td>
</tr>
<tr>
<td>Thakur (2014)</td>
<td>10 adults</td>
<td>KD</td>
<td>Super refractory SE</td>
<td>1–7 days</td>
</tr>
<tr>
<td>O'Connor (2014)</td>
<td>5 children</td>
<td>KD</td>
<td>Mito, myoc, FIRES?</td>
<td>2–8 days</td>
</tr>
<tr>
<td>Caraballo (2015)</td>
<td>10 children (7/10)</td>
<td>KD</td>
<td>Focal seizures</td>
<td>5–7 days</td>
</tr>
<tr>
<td>Lin (2015)</td>
<td>1 child</td>
<td>KD*</td>
<td>Focal with Gen</td>
<td>2–3 days</td>
</tr>
<tr>
<td>Caraballo (2015)</td>
<td>2 children</td>
<td>KD</td>
<td>Myoclonic status (EE in 1)</td>
<td>3 days</td>
</tr>
</tbody>
</table>
SE of mitochondrial diseases and POLG mutations (Martikainen et al., 2012, Desguerres et al., 2014) and of myoclonic atatic epilepsy, or Doose syndrome (Kelley et al., 2010, Caraballo et al., 2013), and in patients with myoclonic SE from unknown etiologies (Caraballo et al., 2015). KD efficacy was also reported in patients with electrical SE during slow sleep (ESES) (Reyes et al., 2015; Veggiotti et al., 2012).

**CHALLENGES OF KETOGENIC DIET USE IN STATUS EPILEPTICUS**

The challenges of the KD might raise some concerns that limit its widespread use for SE in ICUs. Implementation of the diet could be a complex issue in ICUs, especially in centers lacking KD teams. The availability of the KD team and the daily communication between the neurologists, child neurologists, dietitians, and ICU teams are strongly recommended, enabling initiation of the diet under optimal conditions. This daily communication makes it possible to respect the follow-up of the diet, thus avoiding any additional glucose load from fluids and concomitant medications. Enteral feeding should be privileged, since parenteral feeding cannot achieve a high-ratio KD.

Enteral feeding is usually well tolerated in our experience, especially when initiated with continuous infusion slowly increasing the feeding rate to achieve the total caloric intake within 48–72 hours. The major steps for successful implementation in the ICU are summarized in Figure 9.1.

Another limiting factor for the use of the diet might be the time lag for efficacy. In the reported series, ketosis appears within 24 to 72 hours and seizure reduction within the first week (Table 9.1). This time lag is difficult to accept in a severe condition such as SE. Although many studies confirmed the main role of underlying etiology in the cognitive outcome of SE, the long duration of SE might also negatively impact the long-term outcome (Kilbride et al., 2013). However, after the first- and second-line therapies for SE, treatment alternatives are scarce and the use of an anesthetic agent is usually the main strategy left (Ferlisi and Shorvon, 2012). Anesthetic agents are potent seizure suppressors and might help to shorten the SE but their use is based on expert opinion and is not evidence based. In addition, some concerns have been raised about possible worsening of the outcome of refractory SE after the use of anesthetic agents (Sutter et al., 2014, 2015, Ferlisi et al., 2015). Limiting dextrose containing IV fluids early in the course of the SE

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**FIGURE 9.1** Steps to implement the diet in ICU.
after blood glucose has been controlled, and initiating the diet as soon as inborn errors of fat metabolism are ruled out might help to shorten the delay of ketosis and improve the efficacy of KD. Along these lines, some medications used in the setting of SE and refractory SE, such as steroids, can delay ketosis. They should be avoided when medically unnecessary (Nabbout et al., 2010).

The reports of KD in SE are mainly retrospective, reporting small numbers of individuals treated and rarely referring to patients where the diet failed. In addition, comedication and changing doses of comedication with the possible time to achieve efficacy of the diet might make causal attribution debatable. Finally, reports lack the long-term follow-up studies reporting cognitive and neurological outcome, which is, apart from mortality, the major endpoint of any treatment of refractory SE. Indeed, KD reports share the same weakness with all third-line therapies in refractory SE where no drug or therapy has achieved a high level of evidence-base medicine (Ferlisi and Shorvon, 2012).

The KD is well tolerated with low rates of side effects in the short and long term in the ICU setting, as detailed in different reports (Table 9.1). The increasing number of reports worldwide demonstrates its possible implementation in ICUs. Its efficacy in inflammatory SE or in SE from other etiologies, convulsive and nonconvulsive, should make it a therapeutic option in the treatment of refractory SE. Recently a few data on possible improvement of cognitive outcome are emerging in patients with FIRES (Kramer et al., 2011, Singh et al., 2014).

A prospective, randomized controlled trial is necessary to validate this treatment option, as for all third-line therapies for refractory SE. This is important for physicians—at least one patient has died after the KD was stopped following seizure arrest because “this indication was not considered as good clinical practice” (Nabbout et al, 2010). This trial should evaluate efficacy and tolerance and would be mandatory for the acceptance of KD by physicians and by health authorities and institutions. Outcomes should be evaluated in the short term - aiming for control of SE - and also in the long-term (at a few months or longer) to evaluate seizure control as well as cognitive outcomes. Pending the results of such a trial, KD should be available in ICUs and be part of the treatment arsenal of refractory SE, a critical situation where evidence-based medicine is dramatically lacking to date.

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Preventing Side Effects and Diet Discontinuation

CHERIE L. HERREN, MD AND RANA R. SAID, MD

INTRODUCTION
In general, the ketogenic diet (KD) is well tolerated. On average, 60% of patients remain on the diet for over 6 months. Those who stop the diet typically do so due to lack of efficacy rather than tolerability (Keene et al., 2006). Adverse effects of KD therapy can be divided into common and serious. They can also be categorized according to timing during KD therapy (during initiation, during maintenance, or during discontinuation). Common side effects include constipation, vomiting, acidosis, and vitamin/mineral deficiencies. More significant side effects are rare, but include pancreatitis, hepatitis, kidney stones, and cardiomyopathy. During initiation, transient side effects can include dehydration, hypoglycemia, acidosis, vomiting, and refusal to eat. Children need to be monitored closely during initiation so that these adverse effects can be avoided and treated. Fasting and fluid restriction may aggravate these side effects and are not uniformly implemented in KD programs. Fluid restriction contributes to dehydration and metabolic acidosis, which subsequently can cause vomiting and lethargy. With appropriate monitoring and supplementation, these adverse effects can be minimized so the patient can remain on the diet as long as indicated. In addition, there may be social issues, including refusal to eat and managing special occasions and holidays. With support and resources, most families are able to overcome these obstacles. When patients come off dietary therapy, this must be done gradually and with close supervision, as there may be an increase in seizures. Patients must be provided support and direction on how to safely discontinue the diet.

SCREENING
One of the most important steps in avoiding potential side effects (potentially severe or life threatening) is proper screening of patients. Patients with disorders of fat metabolism could present with severe and potentially fatal complications if started on the KD, as the diet will require a shift from carbohydrate metabolism to fat metabolism for energy. All patients should have a thorough metabolic assessment prior to initiation of dietary therapy as detailed in table 10.2. This screening should include electrolytes, blood counts, lipids, acylcarnitine panel, serum amino acids, urine organic acids, liver and kidney function, uninalysis, and urine calcium and creatinine (Kossoff et al., 2009). Patients with disorders of fat metabolism with impaired fatty acid transport and oxidation should not be started on the KD. There are other specific diagnoses for which the KD should not be used including (but not limited to) pyruvate carboxylase deficiency, porphyria and primary carnitine deficiency.

SOCIAL
To avoid complications it is imperative to screen patients both medically and socially. Patients and families should be adequately educated about what to expect while on the diet and the follow-up that will be needed (typically every 3–6 months). For those unwilling or unable to comply with the needed follow-up, careful consideration should be taken to determine whether the diet is an appropriate treatment option. Once patients are started on the diet, good social support can be beneficial to families to maintain adherence and can increase the likelihood that they continue on the diet. This support often comes from medical staff including neurologists, dietitians, and nurses who are well educated on the diet and the demands it places on families. Engagement and education of teachers and schools is vital to the success of KD therapy in school-age children. Reassurance and encouragement can also come from support groups designed specifically for families and KD-specific functions, such as Halloween parties. Special occasions tend to be especially difficult, but families may take comfort in knowing there are numerous recipes available for everything from ice cream to birthday cake! Some centers also use parent coaches,
who can help families navigate issues such as food refusal and menu ideas in real life.

**VOMITING**

Vomiting is one of the more common side effects seen in patients on the KD and is estimated to occur in 5% of patients (Keene et al., 2006). This is most commonly seen during the initiation phase of the diet. Metabolic acidosis may contribute to vomiting, and in turn, vomiting may worsen acidosis, creating a worsening cycle of symptoms. In many cases reversing metabolic acidosis will alleviate nausea and vomiting. In patients who are experiencing vomiting, maintaining proper hydration, either enterally or IV, is an important factor in breaking this cycle. Initiation is a common time for patients to experience vomiting related to the diet itself; however, vomiting can occur for other reasons (infectious, etc.) at any time while on the diet. Families and providers should be watchful for vomiting and have a low threshold to provide intervention. In those patients who cannot adequately maintain oral hydration due to vomiting or in those who have increasing lethargy, intravenous fluids may be required. Monitoring for constipation is also important, as children with constipation may be predisposed to vomit.

Families often are concerned about whether to re-dose antiseizure medications with emesis; we advise our parents to repeat the dose if vomiting occurred less than 30 minutes from dose administration. Management of constipation with polyethylene glycol is well tolerated. Some children require slower delivery of feeds, either with more frequent, smaller meals or, if gastrostomy tube fed, with slower rates of infusion with a feeding pump. For short-term vomiting around times of illness, use of Powerade Zero or Propel Zero can provide hydration and electrolytes, without carbohydrates. In instances of more prolonged feeding intolerance, half strength Pedialyte to provide a source of calories is usually well tolerated, without significant reduction in ketosis.

**ACIDOSIS**

Metabolic acidosis can be found in patients on the KD and can present with lethargy and vomiting. This will typically present during initiation and be minimized with the use of buffering agents such as sodium bicarbonate or Polycitra K (Cypress Pharmaceuticals, Madison, MS, USA). Practitioners should be aware of medications that could worsen acidosis, especially common antiseizure medications, such as topiramate and zonisamide. In some cases, it may be necessary to decrease these medications in order to manage acidosis. During initiation, acidosis should be treated with aggressive hydration and use of oral alkalinizing agents. Rarely would IV alkalization be indicated. Once the patient is on a stable diet ratio, with adequate doses of alkalinizing agents, acidosis becomes less problematic. However, during times of illness, with decreased oral intake and/or vomiting, acidosis can again become problematic and typically can be managed with hydration.

**CONSTIPATION**

Given the composition of the KD, an increase in constipation would not be unexpected. Constipation is common and occurs in up to 65% of patients (Wibisono et al., 2015). This can be particularly problematic in those who had preexisting constipation, and for those who have impaired mobility. Increased hydration may help with symptoms, but many patients will require medications to manage their constipation. Polyethylene glycol (Miralax) is a common choice for treatment as it is not significantly absorbed in the gut and is

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**TABLE 10.1** TIME PATIENT REMAINS ON KETOGENIC DIET (KEENE, 2006)

<table>
<thead>
<tr>
<th>Author</th>
<th>Total sample</th>
<th>% Sample at 3 months</th>
<th>% Sample at 6 months</th>
<th>% Sample at 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>DiMario [2002]</td>
<td>48</td>
<td>?</td>
<td>50</td>
<td>37</td>
</tr>
<tr>
<td>Coppola [2002]</td>
<td>56</td>
<td>75</td>
<td>38</td>
<td>7</td>
</tr>
<tr>
<td>Maydell [2001]</td>
<td>147</td>
<td>80</td>
<td>66</td>
<td>48</td>
</tr>
<tr>
<td>Hassan [1999]</td>
<td>52</td>
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safe for patients on the KD. Glycerin suppositories, increased dietary fiber, medium chain triglyceride (MCT) oil, or mineral oil could also be considered.

HYPOGLYCEMIA

Like many other potential side effects, the risk for hypoglycemia tends to be highest during the initiation phase of the diet. If patients have severe hypoglycemia (<30 mg/dL) or symptomatic hypoglycemia (<40 mg/dL with lethargy, vomiting, diaphoresis, seizures, or shakiness), small amounts of juice (10–20 mL) or dextrose (D5W infusion until blood glucose is >60 mg/dL) can be given to correct the blood sugar. If hypoglycemia is a persistent problem, a reduction in ratio should be considered. Once stable on the diet, glucose tends to be on the low end of normal (50–70 mg/dL), but is overall very stable. Fasting prior to initiation of KD therapy may increase the chances of hypoglycemia (Bergqvist et al., 2005) and therefore close glucose monitoring in these patients is recommended.

KIDNEY STONES

Renal calculi have been reported in 3%–7% of children on the KD (Kielb et al., 2000). These typically form, on average, after 18 months of KD therapy. However, there have been reports of development of urolithiasis after just 1 month on the diet. The majority of stones are uric acid stones, however, calcium oxalate, calcium phosphate, and mixed calcium/urate acid stones are seen. Approximately 50% of stones are uric acid stones (Kielb et al., 2000). This is likely due to KD lowering urinary pH, which facilitates formation of uric acid crystals, as the solubility of uric acid decreases as pH drops. The KD also has been associated with uric acidemia and increased urinary uric acid, which further predisposes to the development of uric acid stones. This can be compounded by fluid restriction, which produces a more acidic urine and decreased urine flow, with precipitation of urate crystals.

The KD has also been associated with hypercalciuria, which can result in calcium crystal formation. Hypocitraturia also occurs due to chronic metabolic acidosis. Urinary citrate is an inhibitor of calcium crystal formation; therefore, low urinary levels increase the risk of calcium stone formation. Fluid restriction serves to increase this risk (Herzberg, 1990).

Children typically present with gross or microscopic hematuria, therefore patients require regular screening urinalysis. They may also, less commonly, have gritty urine or pain. There may be a role for periodically checking serum uric acid and urinary calcium/creatinine ratios. Renal ultrasound is necessary if there is evidence of hematuria. Children with family histories of kidney stones receiving carbonic anhydrase inhibitors (topiramate, zonisamide, or acetazolamide) are also at higher risk for stones (Furth et al., 2000 and Kossoff, 2002). Treatment includes fluid liberalization and urinary alkalization with bicarbonate (Sampath, 2007). In children with a higher risk for kidney stone formation, prophylactic treatment with Polycitra K has been shown to reduce the incidence of kidney stones in this susceptible population (McNally et al., 2009).

INCREASED INFECTIONS

Several studies have reported rates of increased infections in up to 2%–4% of children on the KD (Woody et al., 1989; Vining et al., 1998; Summ et al., 1996). Woody et al. assessed neutrophil function in nine children on the KD, demonstrating that while ketotic, these patients had significantly less bacterial phagocytosis and killing. These effects were reversible, with resolution when the diet was discontinued (Woody et al., 1989). Other conditions associated with ketosis, such as diabetes mellitus, alcoholism, glycogen storage disease, protein-calorie malnutrition, certain carbohydrate-restricted diets, and intralipid infusions, have also reported impairments in neutrophil function. The exact mechanism is not well understood but is likely related to serum metabolites that affect early processes in phagocytosis (Woody et al., 1989). Interestingly, only one of Woody’s patients experienced serious bacterial infections.

VITAMIN DEFICIENCIES

Due to the restrictive nature of the diet (with limited vegetables and grains), it is known to be deficient in several vitamins and minerals, especially B vitamins, calcium, and vitamin D. The International Ketogenic Study Group (Kossoff et al., 2009) made it a universal recommendation that all patients on the KD should be on a multivitamin with minerals and receive calcium with vitamin D supplements. In the study, some did suggest additional supplementation may be needed for zinc, magnesium, selenium, and phosphorous, but this was not a universal recommendation, and may be considered on an as-needed basis depending on laboratory values. Care should be taken to select a carbohydrate-free or
low-carbohydrate multivitamin such as NanoVM (Solace Nutrition, Rockville, MD, USA), FruitiVits (Vitafo, Alexandria, VA, USA), Centrum (Wyeth, Madison, NJ, USA), or Bugs Bunny Sugar Free (Bayer, Morristown, NJ, USA). Vitamin levels should be followed on a routine basis during follow-up, and early supplementation to avoid deficiencies is optimal.

ELECTROLYTE IMBALANCES
Electrolytes such as calcium, magnesium, sodium, potassium, and phosphorus are often depleted or deficient in children on KD therapy. These laboratory values must also be monitored closely and adequately supplemented. In children on KD formulas, many of these electrolytes may be included in the formula. However, children on solid diets will often need additional supplements. These can be in the form of dietary supplements (such as Morton’s Lite Salt) or prescription (K Phos Neutral).

HYPERLIPIDEMIA
Studies show that between 30% and 60% of patients on the KD develop hypercholesterolemia (Wibisono et al., 2015; Nizamuddin et al., 2008). Nizamuddin found this was increased from 25% at preinitiation (baseline). Notably, patients eating a solid diet had a greater risk for developing high cholesterol compared with those who consumed a formula-based diet, likely secondary to an increase in saturated fats in solid foods compared with the liquid formulation. The dyslipidemia seen improved spontaneously and without intervention in about half of patients, suggesting patients are better able to metabolize the fat over time (Nizamuddin et al., 2008). It is unclear whether the dyslipidemia seen with the diet has any long-term cardiovascular or atherosclerotic effects, but given the temporary use of the diet it appears to be low (Berry-Kravis et al., 2001). Nonetheless, routine monitoring of liver enzymes is recommended, and in the event that elevations are noted, hepatic ultrasound and comanagement with gastroenterology should be considered, including possible diet discontinuation.

CARDIAC SIDE EFFECTS
Cardiac complications have been reported in children on the KD, including cardiomyopathy and prolonged QT interval (Neal, 2012). These risks may be related to carnitine and selenium deficiencies associated with the KD. Bergqvist et al. (2003) reported a child with cardiomyopathy in association with selenium deficiency and other children who had lowering of selenium levels while on the KD. In a study of 20 children on the KD, 15% (three children) had prolongation of the QTc interval. Of these three children, two had preinitiation electrocardiograms (EKGs), which confirmed that the QTc abnormalities developed while they were on the diet. Two of the patients had left atrial and left ventricular enlargement, and one had severe ventricular dilatation and dysfunction, with associated symptoms of heart failure. Interestingly, all of the patients in this series had normal selenium levels. It was postulated that higher beta-hydroxybuturate levels and more significant metabolic acidosis may be associated with the development of cardiac complications.

There are also reports of two children developing severe dilated cardiomyopathy while on the KD (Ballaban-Gil et al., 1999). In these cases, the cardiomyopathy resolved following discontinuation of the KD. It is suggested that baseline EKGs and echocardiograms be completed prior to initiation of pancreatitis while on the KD would necessitate coming off the diet (Stewart et al., 2001). Parenteral KD therapy with intralipids also places children at high risk for developing pancreatitis and should be used judiciously.

HEPATITIS
While rare, hepatitis is a potentially significant side effect of the KD. It has been suggested that concomitant use of VPA may increase the risk of hepatitis, however Kang et al. (2004) did not find a statistically significant increase in hepatitis for those on VPA, compared with those not taking this antiseizure medication. Decreased carnitine levels may also increase the risk for liver dysfunction, however the risk appears to be low (Berry-Kravis et al., 2001). Nonetheless, routine monitoring of serum carnitine and carnitine supplementation is recommended. Regular monitoring of liver enzymes is recommended, and in the event that elevations are noted, hepatic ultrasound and comanagement with gastroenterology should be considered, including possible diet discontinuation.

PANCREATITIS
Hyperlipidemia and hypertriglyceridemia are risk factors for development of pancreatitis, which are also potential complications of KD therapy. Concomitant therapy with valproic acid (VPA) may increase this risk. There has been a case report of a 9-year-old who died from acute hemorrhagic pancreatitis while on KD therapy. The development of pancreatitis while on the KD would necessitate coming off the diet (Stewart et al., 2001). Parenteral KD therapy with intralipids also places children at high risk for developing pancreatitis and should be used judiciously.
of the KD and while on the diet, including carnitine and selenium levels. Supplementation with carnitine and selenium should be strongly considered.

**GROWTH**

There are conflicting reports of how the KD affects growth—both linear and weight—in children on the diet. Williams et al. (2002) retrospectively reviewed 21 children treated with the KD for 9.6 months to 2 years, specifically looking at linear growth velocity. Eighteen children (86%) had a drop from their original height percentiles while on the diet. This was independent of mean age, duration on the diet, protein intake, or calories consumed per body weight while on the diet. They concluded that decreased linear growth might be secondary to poor nutritional status. While these children received the recommended nutrient intake for protein, calories were typically restricted to 75% of recommended daily intake. The authors postulated that dietary protein was needed to fill the calorie gap for energy and gluconeogenesis, resulting in relative protein insufficiency to support growth (Williams et al., 2002).

Vining et al. prospectively reviewed the growth of 237 children treated with the KD. They found that older children grew taller “almost normally” but younger children fell more than two standard deviations below the mean in height (Vining et al., 2002). However, Couch et al. (1999) retrospectively evaluated the nutritional status and growth of 21 children on the KD for 6 months. They found a statistically significant increase in both height and weight after 6 months on the diet. Six children had a decrease in mean percentile standard weight for height, but no one fell below their pre-KD height for age percentile after 6 months on the diet. However, the study was limited by the short duration of follow-up (Couch et al., 1999).

Children on the KD need to be monitored closely for adequate protein intake and growth parameters. Often children with intractable epilepsy become more physically active as seizures improve, and energy needs must be recalculated. Protein intake must be adequate and regular laboratory studies to assess protein, albumin, and pre-albumin are necessary. Lack of weight gain can be commonly seen; however, inadequate or slowed growth (which is more problematic in infants) and inappropriate weight loss occur less frequently.

**OSTEOPENIA**

Baseline bone mineral content may be low in patients starting the KD, with only an estimated 54%–63% of patients receiving adequate calcium and vitamin D through their preinitiation diet (Bergqvist et al., 2008). The poor bone health seen may be secondary to antiseizure medication (ASM) use, which can interfere with calcium and vitamin D metabolism and directly impacts the cells of bone formation and absorption. Despite adequate supplementation with calcium and vitamin D, and an overall reduction in ASMs, once patients were started on the KD there was further decline in bone mineral content. Chronic acidosis may contribute to the decline in bone health, and buffering agents such as sodium bicarbonate or sodium citrate/citric acid may be beneficial in reducing the risk of osteopenia and fractures. Bone density scans may not be indicated for all patients, but could be considered in those at higher risk (younger nonambulatory patients) or for those who have experienced pathologic fractures.

**FOOD REFUSAL**

Tolerability of the KD is the single most important factor limiting individual acceptance for initiation, however, 60% of children who start the KD remain on it at 6 months follow-up (Table 10.1). The most common reason for discontinuation of the diet is not food refusal or concerns with palatability of the diet, but lack of efficacy.

Food refusal is typically related to a limited repertoire of foods. Families are encouraged to keep several menus on rotation to minimize food fatigue. Even changing presentation of meals can help with tolerability; for example, one family used a child’s tea service, with its smaller plates to make meals more enjoyable. Close communication with the KD registered dietitian is vital as they have a wealth of suggestions for improved food intake. Parent KD coaches are also a tremendous resource. Food refusal may also represent a food intolerance; changing to a lactose-free formula may be helpful. If food refusal becomes a significant battle, reducing the KD ratio or changing to an MCT-based diet to increase amounts of carbohydrates may be necessary. At times, changing to other dietary therapies, such as the modified Atkins diet or low glycemic index therapy, is warranted.

**DISCONTINUING THE KETOGENIC DIET**

There are many reasons why the KD is discontinued, the most common being lack of efficacy after 3–6 months or if seizures worsen. After 2 years of effective use of the KD, most neurologists would consider discontinuation. Gradually lowering the ratio of fat to protein and carbohydrate, then
relaxing the weighing of ingredients, and finally adding new carbohydrate foods while keeping calories constant is typical. This is followed by relaxation of calorie restriction, then gradual introduction of carbohydrate-containing foods. The ideal weaning speed of the KD is not clear, specifically as it pertains to the risk of increased seizures. Worden et al. (2011) retrospectively reviewed 183 children who discontinued the KD. There was no significant difference in the incidence of seizures worsening between immediate (<1 week), quick (1–6 weeks), or slow (>6 weeks) rates. However, there was an increased risk of seizures worsening in those patients specifically with a 50%–99% seizure reduction and those who were receiving more antiseizure medications (Worden et al., 2011).

Approximately 80% of children will remain seizure-free when tapered off the KD if they have completely responded to diet therapy and are medication free. Factors associated with a higher risk of recurrence include an epileptiform electroencephalogram (EEG), focal abnormalities on neuroimaging, and tuberous sclerosis complex. Approximately 50% of children with recurrence are difficult to control once again, even with resumption of the KD (Martinez et al., 2007).

CONCLUSION
While the KD is an effective therapy for refractory epilepsy and certain metabolic disorders, there are potential adverse effects. Providers must be aware of these risks for proper monitoring of children and counseling families. Like all medical therapies, potential risks of treatment are weighed against benefits of the diet.

REFERENCES


Chapter 10: Preventing Side Effects and Diet Discontinuation


SECTION II

Ketogenic Diet

Emerging Clinical Applications
and Future Potential

JONG M. RHO, MD, SECTION EDITOR
The ketogenic diet (KD) is a proven therapy for drug-resistant epilepsy (Vining et al., 1998; Neal et al., 2008), and while the mechanisms underlying its antiseizure effectiveness remain unclear (Masino and Rho, 2012), there is growing experimental evidence for the neuroprotective properties of this metabolism-based treatment (Barañano and Hartman, 2008; Maalouf et al., 2009; Gano et al., 2014). Although definitive clinical evidence for use outside of epilepsy is presently lacking, there are nevertheless ongoing human trials for a few neurological conditions (www.ClinicalTrials.gov) assessing whether a ketogenic therapy can ameliorate symptoms and even the disease processes themselves. Much of the experimental evidence has been converging on the notion that the broad efficacy of the KD is due in major part to normalization of aberrant energy metabolism (Masino and Rho, 2012). The concept that multiple neurological disorders are pathophysiologically linked to energy dysregulation (Pathak et al., 2013) has been strengthened by numerous studies evaluating brain bioenergetics, and provides a solid rationale for how dietary treatments that ameliorate underlying dysfunction can treat or even prevent disease symptomatology.

In this section, “Ketogenic Diet—Emerging Clinical Applications and Future Potential,” leading investigators in the field of ketogenic therapies provide up-to-date reviews summarizing the evidence that the KD may prove useful in the treatment of a number of neurological conditions. Drs. Thomas Seyfried, Adrienne Scheck, and colleagues put forward a compelling case for cancer as being in part a metabolic (and perhaps more specifically, a mitochondrial) disorder, and that the KD should be strongly considered as adjunctive treatment for malignant brain cancer (Seyfried and Shelton, chapter 12; Woolf and Scheck, chapter 13). These chapters are followed by a comprehensive review by Dr. Ning Cheng and co-authors (chapter 14) of metabolic treatments for autism spectrum disorder, whose prevalence has been rising rapidly throughout the world. Next, Dr. Stephen Cunnane and collaborators (chapter 15) provide a unique translational perspective on brain metabolism—using novel positron emission tomography techniques to make the case that nutritional ketosis, achieved through several currently available strategies, can correct the underlying problem of declining brain fuels during aging. Dr. Wolf Tetzlaff and coauthors (Streijger et al., chapter 16) then review the evidence that the KD (and in particular, ketone bodies) can prevent the metabolic, oxidative, and inflammatory cascades that follow after spinal cord injury and traumatic brain injury. The next chapter, by Drs. Nina Dupuis and Stéphane Auvin (chapter 17), reviews the mounting evidence that the KD possesses anti-inflammatory properties, relevant not only to the pathophysiology of pain syndromes and multiple sclerosis but also to other neurological disorders. Finally, Dr. Carl Stafstrom (chapter 18) covers the current evidence that the KD may also afford beneficial effects against two neurodegenerative disorders (amyotrophic lateral sclerosis and Parkinson’s disease), mood disorders, and migraine. Throughout all of these apparently distinct neurological conditions, it should become clear to the reader that metabolic derangements are now considered key to the pathophysiology of these disorders, and that a dietary treatment such as the KD can potentially benefit patients afflicted with any one of several neurological diseases. It is also the hope of the aforementioned authors that those curious about how altered metabolism can both induce and mitigate neurodegeneration and brain dysfunction will be compelled to pursue further laboratory and/or clinical research in this growing and fascinating area of translational neurosciences.
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Metabolism-Based Treatments to Counter Cancer

Scientific Rationale

THOMAS N. SEYFRIEND, PHD AND LAURA M. SHELTON, PHD

INTRODUCTION
Cancer has long been recognized as a genetic disease involving mutations in oncogenes and tumor suppressor genes that reside in the tumor cell nucleus. The nuclear gene mutations found in nearly all types of tumors are considered the primary cause of the cancer's hallmarks, including (1) sustained proliferative signaling, (2) evasion of growth suppressors, (3) resistance to cell death, (4) replicative immortality, (5) enhanced angiogenesis, and (6) activation of invasion and metastasis (Hanahan and Weinberg, 2000, 2011). The somatic mutations in tumor cells arise randomly during DNA replication in normal nontumorigenic stem cells and are thought to be the origin of cancer (Tomasetti and Vogelstein, 2015).

The somatic mutation theory is the most widely accepted explanation for the origin of cancer, and is the justification for developing targeted or personalized approaches for treating various forms of the disease (Hou and Ma, 2014; McLeod, 2013; Seyfried, 2015; Vaux, 2011). Despite numerous inconsistencies associated with the somatic mutation theory (Baker and Kramer, 2007; Burgio and Migliore, 2015; Cao et al., 2015; Rous, 1959; Sonnenschein and Soto, 2000; Soto and Sonnenschein, 2004), this theory is presented as if it were dogma in most current college textbooks of genetics, biochemistry, and cell biology, and is the mainstay of the National Cancer Institute's position which states, “Cancer is a genetic disease—that is, it is caused by changes to genes that control the way our cells function, especially how they grow and divide” (http://www.cancer.gov/cancertopics/what-is-cancer) (Seyfried, 2015). It should be recognized, however, that there is no place in science for epistemological dogmatism, as dogma tranquilitizes creative thinking and blocks consideration of viable alternatives (Rous, 1959; Sonnenschein and Soto, 2000).

IS CANCER A GENETIC DISEASE OR A MITOCHONDRIAL METABOLIC DISEASE?
As an alternative to the somatic mutation theory, emerging evidence suggests that cancer is primarily a mitochondrial metabolic disease (Bartesaghi et al., 2015; Hu et al., 2012; Seyfried, 2015; Seyfried et al., 2014; Seyfried and Shelton, 2010; Verschoor et al., 2013). This concept originated from the experiments of Otto Warburg (Burk et al., 1967; Warburg, 1956a, 1956b). According to Warburg's theory, respiratory insufficiency is the origin of cancer. All other phenotypes of the disease, including the somatic mutations and aerobic fermentation, arise either directly or indirectly from insufficient respiration (Seyfried, 2012a; Seyfried et al., 2014; Warburg, 1956a). Warburg's metabolic theory was also in line with the concepts of C. D. Darlington and others showing that cancer is largely a cytoplasmic mitochondrial disease (Darlington, 1948; Seeger, 1959; Seyfried, 2012a, 2015; Seyfried and Shelton, 2010; Woods and du Buy, 1945). Advocates of the somatic mutation theory, however, consider the abnormal energy metabolism of tumor cells as simply another phenotype that “is programmed by proliferation-inducing oncogenes and defective tumor suppressor genes” (Hanahan and Weinberg, 2011; Seyfried, 2015). The gene theory of cancer, however, is inconsistent with the data obtained from the nuclear-cytoplasm transfer experiments.

A recent review of data from a broad range of nuclear-cytoplasm transfer experiments provides the most compelling evidence to date showing that somatic mutations alone cannot account for the origin of cancer (Seyfried, 2015). These experiments showed that cytoplasm containing normal mitochondria suppressed tumorigenesis in cells with tumor-derived nuclei. In contrast, cytoplasm
containing abnormal mitochondria from tumor cells enhanced tumorigenesis in cells with normal nuclei. Viewed in the light of Warburg’s central theory, the nuclear-cytoplasm transfer experiments show that cancer originates from damage to the mitochondria in the cytoplasm rather than from damage to the genome in the nucleus. The genomic instability seen in tumor cells follows, rather than precedes, the disturbances in cellular respiration.

Seyfried et al. described how the common pathophysiological mechanism underlying cancer, that is, “the oncogenic paradox,” was better explained when cancer is considered as a mitochondrial metabolic disease than when considered as a genetic disease (Seyfried, 2012c; Seyfried et al., 2014; Seyfried and Shelton, 2010), and recently outlined how all roads to the origin and progression of cancer pass through the mitochondria (Seyfried et al., 2014). Hence, novel therapeutic strategies for management and prevention emerge when cancer becomes recognized as a mitochondrial metabolic disease.

CANCER CELL METABOLISM

It is well known that lactate fermentation is the common metabolic malady seen in most if not all cancer cells (Seyfried et al., 2014; Warburg, 1931, 1956a, 1956b, 1969). Indeed, lactate fermentation can be detected even in the slowest growing tumors (Burk and Schade, 1956; Burk et al., 1967). The greater the lactate production, the faster is the rate of tumor growth. In addition to expressing robust fermentation in hypoxic environments, cancer cells also ferment lactate in aerobic environments. Aerobic fermentation is the antithesis to the Pasteur effect (suppression of lactate fermentation in the presence of oxygen) and has become known as aerobic glycolysis or the Warburg effect (Racker, 1972). Lactate production is greater in cancer cells than in normal cells, because the efficiency of oxidative phosphorylation (OxPhos) is less in cancer cells than in normal cells. Reduced OxPhos efficiency arises from any type of defect in the number, structure, or function of mitochondria (Arismendi-Morillo and Castellano-Ramirez, 2008; Elliott et al., 2012; Feichtinger et al., 2015; Feichtinger et al., 2010; Kiebish et al., 2008; Pedersen, 1978; Seyfried et al., 2014; Singh et al., 2015; Srinivasan et al., 2015). Mitochondrial structure is linked directly to mitochondrial function (Lehninger, 1964). No malignant tumor tissue has yet been found that contains mitochondria of normal number or structure when evaluated under electron microscopy.

Although the evidence supporting mitochondrial abnormalities and OxPhos insufficiency in cancer is now overwhelming, some metabolic studies on cultured tumor cells can be inconsistent with the findings from intact tumor tissue. The unnatural conditions of the in vitro growth environment (Crabtree effect) can make some cancer cells appear to have normal respiration despite their continued production of lactate. The information on tumor cell energy metabolism obtained from in vitro studies, however, is sometimes at odds with the data from in vivo studies. The results from many in vitro studies suggest that normal cells and tumor cells have similar mitochondrial energy metabolism (Cairns, 2015; Koppenol et al., 2011). If mitochondrial structure is abnormal in the vast majority of tumor cells seen in various cancerous tissues, how is it possible that mitochondria and OxPhos could be normal in cultured cells from the tumors? Seyfried et al. recently addressed several confounding issues related to tumor cell energy metabolism that is measured in cultured cells (Seyfried et al., 2014).

The reduced efficiency of mitochondrial OxPhos for ATP production in tumor cells is compensated for with an increased dependency on substrate level phosphorylation for energy production. Substrate level phosphorylation occurs at steps 7 and 10 in the glycolytic pathway, and at the succinyl-CoA synthase reaction (Step 5) of the citric acid cycle. Only those cells that can transition their energy production from OxPhos to substrate level phosphorylation can become tumor cells. Cells that cannot make this transition will die and can never become tumor cells (Warburg, 1956a). As substrate level phosphorylation is less efficient in producing ATP than is OxPhos, cancer cells must consume greater amounts of fermentable fuels than normal cells in order to maintain an adequate supply of ATP for viability (Seyfried and Shelton, 2010).

The increased consumption of glucose is clearly seen with positron emission tomography (PET) scanning using radiolabeled glucose for detecting cancer in the body (Vander Heiden et al., 2009). Elevated glucose consumption leads to an increase in lactate production and acidification of the tumor cell microenvironment (Gatenby and Gillies, 2004). Microenvironment acidification increases inflammation and angiogenesis, which ultimately increases tumor progression. Succinate, produced through amino acid fermentation (Hochachka et al., 1975), can also contribute to microenvironment acidification and inflammation (Tannahill et al., 2013). Hence, the
majority of cancer characteristics can be linked directly to mitochondrial dysfunction coupled with increased glucose consumption for compensatory fermentation.

In addition to a reliance on glucose, cancer cells also depend heavily on glutamine for growth and survival (DeBerardinis and Cheng, 2010; Yuneva, 2008). Glutamine is anaplerotic and can be rapidly metabolized to glutamate and then to α-ketoglutarate for entry into the TCA cycle. In addition to serving as a carbon/nitrogen source for tumor cell growth, glutamine may also play a role in cancer cell survival and growth through enzymatic release of ammonia into the microenvironment (Huang et al., 2013). Glucose and glutamine act synergistically for driving rapid tumor cell growth. Glutamine can produce some ATP under aerobic conditions and can serve as fuel for lipid synthesis under hypoxic conditions (Ta and Seyfried, 2015). In contrast to previous suggestions (DeBerardinis et al., 2007), we found that only minor amounts of glutamine are metabolized to lactate under either normoxia or hypoxia in the VM-M3 invasive glioblastoma cells (Ta and Seyfried, 2015). We suggest that glucose and glutamine can be either oxidized or fermented depending on the physiological state of the tumor microenvironment. Our findings suggest that glutamine targeting can be effective in managing systemic metastatic cancer (Shelton et al., 2010b).

Besides glucose and glutamine, other metabolic fuels might also play a role in tumor cell metabolism. It has been shown recently that in the presence of electron transport chain (ETC) abnormalities, aspartate can rescue cell proliferation without increasing ATP synthesis, suggesting that the redox reactions of the ETC are required for survival (Sullivan et al., 2015). In cancer cells, this acts as a potential target for metabolic therapies because aspartate is central to the de novo synthesis of purines and pyrimidines and proteins, and for maintaining cellular redox balance. While aspartate itself is not necessarily used for ATP synthesis, a number of amino acids such as serine, glycine, and the branched chain amino acids can be catabolized to pyruvate, succinate, and fumarate, all of which participate in the metabolic pathways required for ATP generation and anaplerosis. Additionally, there is evidence suggesting that in the absence of oxygen, Complex III of the ETC can run in reverse, effectively reducing fumarate to succinate, which will accumulate as a fermentation byproduct (Hochachka et al., 1975). This will maintain the redox balance and result in ATP synthesis.

Several byproducts of amino acid fermentation can also accumulate in the tumor microenvironment including acetate, glutamate, alanine, succinate, and ammonia. Although acetate has been considered a potential fuel for supporting tumor cell growth (Comerford et al., 2014; Hosios and Vander Heiden, 2014), acetate levels are generally low in the circulation (Ballard, 1972). Jaworski et al. recently provided a comprehensive discussion on the potential role of acetate in tumor metabolism (Jaworski et al., 2015). It should be recognized that with the exception of glucose and glutamine, none of the other potential fuels for tumor cell metabolism would be present in sufficiently high quantities to maintain robust tumor cell growth. Hence, the restriction of glucose and glutamine becomes of prime importance for targeting tumor cell growth and survival.

**CALORIE RESTRICTION**

Calorie restriction (CR) with adequate nutrition or underfeeding has been long known to limit tumor growth (Hursting et al., 2010; Kritchevsky, 2001; Mukherjee et al., 1999; Tannenbaum, 1942). The mechanisms by which CR reduces tumor growth have been described. Calorie restriction targets several of cancer’s hallmarks, including angiogenesis, inflammation, edema, proliferation, and distal invasion (Jiang and Wang, 2013; Mukherjee et al., 1999; Mulrooney et al., 2011; Shelton et al., 2010a; Thompson et al., 2004; Woolf et al., 2015). Calorie restriction lowers the level of circulating glucose, the prime fermentable fuel for driving tumor growth (Seyfried et al., 2003). In addition, CR or therapeutic fasting, elevates the level of circulating ketone bodies, D-β-hydroxybutyrate and acetoacetate. Ketone bodies serve as a non-fermentable super fuel for functional mitochondria that also reduce reactive oxygen species while increasing the ΔG’ of ATP hydrolysis and metabolic efficiency (Seyfried et al., 2014; Seyfried et al., 2003; Veech, 2004). Based on these observations, Cahill and Veech characterized ketone bodies as “Good Medicine” (Cahill and Veech, 2003). In contrast to normal cells, which transition to ketone bodies for energy when glucose becomes limiting, tumor cells cannot use ketone bodies effectively for energy (Fredericks and Ramsey, 1978; Magee et al., 1979; Maurer et al., 2011; Tisdale, 1984). Ketone bodies can even be toxic to some tumor cells (Poff et al., 2015; Skinner et al., 2009). Shimazu et al. recently suggested that high-dose D-β-hydroxybutyrate could act as a histone deacetylase inhibitor (Shimazu et al., 2013).
The multiple defects in mitochondrial structure and function are considered responsible for failure of tumor cells to effectively use ketone bodies for energy (Seyfried and Mukherjee, 2005; Seyfried and Shelton, 2010).

Notwithstanding this growing experimental literature, the therapeutic benefit of a 40% CR for managing cancer in rodents would not be the same as for managing cancer in humans. The basal metabolic rate in humans is about seven times less than that of mice (Terpstra, 2001). Consequently, water-only therapeutic fasting for 2–3 weeks would be predicted to produce a therapeutic benefit similar to that seen in rodents under 40% CR (Mahoney et al., 2006). As a ketogenic diet (KD) can sometimes replicate the physiological conditions of therapeutic fasting (Seyfried et al., 2009), it will be easier for most cancer patients to use a KD than to use therapeutic fasting for tumor management.

THE KETOGENIC DIET
The KD is a high-fat and low-carbohydrate diet that is widely used to reduce refractory epileptic seizures in children (Freeman and Kossoff, 2010; Kossoff and Hartman, 2012), and is also effective in managing symptoms of a wide range of neurological and neurodegenerative diseases (Seyfried, 2014). The KD is also gaining recognition as an effective therapy for any cancer that expresses the Warburg effect, which includes the majority of metastatic cancers. The anticancer mechanism of action for the KD is potentially very simple. The KD lowers the prime fermentable fuel (glucose) needed for driving the Warburg effect, while elevating blood ketone bodies that require mitochondrial function for effective metabolism. As mitochondrial defects are ultimately responsible for the reliance of tumor cells on fermentation, the KD targets the Warburg effect and tumor cell energy metabolism. The KD can more effectively reduce glucose and elevate ketone bodies than can CR alone, making the KD potentially more therapeutic against tumors than CR. There are no anticancer drugs presently known that can target tumor cells while enhancing the metabolic efficiency of normal cells, as can the KD.

It is important to mention, however, that the unrestricted feeding of KDs can cause weight gain, insulin insensitivity, and dyslipidemia in some strains of mice, leading to elevated blood levels of insulin and glucose (Meidenbauer et al., 2014). Dyslipidemia from excessive consumption of KD can occur despite the very low amounts of carbohydrate in the diet. This could account in part for our failure to detect a therapeutic benefit against either naturally occurring seizures in EL mice, or against astrocytoma growth in mice that were fed KDs in unrestricted amounts (Mantis et al., 2004; Seyfried et al., 2003; Zhou et al., 2007).

Reduced blood glucose is considered essential for managing either epileptic seizures or cancer. As high-fat diets will elevate the appetite suppressor hormone, cholecystokinin, it is possible that dyslipidemia might not occur in most cancer patients on a KD. A therapeutic KD should lower glucose, cholesterol, and triglycerides, while elevating ketone bodies and high-density lipoprotein (Meidenbauer et al., 2014). The body weight loss associated with KD is considered therapeutic, in contrast to the weight loss associated with cachexia or toxic cancer therapies, which is pathological. In contrast to the therapeutic shift in blood metabolites associated with the KD weight loss, cachexic weight loss is associated with elevations of blood glucose and insulin insensitivity that can stimulate tumor growth (Kotler, 2000). High-dose steroids (dexamethasone), which elevate blood glucose levels, are often given to cancer patients undergoing chemotherapy to reduce vomiting and to improve weight gain (Seyfried et al., 2015). It is clear from an understanding of cancer metabolism that that steroid administration for improved weight gain would not be in the best interest of the cancer patient (Wong et al., 2015). Hence, fundamental metabolic differences exist between therapeutic weight loss associated with a KD and the pathological weight loss associated with cachexia and toxic cancer therapies.

The protein and fat composition of the KD differs from that of Atkins-type diets in having less protein and more fat than the Atkins diets. This is important as several amino acids found in proteins can be de-aminated to form pyruvate, which is then metabolized to form glucose through gluconeogenesis (Burt et al., 1982). The fats in KDs also contain more saturated medium chain triglycerides than do Atkins diets. Consequently, blood glucose levels will be lower and ketone body levels will be higher with KDs than with Atkins-type diets. We recently developed the Glucose/Ketone Index calculator (GKIC) to assess the potential therapeutic effects of various low-carbohydrate and KDs for cancer management (Meidenbauer et al., 2015). The GKIC is a simple tool that measures the ratio of blood glucose to blood ketones and can help monitor the efficacy of metabolic therapy in preclinical animal models and in clinical trials for malignant brain cancer or for any
Chapter 12: Metabolism-Based Treatments to Counter Cancer

83

cancer that expresses aerobic fermentation. The GKI can therefore serve as a biomarker to validate the therapeutic efficacy of various diets in targeting cancer cell energy metabolism.

THE FUTURE OF KDS FOR CANCER MANAGEMENT AND PREVENTION
It is our view that reduced glucose with therapeutic ketosis, achieved through low-carbohydrate KDs, can serve as an alternative or complementary approach to cancer management. Evidence also suggests that the therapeutic efficacy of the KD against tumor growth can be enhanced when combined with certain drugs and procedures under a “press-pulse” paradigm as we previously mentioned (Seyfried et al., 2014). For example, therapeutic synergy was seen in combining the calorie restricted KD with the glycolysis inhibitor, 2-deoxyglucose, for management of the syngeneic CT-2a glioma stem cell tumor (Marsh et al., 2008), and in combining the KD with hyperbaric oxygen therapy and ketone esters for the management of systemic metastasis of the syngeneic VM-M3 tumor (Poff et al., 2013; Poff et al., 2015). Scheck and colleagues also showed that the KD could act synergistically with radiation therapy for management of the GL261 mouse model of glioma (Abdelwahab et al., 2012).

The therapeutic effects of KDs seen in these preclinical brain tumor studies have been corroborated in preclinical studies for several other cancer models including neuroblastoma, lung cancer, prostate cancer, and breast and ovarian cancers (Allen et al., 2013; Ly et al., 2014; Mavropoulos et al., 2009; Morscher et al., 2015; Zhuang et al., 2014). These preclinical studies are also motivating case reports and pilot studies in humans with brain cancer and other cancers (Champ et al., 2013; Champ et al., 2014; Maroon et al., 2015; Rieger et al., 2014; Schmidt et al., 2011; Zuccoli et al., 2010). It is clear from these studies, and from the original observation of Linda Nebeling and colleagues (Nebeling et al., 1995), that treatment of cancer patients with KDs is generally well tolerated, which is consistent with decades of research obtained from the evaluation of children treated with KDs for epilepsy management.

In recognizing cancer as a mitochondrial metabolic disease, we suggested that protecting cellular mitochondria from toxic or metabolic stress could best prevent cancer (Seyfried, 2012b). Calorie-restricted KDs and therapeutic fasting are excellent ways to reduce oxidative stress in mitochondria. Metabolism of the ketone body β-D-hydroxybutyrate maintains the coenzyme Q couple in an oxidized state thus reducing the production of damaging ROS (Veech, 2004). Therapeutic water-only fasting or restricted KDs reduce tissue inflammation (Mulrooney et al., 2011; Youm et al., 2015). Chronic inflammation is known to produce mitochondrial stress and cancer (Bissell and Hines, 2011; Coussens and Werb, 2002; Fosslien, 2008; Kamp et al., 2011). It is our contention that glucose/ketone ratios (GKI values) of 1.0 or below could help reduce systemic inflammation. The GKI might therefore serve as an effective biomarker, along with C-reactive protein, for assessing systemic inflammation and for reducing mitochondrial stress that can prevent cancer. Further studies will be needed to validate this concept.

It has not escaped our attention that KDs could have a significant impact on global budgeting contracts for patient care. It is now recognized that global budget contracts with quality incentives encourage changes in practice patterns that can help reduce spending and improve quality of general health (Song et al., 2014). The KDs are well positioned to serve as a low-cost, nontoxic alternative for both the prevention and management of cancer. In addition to cancer, we recently showed how therapeutic ketosis could be effective for the management and prevention of a broad range of chronic inflammatory diseases including obesity, type 2 diabetes, cardiovascular disease, epilepsy, Alzheimer’s disease, Parkinson’s disease, and traumatic brain injury (Seyfried, 2014). Hence, KDs could have utility for improving health and reducing costs for many of the most challenging diseases seen in Western societies.

ACKNOWLEDGMENTS
This work was supported in part by the NIH, Grants (HD-39722, NS-108055195, and CA-102135); and grants from the American Institute of Cancer Research; the Single Cause, Single Cure Foundation; the George Yu Foundation; and the Boston College Research Expense Fund.

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Ketogenic Diet as Adjunctive Therapy for Malignant Brain Cancer

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INTRODUCTION
Human malignant glioma is a uniformly fatal disease due in part to the limitations of currently available treatments, which include surgery, chemotherapy, and radiation therapy. The average survival of patients with glioblastoma multiforme (GBM) is 1.5 years. It is therefore of paramount importance that new therapeutic strategies for brain cancer patients be developed, especially those that can enhance the efficacy of current treatment options without damaging normal brain tissue. Advances in our understanding of the biology of these tumors have led to an increase in the number of targeted therapies in preclinical and clinical trials (Nicholas et al., 2011; Niyazi et al., 2011; Roesler et al., 2010). While these therapies may prove somewhat effective, the heterogeneity of this tumor often precludes the targeted molecules from being found on all cells in the tumor, thus reducing the efficacy of these treatments. In contrast, one trait shared by virtually all tumor cells is altered metabolism.

TUMOR METABOLISM
Alterations in the metabolism of cancer cells, what we now call the “Warburg effect” or aerobic glycolysis, was first described by Otto Warburg in 1927 (Warburg et al., 1927). Cancer cells use glycolysis to provide energy and biomolecules regardless of the availability of oxygen. This results in the production of fewer ATP molecules per molecule of glucose, and thus tumor cells require large amounts of glucose. This shift toward increased glycolytic flux in the cytosol and away from the tricarboxylic acid cycle and oxidative phosphorylation in the mitochondria occurs very early in tumorigenesis. This allows for rapid cell proliferation even under conditions of hypoxia and in the presence of dysfunctional mitochondria. Since Warburg’s discovery, metabolism has been of interest in the cancer field, but it often seemed overshadowed by discoveries of oncogenes, tumor suppressor genes, growth factor pathways, molecular subtypes of cancers, and so forth. There is a resurgence of interest in metabolism as a central theme in cancer, and we continue to find that metabolic pathways intersect and often regulate key components of tumor initiation, progression and therapy response (Wolf et al., 2010; Nijsten and van Dam, 2009). In fact, altered metabolism itself has been referred to as a hallmark of cancer (Cantor and Sabatini, 2012; Ward and Thompson, 2012), in addition to being involved in virtually all of the cancer hallmarks described in the seminal paper by Hanahan and Weinberg (Hanahan and Weinberg, 2011) (see Figure 13.1; Lewis and Abdel-Haleem, 2013).

The term “metabolic remodeling” has been used to describe metabolic changes that can occur in cancer cells (Obre and Rossignol, 2015), and oncogene-associated pathways are now known to intersect with and alter metabolic pathways. For example, the tumor suppressor protein p53, which plays a pivotal role in the cellular responses to hypoxia, DNA damage, and oncogene activation, is now known to regulate glycolysis and assist in maintaining mitochondrial integrity (Puzio-Kuter, 2011). Another important connection between metabolism and tumor growth is through regulation of c-MYC. Overexpression of c-MYC occurs in a wide variety of cancers, including gliomas. C-MYC is a multifunctional transcription factor, and the list of its target genes include those involved in both cell proliferation and cell metabolism (Miller et al., 2012). In addition to stimulating glycolysis, c-MYC has been found to activate glutaminolysis and lipid synthesis from citrate (Obre and Rossignol, 2015).

With the advent of molecular analyses, studies of growth factor pathways seemed to overshadow the influence of metabolism on cancer growth.
Overactivation of the stress-responsive PI3K/AKT signaling pathway is typical in many cancers and often due to activation of growth factor signaling pathways involved in glioma growth, such as platelet-derived growth factor, epidermal growth factor, and insulin growth factor. We now know that these growth factor pathways are intertwined with metabolic signaling pathways (Dibble and Cantley, 2015; Courtnay et al., 2015; Roberts and Miyamoto, 2015; Martini et al., 2014; Iurlaro et al., 2014). The PI3K/AKT signaling pathway has been closely linked to metabolism, and under low glucose conditions it results in rapid tumor cell death (Marie and Shinjo, 2011; Robey and Hay, 2009; Yang et al., 2009).

Another important “hub” linking metabolism and cancer is hypoxia-inducible factor 1 (HIF-1). The expression of HIF-1 is activated by hypoxia, which is typically found in high-grade gliomas and other cancers. HIF-1 is a heterodimeric transcription factor that induces the transcription of a variety of genes involved in angiogenesis (vascular endothelial growth factor [VEGF] and other cytokines) in an attempt to improve tissue perfusion. This results in the formation of abnormal blood vessels that can increase inflammation and edema in brain tumors, as well as induction of the transcription of a variety of genes that promote invasion, migration, and tumor growth (Fischer et al., 2002; Fujiwara et al., 2007; Hayashi et al., 2007; Horing et al., 2012; Justus et al., 2015; Kaur et al., 2005; Masson and Ratcliffe, 2014; Mou et al., 2010; Proescholdt et al., 2012; Yang et al., 2012). In addition to specific actions that relate to the tumor cell's response to oxygen availability, HIF-1 interacts

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**FIGURE 13.1** An illustration of the interconnectedness of tumor metabolism with Hanahan and Weinberg’s hallmarks of cancer (Lewis and Abdel-Haleem, 2013).
with the PI3K/AKT signaling path to act as a regulator of cancer metabolism, proliferation, and glycolysis (Courtnay et al., 2015; Justus et al., 2015; Wei et al., 2013; Pore et al., 2006). It also affects the activation of nuclear factor–kappa B (NF-κB), a transcriptional activator that is central to the regulation of various signal transduction pathways and to transcriptional activation events that mediate inflammation, cell proliferation, cell migration, and angiogenesis. HIF-1 may, at least in part, provide the molecular basis for the Warburg effect by “reprogramming” cellular metabolism in response to oxygen availability (Corbet and Feron, 2015; Courtnay et al., 2015). HIF-1 also is a central figure in alterations to the tumor microenvironment, which affects not only tumor cell growth but also response to therapy (Azzi et al., 2013; Bayley and Devilee, 2010; Bruzzese et al., 2014; Casey et al., 2015; Danhier et al., 2013; Dewhirst, 2009; Hattingen et al., 2011; Horing et al., 2012; Hu et al., 2012; Joon et al., 2004; Justus et al., 2015; Marie and Shinjo, 2011; Masson and Ratcliffe, 2014; Amberger-Murphy, 2009; Metallo et al., 2011; Semenza, 2013; Shweiki et al., 1995; Stegeman et al., 2014; Wei et al., 2011; Yamada et al., 1999; Yang et al., 2012).

The molecular background of a tumor cell can also affect the regulation of the pathways already described. Loss of function of phosphatase and tensin homolog (PTEN) or mutation of p53 also increase HIF-1, as does the accumulation of reactive oxygen species (ROS). ROS are multifaceted effector molecules involved in numerous cellular pathways, including those regulating autophagic/apoptotic responses to genotoxic stress, hypoxia, and nutrient deprivation. Cancer cells often have increased levels of ROS (Fruehauf and Meyskens, 2003), and they have been implicated in angiogenesis induction and tumor growth through the regulation of VEGF and HIF-1 (Weinberg and Chandel, 2009).

It is clear that cancer cell metabolism is far more complex than originally thought. A number of cancer-associated mutations affect metabolism, and defects in mitochondria are seen in cancer that also link metabolism with cancer initiation and progression. Although some of these interactions are mentioned above, in-depth discussions of all of the interactions that occur between cancer and metabolism are beyond the scope of this chapter and the reader is referred to a number of reviews on these subjects (Cantor and Sabatini, 2012; Gatenby and Gillies, 2004; Semenza, 2013; Vander Heiden et al., 2009; Ward and Thompson, 2012; Robey et al., 2015; Gaude and Frezza, 2014; Masson and Ratcliffe, 2014). The fact that metabolic dysregulation is seen in virtually all tumor cells has led to suggestions that a promising therapeutic strategy may be to exploit this feature. One potential way to achieve this goal is through the use of the therapeutic ketogenic diet (KD) or physiologically similar methods, such as caloric restriction or intermittent fasting.

THE KETOGENIC DIET

The KD is more correctly referred to as “metabolic therapy” rather than a “diet.” This high-fat, low-carbohydrate diet is used to treat medically refractory epilepsy (Kim and Rho, 2008; Cross, 2013) in children and, more recently, in some adults. The diet is not without side effects; however, these are typically readily managed when the patient has appropriate supervision by a multidisciplinary team (i.e., dietitian, nurse, and physician) skilled in its use. The KD has been shown to have neuroprotective effects, and there are now studies to determine its efficacy for a number of neurological disorders, including Alzheimer’s disease, traumatic brain injury, and amyotrophic lateral sclerosis (Maalouf et al., 2009; Stafstrom and Rho, 2012).

The KD increases blood ketones and decreases blood glucose by simulating the physiological response to fasting, thus leading to high rates of fatty acid oxidation and an increase in the production of acetyl coenzyme A (acetyl-CoA), When the amount of acetyl-CoA exceeds the capacity of the tricarboxylic acid cycle to utilize it, there is an increase in the production of the ketone bodies β-hydroxybutyrate (βHB) and acetoacetate (ACA), which can be used as an energy source in the normal brain (Cahill and Veech, 2003; Morris, 2005; Vanitallie and Nufert, 2003; Veech et al., 2001; Gasior et al., 2006). Since normal cells readily use ketones as an alternate energy source, they are unlikely to be adversely affected by reduced glucose. In contrast, the metabolic alterations found in cancer cells are generally thought to reduce their ability to be “flexible” regarding their primary energy source, and thus they require glucose (Seyfried et al., 2011; Maurer et al., 2011; Zhou et al., 2007; Seyfried and Mukherjee, 2005; Tisdale and Brennan, 1983; Fredericks and Ramsey, 1978; Seyfried, 2012). The reduction in glucose that results from the KD essentially “starves” the tumor cells and thus inhibits their growth (Baranano and Hartman, 2008; Bozzi and Zupec-Kania, 2015; Freedland et al., 2008; Klement, 2013; Maroon et al., 2015; Seyfried et al., 2009; Seyfried et al., 2015; Simone et al., 2013; Wallace et al., 2010).
Thus, when used as a therapy, the KD can take advantage of the Warburg effect.

In addition to the effects mediated by glucose reduction, work in the epilepsy field and more recent work in cancer research has shown that the KD can exhibit antitumor effects even in the absence of glucose reduction. We demonstrated the effect of adding ketones to media containing glucose in vitro using the AO2V4 cell line (Scheck et al., 2012). This cell line was derived from a recurrent human glioblastoma and is grown in Waymouth’s MAB 87/3 media containing 28 mM glucose and supplemented with 20% fetal calf serum. When 5 mM βHB plus 5 mM ACA was added to complete media, cell growth was significantly inhibited. When 1,3-bis(2-chloroethyl)-1 nitrosourea (BCNU, carmustine), one of the chemotherapeutic agents given to this patient prior to tumor recurrence, was used in addition to ketones there was additional growth inhibition compared to either ketones or BCNU alone. More recent work has shown that the ketones themselves exert antitumor effects separate from the effects of reduced blood glucose (Magee et al., 1979; Scheck et al., 2012; Skinner et al., 2009). The ketone βHB has recently been shown to inhibit histone deacetylases (HDACs), which can result in epigenetic suppression of the expression of a variety of genes. Thus, ketones may provide a link between metabolism and tumorigenesis, although the precise nature of these changes is as yet unknown (Newman and Verdin, 2014a, 2014b; Sassone-Corsi, 2013; Shimazu et al., 2013). The remainder of this chapter addresses the utility of increasing blood ketones and reducing blood glucose for the treatment of brain tumors.

CURRENT STATUS OF KNOWLEDGE

While the idea that metabolism may be a therapeutic target for the treatment of cancer originated with Otto Warburg, there have been only a limited number of case reports describing the use of a KD for cancer therapy. Similarly, preclinical studies of efficacy and mechanisms of action were limited until recently.

Preclinical Evidence

The use of metabolic alteration for the therapy of brain tumors has been championed by Seyfried and colleagues. They used the VM (Shelton et al., 2010) and CT-2A (Marsh et al., 2008) mouse tumor models to show that a KD, especially when given in restricted amounts, extends survival. D’Agostino and colleagues have added hyperbaric oxygen and ketone supplementation to demonstrate reduced tumor cell growth and metastatic spread in the VM metastatic tumor model (Poff et al., 2015; Poff et al., 2014). Scheck et al. used the syngeneic intracranial GL261-luc/albino C57/Bl6 model to demonstrate that caloric restriction was not necessary for the antitumor effect of the KD (Stafford et al., 2010), particularly when a 4:1 fat:carbohydrate plus protein formulation is used (Scheck et al., 2012).

The KD and similar diets used as a monotherapy have a pluripotent effect on the growth of tumors both in vitro and in vivo. This may depend, at least in part, on the model system, the specific metabolic intervention, and the molecular underpinnings of the tumor itself (Cas o et al., 2013; Freedland et al., 2008; Hao et al., 2015; Kim et al., 2012c; Lv et al., 2014; Mavopoulos et al., 2009; Otto et al., 2008; Poff et al., 2015; Poff et al., 2013; Simone et al., 2013; Stafford et al., 2010; Woolf et al., 2015). The striking feature of the work done to date is that alterations in metabolism have a far-reaching effect on tumor cells, tumors, and the tumor microenvironment. Studies have shown reductions in growth rate as one might expect; however, there are also changes in the formation of ROS and oxidative stress (Allen et al., 2013; Stafford et al., 2010; Milder and Patel, 2012), angiogenesis (Puchowicz et al., 2008; Woolf et al., 2015; Seyfried et al., 2015; Zhou et al., 2007), hypoxia (Maurer et al., 2011; Poff et al., 2015; Woolf et al., 2015), inflammation and peritumoral edema (Kim et al., 2012a; Mavopoulos et al., 2009; Woolf et al., 2015), metastasis and invasion (Amann and Hellerbrand, 2009; Gluschnaider et al., 2014; Hao et al., 2015; Lv et al., 2014; Otto et al., 2008; Poff et al., 2015), and the expression of various transcriptional modulators such as NF-κB (Woolf et al., 2015) and microRNAs (Wang et al., 2015a; Wang et al., 2015c; Irani and Hussain, 2015; Bishop and Ferguson, 2015; Wang et al., 2015b; Chan et al., 2015).

Ketogenic Diet in Combination with Standard Therapies

Although evidence suggests that the KD provides antitumor benefits on its own, perhaps the most effective use of the KD is in combination with standard cancer therapies such as radiation and chemotherapy (Allen et al., 2014). The KD greatly enhanced survival in a mouse model of malignant glioma when combined with TMZ when compared with either treatment alone (Figure 13.2) (Scheck et al., 2011). Using a bioluminescent, syngeneic intracranial model of malignant glioma, the KD
was shown to significantly potentiate the anti-tumor effect of radiotherapy. In fact, 9 out of 11 animals maintained on the KD and treated with radiation had complete and sustained remission of their implanted tumors, even after being switched back to a standard rodent diet (Figure 13.2) (Abdelwahab et al., 2012). Allen and colleagues reported similar results when the KD was combined with radiation and chemotherapy in a lung cancer xenograft model (Allen et al., 2013). That is, they found decreased tumor growth rate and increased survival. Calorie restriction and short-term fasting have also been found to be synergistic with radiation and other anticancer therapeutics in both preclinical and clinical studies (Raffaghello et al., 2008; Lee et al., 2010; Seyfried et al., 2012; Lee et al., 2012; Safdie et al., 2012; Saleh et al., 2013; Champ et al., 2013; Champ et al., 2014; Klement and Champ, 2014; Poff et al., 2013; Raffaghello et al., 2010).

The effectiveness of radiation therapy is due to a number of factors including relative damage done to tumor cells versus normal tissue and the ability of normal cells and tumor cells to repair the damage (Klement and Champ, 2014). Radiation works, in part, by creating ROS through the radiolysis of water. The ROS damage the DNA and other macromolecules, causing sublethal damage that can become lethal if not repaired. The potentiation of radiation therapy by the KD or CR seems paradoxical in light of our data demonstrating a reduction in ROS in tumors from animals maintained on a KD (Stafford et al., 2010). However, radiation effects do not only occur through ROS, and radiation can directly damage DNA and other cellular macromolecules. Furthermore, in addition to ROS, radiation causes the production of reactive nitrogen species (RNS), a potential source of macromolecular damage following radiation (Saenko et al., 2013). Whether the KD or CR reduces the formation of RNS is as yet unknown. In fact, the main effect of the KD or CR may not be in altering the amount of radiation-induced damage, but may in fact be in modulating the ability of tumor and normal cells to repair radiation-induced damage (Klement and Champ, 2014; Santivasi and Xia, 2014). Studies have shown that CR can enhance DNA repair in normal cells (Heydari et al., 2007); however, this may not be the case in tumor cells, and the differential response of tumor cells and normal cells to genotoxic stress may be mediated by reduced IGF1 and glucose in the tumor cells. We and others have shown that insulin-like growth factor is reduced in animals maintained on a KD (Freedland et al., 2008; Klement and Champ, 2014; Mavropoulos et al., 2009; Scheck et al., 2012). In addition, a number of studies have shown that reduced activation of the PI3K/Akt pathway, activation of the adenosine monophosphate-activated protein kinase (AMPK) signaling pathway, and reduction of receptor tyrosine kinase growth factor pathways can all reduce radioresistance in tumor cells (Zhang et al., 2014; Choi et al., 2014; Medova et al., 2013; Gil Del Alcazar et al., 2014; Munshi and Ramesh, 2013; Li et al., 2014; Sanli et al., 2014; Danhier et al., 2013; Wang et al., 2013).

Finally, ketones and the KD have been shown to affect the immune system (Rahman et al.,

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**FIGURE 13.2** Kaplan-Meier survival plot of animals implanted intracranially with GL261-luc2 malignant glioma cells and (A) maintained on KetoCal® (KC, the 4:1 fat:carbohydrate plus protein formulation of the ketogenic diet) versus standard diet (SD); (B) treated with 2 × 4 Gy radiation versus KC plus radiation, and (C) treated with 50 mg/kg temozolomide (TMZ) versus KC plus TMZ. Animals on KC survived significantly longer when treated with KC alone, when KC was combined with radiation, and when KC was combined with TMZ (Abdelwahab et al., 2012; Scheck et al., 2011).
2014; Husain et al., 2013; Kim et al., 2012b; Youm et al., 2015), and we have shown that the KD also reverses tumor-mediated immune suppression in a mouse model of malignant glioma (Lussier, Woolf et al., 2016). As radiation-induced tumor killing is known to expose the immune system to a greater diversity of tumor antigens, it is possible that the KD as an adjuvant works to augment the effect of radiation in part by enhancing immunity against GBM.

The variety of effects seen when glucose is lowered and/or ketones are increased suggests that this may also potentiate other therapies, including newer immune- and targeted therapies. Concerns that potentiation of the antitumor effect of a particular therapy may also increase its effect on normal brain are valid. However, we and others have shown that the gene expression changes seen in tumor are different from those seen in normal brain (Stafford et al., 2010; Chang et al., 2013; Maurer et al., 2011). Further, the KD is known to have neuroprotective effects (Lund et al., 2009; Maalouf et al., 2009; Hartman, 2012; Puchowicz et al., 2008), and thus it has been postulated that this may actually help to protect the normal brain from the deleterious effects of radio- and chemotherapy. Taken together, the preclinical data provides strong support for the clinical use of the KD or CR as an adjuvant therapy for the treatment of gliomas and other cancers.

**KETOGENIC DIET IN HUMANS**

Studies of glucose utilization in cancer go back prior to the 1980s, including studies of metabolism and cancer cachexia (Fearon et al., 1988; Tisdale et al., 1987). These and other studies suggested that a KD consisting of a high percentage of medium chain triglycerides (MCT) along with various supplements resulted in weight gain and improved nitrogen balance in both animals and humans. In 1995, Nebeling and colleagues published a case report in which they used a similar KD based on MCT oil to treat two female pediatric patients with advanced stage malignant brain tumors (Nebeling and Lerner, 1995; Nebeling et al., 1995). They demonstrated that dietary-induced ketosis decreased the availability of glucose to the tumor without causing a decrease in patient weight or overall nutritional status. Furthermore, both children had long-term tumor management (Nebeling et al., 1995). The second case report was published in 2010 by Zuccoli and coworkers (Zuccoli et al., 2010). This patient was a 65-year-old female with a multicentric glioblastoma. She was put on a 4:1 (ratio of fats:carbohydrate plus protein) calorie-restricted (600 kcal/day) KD during radiation and chemotherapy. During this time, her body weight dropped by 20%, she had reduced blood glucose, increased urinary ketones, and, most importantly, no observable brain tumor detectable by either fluoro-deoxyglucose positron emission tomography (FDG-PET) or magnetic resonance imaging (MRI). The tumor recurred 10 weeks after the patient resumed her normal eating habits, and she succumbed to her disease less than 2 years after diagnosis. While this patient did not experience long-term tumor control after cessation of the diet, this report demonstrated that the diet could be tolerated, even when used in a calorie-restricted setting.

Results of a phase 1 clinical trial were reported in 2011 by a German group (Schmidt et al., 2011). Tolerability of a restricted calorie KD was tested in 16 patients with a variety of advanced (end-stage) cancers. There were no severe side effects, and 5 of the 16 patients were able to complete the 3-month treatment. These five patients had stable disease while on the diet. Two of the 11 remaining patients died early following the beginning of the trial, one was unable to tolerate the diet and dropped out immediately, two patients dropped out for personal reasons, one was unable to continue the diet for more than a month, three had disease progression within less than 2 months of starting the diet, and one dropped out to resume chemotherapy. While this trial demonstrated tolerability and a favorable side-effect profile, the antitumor efficacy could not be assessed due to the variety and severity of disease in the patients. Recently, Schwartz et al. reported on two patients with recurrent GBM treated with a calorie-restricted KD as monotherapy, and although the diet was tolerated, both patients showed tumor progression—the first within 4 weeks and the second within 12 weeks of beginning the protocol (Schwartz et al., 2015). More recently, a number of prospective clinical trials have been initiated which have been summarized in Table 13.1. These trials include studies of up-front treatment using the KD in addition to standard radiation and chemotherapy in patients diagnosed with GBM.

The case reports described above, along with numerous anecdotal reports, suggest that the KD may be a promising anticancer therapy. However, more work is needed to determine how to best utilize the KD and other metabolic therapies for the treatment of tumors. Most of the information regarding the most effective way to use the KD comes from the epilepsy literature. Further
research is needed to determine optimum blood ketone and glucose levels for anticancer effects. In addition, a variety of KDs are used for seizure control, and it is not clear whether one or more of the different formulations will provide the best results for cancer patients. Finally, while the KD has a long record of safety in the epilepsy community, side effects that occur when used in combination with cancer therapies may differ in type or severity. These data will come from carefully controlled clinical trials that include input from healthcare professionals well versed in the use of the KD. Furthermore, patient enrollment into clinical trials requires “buy-in” from the medical community. Physicians must be educated on the therapeutic benefits of metabolic alteration as an adjuvant therapy. As with any decision regarding therapy, the patient’s overall condition, including nutritional status, must be taken into account. As suggested by Klement and Champ (2014), cancer patients should be comprehensively assessed for nutritional needs and tolerability of such interventions.

Concern about patients’ quality of life is sometimes given as a reason not to employ the KD. Compliance can be made more difficult by the use of steroids (prescribed for peritumoral edema) that often increase hunger and raise blood glucose levels. To address this, at least one clinical trial (NCT02046187) includes an analysis of both patient and caregiver quality of life. Quality of life measurements are being added to more clinical trials, as the importance of this has become recognized at the national level (Dirven et al., 2014; Boele et al., 2013; van den Bent et al., 2011). While some clinicians are concerned compliance will reduce quality of life, the patients that do remain on the KD often comment that this allows them to participate in their own therapy. Despite these caveats, the existing preclinical data suggesting antitumor efficacy and a synergistic effect with standard therapies provides a strong impetus to conduct controlled clinical trials, particularly those that will shed light on the interactions between the KD and other therapies.

**CONCLUSION**

Improvements in the survival and quality of life for patients with malignant brain tumors require the implementation of new therapeutic modalities, especially those that increase the efficacy of current therapies without increasing toxic side effects. While the rapid accumulation of data defining the molecular and genetic aberrations present in these tumors has suggested a host of targets for the development of new treatments, targeted therapies tried to date have met with limited success. This is at least in part due to the molecular heterogeneity of these tumors that prevents any one target from being present in all cells. In contrast, metabolic dysregulation is present in virtually all tumor cells and there is rapidly increasing interest in using metabolic therapies such as the KD for the treatment of various cancers, especially brain tumors. Preclinical data have demonstrated that the antitumor effects of the KD and CR are multifaceted, and alterations in energy metabolism can inhibit cancer cell growth and increase the tumor’s response to therapy. This provides a strong impetus to continue work designed to elucidate the mechanisms through which the KD exerts its anticancer effects, as well as suggesting the need for the design of controlled clinical trials that will shed light on the most effective ways to implement metabolic therapies in combination with standard therapies for the treatment of malignant disease. This is a novel therapeutic paradigm, and we have only begun to scratch the surface in terms of its potential.

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cycles retard growth of tumors and sensitize a range of cancer cell types to chemotherapy. Sci Transl Med 4, 124ra27.


OVERVIEW OF AUTISM SPECTRUM DISORDER

Autism spectrum disorder (hereafter referred to as "ASD") is a group of complex disorders of neurodevelopment. It is characterized by persistent deficits in social communication and interaction across multiple contexts, as well as restricted and repetitive patterns of behavior and interests (DiCicco-Bloom et al., 2006; Lai et al., 2014; Llaneza et al., 2010). Further, it is well known that ASD is a multorgan disorder that can affect the normal function of the gastrointestinal, immune, hepatic, and endocrine systems (Frye et al., 2015; Goines and Van de Water, 2010; Hsiao, 2013; Mayer et al., 2015; Patterson, 2011). The term “spectrum” refers to the wide range of symptoms and levels of impairment that can occur in individuals with ASD. Severity of language impairment of the patients, for example, can range from deficits in pragmatic use of language to complete lack of spoken language. Another level of heterogeneity is the existence of many comorbidities that include both neurologic conditions such as epilepsy, sleep impairment, delays and deficits in motor functions, and psychiatric dysfunctions including depression, anxiety, irritability, attention deficit hyperactivity disorder, as well as physical health issues such as gastrointestinal disturbances. The co-occurrence rate of one or more non-ASD developmental diagnoses is as high as 83% (Levy et al., 2010). By contrast, some people with ASD show extraordinary talents in math, music, and art.

According to epidemiological studies, ASD occurs in all racial, ethnic, and socioeconomic groups, and is highly prevalent. It affects tens of millions individuals worldwide and costs a family approximately $60,000 USD a year on average. In the United States, the incidence of ASD is 1 in 68 children (1 in 42 boys and 1 in 189 girls) based on the data released by the Centers for Disease Control and Prevention in 2014. For controversial reasons, the prevalence of ASD appears to be on the rise (a 10-fold increase in 40 years), which may be explained only partly by improved diagnosis and awareness (Hansen et al., 2015). Developmental delay in ASD can be detected as early as 6 months of age, a critical time for the development of higher-order social, emotional, and communications functions (Courchesne et al., 2007), and increasingly, the importance of early intervention is being recognized (Orinstein et al., 2014). However, on average, children identified with ASD were not diagnosed until after 4 years of age (CDC, 2014), even though patients can be reliably diagnosed as early as 2 years of age (Kleinman et al., 2008; Lord et al., 2006).

Because ASD has broad and heterogeneous manifestations and has been associated with a plethora of possible etiological factors that are both genetic and environmental, ASD remains a clinical syndrome, but not a specific etiologically defined disorder. The prominent heterogeneity in autism has made it a challenge to investigate its neurobiological mechanisms and find interventions for the affected individuals. Currently, only comorbid manifestations of the disorder can be alleviated, such as epileptic seizures, psychiatric disturbances, hyperactivity, sleep disturbances, and digestive issues, but not the core symptoms (DiCicco-Bloom et al., 2006; Lai et al., 2014; Llaneza et al., 2010).

Despite much research, there remains limited knowledge about the pathophysiological mechanisms underlying ASD. It is generally accepted that genetic susceptibility factors and environmental influences both contribute to ASD (Chaste and Leboyer, 2012; Kim and Leventhal, 2015; Sandin et al., 2014; Tordjman et al., 2014), but many questions still remain unanswered. Recent efforts using genome screening and sequencing have identified
rare chromosomal abnormalities and copy number variations, as well as hundreds of rare gene mutations associated with autism (Baker and Jeste, 2015; Devlin and Scherer, 2012; Huguet et al., 2013; Jeste and Geschwind, 2014). A small number of these genetic deficits appear highly penetrant and sufficient to cause autism by themselves. However, the majority of the genetic changes associated with ASD appear to only increase the risk to varying degrees. In these cases, environmental factors influencing early brain development are thought to play a significant role in the etiology of the disorder. In other words, in the presence of a genetic predisposition to autism, a number of prenatal, perinatal, and postnatal risk factors appear to further increase a child’s risk. These include advanced parental age at time of conception (both mother and father), maternal illness during pregnancy, birth complications, and exposure to toxins and/or drugs during early brain development (Christensen et al., 2013; Durkin et al., 2008; Gardener et al., 2011; Stromland et al., 1994). It is important to note that these factors do not cause ASD by themselves. Rather, they seem to modestly increase the risks of developing the disorder in combination with genetic predispositions.

In summary, ASD inflicts a grave burden on affected individuals, their families, and society as a whole. Currently, ASD remains a syndrome characterized by behaviors, but not specific etiologies. Its heterogeneity in terms of both risk factors and clinical presentations makes it a great challenge to unravel the pathophysiological mechanisms and find therapeutic treatments.

**MITOCHONDRIAL AND METABOLIC DYSFUNCTION IN AUTISM SPECTRUM DISORDER**

Because a combination of diverse factors such as genetic background and environmental exposure are thought to contribute to the development of ASD, it has been hypothesized that these heterogeneous influences elicit similar developmental outcomes by modulating a common central nexus, a perturbation of which leads to ASD (Berg and Geschwind, 2012; Geschwind, 2008). Identifying such a node would provide novel insights into the development of ASD, while targeting this pathway could form the basis of therapeutic approaches for various forms of the disorder. A candidate for this central node is mitochondrial function, which is integrated into and key to many cellular pathways. For example, in addition to the well-known role as the “powerhouse of the cell,” producing the bulk of the cellular energy, mitochondria are also critically involved in cellular metabolism, intracellular calcium signaling, generation of reactive oxygen species, and apoptosis (Antico Arciuch et al., 2012; Murphy, 2009; Palmieri et al., 2010; Rizzuto et al., 2012; Suen et al., 2008), as well as in the regulation of innate and adaptive immunity (Weinberg et al., 2015). Furthermore, mitochondria are known be affected by many of the same endogenous and exogenous risk factors of ASD, such as toxins, drugs, immune activation, and metabolic disturbances (Frye and Rossignol, 2011). Thus, understanding the role of mitochondrial dysfunction in ASD may help unify our understanding of this complex disorder.

Mitochondria play a vital role especially in the nervous system. The brain has very high energy demands, as it consumes approximately 20% of the basal oxygen supply in humans, and hence, calories consumed by the body, while accounting for only 2% of the body weight (Raichle and Gusnard, 2002). This is mainly because cells in the nervous system need a great amount of adenosine triphosphate (ATP) to maintain ionic gradients across cellular membranes, which are essential to generate membrane excitability for neurotransmission and plasticity (Harris et al., 2012). In addition, mitochondria are involved in various aspects of neurodevelopment such as the proliferation, differentiation, and maturation of neural stem cells; formation of dendritic arbors and spines; developmental and synaptic plasticity; and the determination of cell survival and death (Kann and Kovacs, 2007; Kimura and Murakami, 2014; Li et al., 2004; Mattson et al., 2008; Xavier et al., 2015). Thus, it is not surprising that there are now multiple lines of evidence from clinical, genetic, and biochemical studies—in both humans and animal models—supporting a role for mitochondrial dysfunction in the etiology of ASD (Dhillon et al., 2011; Frye and Rossignol, 2011; Haas, 2010; Legido et al., 2013; Rossignol and Frye, 2012).

Clinical evidence points to the involvement of mitochondrial disease and dysfunction in ASD. The prevalence of mitochondrial disease in the ASD population is estimated to be about 5%, 500 times higher than that found in the general population (≈0.01%). The prevalence of abnormal values of biomarkers related to mitochondrial function in the ASD population may be even higher, suggesting that as many as 30% of children with ASD may experience mitochondrial dysfunction. For example, almost one-third of autistic children had elevations in plasma lactate and/or the lactate-to-pyruvate ratio, and the levels of...
many other mitochondrial biomarkers (pyruvate, carnitine, and ubiquinone) are significantly different between ASD and controls (Rossignol and Frye, 2012). Conversely, the rate of ASD is higher among children with mitochondrial disease (Randolph-Gips and Srinivasan, 2012). Together, these findings suggest that mitochondrial dysfunction likely represents a significant subclass of ASD.

Furthermore, common comorbidities of ASD also suggest the involvement of mitochondrial dysfunction. One of the most significant comorbidities associated with ASD is epilepsy. A number of studies suggest that epilepsy affects a great proportion of individuals with ASD, with the reported prevalence of epilepsy in the ASD population ranging from 5% to 38%, which is much higher than the 1%-2% prevalence in the general population of children (Frye, 2015). In contrast, seizures have been reported to occur in about 35% to 60% of individuals with biochemically confirmed mitochondrial disease (Rahman, 2012). Thus, shared symptoms suggest a common etiopathology. Similarly, gastrointestinal dysfunction, a frequent comorbidity of ASD (Chaidez et al., 2014), is also commonly reported in mitochondrial disease (Frye et al., 2015).

Interestingly, many animal models of ASD also display mitochondrial dysfunction. These models include ones based on both susceptibility genes such as MECP2, UBE3A, and SLC25A12 as well as environmental risk factors including maternal immune activation, and exposure to propionic acid and valproic acid. The current evidence linking mitochondrial perturbations to ASD is summarized in Table 14.1. Taken together, we believe that mitochondria act as a central nexus responding to and regulating many domains of cellular biology that have been implicated in ASD.

**KETOGENIC DIET AS A PROMISING THERAPY FOR AUTISM SPECTRUM DISORDER**

The ketogenic diet (KD) is a special high-fat, low-carbohydrate diet, which is a remarkably effective nonpharmacological treatment for patients with medically intractable epilepsy (Neal et al., 2008). Based on the historical observation that fasting or starvation can render antiseizure effects, the KD was designed to reproduce the biochemical changes seen in these physiological states (Masino and Rho, 2012). Recently, dietary and metabolic therapies have been attempted in a wider variety of neurological diseases including Alzheimer’s disease, Parkinson’s disease, multiple sclerosis, sleep disorders, and brain cancer (Frye et al., 2013; Napoli et al., 2014; Stafstrom and Rho, 2012).

There are a number of mechanisms through which the KD may provide neuroprotective activity. Two hallmark features of biochemical changes after the KD treatment are the increase in ketone body production by the liver through fatty acid oxidation and a reduction in blood glucose levels (Stafstrom and Rho, 2012). Specific polyunsaturated fatty acids such as arachidonic acid, docosahexaenoic acid, and eicosapentaenoic acid might themselves regulate neuronal membrane excitability (Voskuyl and Vreugdenhil, 2001), reduce inflammation (Cullingford, 2008; Jeong et al., 2011), or decrease the production of reactive oxygen species (ROS) by mitochondria (Kim do and Rho, 2008). Additionally, ketone bodies have been shown to possess neuroprotective properties through improved bioenergetics. They have been reported to raise ATP levels and reduce ROS production through enhancement of nicotinamide adenine dinucleotide (NADH) oxidation and inhibition of mitochondrial permeability transition (Kim do et al., 2007; Kim do et al., 2015). Furthermore, the KD has been shown to stimulate mitochondrial biogenesis (Ahola-Erkkila et al., 2010; Bough et al., 2006).

The second major biochemical feature of the KD is the reduction of glycolysis. In addition to suppressing seizures, it also improves mitochondrial function and decreases oxidative stress, reduces activity of pro-apoptotic factors, and inhibits inflammatory mediators such as interleukins and tumor necrosis factor alpha (Maalouf et al., 2009).

Beyond enhancing bioenergetics and mitochondrial function, the KD has also been shown to modulate the metabolism of γ-aminobutyric acid (GABA) and acetylcholine, two major central nervous system neurotransmitters (Napoli et al., 2014), as well as purines (such as ATP and adenosine) that have pleiotropic neural modulatory roles (Masino et al., 2010; Masino et al., 2009). In addition, the KD has also been reported to regulate energy-sensing pathways such as those involving the insulin-like growth factor and the mammalian target of rapamycin (Napoli et al., 2014).

As mentioned above, mitochondrial and metabolic dysfunction may play a key role in the pathophysiology of ASD. Based on research showing that the KD provides numerous neuroprotective effects through modulating mitochondrial and
TABLE 14.1 STUDIES SHOWING A LINK BETWEEN ASD AND MITOCHONDRIAL DYSFUNCTION

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cases</th>
<th>Evidence of mitochondrial dysfunction</th>
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<tbody>
<tr>
<td>Al-Mosalem et al., 2009</td>
<td>30</td>
<td>Increased plasma lactate levels and activity of creatine kinase</td>
</tr>
<tr>
<td>Boccuto et al., 2013</td>
<td>87</td>
<td>Decreased tryptophan metabolism in lymphoblastoid cell lines</td>
</tr>
<tr>
<td>Celestino-Soper et al., 2012</td>
<td>909</td>
<td>The deficiency of the trimethyllysine hydroxylase epsilon, which encodes the first enzyme in carnitine biosynthesis, was more frequent in probands from male-male multiplex ASD families</td>
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<tr>
<td>Chen et al., 2015</td>
<td>78</td>
<td>Mitochondrial DNA copy number in peripheral blood cells was elevated</td>
</tr>
<tr>
<td>Cohen et al., 1976</td>
<td>25</td>
<td>Increased serum creatine phosphokinase levels</td>
</tr>
<tr>
<td>Correia et al., 2006</td>
<td>241</td>
<td>Increased plasma lactate levels and lactate/pyruvate ratio, but not associated with the variation at the SLC25A12 gene</td>
</tr>
<tr>
<td>Filipek et al., 2004</td>
<td>100</td>
<td>Reduced levels of carnitine and pyruvate, increased levels of alanine and ammonia in serum</td>
</tr>
<tr>
<td>Frye et al., 2013</td>
<td>213</td>
<td>Abnormal acyl-carnitine panels and glutathione metabolism in blood samples</td>
</tr>
<tr>
<td>Glessner et al., 2009</td>
<td>859</td>
<td>Copy number variations in genes involved in the ubiquitin degradation were implicated in susceptibility for ASD</td>
</tr>
<tr>
<td>Goh et al., 2014</td>
<td>75</td>
<td>Lactate doublets detected by brain magnetic resonance spectroscopic imaging were present at a higher rate</td>
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<tr>
<td>Kent et al., 2006</td>
<td>129</td>
<td>The 3243A&gt;G mitochondrial DNA mutation was concluded to be a rare cause of isolated Asperger syndrome</td>
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<tr>
<td>Kim et al., 2011</td>
<td>Multiple families</td>
<td>Polymorphism in a mitochondrial aspartate/glutamate carrier gene (SLC25A12) is found to be associated with restricted repetitive behavior in autism</td>
</tr>
<tr>
<td>Silverman et al., 2008</td>
<td>25</td>
<td>Higher plasma levels of arginine and taurine, and lower levels of 5-oxoproline and lactic acid</td>
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<tr>
<td>Kuwabara et al., 2013</td>
<td>30</td>
<td>Increased serum lactate and pyruvate levels</td>
</tr>
<tr>
<td>Laszlo et al., 1994</td>
<td>127</td>
<td>Gene encodes an inner mitochondrial membrane protease-like protein (IMMP2L) was implicated in susceptibility for ASD</td>
</tr>
<tr>
<td>Moreno et al., 1992</td>
<td>60</td>
<td>Increased lactate and pyruvate levels</td>
</tr>
<tr>
<td>Oliveira et al., 2005</td>
<td>69</td>
<td>20% of ASD patients showed hyperlactacidemia, while 7% were classified with definite mitochondrial respiratory chain disorder</td>
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<tr>
<td>Poling et al., 2006</td>
<td>159</td>
<td>Increase blood aspartate aminotransferase and creatine kinase levels</td>
</tr>
<tr>
<td>Rose et al., 2012</td>
<td>43</td>
<td>Primary immune cells in the blood have a more oxidized intracellular and extracellular microenvironment and a deficit in glutathione-mediated redox/antioxidant capacity</td>
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<tr>
<td>Rose et al., 2014</td>
<td>25</td>
<td>Mitochondrial dysfunction observed in a subset of autism lymphoblastoid cell lines</td>
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<tr>
<td>Ramoz et al., 2004</td>
<td>Multiple families</td>
<td>Polymorphism in a mitochondrial aspartate/glutamate carrier gene (SLC25A12) is found to be associated with autism</td>
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<tr>
<td>Segurado et al., 2005</td>
<td>45</td>
<td>Mitochondrial function and intracellular redox status were compromised in the pyramidal neurons of the temporal cortex</td>
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<td>Turunen et al., 2008</td>
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<td>Tang et al., 2013</td>
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<tr>
<td>Ahn et al., 2014</td>
<td></td>
<td>Mitochondrial dysfunction in a rat model of ASD using prenatal VPA exposure</td>
</tr>
<tr>
<td>Jin et al., 2015</td>
<td></td>
<td>Mecp2, whose mutations cause Rett syndrome, was observed to regulate mitochondrial bioenergetics through a glutamine transporter in microglia.</td>
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<tr>
<td>Kriaucionis et al., 2006</td>
<td></td>
<td>Mitochondrial abnormalities observed in Mecp2-null mouse, a model of Rett syndrome.</td>
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<tr>
<td>Macfabe et al., 2012</td>
<td></td>
<td>Mitochondrial dysfunction observed in a rat ASD model in which propionic acid, an enteric bacterial fermentation product, is infused intracerebroventricularly</td>
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*(continued)*
metabolic pathways, it is logical to hypothesize that this diet could prove to be beneficial for autistic individuals.

**THE EFFECTS OF THE KETOGENIC DIET ON AUTISM SPECTRUM DISORDER PATIENTS**

To date, there have been limited trials of treating autism patients with the KD. Evangeliou and colleagues carried out a pilot prospective study on autistic children aged between 4 and 10 years to investigate the role of the KD, specifically a modified medium chain triglyceride (MCT) diet (Evangeliou et al., 2003). Of the 18 patients who adhered to the diet, improvement was recorded in most individuals, based on several parameters and in accordance with the Childhood Autism Rating Scale (CARS). Significant (>12 units of decrease in CARS) and average (>8–12 units of decrease in CARS) improvement was recorded in two and eight patients, respectively, while minor (2–8 units of decrease in CARS) improvement was reported in the remaining eight patients.

In a separate study, Herbert and Buckley reported the history of a 12-year-old child with autism and epilepsy treated with a gluten- and casein-free KD with fats composed mostly of medium chain triglycerides (Herbert and Buckley, 2013). The patient showed significant improvement in seizure activity, resolution of morbid obesity, and improvement in cognitive and behavioral functioning. Over the course of several years following her initial diagnosis, the child’s CARS score decreased from 49 to 17, representing a change from severe autism to a nonautistic state, and her intelligence quotient increased 70 points.

Finally, Spilioti and colleagues reported the effects following the KD treatment in a group of Greek children with ASD aged between 3.5 and 6 years (Spilioti et al., 2013). Of the 6 patients who successfully implemented the diet, significant and average improvement was recorded in one and two patients, respectively, while minor improvement was reported in the remaining three patients.

In summary, results from these few studies show that more than 50% of the autism patients who received the KD treatment showed moderate-to-significant improvement, while the remaining displayed minor improvement. These preliminary studies suggest that the KD could be a promising therapy for ASD; more and larger clinical studies are needed.

**TREATING ANIMAL MODELS OF AUTISM WITH THE KETOGENIC DIET**

Experiments using animal models of human diseases have contributed much to our understanding of the mechanisms of disease, and the efficacy of potential therapeutics in preclinical settings. Due to the complex etiology of ASD, many animal models exist, and several of them have been used to test the effects of the KD. For example, succinic semialdehyde dehydrogenase (SSADH) deficiency is a rare autosomal recessive condition

<table>
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<tr>
<th>Reference</th>
<th>Cases</th>
<th>Evidence of mitochondrial dysfunction</th>
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<tbody>
<tr>
<td>Naviaux et al., 2013</td>
<td>Antipurinergic therapy improves the autism-like features in the maternal immune activation mouse model and the Fragile X mouse model</td>
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<tr>
<td>Naviaux et al., 2014</td>
<td>Dietary therapy with triheptanoin enhanced mitochondrial substrate use and improved metabolism and behaviors of MeCP2-null mouse model of Rett syndrome</td>
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<tr>
<td>Park et al., 2014</td>
<td>Loss of a mitochondrial aspartate-glutamate carrier gene results in hypomyelination. Myelin deficits in slice cultures from KO mice are reversed by administration of pyruvate</td>
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<tr>
<td>Sakurai et al., 2010</td>
<td>Mitochondrial dysfunction observed in hippocampal neurons of the UBE3A deficient mouse model for Angelman syndrome</td>
<td></td>
</tr>
<tr>
<td>Su et al., 2011</td>
<td>ASD-like features observed in neuronal glucose transporter isoform 3 deficient mice</td>
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</table>

*Only studies reporting more than 25 subjects are included in this table*
that results in mild-to-moderate mental retardation, disproportionate language dysfunction, seizures, hypotonia, hyporeflexia, hallucinations, and autistic behaviors (Pearl et al., 2003). In an animal model of SSADH deficiency, the SSADH knockout mouse, Nylen and colleagues found that KD treatment normalized electroencephalogram (EEG) activity. In addition, the decrease in miniature inhibitory postsynaptic currents in pyramidal cells of CA1 hippocampal slices observed in the mutant animals was completely restored by the KD when compared with wild-type controls. In contrast, there were no significant differences between the groups in terms of miniature excitatory postsynaptic currents. Moreover, GABA$_\text{A}$ receptor-associated chloride channel binding of [35S]tert-butylcyclophosphorothionate was restored fully in hippocampus and frontoparietal cortex, but not in amygdala or thalamus, of KD-fed mutant mice. Behaviorally, KD-treated mutant animals experienced significantly fewer seizures compared with mutant animals fed with the control diet (Nylen et al., 2008).

Another study examined the effects of restricted standard and ketogenic diets on a mouse model of Rett syndrome. Rett syndrome is a neurodevelopmental disorder characterized by normal early growth and development followed by a slowing of development, impairment of motor functions, seizure susceptibility, and intellectual disability. In most cases, it is caused by mutations in the methyl-CpG-binding protein 2 (MECP2) gene (Amir et al., 1999). Children with Rett syndrome often exhibit autistic-like behaviors in the early stages (Percy, 2011). By feeding animals with standard chow in unrestricted or restricted amounts or a KD in restricted amounts, Mantis and colleagues found that performance in assays of motor behavior and anxiety was significantly worse in MeCP2 mutant mice compared with wild-type animals, and restriction of either the standard diet or the KD improved motor behavior and reduced anxiety in the mutant animals (Mantis et al., 2009).

More recently, using the BTBR T+tf/J (BTBR) mouse model of autism, Ruskin and colleagues investigated the effects of the KD on core behavioral abnormalities that define autism (Ruskin et al., 2013). The BTBR inbred strain is one of the most clinically relevant animal models of autism, mainly because it displays all the core behavioral features that define the disorder—specifically, impaired social behaviors and communication as well as stereotyped behaviors and resistance to change (Ellegood and Crawley, 2015; McFarlane et al., 2008; Meyza et al., 2013; Moy et al., 2007; Ruskin et al., 2013; Smith et al., 2014). This model was identified in an extensive effort to characterize 10 inbred mouse strains in terms of possessing autism-like behaviors (Moy et al., 2007), and was later shown to display behavioral phenotypes relevant to all three core diagnostic symptoms of ASD (McFarlane et al., 2008). Additional tests conducted in multiple independent laboratories further confirmed that BTBR animals display prominent deficits in multiple social interaction and communication assays, and exhibit repetitive and stereotyped behaviors. Thus, the BTBR strain has been widely regarded as a consistent and robust animal model of autism, reflecting more common forms of ASD (Gaugler et al., 2014). During the relatively short time since its discovery as an ASD model, the BTBR strain has been increasingly used to study the etiology and potential intervention of ASD (Llaneza et al., 2010; McFarlane et al., 2008; Moy et al., 2007; Ruskin et al., 2013). These studies have identified alterations in BTBR animals at many levels, including genetic and epigenetic; synaptic; neuroanatomic and functional connectivity; and immunological—all of which have been implicated in individuals with ASD (Ellegood and Crawley, 2015; Llaneza et al., 2010; Meyza et al., 2013; Shpyleva et al., 2014). To test the effects of the KD on the BTBR model, Ruskin and colleagues fed the animals with the diet for 3 weeks and then performed an array of behavioral assays. They reported that the BTBR mice showed increased sociability in a three-chamber test, decreased self-directed repetitive behavior, and improved social communication in a food-preference assay (Ruskin et al., 2013). In addition, the authors showed that the behavioral improvements were probably not related to any antiseizure effect of the diet, because no spontaneous seizures or abnormal EEG features were observed in the BTBR animals. These results strongly suggest that the KD may be able to improve the core behavioral symptoms of autism.

As mentioned earlier, both genetic and environmental factors contribute to the risks of developing ASD. An example of environmental risk factors is exposure to exogenous chemicals such as valproic acid (VPA) during pregnancy. Valproic acid is a pharmacological anticonvulsant used in humans primarily for the treatment of epilepsy and migraine. Epidemiological studies showed that maternal use of VPA during pregnancy was associated with a significantly increased risk of the offspring being autistic (Bromley et al., 2013; Christensen et al., 2013). The model of VPA exposure is one of the most frequently studied animal
models of ASD (Chomiak et al., 2013; Roullet et al., 2013). This model exhibits many similar structural and behavioral features of ASD individuals. For example, it has been shown that VPA exposure in both rats and mice leads to autistic-like behavioral impairment including decreased social interactions, increased repetitive behaviors, and deficits in communication, as well as increased anxiety and increased sensitivity to both painful and nonpainful stimuli. Overall, the VPA rodent model possesses construct, face, and predictive validity for ASD studies. Using this model, Ahn and colleagues found that the KD treatment recovered part of the play behavior of juvenile rats exposed to VPA prenatally, shown by increased number of play initiations/attacks. However, the diet did not change the disrupted pattern of play responses (Ahn et al., 2014). Interestingly, the authors also found that prenatal exposure to VPA altered mitochondrial respiration, and the KD was able to partially restore it. Specifically, basal and ATP-linked respiration was significantly increased in the VPA animals, but was partly normalized by the KD (Ahn et al., 2014).

**FUTURE RESEARCH DIRECTIONS USING METABOLIC THERAPY**

Although strong evidence exists that mitochondrial and metabolic dysfunction may play a pivotal role in the complex pathophysiology of ASD, the exact mechanisms remain undefined. For example, is mitochondrial dysfunction a cause, consequence, or epiphenomenon of the disrupted neurodevelopment observed in ASD? Considering that ASD is a multisystem disorder, in which tissue, and more specifically, in which cell types is mitochondrial dysfunction relevant to the disease state? Can impaired mitochondrial function define a subtype of the otherwise heterogeneous patient population? Is the severity of mitochondrial and metabolic disturbance correlated with any behavioral abnormalities or comorbidities? When mitochondrial function is improved, can it ameliorate the general features of ASD? Answering these questions will require the collective efforts of many basic, translational, and clinical researchers, as well as investigators with diverse expertise in multiorgan dysfunction, metabolism, and both genetic and environmental risk factors.

Similarly, although the KD has shown promising results from limited studies in both patients and animal models, a mechanistic understanding of its effects in ASD is lacking. Borrowing from the rich literature on the KD treatment for epilepsy, shifts in energy metabolism and the direct actions of the ketone bodies on the mitochondria are two of the promising candidate mechanisms. In addition, the optimum formulation of the KD needs to be established, which may be different for ASD compared with epilepsy, and distinct biomarkers need to be developed that will accurately track behavioral symptoms as well as disease ontogeny. Last but not least, the potential side effects of the KD should be carefully investigated, especially for ASD patients who may also suffer from intrinsic metabolic derangements.

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Glucose and Ketone Metabolism in the Aging Brain

Implications for Therapeutic Strategies to Delay the Progression of Alzheimer’s Disease

STEPHEN C. CUNNANE, PHD, ALEXANDRE COURCHESNE-LOYER, MSC, VALERIE ST-PIERRE, BSC, CAMILLE VANDENBERGHE, BSC, ETIENNE CROTEAU, PHD, AND CHRISTIAN-ALEXANDRE CASTELLANO, PHD

INTRODUCTION

The impact of deteriorating cognitive function in older age has become a major sociomedical pre-occupation worldwide. Alzheimer’s disease (AD), the most common form of impaired cognitive function in older age, is debilitating and dehumanizing, and is expensive to manage. To make matters worse, there is currently no effective treatment. Despite considerable effort and expense in the development of anti-amyloid medications, there is no clear sign that this treatment strategy will be effective in the near future (Jack et al., 2009; Rafii and Aisen, 2009). Some major lifestyle risk factors for AD are well known and include type 2 diabetes, hypertension, and sedentarity. Whether inadequate intake of certain nutrients such as omega-3 fatty acids and certain B vitamins also contributes to AD is receiving considerable attention but remains unclear and controversial (Douaud et al., 2013; Jerneren et al., 2015; Quinn et al., 2010). As a general rule, “prevention” strategies based on reducing the impact of lifestyle risk factors can be implemented and have been shown in clinical trials to be beneficial in reducing the progression of not only prodromal AD but also other lifestyle-related chronic diseases (Brown et al., 2010; Ngandu et al., 2015; Okonkwo et al., 2014). We speculate that effective strategies to delay the progression of AD may have at least one feature in common—that of improving brain energy metabolism in older people.

It is well understood that the brain requires a disproportionately large amount of energy; whereas the adult brain represents about 2% of adult body weight, it consumes about 20%–23% of whole body energy requirements. Glucose is the brain’s predominant fuel, but, like other organs, the brain has a back-up fuel for occasions when glucose supply is insufficient, for example, during fasting, starvation, strenuous exercise, or malnutrition. While other organs use free fatty acids directly to replace insufficient availability of glucose, ketones (also known as ketone bodies) are the only significant alternative fuel to glucose for the brain. The two ketones that replace glucose for the brain are beta-hydroxybutyrate and acetacetate. As the decarboxylation product of acetacetate, acetone appears to mainly be excreted on breath but can also be metabolized through other pathways.

For over 30 years now it has become increasingly well established that brain glucose uptake and metabolism are defective in AD, a problem that is particularly evident in the parietal and temporal cortex (reviewed in Cunnane et al., 2015; Cunnane et al., 2011). These results have generally been interpreted to mean that low brain glucose uptake in AD is a consequence of neuronal failure and death. While fewer functioning neurons would indeed diminish the need for glucose, this perspective does not account for multiple examples of conditions in which regional brain glucose hypometabolism is present presymptomatically in individuals at elevated risk of AD, that is, before the clinical (cognitive) onset of the disease. An emerging literature is discussed here that provides several clear examples in which individuals at genetic or lifestyle risk of AD have impaired brain glucose presymptomatically. Given that brain function is acutely dependent on a constant supply of glucose and oxygen, it is crucial to know whether latent presymptomatic deterioration in brain glucose...
uptake and/or utilization could be contributing to the development of AD, because this could have an impact on prevention and treatment strategies.

We have developed a positron emission tomography (PET) research program using the ketone tracer, $^{11}$C-acetoacetate (AcAc), to better understand the relation between brain fuel uptake and brain function in people with or at risk of AD. In each individual studied, we use this dual tracer PET protocol to compare the brain uptake of $^{11}$C-AcAc with that of the glucose tracer, $^{18}$F-fluorodeoxyglucose (FDG). We quantify the magnitude of glucose and ketone uptake regionally throughout the brain. Clinical studies are underway to test the potential therapeutic utility of ketogenic supplements based primarily on medium chain triglycerides (MCT), with the dual tracer PET imaging done before and after the interventions. Here, we review the rationale for these studies with the objective of highlighting the main points supporting a strategy that implicates maintenance of brain fuel supply to delay the onset and/or progression of AD.

**ARTERIOVENOUS DIFFERENCE STUDIES ACROSS THE BRAIN: THE FOUNDATION**

The PET-FDG protocol was first used to image brain glucose metabolism in the late 1970s with reports on AD first being published in the early 1980s (Benson et al., 1983). Since then, PET-FDG has been a cornerstone of human and animal studies on brain energy metabolism in aging and AD (reviewed in Cunnane et al., 2011). Indeed, without PET-FDG, it is doubtful that brain glucose hypometabolism in AD would have become widely studied, because the only option besides PET, the arteriovenous difference (AVD) method, is highly invasive and used increasingly less in research. Nevertheless, it was the AVD method that first showed that brain oxygen and glucose uptake were unaffected by healthy aging (Dastur, 1985). This method produced several groundbreaking reports comparing brain ketone and glucose uptake that laid the foundation for our current understanding that ketones are an essential physiological fuel working daily in tandem with glucose to assure brain energy requirements are being met.

The first of these pioneering AVD reports was by Owen et al. (1967), who showed that ketones could supply about two-thirds of the brain’s fuel requirement when glucose availability was severely limited during medically supervised starvation. This experiment was done in three obese individuals who underwent total dietary deprivation for 40 days. At that time, it was suspected that some other fuel was helping to meet the brain energy requirements during extreme hypoglycemia because brain oxygen consumption was not decreased as much as glucose uptake, but the replacement fuel was still unknown. The report by Owen et al. (1967) was the first to demonstrate that ketones were the unknown replacement fuel for glucose for the brain, that is, that ketones played a physiologically important role in the adult brain rather than simply being a pathological marker of severe insulin deficiency in decompensated type 1 diabetes. This paper also proposed that brain ketone uptake was proportional to blood ketone concentration. Owen et al. (1967) did not describe brain ketone uptake under less extreme conditions, but shortly after their paper was published, Gottstein et al. (1971) used AVD to show that the brain also consumed ketones under the more physiological conditions of a 12- to 16-hour overnight fast. Hence, ketones contribute to brain energy metabolism on a daily basis, not just when brain glucose availability is severely limited during chronic hypoglycemia. Drenick et al. (1972) were the first to note that the symptoms of severe glucopenia (as low as 0.5 mM plasma glucose) induced by controlled insulin infusion could be avoided by up to 60 days of medically supervised starvation. Their study showed that ketones could account for ≥85% of brain fuel requirements in obese adults. Drenick et al. (1972) also used AVD to show that skeletal muscle of the forearm was not using ketones at all during prolonged starvation. These starvation studies were clearly extreme, but they unequivocally demonstrated the physiological role of ketones particularly if not uniquely for the brain.

The first AVD report on brain energy metabolism in AD was by Lying-Tunell et al. (1981), who showed that brain glucose uptake was impaired by 26% but that brain ketone uptake was still normal (Table 15.1). This report confirmed earlier reports using AVD that had demonstrated lower brain glucose uptake in AD and provided the first suggestion that the disease did not necessarily adversely affect the brain ketone utilization to the same extent as glucose, even when the cognitive deficit became severe. Hoyer et al. (1988) also used the AVD method and demonstrated 45% lower brain glucose utilization in suspected (i.e., early stage) AD. Unlike in more advanced AD,
they showed that neither blood flow to the brain nor oxygen uptake was adversely affected early in AD. Hoyer et al. (1988) surmised from their results that the problem with brain glucose utilization in AD probably involved impaired glycolysis, particularly the conversion of glucose to pyruvate via pyruvate dehydrogenase (Sorbi et al., 1983). Hoyer et al. (1992; 1988) were the first to conceptualize the significance of lower brain glucose utilization as an early and possibly presymptomatic problem in AD. However, they did not report brain ketone uptake.

The second pioneering AVD paper that addressed brain energy metabolism in AD was by Ogawa et al. (1996), who confirmed the report by Lying-Tunell et al. (1981) showing that AD adversely affected brain glucose but not brain ketone uptake (Table 15.1). Unlike Lying-Tunell et al. (1981) but similar to Hoyer et al. (1988), Ogawa et al. (1996) found that brain oxygen uptake was less adversely affected in AD than brain glucose uptake. These AVD studies raised the possibility that brain glucose hypometabolism was a relatively early problem in AD, and that this problem was apparently not seen with brain ketone uptake.

The CMR of glucose (CMRg) is measured less and less because, ideally, it requires blood sampling from a catheter inserted into the brachial artery as well as exhaustive data processing. As a result of declining quantification of CMRg, the actual deficit in brain glucose uptake in people at risk of AD is now only infrequently measured; rather, PET-FDG data are increasingly expressed using a statistical parametric map. Statistical parametric mapping clearly identifies the regions of the brain with FDG uptake below a certain statistical threshold, but the actual magnitude of that difference compared with the controls is generally unknown (Baker et al., 2011; Kalpouzos et al., 2009). Hence, although the regional nature of the deficit in brain FDG uptake in AD can be clearly observed in some detail with PET (which is impossible with AVD), there are fewer and fewer quantitative PET-FDG reports, so we have actually learned very little about the magnitude of the regional deficit in brain glucose uptake in AD or how it changes as AD progresses since the AVD studies and insights of Hoyer et al. (1988). Furthermore, with the focus on glucose as the representative brain fuel using PET-FDG, until recently, the possibility that brain ketone uptake could be less affected in AD and hence possibly part of a treatment strategy has largely been overlooked. However, knowing the magnitude of the deficit in brain glucose uptake in AD and the extent to which it continues to worsen without treatment provides critical information about existing treatments that may fortuitously improve brain glucose uptake, namely, acetylcholinesterase inhibitors (Nordberg, 2006) or NMDA receptor antagonists (Wang et al., 2013), or new treatments aiming to bypass or correct this problem early enough to delay the progression of AD.

<table>
<thead>
<tr>
<th>Fuel</th>
<th>Controls</th>
<th>Mild Alzheimer’s</th>
<th>p value (versus Control)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (µmol/100 g/min)</td>
<td>24.9±7.2</td>
<td>11.6±4.0</td>
<td>&lt;0.01</td>
<td>Lying-Tunell et al., 1981</td>
</tr>
<tr>
<td>Ketones (µmol/100 g/min)</td>
<td>0.14±0.08</td>
<td>0.11±0.06</td>
<td>ns</td>
<td>Ogawa et al., 1996</td>
</tr>
</tbody>
</table>

ns—not significant between Controls and mild Alzheimer’s
**PRESYMPTOMATIC BRAIN GLUCOSE HYPOMETABOLISM: MULTIPLE EXAMPLES**

While AVD has largely become a technique of the past, it helped seed our interest in applying PET-FDG to the question of causality—is brain glucose hypometabolism a consequence of AD or part of the cause or, indeed, both? The stage of AD can be difficult to determine precisely, which in turn means that it can be difficult to determine whether those on the threshold or at the early stages of the disease have a specific deficit in brain glucose uptake or a broader deficit that also includes impaired oxygen uptake and lower blood flow to the brain. Assessing the presence of a possible presymptomatic neurometabolic deficit in a disease of uncertain and gradual onset like AD therefore requires a different strategy such as studying those at elevated risk of the disease but in whom cognitive scores are still normal.

Carriers of the presenilin-1 mutation are at very high, almost certain, risk of developing AD (Blennow et al., 2006; Fox et al., 1997; Scholl et al., 2011). Carriers of the apolipoprotein E4 allele and persons with a family history of AD, especially on the maternal side, are also at increased risk of AD (Bu, 2009; Kennedy et al., 1995; Mosconi et al., 2005; Reiman et al., 2004), although their risk is still relatively low compared with presenilin-1 carriers. Type 2 diabetes ranks among the most important of the nongenetic or lifestyle risk factors for AD. In presymptomatic individuals with these aforementioned conditions in whom the results on cognitive batteries are still normal, PET-FDG clearly shows that regional brain glucose hypometabolism is present before the onset of cognitive deficit (Table 15.2). These examples

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**TABLE 15.2 PRESYMPTOMATIC BRAIN GLUCOSE HYPOMETABOLISM IN PERSONS AT RISK OF ALZHEIMER’S DISEASE (AD)**

<table>
<thead>
<tr>
<th>Group at risk of AD</th>
<th>Mean age (y)</th>
<th>Brain region affected</th>
<th>Magnitude of brain glucose hypometabolism (% difference from control)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young adult carriers of Presenilin-1</td>
<td>30</td>
<td>Posterior cingulate</td>
<td>–14 to –25</td>
<td>Schöll et al., 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parietal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Temporal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young adult carriers of Apolipoprotein-E4</td>
<td>31</td>
<td>Parietal cortex</td>
<td>–9 to –11</td>
<td>Reiman et al., 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Temporal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posterior cingulate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prefrontal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal family history of AD</td>
<td>43</td>
<td>Parietal cortex</td>
<td>–12 to –21</td>
<td>Mosconi et al., 2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Temporal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hippocampus</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Entorhinal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posterior cingulate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prediabetic older persons</td>
<td>74</td>
<td>Temporal cortex</td>
<td>N/A</td>
<td>Baker et al., 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parietal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posterior cingulate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Precuneus</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prefrontal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin resistant young women with polycystic ovary syndrome</td>
<td>25</td>
<td>Frontal cortex</td>
<td>–9 to –14</td>
<td>Castellano et al., 2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle temporal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cognitively healthy older adults</td>
<td>72</td>
<td>Frontal cortex</td>
<td>–10 to –18</td>
<td>Nugent et al., 2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Temporal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anterior cingulate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Putamen</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thalamus</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N/A—not available
are a good basis for concluding that presymptomatic brain glucose hypometabolism can be present and could therefore be contributing to the development of AD.

Aging is considered to be the most significant risk factor for AD, so we have extensively studied brain energy metabolism in cognitively normal older persons. Using quantitative PET-FDG, we have shown that in cognitively normal people aged 72 years, the CMRg in the superior frontal cortex is about 35±5 instead of the 40±7 mmol/100 g/min seen in young adults, that is, the glucose uptake deficit in this brain region is of the order of 12%–13% (Nugent et al., 2015; Nugent et al., 2014a; Nugent et al., 2014b). The CMRg did not differ significantly between young and older adults in either white or gray matter as a whole nor did it differ in the temporal or parietal cortex where CMRg decreases in AD. Lower CMRg that is present mostly if not exclusively in the frontal cortex of cognitively normal older people has been reported on several previous occasions (Cunnane et al., 2011; Kalpouzos et al., 2009). Thus, lower CMRg in the temporal and parietal cortex in AD is a fundamentally different situation (but perhaps related to) lower frontal CMRg during aging. Incidentally, in older people with normal cognition, cortical thickness and regional volumes are lower in many more brain regions than CMRg (Nugent et al., 2015). Thus, although lower frontal CMRg is clearly presymptomatic in relation to aging-associated cognitive decline, it is by no means the only or even the most significant change occurring in the brain during aging.

We have also observed a deficit of about 14% in CMRg in the superior and middle frontal cortex in 24-year-old women with polycystic ovary syndrome (Castellano et al., 2015a). Polycystic ovary syndrome is a multifactorial endocrine disease involving infertility, hyperandrogenism, and mild-to-moderate insulin resistance. It was the mild insulin resistance that was of particular interest to us because of its association with increased risk of AD (Baker et al., 2011; Craft, 2009, 2012; Matsuzaki et al., 2010; Ronnemaa et al., 2008; Schrijvers et al., 2010). These women with polycystic ovary syndrome were of normal weight and body-mass index, so this condition represents a possible model of the impact of mild insulin resistance on brain glucose metabolism that is independent of age and obesity. The mild insulin resistance in women with polycystic ovary syndrome was significantly inversely correlated to CMRg in several brain regions. These women also had borderline low-normal scores on a test of working memory, suggesting that insulin resistance can adversely affect both brain glucose metabolism and possibly cognitive performance in young adults (Castellano et al., 2015a). Whether women with polycystic ovary syndrome are predisposed to a higher risk of cognitive decline as they age remains to be seen but deserves further attention.

These examples demonstrate that regional brain glucose hypometabolism can be present in those in whom cognition is still normal even though they are at risk of AD due to old age, or certain genetic or lifestyle factors associated with AD. The presymptomatic glucose uptake deficit is commonly but not exclusively in the frontal cortex, and its magnitude is of the order of 12%–15% (Castellano et al., 2015b; Cunnane et al., 2011; Nugent et al., 2015). These observations run counter to the widespread perception that brain glucose hypometabolism is uniquely a consequence of synaptic dysfunction and neuronal failure or death. Indeed, they suggest that a vicious cycle can develop in which latent presymptomatic brain glucose hypometabolism develops and leads to chronic brain energy deficit, deteriorating neuronal function, further decline in demand for glucose, and further cognitive decline (Figure 15.1; Cunnane et al., 2011). The presence of presymptomatic brain glucose hypometabolism does not necessarily mean that it is a cause of AD, nor that neuronal function is necessarily normal, nor even that glucose hypometabolism is the first abnormality detectable in those at risk of AD. Nevertheless, knowing that brain glucose hypometabolism can

FIGURE 15.1 Schematic representation of latent brain glucose hypometabolism leading to a vicious cycle of accelerating metabolic deterioration and neuronal dysfunction that increases the risk of developing Alzheimer’s disease (from Cunnane et al., 2015).
be present in people at risk of AD before the onset of measurable clinical (cognitive) deficit has implications for potential therapeutic strategies (Blass, 2008; Cunnane et al., 2015; Cunnane et al., 2011; Gibson et al., 2000; Henderson et al., 2009; Veech et al., 2001).

**BRAIN GLUCOSE UPTAKE OR METABOLISM OR BOTH?**

The AVD studies show the magnitude of the lower glucose “disappearance” into the brain in AD. However, they do not establish whether the problem is with brain glucose uptake, that is, its transport into the brain, or with glycolysis, that is, glucose metabolism within the brain, or both. The PET studies clearly show that there is a problem in AD at the level of glucose (FDG) uptake, that is, with glucose transport into the brain and its conversion to glucose-6-phosphate by hexokinase. However, PET-FDG provides no information about whether, in addition to defective glucose transport, the glycolytic steps are also altered. It may not matter to the brain whether the problem with glucose hypometabolism is with brain glucose uptake per se or with glucose metabolism via glycolysis; either way, if uncorrected, the brain’s main energy source is compromised and it is at increased risk of chronic energy deficit and eventual exhaustion. In fact, the energy supply problem seems to be at the level of both brain glucose uptake and metabolism. In vitro studies show that several enzymes in glycolysis are markedly impaired in AD, including phosphofructokinase (Bowen et al., 1979; Iwangoff et al., 1980), alpha-ketoglutarate dehydrogenase complex (Gibson et al., 2000), and pyruvate dehydrogenase (Cunnane et al., 2011; Perry et al., 1980; Sorbi et al., 1983).

**KETONES—THE BRAIN’S PHYSIOLOGICAL ALTERNATIVE FUEL**

The now classic studies by Owen et al. (1967) and Drenick et al. (1972) demonstrated that ketones are the main reserve fuel for the brain when glucose supply is compromised by starvation. The human liver can produce ketones at a rate of 100–150 g/day (Flatt, 1972; Reichard et al., 1974), which is more than sufficient to account for the brain’s ketone utilization even during prolonged starvation. The energy cost to the liver of producing ketones is mostly supplied by gluconeogenesis, the rate of which parallels and may eventually actually limit ketone production (Flatt, 1972; Garber et al., 1974).

In adults, ketogenesis is principally from long chain fatty acids stored in adipose tissue, the release of which is controlled by insulin. The branched chain amino acids, isoleucine and leucine, are also ketogenic (Mitchell et al., 1995). During fasting, blood glucose and insulin decrease, which releases the inhibition on lipolysis in adipose tissue, thereby allowing plasma free fatty acids to increase. This increase in plasma free fatty acids helps meet the need for an alternative fuel to glucose for most tissues with the notable exception of the brain. The increased supply of fatty acids entering the liver leads to ketogenesis by condensation of two acetyl-CoAs, which are present in excess due to fatty acid beta-oxidation (Figure 15.2).

Implicitly, if insulin controls the ebb and flow of plasma glucose, free fatty acids, and ketones, insulin sensitivity becomes an important parameter in the process. Indeed, conditions involving decreased insulin sensitivity, that is, insulin resistance, also impair tissue glucose uptake and are associated with a vicious cycle of hyperglycemia, hyperinsulinemia, and an eventual trend toward type 2 diabetes, which is a major risk factor for AD (Baker et al., 2011; Craft, 2009). Postprandial hyperinsulinemia inhibits fatty acid release from adipose tissue and ketogenesis, which is appropriate as long as insulin sensitivity and tissue glucose uptake are normal, that is, when and insulin returns to normal 3–4 hours postprandially. However, chronic sedentarity commonly leads to chronic hyperinsulinemia and insulin resistance, which not only compromise tissue glucose uptake but also ketogenesis and ketone metabolism (Bickerton et al., 2008; Fukao et al., 2004). In effect, this puts the aging brain in double jeopardy because now it is not only getting insufficient glucose but is also getting less of the main alternative fuel, ketones (Cunnane et al., 2015; Cunnane et al., 2011; Mamelak, 2012).

**KETONE KINETICS AND TRANSPORT IN ADULT HUMANS**

Elegant kinetic studies show that ketone utilization essentially matches synthesis in normal weight adults fasted overnight (Avogaro et al., 1990; Balasse and Fery, 1989; Hall et al., 1984). The rapid utilization of ketones as they are produced during short-term fasting generally keeps plasma ketones ≤0.3 mM (Table 15.3). Exercise for 30 minutes has little or no effect on ketone synthesis, utilization or clearance. After 3–5 days fasting, plasma ketones rise about 10-fold due mainly to increased synthesis and lower clearance. In obese adults, overnight
or 3–5 days fasting has little to no effect on these kinetic parameters, but synthesis may exceed utilization, thereby raising plasma ketones a bit more than in normal weight adults. In type 1 diabetes, 12 hours of fasting increases plasma ketones about 10-fold more than in nondiabetic adults, but it is not clear why, as utilization appears to keep pace with synthesis, both of which are similar to values seen in nondiabetic adults after 12 hours of fasting (Table 15.3).

As far as is known, ketones can be transported into and catabolized by all tissues except the liver. However, during extended fasting, free fatty acids compete with ketones and become the main fuel for some tissues such as skeletal muscle, leaving most if not all of ketone production available to complement the available glucose in meeting the energy needs of the brain (Drenick et al., 1972; Owen and Reichard, 1971). Ketone transport into tissues occurs via monocarboxylic acid transporters, of which there are at least six subtypes (Simpson et al., 2007). Several of the monocarboxylic acid transporters are expressed in the brain. Monocarboxylic acid transporter

![Diagram of ketogenesis](image_url)

**FIGURE 15.2** Ketogenesis arising from the condensation of two acetyl-CoAs to form the ketones, acetoacetate, beta-hydroxybutyrate, and acetone. HMG-CoA: 3-hydroxy-3-methylglutaryl-coenzyme A.
expression responds rapidly to hyperketonemia (Pan et al., 2001), so, under normal conditions, brain uptake of ketones is directly proportional to their plasma concentration over about a 600-fold range of beta-hydroxybutyrate (0.02–12 mM; Courchesne-Loyer et al., 2013; Cunnane et al., 2015; Cunnane et al., 2011). However, as with brain glucose uptake, the transport of ketones into the brain may not be the limiting variable in their uptake. Rather, ketones are “pushed” into the brain in proportion to their plasma ketone concentration, which usually only increases when plasma glucose decreases. This contrasts with glucose, which is “pulled” into the brain in proportion to its utilization. Hence, the “push-pull” strategy ensures that under conditions in which glucose availability decreases, ketone synthesis would normally be stimulated and the brain’s energy supply would be maintained (Figure 15.3).

### TABLE 15.3 KETONE (BETA-HYDROXYBUTYRATE + ACETOACETATE) KINETICS IN HUMANS SUMMARIZED FROM A SERIES OF PUBLISHED STUDIES

<table>
<thead>
<tr>
<th>Condition</th>
<th>Fasting period</th>
<th>Plasma ketones (mM)</th>
<th>Utilization (µmol/kg/min)</th>
<th>Synthesis (µmol/kg/min)</th>
<th>Metabolic clearance (mL/kg/min)</th>
<th>Urinary excretion (µmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy adults</td>
<td>12–16 h</td>
<td>0.1–0.3</td>
<td>3–5</td>
<td>2–5</td>
<td>18</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.5</td>
<td>—</td>
<td>10</td>
<td>—</td>
<td>4</td>
</tr>
<tr>
<td>Healthy adults + 30 min exercise</td>
<td>16 h</td>
<td>0.2–0.4</td>
<td>6</td>
<td>6</td>
<td>21</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>3–5</td>
<td>4–5</td>
<td>20</td>
<td>22</td>
<td>4–6</td>
<td>—</td>
</tr>
<tr>
<td>Obesity</td>
<td>12–16 h</td>
<td>0.4–0.7</td>
<td>4</td>
<td>3–8</td>
<td>12</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>≥ 3 d</td>
<td>3–7</td>
<td>9</td>
<td>5–25</td>
<td>3</td>
<td>80–240</td>
</tr>
<tr>
<td>Type I diabetes</td>
<td>12 h</td>
<td>2.5</td>
<td>6</td>
<td>6</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

h—hours  
d—days  
A (Hall et al., 1984)  
B (Reichard et al., 1974)  
C (Balasse, 1979)  
D (Garber et al., 1974)  
E (Owen et al., 1967)  
F (Fery and Balasse, 1983)  
G (Avogaro et al., 1990)  
H (Balasse et al., 1978)  
I (Balasse, 1986)  
J (Fery and Balasse, 1986)

**Figure 15.3** The contrasting “push-pull” mechanism of brain fuel supply. Glucose is pulled from the blood into the brain as a function of the brain’s metabolic demand during neuronal activation. Under normal conditions (excluding insulin resistance), ketones are pushed from the blood into the brain in direct proportion to their plasma concentration.
Chapter 15: Glucose and Ketone Metabolism in the Aging Brain

**REGIONAL BRAIN KETONE UPTAKE IN EARLY ALZHEIMER’S DISEASE**

Positron emission tomography provides a means of directly quantifying and comparing brain glucose and ketone uptake. Using PET, Blomqvist et al. (2002; 1995) reported the brain metabolism of $^{11}$C-beta-hydroxybutyrate in humans and confirmed the earlier AVD studies showing that the uptake was directly proportional to plasma beta-hydroxybutyrate even at very low plasma ketone concentrations (Cunnane et al., 2011; Lying-Tunell et al., 1981; Figure 15.4). We found that synthesizing $^{11}$C-AcAc was easier than synthesizing $^{11}$C-beta-hydroxybutyrate (Tremblay et al., 2007), and since acetoacetate is the ketone that actually enters the mitochondria and is catabolized to acetyl CoA, we worked with it as our brain ketone PET tracer. We developed a PET protocol in which $^{11}$C-AcAc is injected intravenously first, followed by a wash-out period, and then FDG is injected. We have applied this protocol in human (Castellano et al., 2015b; Nugent et al., 2014b) and animal (Pifferi et al., 2011; Roy et al., 2012) studies. This dual tracer technique allows for a quantitative comparison of brain uptake of glucose and ketones with a delay of no more than 2 hours between the two tracer infusions, which avoids the potentially greater biological variability between scans done on different days.

This dual tracer technique permitted us to compare brain uptake of FDG and $^{11}$C-AcAc in early AD (Castellano et al., 2015b). The aim was three-fold: (1) to confirm the AVD reports of normal ketone but low brain glucose uptake early in AD (Ogawa et al., 1996), (2) to assess brain fuel metabolism in early AD rather than the more advanced stages previously reported (Lying-Tunell et al., 1981), and (3) to quantify the regional pattern of brain uptake of both fuels under postprandial conditions. We confirmed that global brain FDG uptake (CMRg) was 14% lower in early AD versus cognitively normal controls, and that this global deficit was primarily confined to the parietal cortex, posterior cingulate, and thalamus. However, neither $^{11}$C-AcAc uptake (CMRa) nor the acetoacetate uptake constant ($K_a$) were significantly different

**FIGURE 15.4** Direct, linear relation between plasma β-hydroxybutyrate (β-HB), brain ketone uptake (left-hand Y axis), and percentage contribution to total brain energy requirement (right-hand Y axis) in adults. Two relationships are shown, one for plasma β-HB versus the rate of brain β-HB uptake (solid line, $R^2 = 0.97$; $Y = 1.57X - 0.20; p < .0001$), and the other for plasma acetoacetate versus the rate of brain acetoacetate uptake (Castellano et al., 2015b) (dotted line, $R^2 = 0.83; Y = 3.46X - 0.03; p < .0001$). Alzheimer’s disease (AD), ◦ cognitively healthy age-matched controls. Units are the same for both ketones—cerebral metabolic rate (CMR; μmol/100 g/min). β-HB data have been combined from several sources: □ postprandial state (Blomqvist et al., 1995), ▼ (Blomqvist et al., 2002) β-HB infusion, ◇ AD and ◯ healthy older controls (Lying-Tunell et al., 1981), ▲ 40-day fast (Owen et al., 1967), ■ 60-day fast (Drenick et al., 1972), and ▼ AD and ▲ healthy older controls (Ogawa et al., 1996; indicated by the arrow). All the β-HB data are from arteriovenous difference studies except for one report, which used β-HB-PET (Blomqvist et al., 1995). The AcAc data were obtained using $^{11}$C-AcAc PET (Castellano et al., 2015b). Each symbol represents a single individual except when not available in the original publication: Drenick et al. (1972), for which ■ represents the mean of $n = 5$ participants, and Ogawa et al. (1996), for which ◇ and ▲ both represent the mean of $n = 7$. The relationship between plasma β-HB and the percent of brain energy consumption supplied by β-HB in adults is as follows: at plasma β-HB values around 0.1 mM, ketone supply >5% of brain energy; at 1 mM β-HB, they supply about 10%–15%; at 5–7 mM β-HB, 50%–65% and over 7–8 mM β-HB, >75% of brain energy consumption. For a given plasma AcAc concentration, AcAc is taken up by the brain more rapidly than β-HB, which is why the dotted regression line lies above that of the solid line for β-HB.
in the brain as a whole or in any brain region in AD versus the age-matched controls (Figure 15.5). The kinetics of $^{11}$C-AcAc metabolism (or $^{11}$C-beta-hydroxybutyrate metabolism; Blomqvist et al., 1995) suggest a one-compartment model in which brain utilization essentially matches brain uptake. Plasma acetoacetate and CMRa were significantly positively correlated, the slope of which did not differ between controls and early AD. This implies that brain ketone utilization in AD was still proportional to plasma concentration on the same slope as in controls (Figure 15.6). These results confirm those of Lying-Tunell et al. (1981) and Ogawa et al. (1996). Unlike with FDG, the $^{11}$C-AcAc PET tracer is chemically identical to acetoacetate produced by the body, so it is possible to interpret our results as showing that both uptake and metabolism of $^{11}$C-AcAc were normal in early AD. Unlike for glucose, which generates ATP by both oxidative phosphorylation and aerobic glycolysis, production of ATP from ketones is exclusively via the citric acid cycle and oxidative phosphorylation. Since we observed that brain $^{11}$C-AcAc metabolism was normal in early AD, our results with brain ketone PET therefore indirectly support the speculation by Hoyer et al. (1988) that oxidative phosphorylation may still be normal early in AD. These observations provide a rationale for the concept that if provided with more ketones, the aging brain would be able to use them which, in turn, might help it overcome its deficit in glucose uptake and metabolism, thereby delaying brain energy exhaustion and decreasing the risk of cognitive decline and AD.

**FIGURE 15.5** Whole-brain $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) uptake is 14% lower in mild Alzheimer’s disease (white bars; $n=10$) compared with cognitively normal age-matched controls (black bars; $n=30$; * $p=0.003$). In contrast to FDG, whole-brain $^{11}$carbon-acetoacetate ($^{11}$C-AcAc) uptake is not significantly different in mild Alzheimer’s disease compared with cognitive normal, age-matched controls (from Castellano et al., 2015b).

**FIGURE 15.6** The direct, linear relationship between plasma ketone (acetoacetate; AcAc) concentration and brain ketone uptake is not altered in mild Alzheimer’s disease (white triangles, dotted line; $n=10$) compared with cognitively normal age-matched elderly (black squares, solid line; $n=30$) (from Castellano et al., 2015b).
milk of medium chain fatty acids, that is, fatty acids of 6–12 carbons (Cunnane and Crawford, 2014). Medium chain fatty acids are mostly absorbed through the portal vein, hence gaining direct and more rapid access to the liver than long chain fatty acids, which are absorbed into the peripheral circulation via the lymph. Medium chain fatty acids are also beta-oxidized without needing to be activated by carnitine. The net result is rapid beta-oxidation and ketogenesis. With rare exceptions, there is normally no further opportunity to consume medium chain fatty acids from the diet once breast-feeding is terminated. The exceptions are coconut oil and palm kernel oil, in which medium chain fatty acids make up about 15% and 10% of the fatty acid composition, respectively. The fraction of these oils that contains these medium chain fatty acids can be concentrated, resulting in a generic product containing mostly fatty acids of eight (octanoic or caprylic acid) and 10 carbons (decanoic or capric acid) called medium chain triglycerides (MCTs). The ratio of these two principal fatty acid components and their proportion of the total can vary widely from one MCT product to another. The MCTs are well known to be ketogenic (Bach and Babayan, 1982; Freund and Weinsier, 1966). Notwithstanding the generic nature of MCTs and different study designs to assess their metabolism, there is a remarkably good correlation in the literature between the maximal plasma ketone levels achieved on oral doses of MCTs from 10 to 70 g (Figure 15.7). These dose-response studies show similar results whether a single or multiple MCT doses are given, probably because of rapid plasma ketone clearance.

Healthy older people have the same level of hyperketonemia and 13C-ketone oxidation to 13C-CO2 after a standard high-fat ketogenic breakfast containing MCTs as middle aged or young adults (76 years old versus 50 or 23 years old, respectively; Freemantle et al., 2009; Figure 15.8). Indeed, hyperketonemia after 18 hours of fasting may actually be somewhat higher in the seventh to eighth decade of life compared with younger adults (London et al., 1986). Hence, the capacity to produce and utilize ketones does not appear to be reduced and may actually be increasing somewhat during healthy aging. These studies did not distinguish between ketone utilization by the brain versus other organs, but our brain ketone PET results confirm that the brain has similar ketone uptake and utilization in healthy older compared with younger persons (Nugent et al., 2014b).

**Clinical Studies of Hyperketonemia in Conditions of Cognitive Deficit**

Several products based on MCTs are now available on the market or by prescription in the United States and in Europe. The arrival of these products was greatly stimulated by reports that MCTs had beneficial effects on cognitive outcomes in mild-moderate AD after a single dose (Reger et al., 2004) or
with regular consumption over several months (Henderson et al., 2009). Improved cognitive outcomes after MCTs are also seen in type 1 diabetics during controlled acute hypoglycemia caused by insulin infusion (Page et al., 2009). The very high-fat ketogenic diet has been reported to have a similar beneficial effect on cognitive and cardiovascular outcomes in mild cognitive impairment, the prodromal state to AD (Krikorian et al., 2012). These reports complement the studies showing that autonomic and neurological symptoms of acute severe experimental hypoglycemia and starvation can be avoided by ketone infusion (Table 15.4).

Acetoacetate and beta-hydroxybutyrate can also be directly administered orally or by infusion. This requires that they be provided as salts or as esters (Clarke et al., 2012; Hasselbalch et al., 1996; Plecko et al., 2002), with the esters being more practical for long-term use. The safety (Clarke et al., 2012) of a beta-hydroxybutyrate-monoester and its anecdotal utility in improving some aspects of cognitive function in an advanced case of early-onset Alzheimer's disease have recently been reported (Newport et al., 2015). The results of these clinical studies are still preliminary, but they support the hypothesis that brain glucose deficit contributes to impaired cognition associated with aging and that this deficit can at least in part be reversed or bypassed by either a ketogenic supplement containing MCTs, a ketone ester or a very high-fat ketogenic diet.

**ANAPLEROSIS**

During prolonged starvation, ketones can supply upward of 70% of the energy requirement of the adult brain (Drenick et al., 1972; Owen et al., 1967) but glucose is still essential in the process. In addition to being the main engine to generate ATP, the citric acid cycle also spins off molecules needed for brain function including the neurotransmitters GABA and acetylcholine, a process known as cataplerosis (Owen et al., 2002). Cataplerosis would rapidly deplete the citric acid cycle of its intermediates except that, via oxaloacetate, glucose supplies carbon to replace those intermediates, a process known as anaplerosis. Hence, glucose is not only a major fuel but also controls the balance between anaplerosis and cataplerosis, that is, the net flow of carbon out of the citric acid cycle via products other than CO$_2$. Like glucose, ketones generate ATP via the citric acid cycle and oxidative phosphorylation, but, unlike glucose, ketones do not contribute to anaplerosis (Brunengraber and Roe, 2006). In fact, ketones are cataplerotic because they increase citric acid cycle activity (Roy et al., 2015) but do not provide carbon to replace intermediates in the cycle. A metaphor would be the complementary roles of gasoline and motor oil in an engine; glucose provides both the gasoline and the motor oil, so the engine will work efficiently, but ketones only provide the gasoline, so if forced to run excessively on ketones, the engine soon burns out. Therefore, as long as sufficient glucose is available, anaplerosis will be maintained and citric acid cycle function will continue even during extreme ketosis.

During short-term starvation, gluconeogenesis is directly proportional to ketogenesis (Garber et al., 1974). Some of that glucose is needed by the liver (which cannot catabolize ketones; (Fukao

![FIGURE 15.8 Plasma β-hydroxybutyrate (β-HB) response after a high-fat meal in a middle-aged group (gray triangles; 50 years old; n = 18) and older group (white circles; 76 years old; n = 14) compared with young adults (dotted line; 23 years old; n = 22) (from Freemantle et al., 2009).](image-url)
et al., 2004), but part of the resulting glucose production is undoubtedly used to sustain anaplerosis, especially in the brain. During conditions in which glucose supply to the brain is more severely limited, for example, inherited glucose transporter (Glut1) deficiency (Brunengraber and Roe, 2006; Mochel et al., 2005; Roe and Mochel, 2006), there is insufficient glucose to maintain anaplerosis or energy production in the brain, so providing a ketogenic supplement may be beneficial. However, beyond a certain point, supplying more ketones is futile because they further stimulate the citric acid cycle (Roy et al., 2015) without contributing to anaplerosis so, in essence, they burn the cycle out (Brunengraber and Roe, 2006). Whether the situation is analogous in AD remains to be seen, but it is clear that as AD becomes more severe, brain glucose uptake and/or utilization continue to deteriorate thereby further compromising both ATP production and anaplerosis, especially if ketogenesis is stimulated. There is most likely to be a trade-off between supplying more ketones to compensate for the glucose deficit and oversupplying them, thereby further depleting glucose and risking burning out the citric acid cycle.

#### TABLE 15.4 CLINICAL STUDIES IN WHICH HORMONAL AND COGNITIVE RESPONSES SUGGEST THAT ACUTE OR CHRONIC KETOGENIC TREATMENTS MAINTAIN BRAIN FUNCTION BY COMPENSATING FOR HYPOGLYCEMIA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute studies</strong></td>
<td></td>
</tr>
<tr>
<td>Controlled insulin-induced hypoglycemia ± fasting in obesity (n = 9)</td>
<td>Treatment: 2 h insulin infusion ± 60 d fast</td>
</tr>
<tr>
<td></td>
<td>Outcomes: ↓ effect of acute severe hypoglycemia (0.5 mM in one case), including ↓ mental confusion, anxiety, sweating, tachycardia, blood pressure if fasted for 60 d before the insulin infusion</td>
</tr>
<tr>
<td>Controlled insulin-induced hypoglycemia in healthy adults (n = 6)</td>
<td>Treatment: 4 h i.v. β-HB infusion</td>
</tr>
<tr>
<td></td>
<td>Dose: 30 μmol/min/kg body weight</td>
</tr>
<tr>
<td>Controlled insulin-induced hypoglycemia in healthy adults (n = 13)</td>
<td>Treatment: 6 h i.v. β-HB infusion</td>
</tr>
<tr>
<td></td>
<td>Dose: 20 μmol/min/kg body weight</td>
</tr>
<tr>
<td>Controlled insulin-induced hypoglycemia in Type 1 diabetes (n = 11)</td>
<td>Treatment: single oral MCT</td>
</tr>
<tr>
<td></td>
<td>Dose: 40 g in three stages (20, 10, 10 g)</td>
</tr>
<tr>
<td></td>
<td>Outcomes: ↓ cognitive symptoms of acute hypoglycemia.</td>
</tr>
<tr>
<td><strong>Age-associated cognitive decline</strong></td>
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</tr>
<tr>
<td>Mild cognitive impairment (n = 23)</td>
<td>Treatment: 6 weeks high-fat ketogenic diet</td>
</tr>
<tr>
<td></td>
<td>Outcomes: ↑ secondary memory performance</td>
</tr>
<tr>
<td>Mild-moderate Alzheimer’s disease (n = 20)</td>
<td>Treatment: Single dose oral of MCT (95% octanoate)</td>
</tr>
<tr>
<td></td>
<td>Dose: 40 g</td>
</tr>
<tr>
<td></td>
<td>Outcomes: ↑ cognitive score in APOE4(-) patients</td>
</tr>
<tr>
<td>Mild-moderate Alzheimer’s disease (n = 77)</td>
<td>Treatment: 90 days oral MCT (95% octanoate)</td>
</tr>
<tr>
<td></td>
<td>Dose: 20 g/d</td>
</tr>
<tr>
<td></td>
<td>Outcomes: ↑ cognitive score in APOE4(-) patients</td>
</tr>
<tr>
<td>Severe Alzheimer’s disease (n = 1)</td>
<td>Treatment: 20 months oral MCT + coconut oil (4:3)</td>
</tr>
<tr>
<td></td>
<td>Dose: 165 mL/day</td>
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<tr>
<td></td>
<td>Outcomes: ↑ mood, affect, self-care, and cognitive and daily activities</td>
</tr>
</tbody>
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β-HB: beta-hydroxybutyrate

**BRAIN ENERGY STATUS, NEURAL PROTECTION, AND MITOCHONDRIAL FUNCTION**

Regardless of whether glucose or ketones are being catabolized, mitochondria are required to produce the bulk of the ATP being generated. Glucose can produce some ATP via aerobic glycolysis, a
process occurring outside mitochondria, whereas ketones are metabolized in an obligatory manner via oxidative phosphorylation within mitochondria. Although controversial, the enzymes of mitochondrial oxidative phosphorylation may actually continue to function normally, at least early in AD, but ATP production declines because of lower glycolysis to acetyl CoA. If so, there may be increased reliance on lactate as a fuel and even on gluconeogenesis within the brain, with adverse consequences for amino acid and neurotransmitter production including acetylcholine (Hoyer et al., 1988).

Mitochondrial damage and increased production of reactive oxygen species are widely considered to coincide in AD and to exacerbate the disease (Gibson et al., 2000). Mitochondrial dysfunction has been proposed to underlie beta-amyloid accumulation and cognitive deterioration in AD (Swerdlow et al., 2014; Swerdlow and Khan, 2004; Yao et al., 2009). Neural protection by ketones seems to be related to improved mitochondrial function including reduced mitochondrial production of reactive oxygen species in response to glutamate (Maalouf et al., 2009). The energy status of the brain appears to be increased in rats on a ketogenic diet based on ATP levels and brain glucose relative to blood glucose (Cahill, 2006; DeVivo et al., 1978; Veech et al., 2001), as well as indirect measurement of citric acid cycle activity (Roy et al., 2015). These changes could potentially be accompanied by mitochondrial biogenesis and improved respiratory function (Bough et al., 2006). Others have shown that brain glucose uptake and Glut expression increase in rats on a ketogenic diet (Pifferi et al., 2011; Puchowicz et al., 2007; Roy et al., 2012), but this has not been confirmed in humans; in fact; both PET and AVD studies suggest that, in human adults, brain glucose uptake decreases as brain ketone availability increases (Hasselbalch et al., 1995, Courchesne-Loyer et al., 2016).

SAFETY OF MEDIUM CHAIN TRIGLYCERIDES

In doses up to 1 g/kg/d, MCTs have a robust safety record in all species studies including humans (Bach and Babayan, 1982; Traul et al., 2000). Thus, they are not associated with increased risk of cancer, obesity, or other diseases. However, they can have secondary side effects involving gastrointestinal distress, an issue that can be partially mitigated by gradual dose titration. The MCTs are saturated fats and, as such, have long been painted with the brush of increasing cardiovascular risk. However, treatment for 30 days with 30 g/d of MCTs does not adversely affect serum glucose, insulin, triglycerides, cholesterol, free fatty acids, body weight, or body-mass index (Courchesne-Loyer et al., 2013).

EARLY BRAIN DEVELOPMENT AND HUMAN BRAIN EVOLUTION

Although outside the scope of this review, it has become clear over the past 3–4 decades that ketones are much more important to the developing brain than to the adult brain. Unlike in adults, ketones are an essential fuel and brain lipid substrate in infants because there is insufficient glucose available for it alone to meet brain energy requirements in human neonates (Bougueres et al., 1986; Robinson and Williamson, 1980; Settergren et al., 1976). The fatness of human babies makes this essential role of ketones in neonates all the more plausible given that long chain fatty acids stored in adipose tissue are the main substrate for ketogenesis. It is unlikely to be a coincidence that ketones are essential for human brain development and that humans have fat babies; rather, these two conditions probably coevolved during human evolution with the former undoubtedly being facilitated by the latter (Cunnane and Crawford, 2014). The potential utility of ketogenic supplements to the aging brain is perhaps analogous to their important role in the developing brain.

PERSPECTIVES

We propose that AD is in part exacerbated by a form of chronic gradual fuel starvation or exhaustion due to brain glucose deficit and that early in the disease this feature may be amenable to treatments that moderately but safely raise plasma ketones. The broadly similar neurological/cognitive benefit of prolonged fasting, a very high-fat ketogenic diet containing no MCTs, or a regular diet to which MCTs or ketone esters are added (Table 15.4) suggests that the improvement in cognition is related to a common denominator of raised plasma ketones, which bypass chronically impaired glucose uptake and utilization by the AD brain. However, this is still speculative; to date, no study has yet provided a mechanism by which MCT or ketones improve cognitive outcomes. We have deliberately not discussed the beta-amyloid hypothesis of AD, but beta-amyloid accumulation could arise as a result of (Meier-Ruge et al., 1994) or be contributing to (Meier-Ruge and Bertoni-Freddari, 1997) impaired glycolysis in the AD brain. It has also not been established whether certain MCTs (e.g., octanoate vs. decanoate) are more effective for ketogenesis than others. Octanoic...
acid can be taken up by the brain (Ebert et al., 2003; Kuge et al., 1995), so it may have direct effects on brain function including but not limited to conversion to ketones by astrocytes (Auestad et al., 1991). Several clinical trials are underway that combine either a ketogenic supplement or diet with cognitive evaluation (Table 15.5) and should produce results before the end of 2017, which will shed further light on the potential utility of ketotherapeutics in neurodegenerative diseases such as AD.

Attempts to correct or bypass impaired brain glucose metabolism in AD have also been made using several other approaches including supplements of glucose (Korol and Gold, 1998), a cocktail of glucose, malate, and antioxidant (Blass, 2008) or intranasal insulin (Benedict et al., 2011; Craft, 2012). The details of those studies are beyond the scope of this review but they are important because they show that for 20 years now, the metabolic defect in the AD brain has been viewed by many different groups as a logical target for therapeutic strategies in AD (Cunnane et al., 2015; Cunnane et al., 2011; Gibson et al., 2000; Henderson, 2008; Hertz et al., 2015; Kapogiannis and Mattson, 2011; Yao et al., 2011). Whether trying to correct the problem of impaired glucose uptake-metabolism and deteriorating mitochondrial function should be undertaken at the same time as providing a ketogenic supplement remains to be assessed.

**ACKNOWLEDGMENTS**

Christine Brodeur-Dubreuil and Eric Lavallée provided excellent technical assistance. Financial support was provided by a Canada Research Chair and University Research Chair (SCC), CFI, CIHR, NSERC, FRQS, FQRNT, Sojecci II, The Alzheimer Association (USA), and the Université de Sherbrooke. Some MCTs used in our studies were provided as gifts by ABITEC and Nutricia.

**REFERENCES**


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**TABLE 15.5 ONGOING REGISTERED CLINICAL TRIALS INVOLVING KETONE TREATMENTS IN MILD COGNITIVE IMPAIRMENT OR ALZHEIMER’S DISEASE**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Treatment</th>
<th>Reference #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild cognitive impairment</td>
<td>Treatment: 6 months oral MCT beverage &lt;br&gt;Design: Double-blind, randomized, placebo-controlled trial (n = 50/group) &lt;br&gt;Dose: 30 g/d &lt;br&gt;Outcome: Improvement in cognitive performance and change in brain glucose and ketone metabolism</td>
<td>NCT02551419</td>
</tr>
<tr>
<td></td>
<td>Treatment: 6 months oral MCT oil &lt;br&gt;Design: Double-blind, randomized controlled trial (n = 25/group) &lt;br&gt;Dose: 40 g/d &lt;br&gt;Outcome: Improvement in cognitive performance</td>
<td>NCT01669200</td>
</tr>
<tr>
<td>Mild Alzheimer’s disease</td>
<td>Treatment: 14 days oral MCT &lt;br&gt;Design: Double-blind, randomized, placebo-controlled trial (n = 8) &lt;br&gt;Dose: 10, 20, 30, and 40 g/d &lt;br&gt;Outcome: Change in CANTAB task performances and P300 EEG measurement</td>
<td>NCT01702480</td>
</tr>
<tr>
<td>Mild-moderate Alzheimer’s disease</td>
<td>Treatment: ≥ 6 months of AXONA (MCT powder) &lt;br&gt;Design: retrospective trial (n = 200) &lt;br&gt;Outcome: Change in living situation, psychiatric condition, daily living, and cognitive performance</td>
<td>NCT01538212</td>
</tr>
</tbody>
</table>
section II: Emerging Clinical Applications and Future Potential


Ketogenic Diet and Ketones for the Treatment of Traumatic Brain and Spinal Cord Injury

FEMKE STREIJGER, PHD, WARD T. PLUNET, PHD, AND WOLFRAM TETZLAFF, MD, DR. MED, PHD

INTRODUCTION

Every year, over 1,700 Canadians suffer an acute traumatic spinal cord injury (SCI) (Rick Hansen Registry, 2006) (Rick Hansen Registry: http://www.rickhanseninstitute.org/work/our-projects-initiatives/rhscir), and in the United States the number is around 12,500 (National Spinal Cord Injury Center). Spinal cord injury has a devastating impact on the quality of life that goes beyond the obvious paralysis and includes pain, spasticity, sexual dysfunction, respiratory problems, bowel and bladder disorders, skin ulcers, autonomic dysregulation, cardiovascular disease and metabolic disease, leading to a greatly reduced life expectancy (SCIRE, 2105). The economic burden of traumatic SCI in Canada is enormous and ranges from $1.47 million CAD for a person with incomplete paraplegia and close to $3 million CAD for a complete tetraplegic individual over the course of life (when injury is sustained at age 35) (Krueger et al., 2013).

The acute management of SCI is mainly focused on surgical stabilization of the spinal column and surgical decompression of the spinal cord to relieve extrinsic pressure (Fehlings et al., 2012; Furlan et al., 2011), although the benefits from early surgical intervention are challenging to demonstrate (Furlan et al., 2011). There are currently no neuroprotective or neurorestorative therapies available for SCI patients, and the only drug approved by the US Food and Drug Administration (FDA), methylprednisolone, showed only marginal neuroprotective effects when administered within 8 hours after injury (Bracken et al., 1998). Due to its limited efficacy and significant side effects, methylprednisolone is no longer recommended (Evaniew et al., 2015). The reality is that the only effective treatments for people with SCI to date are rehabilitation programs together with medical care for the wide range of secondary complications listed above (SCIRE, 2105). Rehabilitation interventions have led to significant improvements in the neurological outcome after chronic motor incomplete SCI, and more recently promising strides were made in a few individuals with functionally complete SCI (Harkema et al., 2012a; Harkema et al., 2012b). However, there is still a large unmet clinical need for treatments to protect the injured spinal cord from secondary damage in the wake of a primary injury. While little can be done for the immediate necrosis of the tissue due to mechanical destruction by the primary impact, the injury triggers a cascade of secondary damage due to vascular disruption, hemorrhage, edema, and ischemia, leading to energy depletion and ion pump failures, depolarization, excitotoxicity, calcium overload, activation of proteases, free oxygen radical formation, cell membrane compromise, lipid peroxidation and protein nitrosylation, inflammation, and cell death by various mechanisms. Such secondary damage results in oligodendrocyte death with demyelination, neuronal losses, and permanent functional impairments (for reviews see, Oyinbo, 2011).

The quest for treatments to mitigate secondary injury after neurotrauma has led to the preclinical discovery of numerous “neuroprotective” drug candidates as well as metabolic and dietary treatments. The latter include a number of health supplements and dietary regimes that are neuroprotective after acute SCI in rodents including acetyl-L-carnitine (Patel et al., 2012; Patel et al., 2010; Patel et al., 2014), creatine (Rabchevsky et al., 2003), melatonin (Yang et al., 2015), natural polyphenols from green tea, olive oil, resveratrol, turmeric (for review, see Khalatbary, 2014), fish oils (King et al., 2006; Michael-Titus and Priestley, 2014), and inosine (Kim et al., 2013) as
well as multinutrient combinations of DHA (docosahexaenoic acid), EPA (eicosapentaenoic acid), choline, phospholipids, and various vitamins marketed as Fortasyn (Pallier et al., 2015). Many of these approaches with "naturally occurring" nutrients and over-the-counter health supplements appear to have anti-inflammatory, anti-apoptotic, and antioxidant properties by mechanisms that are incompletely understood, while some also enhance synaptic plasticity in the injured spinal cord of rodents (Kim et al., 2013). However, none of these treatments have yet to become clinical standard through validation in clinical trials. As a matter of fact, the nutritional guidelines for acute SCI are merely based on expert opinions and few very small cohort studies (Class III/IV evidence) (Consortium-for-Spinal-Cord-Medicine, 2006; Rodriguez et al., 1997; Thibault-Halman et al., 2011). Nutrition is generally recommended within 24 hours using a balanced enteral formula composed of carbohydrates, fat and protein (Consortium-for-Spinal-Cord-Medicine, 2006; Thibault-Halman et al., 2011).

Contrasting these clinical guidelines, our laboratory discovered in rats that a form of caloric restriction known as intermittent fasting (food withdrawal every other day) improved outcomes from acute cervical and thoracic SCI by promoting neuroprotection and neuroplasticity (Jeong et al., 2011; Plunet et al., 2010; Plunet et al., 2008). These results raise new and interesting questions, especially given the widely appreciated notion that intermittent fasting can have a positive impact on multiple chronic diseases in animal models and on parameters of metabolic, cardiovascular and inflammatory diseases in humans (Brandhorst et al., 2015; Longo and Mattson, 2014; Maalouf et al., 2009). Despite the growing enthusiasm for fasting in the research field in recent years, introducing such a regimen to acutely injured patients is met with little enthusiasm from clinicians, despite the fact that the caloric basal metabolic rate and energy expenditure requirements are drastically reduced after SCI due to paralysis (Cook et al., 2008; Magnuson et al., 2011)—unlike in traumatic brain injury (TBI), where they may be increased (Cook et al., 2008). Fasting affects multiple pathways, including the generation of ketone bodies by the liver, most prominently D-beta-hydroxybutyrate (βHB) and acetoacetate (AcAc), which have become increasingly known to be "neuroprotective" through their intensely studied role in the so-called ketogenic diet (KD) for epilepsy (for reviews, see Gano et al., 2014; Gasior et al., 2006; Rho, 2015). KDs are high in fat, adequate in protein, and very low in carbohydrates, and induce a fasting-like state. KDs or ketones have been shown to be beneficial in models of Alzheimer's disease (Hertz et al., 2015; Imamura et al., 2006; Kashiwaya et al., 2000; Paoli et al., 2014), Parkinson's disease (Cheng et al., 2009; Kashiwaya et al., 2000; Tieu et al., 2003), Huntington's disease (Lim et al., 2011), amyotrophic lateral sclerosis (Zhao et al., 2012; Zhao et al., 2006), stroke (Austin et al., 2012; Gibson et al., 2012; Puchowicz et al., 2008; Rahman et al., 2014; Suzuki et al., 2002), multiple sclerosis (Kim do et al., 2012), and TBI (Appelberg et al., 2009; Davis et al., 2008a; Hu et al., 2009a; Prins et al., 2005). Clinical trials of ketogenic regimens in various forms are ongoing for a wide range of neurological and nonneurological disorders. The former include Parkinson's disease (NCT013644545), Tay Sachs disease (NCT01364545), stroke (NCT01820663), multiple sclerosis (NCT01915433), mild cognitive impairments (NCT02521818), childhood epilepsy (NCT02497105), refractory epileptic status (NCT01796574), adult epilepsy (NCT01906398), autism (NCT02477904), glioblastoma (NCT01865162) and acute TBI in children (NCT02174016). Clinically, KDs are successfully used for drug-resistant epilepsy in children and for a variety of genetic metabolic disorders, notably Glut1 deficiency (Kossoff, 2011).

In this chapter, we review the neuroprotective effects of KDs and ketones as they relate to neurotrauma, specifically SCI and TBI; place them into context with our own data using KDs in rodents with acute SCI; and discuss the potential mechanisms. Due to the overlapping pathophysiology of SCI and traumatic TBI we discuss some of the evidences for possible benefits of KDs in both conditions concurrently.

KETOSGENIC DIET IMPROVES OUTCOME AFTER SPINAL CORD INJURY

The previously established neuroprotective effects of KD/ketone administration and the fact that our rats treated with intermittent fasting after SCI showed ketosis every second day, presented our rationale for a battery of tests of whether KD after rodent SCI could promote neuroprotection and recovery (Streijger et al., 2013). We developed and applied a unilateral hemicontusion model of the cervical spinal cord for adult rats (Lee et al., 2012; Streijger et al., 2013). At 4 hours after injury, access to ad lib KD consisting of a fat:protein+carbohydrate ratio of 3:1 was given and maintained for 12 weeks. Blood βHB...
levels reached 0.7 mmol/L at 24 hours and peaked around 1.8 mmol/L at 7 days post injury. While spinal cord injured rats showed a lower food intake on the first 2 days and lost more weight acutely after SCI, their weight returned to normal values within approximately 2 weeks, and both standard diet (SD) and KD rats gained weight above the preinjury levels thereafter. We ruled out that a 48-hour fast acutely after injury was responsible for the improved recoveries seen with KD (unpublished observation). The KD-fed rats used the forelimb on their injured side more frequently than carbohydrate-based SD-fed controls during vertical exploration of their environment in a cylinder. Importantly, this improvement was maintained beyond week 12, when KD was replaced by SD. The KD-fed rats also displayed a greater active range of forelimb movement during grooming.

Given that hand function is the most coveted function in people with cervical SCI (Anderson, 2004), we performed two different reaching tests, and on both tasks we observed an improvement in rats fed KD in their ability to reach forward and grasp a small pellet compared with SD-fed animals. In addition to scoring reaching success, which indicates little about the strategy the animal uses to obtain the pellet, reaching can be broken down into discrete components of motion in time-lapse video-analysis (Whishaw and Gorny, 1994). In our experiments, KD improved the pellet grasp and the subsequent supination that directs the pellet toward the mouth. Histological analysis revealed that KD treatment resulted in smaller gray matter damage and greater protection of neuronal survival in the vicinity of the lesion, less infiltration by inflammatory cells, and increased expression of the glucose transporter Glut1. An RT-qPCR mRNA analysis did not reveal differences in BDNF, PDGFβ, VEGF, HIF1α, or SDF1α—although a trending threefold increase in SDF1α remains to be revisited.

In agreement with others (Leino et al., 2001), KD treatment significantly increased monocarboxylic acid transporter 1 (MCT1) expression at 2 weeks post injury (Streijger et al., 2013), which coincided with elevated blood βHB levels indicative of increased cerebral uptake of ketones. MCTs facilitate the transport of monocarboxylic acids such as lactate, pyruvate, and ketone bodies across biological membranes (Halestrap and Wilson, 2012). In the brain, MCT1 is predominantly expressed by vascular endothelial cells, astrocytes, and oligodendrocytes (Halestrap and Wilson, 2012; Pierre et al., 2002; Pierre and Pellerin, 2005), while MCT2 is mainly neuronal (Pierre et al., 2002). Interestingly, while KD preserved tissue following SCI, this was abolished following administration of the MCT inhibitor α-cyano-4-hydroxycinnamic acid (4-CIN) beginning after injury. This suggest that transport of ketone bodies and/or lactate plays a role in the observed protection rather than general metabolic effects like low glucose levels or free fatty acid-mediated events (Streijger et al., 2013). The interpretation of this experiment, however, is complicated by the fact that 4-CIN also inhibits the mitochondrial pyruvate transporter (McKenna et al., 2001).

Taken together, manipulating the composition and timing of macronutrient intake can have profound effects on the outcome from spinal cord trauma in rodents, which questions our current, poorly evidenced clinical nutritional guidelines for SCI. However, our understanding of how KD and ketone bodies mediate improved recovery from injury is very incomplete.

**KETOGENIC DIET IMPROVES OUTCOME AFTER TRAUMATIC BRAIN INJURY**

KD has also been shown to improve functional outcome following TBI in adolescent (35 days old) but not in adult rats (75 days old) (Appelberg et al., 2009). After a controlled cortical impact injury (CCI) and subsequent feeding of a 7:1 KD immediately after injury, adolescent rats showed significant improvements in the time to traverse a beam, a measure of fine motor coordination and balance, on postinjury day 3, but not on postinjury days 4–7. Additionally, on post injury day 3 the number of foot slips was reduced in the 35-day-old KD-fed CCI rats (Appelberg et al., 2009). In the Morris Water Maze, 35-day-old KD-fed animals showed significantly shorter escape latencies compared with CCI SD-fed animals. Such motor and cognitive improvements were not observed among 75-day-old animals; this, however, might have been attributable to increased hyperactivity, which has been shown by others in adult rats kept on KD (Murphy and Burnham, 2006; Ziegler et al., 2005). In a preceding study, Prins and coworkers showed that pretreatment with KD reduced CCI lesion volume by 58% in 35-day-old adolescent KD-fed rats and not in the adult KD-fed rats (Prins et al., 2005). There were also fewer Fluoro-Jade positive cells, indicative of better neuronal preservation. Oddly, very young rats 17-days of age did not show a reduction in lesion volume under these conditions (Prins et al., 2005). These findings are independently supported by Hu and coworkers, who...
performed similar KD experiments on 35-day-old TBI rats using a weight drop model (Hu et al., 2009a: Hu et al., 2009b). They found a decreased expression of the pro-apoptotic gene Bax (Bcl-2 Associated X Protein), together with a reduction of several markers for apoptosis as well as less edema (Hu et al., 2009a; Hu et al., 2009b). It is noteworthy to recall that our KD studies in the injured spinal cord were all performed in adult rats weighing around 350 g at time of injury (11–13 weeks old).

**POSSIBLE MECHANISMS OF KETOGENIC DIETS**

**Ketones Provide an Alternative Source of Energy—Anaplerosis**

It is well known that a physiologically low intake of carbohydrates during consumption of KDs or fasting typically causes hepatic ketogenesis fueled by the beta-oxidation of fatty acids, resulting in increased blood levels of the ketone bodies: βHB, acetocetate (AcAc), and acetone (Cahill, 2006). These ketone bodies cross the blood-brain/spinal cord barrier and enter neuronal and glial cells via MCTs (Nijland et al., 2014; Pierre and Pellerin, 2005). Here, ketone bodies are converted into acetoacetyl-CoA by the enzyme succinyl-CoA:3-CoA transferase (SCOT) and are broken down into two molecules of Acetyl-CoA that are subsequently used in the Krebs cycle, (see, e.g., Fukao et al., 2014; Kim do and Rho, 2008). Interestingly, brain astrocytes are an additional site for ketogenesis (Auestad et al., 1991; Guzman and Blazquez, 2001). Taken together, KDs provide an effective source of energy without the need for glycolysis or mitochondrial complex-I and the associated free oxygen radical byproducts. The brain can utilize ketones for up to 60% of its energy demands (Owen et al., 1967). Energy supplies are further boosted by an increase in mitochondrial biogenesis together with an increase of numerous energy metabolism genes in rat hippocampi after several weeks of KD (Bough and Rho, 2007; Bough et al., 2006). This can provide an important alternative source of energy when the activity of pyruvate dehydrogenase is low, as seen after both SCI and TBI (McEwen et al., 2011; Sharma et al., 2009). Pyruvate dehydrogenase converts pyruvate to acetyl-CoA, which is for mitochondrial ATP production. Low activity of this enzyme could trigger an energy crisis. Hence, some of the benefits of KD observed after SCI or TBI (Prins and Matsumoto, 2014) could be due to this metabolic rescue by ketones.

A TBI triggers a transient increase and subsequent depression in glucose uptake accompanied by decreased glycolysis (Prins and Matsumoto, 2014). Oxidative damage-driven activation of the DNA repair enzyme poly ADP-ribose polymerase (PARP) may lead to depletion of NAD+ (Besson et al., 2003; LaPlaca et al., 1999) that would cause a decrease in glyceraldehyde phosphate dehydrogenase activity. Together with an oxidative damage-driven decrease in pyruvate dehydrogenase activity, less glucose can be utilized for ATP generation in the TCA cycle and energy failure ensues (Lee et al., 1999; Sharma et al., 2009; Singh et al., 2006). Exogenously applied βHB is readily taken up by the injured brain after a CCI, and used to restore the depleted ATP stores (Prins et al., 2004). Such uptake is not seen in the uninjured brain, where energy supplies are largely glucose-derived (Prins et al., 2004). However, this depletion of energy stores after contusion injury occurs more slowly in adolescent rats than in adult animals (Deng-Bryant et al., 2011). Administration of βHB results in a more pronounced increase in βHB levels in the brain of younger rats and also restores their energy stores more effectively compared with older rats. This could be explained by the fact that young rats express higher level of MCTs for the uptake of ketone bodies into the brain compared with older rats (Prins and Giza, 2006); the age specific differences in neuroprotection seen with KD treatments after TBI are likely related. In addition, young rats show an elevation of ketone levels within 6 hours, while this takes closer to a day in adult rats (Prins et al., 2005; Prins and Giza, 2006). This time difference might be critical, since injury triggers an immediate secondary cascade and the rescue of neuronal tissue after injury is time sensitive.

This metabolic concept of KD and ketone treatment as key players in protection is supported by the pronounced neuroprotective effect after SCI and TBI of acetyl-L-carnitine, which supplies an acetyl group for mitochondrial acetyl-CoA synthesis used in the TCA cycle (Patel et al., 2012; Scafidi et al., 2010). Like ketones, in the absence of pyruvate, acetyl-L-carnitine can be used for mitochondrial respiration (Patel et al., 2012; Patel et al., 2010). Direct proof for the metabolic role of ketone bodies could be provided with a transgenic mouse line allowing for a cell (tissue)-specific deletion of the mitochondrial enzyme succinyl-CoA:3-CoA transferase (SCOTβIII). This will discern the metabolic role played by ketones as a biofuel for the TCA cycle (anaplerosis) from the nonmetabolic functions of ketones as inhibitors of inflammation and oxidation (see next section).
Non-Anaplerotic Effects of Ketogenic Diet

In addition to the aforementioned anaplerotic effects of ketones as fuel for the TCA cycle, the beneficial effects of KD may also be due to low-normal glucose levels, with reduced glycolysis and/or due to increases in free fatty acids and a myriad of lipid signaling molecules (for review, see Rho, 2015). For example, elevated blood glucose levels are often observed in humans after SCI and have been shown to correlate with less favorable recovery (Kobayakawa et al., 2014). Mice under hyperglycemic conditions show exacerbation of inflammation and secondary damage, activation of NFκB, and inferior functional outcome compared with mice in which glucose levels are kept normal (Kobayakawa et al., 2014). Similar considerations apply to TBI in humans, in particular when hyperglycemia is stress induced (Bosarge et al., 2015), and there is consensus that blood glucose should be carefully controlled (Badjatia et al., 2014). However in the case of our KD experiments in SCI rats, glucose levels were in the normal range and not significantly different from controls, making a low-glucose mediated effect unlikely.

Changes in circulating lipids due to KD may have significant effects on functional outcome following SCI. Lipids are important constituents of all mammalian cells and have diverse biological functions, including as (1) major building material for neuronal and glial membranes; (2) precursors for various messenger molecules such as arachidonic acid (AA), docosahexaenoic acid (DHA), ceramide, 1,2-diacylglycerol (DAG), phosphatidic acid, and lyso-phosphatidic acid; and (3) energy reservoirs such as triglycerides. Free fatty acids increase the expression of mitochondrial uncoupling proteins (UCPs) via activation of peroxisome proliferator activating receptors (PPAR) (Debril et al., 2001; Kiec-Wilk et al., 2005). UCPs regulate the mitochondrial transmembrane potential and prevent free oxygen radical (ROS) production when this potential gets too high. The addition to isolated mitochondria of AA, docosapentaenoic acid (DPA), eicosapentaenoic acid (EPA), palmitoleic acid, myristic acid, and butyric acid all reduced the mitochondrial transmembrane potential and thereby ROS production (Davis et al., 2008b; Sullivan et al., 2004). Both fasting and KD increase mitochondrial uncoupling proteins which are likely responsible for the reduction of mitochondrial ROS production (Davis et al., 2008a). Along the same vein, DHA has been shown on multiple occasions to be neuroprotective after SCI and TBI (Michael-Titus, 2007; Michael-Titus and Priestley, 2014), although its mode of action is very broad.

It is therefore of significant interest to understand how different lipid compositions in varying KD regimens affect their efficacy after neurotrauma. In addition, it would be interesting to see whether KD is beneficial for myelination in the aftermath of injury when lipids and metabolic energy are in high demand. Demyelination is regularly seen after SCI due to apoptosis of oligodendrocytes (Crowe et al., 1997) and remyelination is notoriously sluggish in the CNS (Franklin and French-Constant, 2008).

In addition, it is becoming increasingly clear that ketone bodies have direct non-metabolic actions as ligands for various receptors, transporters, and regulators of enzymes. These likely contribute to the beneficial effects of KDs for a wide range of neurological disorders that are covered in other chapters of this book. Of particular interest in the context of SCI are the anti-excitotoxic, antioxidant, and anti-inflammatory actions of ketone bodies themselves.

**Anti-Excitotoxic**

The anti-excitotoxic mechanisms of KD and ketone bodies have been extensively reviewed on multiple occasions due to their relevance to epilepsy treatment (Gano et al., 2014; Kim do et al., 2015; Kossof and Rho, 2009; Lutas and Yellen, 2013; Masino and Rho, 2012; Rho, 2015). Both TBI and SCI trigger excitotoxic neuronal damage, and a variety of drugs blocking neuronal excitation are neuroprotective after injury (Chen et al., 2014b; Park et al., 2004). However, clinical translation of glutamate receptor blockers has been challenging in contrast to the less specific excitation-dampening drugs like valproic acid, used in epilepsy (Chen et al. 2014b), or riluzole, a nonspecific sodium channel blocker used in ALS (Wilson and Fehlings, 2014). A clinical trial of riluzole for acute SCI is currently under way (Fehlings et al., 2015). However, these anti-excitotoxic treatments for SCI have in common that they need to be administered within hours after SCI in order to be effective. In contrast, the antiepileptogenic effect of KD requires several weeks of treatment to manifest, suggesting that prevention of excessive excitation is not a major player in the neuroprotection provided by KD in the setting of acute SCI.

**Antioxidant**

Oxidative stress and damage occurs after SCI and TBI (for an excellent overview, see Bains and Hall, 2012). ROS lead to peroxynitrite formation and lipid peroxidation, leading to perturbation of
many cellular processes including Ca$^{2+}$ and Na$^+$ / K$^+$ pumps. These failures lead to further Ca$^{2+}$ overload, mitochondrial damage, and secondary ROS production. In both TBI and SCI, the byproducts of oxidative damage can be measured within minutes of injury and both 3-nitrotyrosine and 4-hydroxynonenal peak between 1 and 3 days. This implies some urgency when attempting to prevent secondary injury with an antioxidative treatment. Indeed, in laboratory animals, most neuroprotective treatments are effective if given prior to or at the time of injury but lose efficacy when administered after a delay of several hours (Kwon et al., 2011). Similarly, in humans with SCI, methylprednisolone, an antioxidant with glucocorticoid actions, was only somewhat successful when given within 8 hours post-SCI (Bracken et al., 1998). This implies that any diet-induced benefit provided by the initiation of KD in the acute stage of injury would likely miss much of the oxidative damage during the first day of injury, since it takes 16–20 hours for the endogenous ketone levels to significantly rise.

The antioxidant effects of KDs have been extensively reviewed (Gano et al., 2014). KD up-regulates mitochondrial glutathione (GSH) biosynthesis in the brain (Jarrett et al., 2008), enhances mitochondrial antioxidant status, and reduces ROS production (Maalouf et al., 2007), protecting mtDNA from oxidant-induced damage (Jarrett et al., 2008). The exact mechanism of this GSH increase is unknown. The GSH is synthesized by glutamate cysteine ligase (GCL) forming gamma-glutamylcysteine, to which glycine is added by glutathione synthase. The activity of GCL, which is rate limiting, is increased by KD (Jarrett et al., 2008) and could be mediated by the transcription factor Nrf2 that is found in nuclear fractions of KD-fed rats (Milder et al., 2010). Nrf2 binds to antioxidant response elements (ARE) of several genes including GCL (Milder and Patel, 2012). It is hypothesized that Nrf2 is inactivated by forming a complex with Kelch-like ECH-associated protein 1 under normal physiological conditions. This complex functions as a redox sensor and releases Nrf2 in situations of stress and high ROS levels. It remains to be shown whether a transient stress or ROS response after initiation of KD (Milder et al., 2010) is eliciting an increase in Nrf2 by KD after neurotrauma. Interestingly, there is an increase in Nrf2-ARE regulated antioxidant genes after TBI, but it does not occur until 24–48 hours after injury (Miller et al., 2014), which may be too late to prevent much secondary damage. Acceleration of Nrf2-ARE activation with carnosic acid provides effective protection when given within 15 minutes of injury, and some benefits such as reduced cytoskeletal breakdown are observed with application at 8 hours after TBI (Miller et al., 2015). It remains to be shown whether KD or administration of ketone bodies will be able to elicit such a protective response via this Nrf2-ARE pathway in the time-sensitive setting of acute neurotrauma.

A recently recognized antioxidant function of βHB is that of a direct endogenous inhibitor of class histone deacetylases at concentrations that occur with KD administration (EC$^{50}$ between 2.5 and 5 mM) (Shimazu et al., 2013). Kidneys of mice fasted for 24 hours showed increased acetylation at the Foxo3a and metallothionin 2 (Mt2) promoters due to HDAC1 inhibition by βHB. Both genes protect against oxidative stress, whereby Foxo3a increases the expression of mitochondrial antioxidant manganese superoxide dismutase (MnSOD) and catalase (Shimazu et al., 2013) (for review, see Newman and Verdin, 2014). Whether a similar inhibition of class I HDACs by βHB occurs in the brain or spinal cord and how this relates to the known antioxidant effects of KD has yet to be clarified. What is becoming clear, however, is that ketones may have profound epigenetic effects that reach far beyond anaplerosis.

**Anti-Inflammatory Effects of Ketones**

It is increasingly appreciated that KD has anti-inflammatory effects in models of multiple sclerosis (Kim do et al., 2012), stroke (Rahman et al., 2014), pain (Ruskin et al., 2009), and other disorders (Dupuis et al., 2015). While part of this may be a consequence of reduced excitotoxicity and improved mitochondrial oxidation with less ROS formation, recent research has revealed direct effects of βHB as inflammatory regulators. The βHB inhibits caspase 1 cleavage as part of the NLRP3 inflammasome complex, which ultimately dampens increases in IL1β and IL18 and contributes further to the anti-inflammatory actions of ketones (Youm et al., 2015). The inflammasome is a multiprotein complex involved in caspase 1 activation and cleavage of pro-IL1β and pro-IL18. It is composed of the apoptosis-associated speck-like protein containing a caspase recruitment domain (CARD) termed ASC and a nucleotide oligomerization domain (NOD)-like receptor (e.g., NLRP1 or NLRP3). In vitro, inhibition of procaspase 1 cleavage was shown in lipopolysaccharide-primed macrophages on activation with ATP using concentrations of βHB in the 2- to 10-mM range as they occur during fasting. Importantly, this effect
was not seen with acetoacetate or the related butyrate and was specific to βHB (Youn et al., 2015). The authors tested a battery of other NLRP3 activators, including the fatty acids palmitate, ceramide, and sphingosine and obtained similar results (i.e., inhibition by βHB). Priming macrophages with Toll-like agonists revealed a potent inhibition of Pro-IL-1β cleavage by βHB treatment at similar concentrations. These βHB effects were specific to the NLRP3 inflammasome, and accordingly, the formation of ASC oligomers, aka specks, in primed and ATP-activated macrophages was greatly reduced with βHB addition. In vivo, βHB reduced peritoneal infiltration by neutrophils and IL1β-secreting macrophages following i.p. injection of uric acid crystals in mice.

Mice carrying a Nlrp3 mutation that leads to NLRP3 inflammasome activation show severe peritoneal neutrophilia, which is greatly reduced by feeding of oral ketone esters. Thus, even with levels in the order of 1mM, βHB is an effective and specific inhibitor the NLRP3 inflammasome. Importantly, these actions are independent of the presence of the GPR109a (HCA2) receptor (see below), UC2P, and the metabolic function of βHB in the TCA cycle. SCI leads to a pronounced increase of the NLRP3 inflammasome, including the partnering protein ASC (both mainly in activated microglia) and the well-known activation of caspase-1 and pro-IL1β cleavage (Zendedel et al., 2015). Increased ASC expression, caspase-1 activation, and cleavage of pro-IL1β had previously been described in a moderate cervical spinal cord contusion model at the C5 level in rats (de Rivera Vaccari et al., 2008). However, these authors did not find the NLRP3 increase but detected NLRP1 in co-immunoprecipitations with ASC without increases in NLRP1 expression. These differences might be due to differences in the SCI models used. Importantly, antibody-mediated ASC neutralization reduced caspase-1 activation and cleavage of IL1β and IL18, reducing lesion volume and improving functional recovery in multiple forelimb tests (i.e., grip strength, sticker removal, gait analysis), underscoring the importance of inflammasome activation in secondary injury cascades after SCI. Whether KD or oral ketone esters are mediating their benefits in the injured spinal cord via this mechanism remains unclear.

An additional way to directly modify inflammation by ketones is through activation of the hydroxyl carboxylic acid (HCA2) receptor also known as GPR109A, PUMA-G or niacin receptor. This receptor has received much attention in the past due to its favorable effects on blood lipid levels/composition and anti-inflammatory actions deemed responsible for cardiovascular benefits (Gille et al., 2008; Lukasova et al., 2011). However, βHB is the main known endogenous ligand of the HCA2 receptor at concentrations that are easily reached by the KD (EC_{50} 0.7 mM) (Taggart et al., 2005). While HCA2 is widely expressed in adipose tissue, where it regulates lipolysis (Taggart et al., 2005), it is also found on immune cells such as neutrophils, macrophages, and microglia but not in astrocytes or neurons (Kostylina et al., 2008; Rahman et al., 2014). HCA2 receptor activation promotes neutrophil apoptosis (Kostylina et al., 2008), and on macrophages/monocytes it activates/attracts a neuroprotective Ly-6c subset (Rahman et al., 2014). The latter study is of particular interest, showing that the beneficial effects of KD or niacin in a model of stroke by middle cerebral arterial occlusion (MCAO) in mice were lost when these mice were lacking the HCA2 receptor (HCA2^{-/-}). Transplantation of bone marrow from HCA^{+/+} wild-type into the HCA^{−/−} mice restored this rescue effect, demonstrating that monocyte/macrophage HCA2 expression is required. It was also necessary since the inversion of this transplantation paradigm (i.e., HCA^{−/−} bone marrow into wild-type mice) abolished the neuroprotective effect. This beneficial effect of niacin (and, by inference, βHB) on stroke outcome requires Cox1 and hematopoietic prostaglandin D2 synthase (Rahman et al., 2014). The HCA2 receptor is also activated by dimethylfumarate (DMF), a drug used clinically for MS. The HCA2 receptors have been shown to mediate the inhibition of DMF on neutrophil adhesion and migration in an inflammatory mouse model of MS (Chen et al., 2014a). Interestingly, prostaglandin D2 and its PD1 receptor have been implicated in the mitigation of damage after excitotoxic challenge or stroke (Dore and Shafique Ahmad, 2015) and to dampen seizures (Kaushik et al., 2014). Taken together, these data indicate that βHB at concentrations measured during fasting or KD treatment can modify the molecular phenotype of blood monocytes and macrophages toward an anti-inflammatory (M2-like) tissue protective response via direct activation of the HCA2 receptor. Since neutrophil invasion and M1 macrophage polarization play important damaging roles after SCI (for review, see Donnelly and Popovich, 2008; Kigerl et al., 2009; Popovich, 2014), it is conceivable that the protective effect of the KD is mediated at least in part by these HCA2 receptor triggered mechanisms. This hypothesis remains to be tested, and in light of an arsenal of
HCA2 agonists developed to treat dyslipidemias and atherosclerosis, it is becoming increasingly attractive.

**Translational Consideration of Ketogenic Diet in the Clinical Setting**

KD and ketone bodies have powerful anti-excitotoxic, antiepileptic and anti-inflammatory effects that go far beyond their classic function as an energy-providing metabolic boost and provide neuroprotection by multiple mechanisms. One translational challenge lies in the timing of KD administration, since secondary injury cascades begin within minutes of neurotrauma. As a matter of fact, most neuroprotective treatments are most effective when given prior to injury and lose their effects when administered more than 1–3 hours after injury (Kwon et al., 2011). However, a dietary intervention with KD does not increase ketone body levels until the end of the first day—in particular in the context of injury stress as it occurs after SCI (Streijger et al., 2013). In this regard, the neuroprotective effects of KD after SCI represent one of the more promising interventions currently available. While this may be encouraging, we hypothesize that in the acute setting of neurotrauma, a faster increase in ketone bodies would be greatly advantageous. A carefully monitored direct infusion of βHB for the first days after trauma in parallel with a KD could be one avenue. An alternative approach has been envisioned by Richard Veech and colleagues by suggesting the use of oral ketone-ester supplementation after TBI (Veech et al., 2012). In vivo oral administration of ketone esters readily increases βHB levels to the desired range of 3–4 mmol/L and has been shown to improve cognition and histopathology in 3xTgAD mice that are a model for Alzheimer’s disease (Kashiwaya et al., 2013). Whether or not such supplementation with oral ketone esters after acute TBI and SCI is best combined with KD or will be effective by itself as a “diet in a bottle” remains to be determined.

Our clinical colleagues in Guangzhou (China) recently conducted a clinical trial of KD in patients with acute SCI to evaluate its safety and feasibility (Guo et al., 2014). These investigators introduced KD treatment in the acute phase after injury in 10 patients with SCI. Comparable to what was seen in the rodent models of SCI (Streijger et al., 2013), all patients developed ketone levels above 2 mM (typically ~ 3 mmol/L) while maintaining normal glucose levels (9 out of 10). Routine blood tests for electrolytes and liver and kidney function showed no changes. This demonstrates that diet-induced ketosis in patients with SCI is feasible and that the KD is safe when initiated after SCI in the hospital setting. Yet, there was some transient gastrointestinal discomfort (diarrhea, nausea, poor appetite, gastric pain, and abdominal distension) in five patients and a low enthusiasm for the Chinese KD formulation. This underscores the translatability of this dietary approach, but strongly motivates us to improve this metabolic treatment in order to minimize unpleasant side effects and increase compliance to be able to implement a 2- to 3-month-long regimen after SCI in a human clinical trial.

For the same reason, more “liberal” diets have been explored in the epilepsy field with the hope for similar benefits. These alternatives share restriction in the amount/type of carbohydrates and vary in the lipid and protein content. They include a medium chain triglyceride enriched KD diet (MCT-KD), a low glycemic index treatment (LGIT) and the “modified Atkins diet” (MAD) (Miranda et al., 2012). The latter alternative has been the most extensively studied and was in its original form introduced by Dr. Atkins in the 1970s. However ketosis with the original Atkins is variable and in order to mimic the ketosis of classical KD the amount of carbohydrates are strictly reduced in the MAD to less than 20 g per day (in adults) while the intake in protein is not restricted (unlike in KD). While still ketogenic, this MAD results in low-normal blood glucose levels due to gluconeogenesis from proteins. Compared with the 3:1 ratio (3 g fat to 1 g of protein+carbohydrate) in KD, the ratio in MAD is ~1:1 whereby the carbohydrates provide <6% of the total calories, protein ~25%–35% and the lipids ~60%–70% of the total calories; per gram, fat has more than twice as many calories as protein or carbohydrates. Thus the MAD is much easier to implement and better tolerated by the patients than the 3:1 KD, where <3% of the calories stem from carbohydrates, ~11% from protein and ~88% from fat. MAD appears almost as effective as KD as long as the carbohydrate intake is strictly controlled (Sharma et al., 2013; for review, see Auvin, 2012; Kossoff, 2011, 2013; Miranda et al., 2012). A meta-analysis of close to 20 studies indicates that a 50% rate of seizure reduction with MAD is nearly as good as the 56% rate with classical KD (Kossoff et al., 2013). Whether MAD is as effective as KD in the SCI setting is worth further investigation. It would certainly make a huge difference for individuals with neurotrauma and most likely increase their compliance, if we could trial a MAD instead of a classical KD, even when conducting a dietary treatment for only 2–3 months,
which is the presently anticipated treatment duration after SCI.

We hope to close the “translational gap” from bench to bedside by collecting the necessary data to better understand the underlying mechanisms responsible for the beneficial effects of KDs in the setting of SCI and by conducting the necessary preclinical experiments to decide in what exact form the benefits of the KD and ketone bodies could be used for clinical trials in humans with acute SCI and TBI.

ACKNOWLEDGMENTS

The authors thank Brett Hilton for critically reading this manuscript; he is supported by a Banting and Best Scholarship. Wolfram Tetzlaff holds the John and Penny Ryan British Columbia Leadership Chair in Spinal Cord Injury; the research in his laboratory is supported by the Canadian Institute for Health Research, the Multiple Sclerosis Society of Canada, Wings for Life, the International Spinal Research Trust, Craig H. Nielsen Foundation, and the Rick Hansen Institute and Rick Hansen Foundation.

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Anti-Inflammatory Effects of a Ketogenic Diet

Implications for New Indications

NINA DUPUIS, PHD AND STÉPHANE AUVIN, MD, PHD

INTRODUCTION

The ketogenic diet (KD) is a high-fat, low-carbohydrate diet that results in ketosis, modulation of glycemia, relative caloric restriction, and elevations in the levels of certain fatty acids. In clinical practice, KD is an established treatment for pharmacoresistant epilepsy, and is increasingly being explored for some inflammation-induced epileptic encephalopathies such as fever-induced refractory epileptic encephalopathy in school-age children (FIRES) (Nabbout et al., 2011). The FIRES syndrome begins with severe seizures evolving into status epilepticus, and is preceded by fever or common viral infection but without an identifiable cause (Dupuis and Auvin, 2015). Whether fever itself or some other factor is the trigger remains to be determined. The outcome is very severe with adverse motor and cognitive consequences as well as medically refractory epilepsy (Kramer et al., 2011). It is hypothesized that the synergy between inflammation and status epilepticus, the latter resulting partly from inflammation itself and contributing to the induction of inflammation, generates a vicious cycle that leads to major neurological consequences (Nabbout et al., 2011).

While the antiseizure mechanism(s) remain(s) unclear, new uses for the KD are increasingly suggested, given its broad efficacy in various experimental models, including animal models exhibiting prominent inflammatory changes. Our aim in this chapter is to review the data supporting the anti-inflammatory profile of the KD, and to link improvements in various animal models of neurological disease with KD-induced modulation of inflammation. Finally, we discuss the possible mechanisms underlying the anti-inflammatory properties of the KD.

KETOCGENIC DIET EXHIBITS ANTI-INFLAMMATORY PROPERTIES

The use of the KD in a pain model first led to the idea that this high-fat, low-carbohydrate diet might possess anti-inflammatory properties. In this study, the KD attenuated thermal nociception and decreased peripheral edema (details below) (Ruskin et al., 2009). We recently further established the anti-inflammatory effects of the KD using a rat model of fever (Dupuis et al., 2015). In Wistar rats, 2 weeks of KD treatment before lipopolysaccharide (LPS) i.p. injection reduced the fever response as well as pro-inflammatory cytokine levels. In this fever model, the body temperature did not rise similarly in KD-treated animals compared with the controls. The body temperature was actually significantly lower in the KD group (around 37.6°C vs 38.5°C) from 1 h 30 to 4 h after LPS administration (Dupuis et al., 2015). The fever profile correlated with plasma IL-1β levels: 1 h after injection 130.8 ± 36.3 pg/mL in control versus 51.4 ± 9.1 pg/mL in KD (p < .05); 2 h after injection 203.3 ± 65.8 pg/mL in control versus 171.9 ± 38.8 pg/mL in KD (p < .05). Four hours after LPS injection, IL-1β and TNF-α plasma levels were significantly lower in the KD group compared with the control group, although no difference was observed before LPS injection. In contrast, 4 hours after LPS injection, PGE2 plasma levels were not different between the two groups. Interestingly, 4 hours after LPS injection, the KD group also showed lower IL1β mRNA levels in the hippocampus compared with controls. As expected, total fatty acid levels were increased after 14 days of KD compared with a standard diet. The levels of the long-chain omega-3 (n-3) PUFAs eicosapentaenoic acid (EPA; C20:5 n-3) and
docosahexaenoic acid (DHA; C22:6 n-3), which are precursors of some anti-inflammatory agents, were decreased in the KD group. In contrast, arachidonic acid (AA; C20:4 n-6), an n-6 PUFA that is a precursor for the synthesis of some pro-inflammatory eicosanoids, was also reduced by the KD (Dupuis et al., 2015).

The KD further modulated fever by decreasing peripheral inflammation and brain IL-1β expression. Although we cannot exclude an effect of ketone bodies and/or caloric restriction in our recent study, we propose that the decrease of AA may be key factor in the anti-inflammatory properties of the KD. This suggestion is underscored by our previous report of a decrease in AA levels in patients responding to KD (Porta et al., 2009).

Another recent study evaluated the effect of a KD on fibroblast growth factor 21 (FGF21) and inflammation (Asrih et al., 2015). The authors first established that the KD promoted weight loss through increased energy expenditure and reduced food intake. The mice treated with a KD had increased plasma levels of FGF21; Fgf21 gene expression was also increased in the liver but decreased in white adipose tissue (WAT). The FGF21 is produced principally in the liver and mainly acts on adipocytes. It has been shown that by targeting adipose tissue, FGF21 promotes adiponectin production and secretion; adiponectin subsequently mediates the systemic effects of FGF21 (Holland et al., 2013; Lin et al., 2013). The authors reported an increase in the expression of pro-inflammatory cytokines (Tnfa, Il-6) and macrophage accumulation (Emr1, Cd68, Itgam). In addition, a key mediator of the inflammasome (Nlrp3) was significantly increased in the liver of mice treated with a KD. Interestingly, these inflammatory markers were decreased in adipose tissue of KD-fed mice (Asrih et al., 2015).

**KETOGENIC DIET IMPROVES THE OUTCOME OF NEUROLOGICAL DISEASE ANIMAL MODELS WITH INVOLVEMENT OF INFLAMMATION**

The KD has been widely studied in experimental models of seizure and epilepsy, and has also been explored in other models of neurological diseases, such as pain, multiple sclerosis, and Parkinson's disease. In these latter conditions, the KD appears to render broad neuroprotective effects (Table 17.1).

**Pain**

Chronic pain persists or progresses over a long period of time. It seems that some chronic pain patients suffer from long-term immune-system activation resulting in continuous release of pro-inflammatory cytokines. In contrast, there is evidence that levels of anti-inflammatory cytokines are decreased in patients with widespread pain (Uceyler et al., 2006) and neuropathic pain (Uceyler et al., 2007). This explains why chronic pain may arise through an increase in pro-inflammatory cytokines and the resulting inhibition of anti-inflammatory cytokines.

In a model of pain induced by complete Freund’s adjuvant through intraplantar injection, KD treatment over 3 weeks resulted in a decrease in thermal nociception. This effect was associated with a reduction in the peripheral inflammatory response as measured by paw swelling (volume measure) and plasma extravasation (measurement of the dye Evans Blue from dissected tissue) (Ruskin et al., 2009). In this study, the authors compared the response of adult and juvenile (beginning diet at P21) animals. The KD was effective in both age groups, but both the hypoalgesic effect and the reduction in plasma extravasation were more robust in juveniles. However, the presence of edema is only one indicator of an inflammatory response, so these findings only tell part of the story.

**Multiple Sclerosis**

Multiple sclerosis (MS) is an autoimmune inflammatory disorder of the central nervous system affecting the white matter. This demyelinating disorder results in a wide range of symptoms including motor, cognitive, and sometimes psychiatric problems. The most widely studied animal model of MS is the experimental autoimmune encephalomyelitis (EAE) murine model induced by the subcutaneous injection of myelin oligodendrocyte glycoprotein peptide in complete Freund’s adjuvant, followed by injection of pertussis toxin. This model is characterized by CNS neurodegeneration and synaptic loss, particularly in the hippocampus, with resultant spatial learning and memory deficits and motor impairment. Spatial learning and memory is usually quantified by the Morris water maze test, where mice must repeatedly find a hidden platform in clouded water, and motor impairment is quantified by swim speed (latency) and distance (path length).

In the only such study to date, a KD was given 7 days prior to EAE induction and continued until motor and cognitive disabilities could be assessed.
<table>
<thead>
<tr>
<th>Type of diet</th>
<th>Species</th>
<th>Model</th>
<th>Anti-inflammatory effect of KD</th>
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<tr>
<td>Ruskin et al., 2009</td>
<td>6.6:1 KD</td>
<td>Rats</td>
<td>subcutaneous injection of complete Freund's adjuvant into one hindpaw</td>
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<td>Made by the lab</td>
<td>– 3 weeks before Exp.</td>
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<td>Xan and Chen, 2010</td>
<td>C57BL/6J Mice</td>
<td>MPTP model</td>
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<td>– 2 weeks before Exp.</td>
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<tr>
<td>Kim do et al., 2012</td>
<td>6.3:1 KD (Bio-Serv F3666 diet)</td>
<td>C57BL/6 mice</td>
<td>Experimental autoimmune encephalomyelitis</td>
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<tr>
<td>– 7 days before Exp.</td>
<td>– S.C. myelin oligodendrocyte glycoprotein (MOG)35–55 peptide + complete Freund's adjuvant (CFA)</td>
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<td>– tendency toward increased CD4+ CD25+ Foxp3+ Treg cells</td>
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<td></td>
<td>– I.V. 20 ng of pertussis toxin</td>
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<td>– Lymph node &amp; CNS reduction in cytokines (IL-1β, IL-6, TNF-α, IL-12, IL-17) and chemokines (IFN-γ, MCP-1, MIP-1a, MIP-1b)</td>
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<td>Liver: Increase of expression of Tnfa, Il-6, Emr1, Cd68, Itgam, Nlrp3</td>
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<td>WAT: Decrease of expression of Tnfa, Il-6, Emr1, Cd68, Itgam, Nlrp3</td>
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<td>Asrih et al., 2015</td>
<td>6.3:1 KD (Bio-Serv F3666 diet)</td>
<td>C57BL/6 mice</td>
<td>Liver and White Adipose Tissue (WAT)</td>
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<td>– 4 weeks before Exp.</td>
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<td>Blood: reduce IL-1β, TNF-α</td>
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<td>Brain: reduce IL-1β mRNA</td>
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<td>Dupuis et al., 2015</td>
<td>3:1 KD (Ketocal)</td>
<td>Wistar rats</td>
<td>Fever model. 50 μg/kg of LPS (Escherichia coli 055:B5)</td>
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in comparison with a control group (Kim do et al., 2012). The KD ameliorated both motor function and cognitive impairment; spatial learning-memory function was also significantly improved, as demonstrated with the Morris water maze and measurements of hippocampal long-term potentiation. Further, using 7T-MRI imaging techniques, there was a reduction in the size of periventricular white matter lesions and restoration of hippocampal volume loss after KD treatment in the induced EAE group of mice. Importantly, this constellation of beneficial effects was associated with modulation of systemic inflammation. Specifically, the KD reduced by over twofold the number of CD4-positive T cells and CD11b/CD45-positive macrophages/microglia. The KD-fed EAE mice also showed a tendency toward increases in CD4+ CD25+ Foxp3+ Treg cells. Furthermore, levels of several cytokines (IL-1β, IL-6, TNF-α, IL-12, IL-17) and chemokines (IFN-γ, MCP-1, MIP-1a, MIP-1b) were substantially lower in the KD-treated EAE group compared with control EAE mice. This modulation of cytokine and chemokine levels was observed in both brain and blood (Kim do et al., 2012). Finally, other than this anti-inflammatory modulation of lymphocyte proliferation and cytokine/chemokine expression, the KD decreased overall oxidative stress in the brain (Kim do et al., 2012).

**Parkinson’s Disease**

Parkinson’s disease (PD) is a progressive neurodegenerative disorder characterized by abnormal shaking, rigidity, and slowness. The symptoms arise from the death of dopaminergic neurons in the substantia nigra (SN). Despite abundant clinical information about PD, the mechanisms of neurodegeneration in the SN remain unclear. Nevertheless, there is some evidence for the role on inflammation in PD, since activated microglia have been implicated in the pathogenesis and progression of PD (Hirsch and Hunot, 2009; Hirsch et al., 2012).

The murine MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) model of PD recapitulates the motor dysfunction and neurochemical changes observed in humans diagnosed with this disorder. As with PD patients, MPTP-treated mice are characterized by selective progressive loss of dopaminergic neurons in the SN and their projections to the striatum. Ketogenic diet pretreatment has been shown to provide neuroprotective and anti-inflammatory effects and to alleviate the motor dysfunction induced by MPTP (Yang and Cheng, 2010).

In addition to the improvement in motor function, the KD was found to inhibit degeneration of tyrosine hydroxylase (TH)-positive neurons and preserved dopamine levels in the SN (Yang and Cheng, 2010). This effect was associated with a reduction in activated microglia as shown by iba-1 immunostaining. Moreover, the KD decreased pro-inflammatory cytokine levels (specifically, IL-1β, IL-6, and TNF-α) in the SN of MPTP-treated animals (Yang and Cheng, 2010).

Most of the experimental data thus far has shown a correlation between amelioration of disease symptomatology by the KD and decreased inflammation (mostly with respect to pro-inflammatory cytokine levels). However, whether the beneficial effects of a KD in models of PD are a direct consequence of decreased inflammation has yet to be firmly demonstrated.

**POSSIBLE MECHANISMS SUPPORTING ANTI-INFLAMMATORY PROPERTIES OF THE KETOGENIC DIET**

Although the clinical efficacy of the KD against forms of pharmacoresistant epilepsy is well established, the underlying mechanisms are incompletely understood. There have been a number of intriguing hypotheses advanced to explain KD action (Lutas and Yellen, 2013; Masino and Rho, 2010). However, it is unlikely that a single mechanism can explain all of the clinical effects of the KD. In the context of the present chapter, there are also probably multiple mechanisms that are likely involved in the anti-inflammatory effect of KD (Figure 17.1).

**Ketone Bodies**

The ketone bodies beta-hydroxybutyrate (BHB) and acetoacetate (AcAc) are the products of fatty acid oxidation in hepatic mitochondria. Recently, several lines of research support a direct role for BHB as an anti-inflammatory mediator (Rahman et al., 2014; Youm et al., 2015). Beta-hydroxybutyrate has been shown to directly inhibit the NLRP3 (NOD-, LRR-, and pyrin domain-containing 3) inflammasome assembly, which is responsible for caspase-1 activation and the release of the pro-inflammatory cytokines IL-1β and IL-18. The NLRP3 functions primarily in cells of the myeloid lineage. In mouse bone-marrow-derived macrophages (BMDMs) and human monocytes, BHB inhibited ATP-induced cleavage of caspase-1 and the processing of the active form of IL-1β in LPS-primed cells (Youm et al., 2015). This effect was independent of fasting-regulated mechanisms.
such as reactive oxygen species (ROS) production and inhibition of glycolysis and autophagy, both of which are known to regulate the NLRP3 inflammasome. The inhibitory effect of BHB appears specific to NLRP3 because BHB does not inhibit caspase-1 activation in response to pathogens activating other inflammasomes—namely, NLRC4 (NLR family, CARD domain containing 4) and AIM2 (absent in melanoma 2) (Youm et al., 2015). Additionally, BHB was shown to inhibit caspase-1 activation and IL-1β secretion in mouse models of Muckle-Wells syndrome, a condition associated with a mutation in NLRP3. Finally, the KD and the associated elevation in BHB levels protected mice bearing the missense Nlrp3 mutation that leads to familial cold auto-inflammatory syndrome (Youm et al., 2015).

Beta-hydroxybutyrate has also been shown to be a potent modulator of the HCA2 (hydroxyl-carboxylic acid receptor 2) receptor, and through this target exerts neuroprotective effects in a rodent model of stroke (Rahman et al., 2014). The HCA2 activation by BHB induces Ly-6Clo monocytes and/or macrophages to deliver a neuroprotective signal to the brain (Rahman et al., 2014).

**Caloric Restriction**

The KD is frequently associated with a reduced calorie intake. More recently, it has been shown that KD promotes weight loss through increased energy expenditure and reduced food intake (Asrih et al., 2015). Caloric restriction (CR) is a pragmatic strategy to handle excess energy, stored in adipose tissues, that might contribute to systemic inflammation (Ye and Keller, 2010). Caloric restriction can increase anti-inflammatory protein gene expression of mediators such as NFκB inhibitor alpha (Nfkbia), tissue inhibitor of metalloproteinases-3 (Timp3), and peroxisome proliferator-activated receptors (PPARs) (Sung et al., 2004; Swindell, 2009), and conversely inhibit pro-inflammatory genes such as TNF-α, IL-6, COX-2, iNOS, VCAM-1, and ICAM-1 (Higami et al., 2006; Jung et al., 2009).

Despite these data, evidence for the potential anti-inflammatory mechanisms of CR in humans is more limited, and most of the studies addressing this have been developed in obese patients. In this particular context, CR appears to reduce pro-inflammatory molecules (Lee et al., 2010; Salas-Salvado et al., 2006), but these data reflect more the correction of an abnormal state to the non-pathologic state than the changes induced by CR under healthy conditions.

Overall, the underlying mechanisms explaining the anti-inflammatory effects of CR are not well understood. However, since many nutrients and growth factors are able to activate
mammalian target of rapamycin (mTOR), the anti-inflammatory effect of CR might be in part the result of stimulating this pathway. Laboratory studies have already demonstrated that the KD inhibits mTOR signaling in the brain and liver of normal rats (McDaniel et al., 2011). This inhibition is thought to be related to a decrease in Akt signaling in the brain and the liver. McDaniel and colleagues also showed that the KD prevented mTOR hyperactivation after kainate-induced status epilepticus (SE) (McDaniel et al., 2011). However, this study did not correlate the modulation of the mTOR by the KD with any SE-induced pro-inflammatory changes.

**Polyunsaturated Fatty Acids**

Polyunsaturated fatty acids (PUFAs) are dietary lipids that contain more than one double bond. There are two groups of PUFAs: the omega-3 (n-3) and the omega-6 (n-6) PUFAs. This nomenclature refers to the position of the double bond relative to the methyl terminal of the molecule (Taha et al., 2010). Diet is an important source of PUFAs. Dietary n-3 PUFAs are found in flaxseed, in some nuts, in marine fish, and in marine mammals, such as seals. In contrast, n-6 PUFAs are found in a variety of animal products and in vegetable oils, such as canola and corn oil, and make up the majority of PUFAs in the modern Western diet (Taha et al., 2010).

With normal dietary intake, n-3 PUFAs can decrease the production of inflammatory eicosanoids, cytokines, and ROS and the expression of adhesion molecules. Toward these ends, n-3 PUFAs act both directly (by replacing arachidonic acid as an eicosanoid substrate and inhibiting arachidonic acid metabolism) and indirectly (by altering the expression of inflammatory genes through effects on transcription factor activation). The n-3 PUFAs, particularly eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), also have anti-inflammatory actions, mediated primarily by their hydroxylated metabolites, which include resolvins and docosanoids (Hong et al., 2003). For instance, EPA and DHA have been reported to incorporate into human neutrophil cells and to decrease the production of the pro-inflammatory prostaglandin E2 (PGE2) in a dose-dependent manner (Rees et al., 2006).

Ketogenic diet treatment not only increases fatty acid levels in the blood but also increases levels of specific PUFAs that can bind to and activate peroxisome proliferator-activated receptors (PPARs). The PPARs have been considered as potential drug targets for seizure control (Sampath and Ntambi, 2005). Both PPARα and PPARγ are activated by PUFAs and their eicosanoid derivates, as well as by synthetic ligands such as the fibrates (Kersten et al., 2000). Activation of PPARs stimulates the transcription of the genes involved in fatty acid oxidation via the formation of heterodimeric transcription-factor complexes with the retinoid-X-receptor (RXR) (Smith, 2002). Three types of PPARs have been identified—alpha, beta, and gamma: PPARα is expressed mainly in liver, kidney, and heart muscle; PPARβ is expressed in a number of tissues, such as brain, adipose tissue, and skin; PPARγ is also expressed in the brain. Both ALA and LA have a greater binding affinity for PPARα than EPA or DHA (Lin et al., 1999).

Synthetic PPAR agonists reduce experimentally induced inflammation (Cuzzocrea et al., 2003; Cuzzocrea et al., 2004; Lo Verme et al., 2005). This effect is the result of the inhibition of pro-inflammatory pathways involving nuclear factor κ B, signal transduction and transcription-1, and nuclear factor of activated T-cells (Blanquart et al., 2003).

**Adenosine Modulation**

The KD has been shown to modulate adenosine levels. Astrocytic adenosine kinase (ADK) is an enzyme responsible for adenosine phosphorylation and clearance of adenosine from the extracellular space. Expression of ADK is reduced by the KD, thereby increasing extracellular levels of adenosine and the activation of inhibitory adenosine A1 receptor (A1R) (Masino et al., 2011). Importantly, the effect of the KD on adenosine has been linked to a decrease in electrographic seizure activity (Masino et al., 2011). The anti-inflammatory effects of the KD may be explained in part by adenosine, as this metabolic mediator has long been known to induce anti-inflammatory activity (Kowaluk et al., 1998; Linden, 2001). Along these lines, adenosine has been reported to reduce central and peripheral inflammation. Using the A1R(-/-) genetically engineered mice and a pretreatment with the specific A1R agonist (2’Me-2-chloro-N6-cyclopentyladenosine) in wild-type mice, it was shown that A1R modulates LPS-induced transmigration of polymorphonuclear cells and reduced levels of TNF-α, IL-6, and CXCL2/3 in the lung (Ngamsri et al., 2010). There are also data suggesting that A1Rs are up-regulated in acute forms of neuroinflammation and down-regulated in chronic forms. Activation of cerebral A1R act as a brake for the microglial response after traumatic brain injury (Haselkorn et al., 2010).
Both A1ARs and A2ARs appear to be involved in the inflammatory response, and pharmacological interventions might mitigate this (Lukashev et al., 2005; Sitkovsky and Ohta, 2005). However, A1ARs as well as pharmacological intervention targeting A2ARs might have bidirectional effects on neuroinflammation. Specifically, the local glutamate level following brain injury is one of the crucial factors that determines the direction of an A1AR-mediated effect on neuroinflammation (Dai and Zhou, 2011).

**Reactive Oxygen Species production, Uncoupling Proteins, Mitochondrial Membrane Potential**

Both BHB and AcAc have been shown to induce neuroprotective effects. These effects have been linked to reduction of ROS through enhanced NADH oxidation and inhibition of mitochondrial permeability transition (Kim do et al., 2007; Kim do et al., 2015; Maalouf et al., 2007). Furthermore, the KD has been shown to enhance mitochondrial biogenesis (Bough et al., 2006). The ROS and inflammation are tightly linked through a strong reciprocal relationship; ROS generated by inflammatory cells act as inflammatory signaling molecules and participate in the inflammatory response. Furthermore, ROS play a key role in the control of nuclear factor kappa B (NF-kappaB), activator protein-1 (AP-1), and other transcription factors involved in gene expression of both inflammatory and immune mediators. When sustained, oxidative stress can lead to a chronic inflammatory state and inflammation can produce aggravate ROS production (Harijith et al., 2014).

The KD can reduce both ROS production and inflammation. Recently, it has been shown that ROS can activate the NLRP3 inflammasome protein, a molecular platform activated on signs of cellular “danger” to trigger innate immune defenses through the maturation of pro-inflammatory cytokines such as IL-1β and IL-18 (Zhou et al., 2011). Moreover, oxidative stress can act as a central regulator of HMGB1’s translocation, release, and activity in inflammation and cell death. Thus, modulation of HMGB1 can limit inflammation and reduce tissue damage during infection as well as sterile inflammation (Yu et al., 2015). Ketone bodies now appear to have the ability to target the inflammatory pathways in multiple ways: (1) direct action on NLRP3 as described previously (Youm et al., 2015), and (2) decrease in ROS production and levels (Kim do et al., 2007; Kim do et al., 2015; Maalouf et al., 2007).

**CLINICAL EVIDENCE AND FUTURE INDICATIONS**

There is currently no clinical open label study or controlled trial that demonstrates that the effects of the KD are through an anti-inflammatory mechanism. Notwithstanding this lack of data, it is intriguing to note once again that the KD is an effective treatment for a condition associated with both inflammation and medically refractory seizures—namely, FIRES (Nabbout et al., 2010; Singh et al., 2014). At present, whether the KD would indeed be useful for diverse conditions associated with neuroinflammation—for example, pain, multiple sclerosis, and Parkinson’s disease, among others—remains unclear, but the current evidence pointing to broad anti-inflammatory effects of the KD is compelling, as this metabolism-based treatment could constitute a readily available therapy for neurological disorders associated with inflammation. Detailed preclinical studies in relevant animal models of neurological disease are no doubt of paramount importance to uncover the underlying biology of KD action. Ultimately though, well-designed clinical trials must be implemented to validate readily available therapeutic approaches such as the KD.

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INTRODUCTION
The ketogenic diet (KD) has established efficacy for epilepsy and is best known for this role (Neal et al., 2008; Winesett et al., 2015). Since the 1920s, the KD has been used for treatment of intractable epilepsy, often as a last resort. The mechanism of the KD is complex and not fully elucidated, but likely involves metabolic adaptations in a variety of cellular signaling pathways leading to decreased neuronal excitability and neuroprotection. As such, it is possible that the KD can be used therapeutically in other neurological diseases (Barañano and Hartman, 2008; Paoli et al., 2014; Stafstrom and Rho, 2012; Gano et al., 2014), and small preliminary trials have been performed in brain cancer, Alzheimer disease, autism spectrum disorder, multiple sclerosis, and pain, as discussed elsewhere in this volume.

Here, I review the use of the KD in amyotrophic lateral sclerosis (ALS), Parkinson disease, mood disorders, and migraine. These diverse disorders do not share a pathophysiological basis that is immediately obvious. Amyotrophic lateral sclerosis is a motor neuron disease involving degeneration of alpha motor neurons. Parkinson disease entails excitotoxic degeneration of dopaminergic neurons of the substantia nigra leading to abnormal motor function and cognition. Bipolar disorder and other mood disorders are related to dysfunction of neurochemical balance in the brain, especially monoamines. Migraine is a paroxysmal headache disorder involving abnormal sensitivity of blood vessels and neurons, especially involving the trigeminal system.

Despite this phenotypic and mechanistic heterogeneity, each disorder involves abnormalities in cellular energy utilization, implying that a beneficial effect might be possible by manipulating nutrients and metabolic substrates. Specifically, the role of altered energy metabolism points to altered mitochondrial function as a common factor in pathogenesis (Kunz, 2002). Similar therapeutic efficacy might be achieved through alternative forms of the KD, including the medium chain triglyceride (MCT) diet, modified Atkins diet, low glycemic index treatment, or calorie restriction; these therapies are mentioned only in passing in this chapter, as their role in nonepileptic disorders has barely been explored to date.

THE NEUROPROTECTIVE ROLE OF THE KETOGENIC DIET
Over the past 15 years or so, investigators have identified numerous mechanisms by which the KD diet might provide neuroprotection (Hartman, 2012; Rho and Stafstrom, 2012; Lutas and Yellen, 2013; Danial et al., 2013). This complex pathophysiology is covered in other chapters of this volume. Nevertheless, a brief discussion of pathophysiological mechanisms is warranted here, as a basis for explaining why diverse neurologic disorders could be amenable to KD treatment.

Two hallmark features of KD treatment are the increase in ketone body production by the liver and a reduction in blood glucose levels. Ketone elevation is largely a consequence of fatty acid oxidation. In the setting of high fat and low carbohydrate intake, as in the KD, fatty acids are oxidized in the liver to produce ketone bodies: β-hydroxybutyrate and acetoacetate. These ketones then occasion metabolic adaptations that produce an antiseizure effect. Ketone bodies, as well as specific polyunsaturated fatty acids (PUFAs) such as arachidonic acid, docosahexaenoic acid, and eicosapentaenoic acid might themselves regulate neuronal membrane excitability by (1) directly blocking voltage-gated sodium and calcium
channels (Vreugdenhil et al., 1996); (2) altering the synthesis, degradation, or reuptake of excitatory or inhibitory neurotransmitters (Yudkoff et al., 2001; Juge et al., 2010); or (3) reducing inflammation through activation of peroxisome proliferator-activated receptors (Hajjar et al., 2012).

How could altered neuronal excitability and dysregulated cellular energy metabolism be linked? One way is via ATP-sensitive potassium (K\(_{\text{ATP}}\)) channels. β-hydroxybutyrate increases the probability that K\(_{\text{ATP}}\) channels open, which would decrease cellular excitability and reduce firing rate and seizure occurrence (Ma et al., 2007). Another route is adenosine, which is produced from ATP and causes seizure suppression through activation of inhibitory adenosine A1 receptors. The KD increases ATP and thus adenosine, producing an inhibitory effect on cellular excitability (Masino et al., 2009). Ketone bodies have also been shown to facilitate neuroprotection by raising ATP levels and reducing ROS production (Kim et al., 2007) through enhanced NADH oxidation and inhibition of mitochondrial permeability transition (Kim et al., 2015). Along similar lines of improved bioenergetics, the KD has been shown to stimulate mitochondrial biogenesis, resulting in stabilized synaptic function (Bough et al., 2006). Therefore, altered mitochondrial biogenesis or function represents a mechanistically appropriate target for the KD in both epilepsy and nonepileptiform neurologic disorders (Milder and Patel, 2012; Pani, 2015).

The second major biochemical feature of the KD is decreased glycolytic flux. Reduction of glycolysis is an essential feature of calorie restriction, which has been shown to suppress seizures as well as prolong the lifespan of numerous species, including primates (Pani, 2015). While the link between calorie restriction and KD effectiveness remains controversial, it is clear that both treatments result in reduction of blood glucose, likely involving reduced glycolytic flux. In this regard, 2-deoxy-D-glucose (2-DG), an analog of glucose that blocks phosphoglucone isomerase and hence inhibits glycolysis, has been shown to block epileptogenesis in the rat kindling model by decreasing expression of brain-derived neurotrophic factor and its principal receptor, tyrosine kinase B (Garriga-Canut et al., 2006). 2-DG is also effective in several acute seizure models (Stafstrom et al., 2009). Similarly, fructose-1,6-diphosphate reduces glycolysis by diverting glucose to the pentose phosphate pathway and is neuroprotective in multiple seizure models (Lian et al., 2007; Ding et al., 2013).

Other important mechanisms also contribute to the neuroprotective consequences of calorie restriction, including improved mitochondrial function and decreased oxidative stress (similar to that seen with ketones and PUFAs), decreased activity of pro-apoptotic factors, and inhibition of inflammatory mediators such as interleukins and tumor necrosis factor alpha (Maalouf et al., 2009). Restoring exhausted metabolic substrates may constitute another novel treatment approach (Kovac et al., 2013). In that regard, a biochemical process called anaplerosis aims to replenish tricarboxylic acid cycle intermediates that are depleted during the intense neuronal firing that constitutes seizure activity (Willis et al., 2010).

In the end, numerous mechanisms are likely to contribute to the neuroprotective properties of the KD. While several of these mechanisms are thought to relate principally to the antiseizure activity of the KD, the KD also likely contributes to cellular homeostasis and the prevention of neuronal dysfunction and injury. An important caveat, however, is that yet unidentified mechanisms operate in disorders outside of epilepsy, presenting further opportunities for examining the pleiotropic effects of metabolism-based therapies at a mechanistic level. Furthermore, an important consideration applicable to all neurodegenerative diseases is the timing of intervention that is crucial for a neuroprotective effect to be enabled by KD treatment. Neurological disorders in late stages of progression may have such extreme neuronal dysfunction and death that neuroprotective therapies may no longer work. All of these potential mechanisms are discussed in greater detail in other chapters of this volume.

**Examples of Neurologic Disorders Potentially Amenable to the Ketogenic Diet**

Several neurologic diseases might benefit from dietary therapy, including amyotrophic lateral sclerosis, Parkinson disease, mood disorders, and migraine. These disorders have diverse underlying pathophysiologies, yet each entails some contribution of cellular energy dysfunction.

**Amyotrophic Lateral Sclerosis**

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive disease due to degeneration of motor neurons of the cortex and anterior horn of the spinal cord. As a consequence, voluntary motor activity gradually deteriorates, leaving the affected individual profoundly weak despite largely retained
cognitive function. The essential pathophysiological mechanisms that underlie this relentless disorder are yet to be fully elucidated, and likely involve oxidative damage, glutamate excitotoxicity, inflammation, and mitochondrial membrane dysfunction (Vucic et al., 2014). Similar to other neurodegenerative disorders, energy-producing systems likely play a role and mitochondrial dysfunction probably contributes to disease pathogenesis (Martin, 2011). In this regard, the KD may be a promising adjunctive treatment for ALS (Siva, 2006).

Amyotrophic lateral sclerosis is hereditary in an estimated 10% of cases. Approximately 20% of patients with familial ALS harbor a mutation in the gene encoding copper/zinc superoxide dismutase (SOD1). A transgenic mouse model of ALS has been created in which the gene SOD1-G93A is overexpressed, leading to progressive muscle weakness and death resulting from respiratory failure as in the human disease. Administration of the KD to these mutant mice led to both histological (higher motor neuron counts) and functional (preserved motor function on the rotarod test) improvements compared with non-KD fed SOD1-G93A transgenic animals (Zhao et al., 2006). However, the KD did not extend survival time compared with non-KD fed control mice. Although providing proof-of-principle for using the KD in ALS, this study is limited by a number of factors including very small group sizes and initiation of motor testing before the onset of ALS-type symptoms.

Mitochondria isolated from brains of SOD1-G93A mutant mice had increased levels of ATP and increased rates of ATP synthesis, as well as decreased function of complex I of the electron transport chain. Administration of the β-hydroxybutyrate restored complex I activity in neurons in which complex I function was blocked pharmacologically (Zhao et al., 2006), a result also seen in mitochondria isolated from brains of a Parkinson’s disease animal model (Tieu et al., 2003). An alternative approach involves caprylic triglyceride, a medium-chain triglyceride with antiseizure effects that is metabolized to ketone bodies (Właž et al., 2012). Caprylic triglyceride, when fed to SOD1-G93A transgenic mice, reduced motor neuron loss and preserved motor performance on the rotarod and other tests (Zhao et al., 2012). Finally, a recent study examined SOD1-G93A mutant mice fed a KD variant called the Deanna Protocol (DP, which provides alternative fuels in the form of tricarboxylic acid cycle intermediates such as arginine-α-ketoglutarate), or both the standard KD and the DP together (Ari et al., 2014). Both the KD and DP diets were associated with improved motor scores compared with control-fed mutant mice, and the KD and KD+DP diets prolonged life expectancy. These results raise the possibility that the KD or similar metabolic therapies, by raising ketone levels, might benefit patients with ALS. In a broader sense, the role of calories or simply increasing dietary fat in ALS patients remains a subject of intense interest, since dietary approaches are a rather straightforward way to potentially enhance the quality and duration of life in this devastating disease (Paganoni and Wills, 2013).

Parkinson Disease

The primary pathophysiology in Parkinson disease (PD) is excitotoxic degeneration of dopaminergic neurons in the substantia nigra pars compacta, leading to abnormalities of movement, cognition, and other cortical functions. Seizures are not common in PD (Szot, 2012). Therefore, how could the KD benefit patients with PD? A primary pathogenic mechanism of cell death in PD is thought to be a defect in complex I of the electron transport chain. The KD might bypass this defect and allow oxidative phosphorylation to proceed. In a small clinical study, five of seven PD patients treated with the KD showed improved scores on a standard PD rating scale (Vanitallie et al., 2005). Sample sizes were small, however, and a placebo effect could not be fully excluded. There is also evidence that calorie restriction alone helps patients with PD (Srivastava and Haigis, 2011), and this finding was verified in a primate model (Maswood et al., 2004); these observations support a critical role of energy dysregulation in this disorder.

An animal model of PD is produced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration; this toxin causes death of substantia nigra neurons by inhibiting mitochondrial NADH dehydrogenase (Tieu et al., 2003). β-hydroxybutyrate, elevated during KD treatment, ameliorated MPTP-induced mitochondrial respiratory chain damage in this model by bypassing the defect in complex I activity. Additional support for the potential benefits of ketone bodies in PD comes from in vitro experiments. In substantia nigra neuron precursors, ketone bodies protect against mitochondrial respiratory chain dysfunction induced exogenously by complex I and II inhibitors and provide anti-inflammatory action on MPTP-induced neurotoxicity (Kashiwaya et al., 2000; Yang and Cheng, 2010). As in ALS, the KD effect in PD appears to require intact complex I function (Tieu et al., 2003). In another model of
PD induced by 6-hydroxydopamine neurotoxicity in rats, the KD protected nigral dopaminergic neurons from degeneration (Cheng et al., 2009).

**Mood Disorders**

Beneficial effects of the KD have been hypothesized in mood disorders, based on the observation that other antiseizure agents also stabilize mood (e.g., valproate, carbamazepine, lamotrigine) (El-Mallakh and Paskitti, 2001; Bialer, 2012). In addition, the KD alters levels of metabolites of biogenic amines such as dopamine and serotonin, neurotransmitters critical to the pathophysiology of depression (Murphy et al., 2004; Dahlin et al., 2012; Szot, 2012). The KD increased levels of endogenous norepinephrine levels in rats, which had an antiseizure effect (Weinshenker, 2008).

With respect to mood disorders, only anecdotal clinical studies have been conducted thus far. One brief KD trial on a single patient with depression was reportedly ineffective, although urinary ketosis was not achieved (Yaroslavsky et al., 2002). Two women with bipolar disorder type II were treated with a KD for 2–3 years; both subjects tolerated the diet and demonstrated improved mood (Phelps et al., 2013). Obviously, large-scale studies are needed.

Depression in rats is studied using the forced swim test, in which an animal is placed into a water-filled chamber with smooth walls, from which it cannot escape. After an initial phase of frantic swimming, a rat becomes immobile, simply floating to prevent drowning. Depressed animals have a shorter latency to the immobile phase, regarded as a measure of despair. The role of the KD in depression has been studied using this model. Compared with control-fed rats, those fed the KD spent significantly less time in the immobility phase of the test, reflecting an overall reduction in “behavioral despair” (Murphy et al., 2004). In recent experiments, mice were exposed to the KD prenatally (by feeding dams the KD) and then their behavior was tested in adulthood (Sussman et al., 2015). All rats received the standard (non-KD) diet after birth. Adult KD offspring exhibited reduced susceptibility to anxiety and depression and were physically more active than control mice. The authors concluded that prenatal exposure to a KD was associated with decreased stress responses in adulthood. At present, it is not clear whether there are any practical or translational implications of these results.

**Migraine**

Epilepsy and migraine involve paroxysmal excitability changes in the brain, and many of the same pharmacological agents are used to treat both conditions (e.g., topiramate, valproate, lamotrigine) (Bialer, 2012). Migraine and epilepsy share considerable phenotypic overlap (Rogawski, 2008), although the intrinsic mechanisms underlying seizures and migraine attacks differ in some fundamental respects. There are theoretical reasons to consider the KD for chronic migraine. Migraine involves a complex interplay between genes and environment, and some of the causative genes code for proteins involved in energy metabolism (e.g., ATP1A2 in one form of familial hemiplegic migraine; Gritz and Radcliffe, 2013). Brain function is highly dependent on normal mitochondrial function to produce sufficient energy via oxidative phosphorylation, so disrupted mitochondrial function has been hypothesized as a causative factor in migraine (Roos-Araujo et al., 2014).

It might seem unlikely that an individual with migraine would undertake such a complicated dietary regimen as the KD, but in light of suboptimal alternatives, the KD is at least worth considering in some cases, particularly in persons with headaches that are refractory to conventional agents (Strahlman, 2006; Maggioni et al., 2011). Interestingly, the first report using the KD for migraine appeared in 1928, only a few years after the diet’s first use for epilepsy (Schnabel, 1928). Nine of 28 migraine patients reported “some improvement,” although the validity of this clinical study is uncertain and some patients admitted to poor compliance. In patients with epilepsy, better compliance is often achieved using the less restrictive modified Atkins diet (MAD) (Kossoff and Dorward, 2008). A trial of the MAD was attempted in adolescents with chronic daily headache (Kossoff et al., 2010). Of eight enrolled patients, only three patients were able to complete the 3-month trial. Although there was some improvement in reported quality of life in those three patients, all of them continued to experience chronic daily headaches. These findings suggest that even the MAD is too restrictive for long-term use in adolescents with headache and any therapeutic benefit of ketosis in treating headache is quite modest.

One large prospective observational study of the KD in migraine patients has been reported (DiLorenzo et al., 2015). Ninety-six obese female patients with migraine self-referred to a dietitian for weight control. Patients were randomized to a KD or standard diet for 1 month. The KD group experienced a decrease in mean baseline headaches from 2.9 per month to 0.71 per month; the total number of headache days was also fewer in
the KD group (0.91 headache days for the KD group vs. 5.1 days in controls). When the KD was stopped and the patients returned to a normal diet, the higher migraine headache frequency resumed. A case report of a pair of obese twin sisters with migraines reported an improvement in headache control on a KD, and decreased migraine attacks correlated with the level of ketosis (Di Lorenzo et al., 2013).

Cortical spreading depression (CSD), a wave of depolarization that spreads slowly along the cortical surface (~3 mm/min) and is thought to correlate with migraine aura (Ferrari et al., 2015), is associated with neurogenic inflammation, forming a target for current antimigraine drugs (Cui et al., 2014). Laboratory studies have investigated the effect of the KD on CSD (de Almeida Rabello Oliveira et al., 2008). It was found that short-term treatment (10 days) with either the MCT or long-chain triglyceride forms of the KD resulted in significant reduction in CSD velocity in rats, but long-term treatment (7 weeks) with either diet had no effect on CSD. Although the clinical implications of these results are uncertain, it is clear that the KD reduces cortical excitability in a time-dependent fashion. It is unknown whether the KD will play a role in headache treatment in the future, but the diet may provide some clues as to migraine pathophysiology. For example, mitochondrial dysfunction, leading to decreased ATP production, has been linked to the occurrence of CSD (Sparaco et al., 2006).

CONCLUSIONS

This chapter has considered several neurologic disorders that might be amenable to metabolic treatment with therapies such as the KD and its variants. Despite the relative lack of clinical data supporting the use of KD in these disorders, preliminary studies support the idea that KD-induced metabolic shifts may lead to neuroprotection. How can a simple dietary change lead to improvement in a set of disorders with such a huge span of pathophysiological mechanisms? A common theme appears to be alteration in energy metabolism. So while the mechanisms through which the KD exerts such effects are certainly diverse, there may be a finite set of final common pathways that are shared mechanistically. Ultimately, the details of how such metabolic factors reduce excitability, diminish ongoing neurodegeneration, or mitigate functional disability remain unknown. Herein lie rich opportunities for further investigation in both the laboratory and the clinic.

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Chapter 18: Dietary Therapy for Neurological Disorders


SECTION III

Ketogenic Diet in the Laboratory

DETLEV BOISON, PHD, SECTION EDITOR
Overview of Ketogenic Diet in the Laboratory

Progress on Models and Mechanisms

DETLEV BOISON, PHD

Increased clinical interest in the ketogenic diet and similar metabolic treatments has naturally spurred research into mechanisms underlying their anticonvulsant/antiepileptogenic efficacy; significant progress and new insights are being made after many decades of relative obscurity. This section, “Ketogenic Diet in the Laboratory,” includes discussions of a number of molecular targets that are affected by ketogenic diet feeding. In parallel, several alternative experimental methods to either elucidate or mobilize ketogenic diet mechanisms are explored. Based on this laboratory research, putative and novel uses for metabolic treatments are proposed.

The first published reports in the medical literature of the effect of a ketogenic diet (KD) on epilepsy are two one-page reports by R.M. Wilder in the daily Mayo Clinic Bulletin. The first makes a brief theoretical case for use of a high-fat, low-carbohydrate diet based on prior successful work with fasting in epilepsy (Wilder, 1921a). The second (published the very next day) consists of case descriptions of three patients who became seizure-free after KD treatment (Wilder, 1921b). Interestingly, given that the KD became best known as a treatment for pediatric epilepsy, two of these three patients were adults (aged 23 and 31). Naturally, from the beginning there were questions about the anticonvulsant mechanism of the KD. Wilder’s own speculation was that elevated ketone bodies were crucial, given that this metabolic response is in common between fasting and eating a high-fat, low-carbohydrate diet (Wilder, 1921a). This is still seen as the key mechanism by many investigators, though not without dispute. Other early postulated mechanisms such as dehydration and acidosis have fallen by the wayside. In fact, these latter two are seen now as unnecessary and treatable side effects. One main point of this section, “Ketogenic Diet in the Laboratory,” is to discuss recent mechanistic studies.

Early in vivo work in rodents showed that KD feeding was effective against some but not all types of experimentally induced seizures (Appleton and DeVivo, 1974; Millichap et al., 1964; Uhlemann and Neims, 1972). Later mechanistic work in animals turned to in vitro preparations (e.g., Stafstrom et al., 1999; Thio et al., 2000). Here, Dr. Kawamura’s chapter reviews in vitro methodologies for modeling the KD. These involve direct application of ketone bodies, modulation of glucose and intracellular ATP, and maintaining the metabolic milieu of KD-fed animals in acute brain slices.

Several chapters address anticonvulsant or antiepileptogenic molecular targets for the KD. Dr. Simeone reviews the literature on peroxisome proliferator-activated receptor γ (PPARγ), a fatty acid receptor that is expressed in many brain neurons. He describes the regulation of this nutrient sensor by seizure activity and by KD feeding, and that pharmacological or genetic inactivation of PPARγ blocks the anticonvulsant effect of KD feeding. Drs. Sada and Inoue review the functions of the astrocyte/neuron lactate shuttle, and then discuss their work in brain slices, in which it was shown that lactate reverses the inhibition caused by ketone bodies. Conversely, ketogenic diet feeding lowered hippocampal lactate levels. Drs. Lusardi and Boison describe the epigenetics of epilepsy, review the augmentation of the neuromodulator adenosine by KD treatment, and outline the dual functions of adenosine: anticonvulsant effects via modulating synaptic activity and antiepileptogenic effects via modulating DNA methylation.

Several chapters address mechanistic effects of KD treatment in pathophysologies other than epilepsy. Drs. Curtis, Kemper, et al. review how reactive oxygen and nitrogen species are involved in aging and radiation damage. They then discuss
how ketone body-based metabolism generates NADPH, thereby quenching these species through reactions driven by the [NADP+] /[NADPH] couple and also by increasing transcription of certain antioxidant enzymes. Also in the context of aging, Drs. Xu, LaManna, and Puchowicz describe the beneficial effects of KD treatment on neurodegenerative processes. They propose that these effects arise from ketosis-induced stabilization of hypoxia-inducible factor 1α, a metabolic sensor and transcription factor, and its target genes. Drs. Veech and King review the literature on the pathophysiology of Alzheimer’s disease and present evidence that ketosis-inducing treatments could be beneficial in circumventing the inhibition of pyruvate dehydrogenase found in that disease.

Additional chapters address other physiological effects of the KD. Drs. Banjara and Janigro review the biology of the blood-brain barrier including the process of ketone body uptake. The regulation of glucose and ketone body transporters in the barrier and the suggestion of barrier repair by KD treatment are discussed. Drs. Harney, Gudsnuk, et al. describe delays in puberty and effects on reproduction in female rats fed a strict KD. Effects of KD feeding on spatial reference learning in dams and their pups are also presented.

Three chapters deal with novel uses of KDs that are mostly (or completely) still confined to the laboratory. Such is the case with Drs. Veech and King and Drs. Curtis, Kemper, et al. proposing KDs for treating Alzheimer’s disease and ionizing radiation damage, respectively, both discussed previously. In addition, Dr. Ruskin reviews the evidence for KD treatment being able to alleviate pain and inflammation.

Finally, among these chapters in this section many also address treatment strategies and methodologies to invoke the mechanisms of the KD with nondietary treatments. Drs. Martinez-François, Danial, and Yellen review the functions of BCL-2-associated agonist of cell death (BAD), a protein that regulates glucose metabolism. They describe how genetic manipulation of BAD to reduce glucose metabolism results in mice resistant to seizures, an effect involving ATP-sensitive potassium channels. Drs. Sada and Inoue propose lactate dehydrogenase blockers to reduce activity of the astrocyte/neuron lactate shuttle; Dr. Simeone proposes agonists of PPARγ; Drs. Lusardi and Boison propose blockers of adenosine kinase to boost adenosine and decrease DNA methylation; and Drs. Veech and King and Drs. Curtis, Kemper, et al. propose administration of ketone esters, which are rapidly metabolized to ketone bodies.

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INTRODUCTION

Historical Overview
A switch from a normal Western diet of low-fat and high-carbohydrate/protein consumption to a high-fat and low-carbohydrate/protein ketogenic diet (KD) necessitates an adjustment in the metabolic machinery to handle new primary fuel sources. As detailed elsewhere in this volume, there are three prominent biochemical consequences of a KD: decreased glucose, increased free fatty acids, and increased ketone bodies (i.e., beta-hydroxybutyrate, acetoacetate, and acetone). The lower glucose and higher free fatty acids, of course, directly result from the formulation of the diet. The ketone bodies are a partial breakdown product from using fatty acids for fuel in a low-glucose environment, and themselves can be used as an efficient, deliverable fuel source. Ketone bodies skip glycolysis and enter the tricarboxylic acid cycle at the level of acetyl-CoA.

In order for fatty acids to be used for fuel and for ketogenesis to occur, a series of enzymes and proteins must be present and have increased expression. These include, but are not limited to, beta-oxidation enzymes such as long chain acetyl-CoA synthetase I (ACS1); carnitine palmitoyltransferase (CPT), which commits palmitate to mitochondrial entry; and the ketogenic enzyme mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCS2). For animals that are on a ketogenic diet, this mainly occurs in the periphery in hepatocytes of the liver, but there is evidence that astrocytes are capable of local beta-oxidation and ketogenesis (Auestad et al., 1991; Cullingford et al., 2002a; Cullingford et al., 2002b; Guzmán and Blázquez, 2004; Takahashi et al., 2014).

There are two immediate implications from these findings. One is that dramatic dietary changes in fuel sources somehow coordinate the expression of large numbers of genes. The second implication is that there is(are) nutrient sensor(s) that regulate this shift in gene expression. In hepatocytes, fatty acids are endogenous ligands for peroxisome proliferator activated receptor alpha (PPARalpha), a nuclear receptor transcription factor that regulates genes involved in beta-oxidation and ketogenesis. Logically, it has been hypothesized that the KD engages PPARalpha in the brain, providing increased ability to use fatty acids to produce ATP and ketone bodies, which may be critically important for KD-mediated neuroprotective and antiseizure effects. Indeed, PPARalpha agonists exert antiseizure effects in acute seizure models (Porta et al., 2009; Puligheddu et al., 2013). However, the KD also exhibits anti-inflammatory and antioxidant properties and promotes mitochondrial health, mechanisms with ever-growing empirical support for their importance in controlling seizures in refractory epilepsy. An alternative nutrient-sensing transcription factor that regulates genes in these pathways, as well as genes involved in beta-oxidation and ketogenesis, is PPARgamma. Remarkably, PPARgamma, like the KD, is under intense investigation for therapeutic potential in Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, amyotrophic lateral sclerosis, multiple sclerosis, stroke, and cancer (reviewed in Heneke and Landreth, 2007; Collino et al., 2008; Mandrekar-Colucci and Landreth, 2011; Dineley et al., 2014; Johri et al., 2014; Carta and Simuni, 2015; Corona and Duchen, 2015; Shen et al., 2015). In this chapter, we review PPARgamma function and modulation and the evidence for PPARgamma involvement in the KD mechanism of action.

What Are Peroxisome Proliferator Activated Receptors?
Peroxisome proliferator activated receptors (PPARs) are ligand-inducible transcription factors that belong to the superfamily of nuclear receptors (NR), which comprises 48 transcription factors including receptors for endogenous steroid...
hormones, thyroid hormone, lipophilic vitamins, and cholesterol metabolites (Burris et al., 2013). Functional diversity between the 48 NRs is provided by distinct preferences for both ligands and DNA sequences recognized as response elements within the genome. In most cases the NRs function as dimers, either homodimers or heterodimers, and bind response elements in the absence and presence of ligand. The NR binding in the absence of ligand may have passive or active (i.e., constitutive basal transcription or silencing) consequences on target gene regulation (Burris et al., 2013). As a group, NRs are a significant and important therapeutic target for human disease, which is illustrated by the fact that 10%–15% of US Food and Drug Administration-approved drugs target NRs (Overington et al., 2006).

There are three different PPAR isoforms, PPARalpha (also known as NR1C1), PPARbeta (also known as delta and NR1C2), and PPARgamma (also known as NR1C3), which are encoded by genes located on human chromosomes 22, 6, and 3, respectively (Collino et al., 2008). The PPARs are involved in several aspects of development, such as differentiation of adipose tissue, brain, placenta, and skin; and the three isoforms display unique tissue- and temporal-dependent expression patterns during development in cell types having ectodermal, mesodermal, and endodermal embryonic origins (Desvergne and Wahli, 1999). Postnatally, the three isoforms exhibit considerable tissue-specific expression. PPARalpha is highly expressed in tissues that catabolize fatty acids (e.g., hepatocytes, cardiomyocytes, enterocytes, and kidney proximal tubule cells). PPARbeta is ubiquitously expressed in all cell types with higher levels in proliferating and differentiating cells. PPARgamma is strongly expressed in adipose tissue and the immune system. All PPARs have roles in lipid and glucose metabolism and homeostasis, inflammation, cell proliferation and differentiation, and vascular biology (Braissant et al., 1996; Tontonoz and Spiegelman, 2008); however, a role in peroxisome proliferation is species-dependent and it remains unclear whether PPARs are involved in this function in humans (Schrader et al., 2015).

Members of the nuclear hormone receptor superfamily contain four domains (Figure 20.1A). The N-terminal transactivation domain (activation function 1, AF1) is involved in ligand-independent transactivation, interaction with transcriptional coactivators and influences ligand-binding affinity of the ligand binding domain (LBD). The AF1 sequence has minimal homology between PPAR isoforms, which underlies the differential biological function of the isoforms. The DNA binding domain (DBD) contains two highly conserved zinc fingers. There is a hinge region between the DBD and the C-terminal LBD, which is required for receptor dimerization. The LBD contains the ligand-dependent transactivation domain AF2 and is less homologous between the isoforms, resulting in isoform specificity and sensitivity to ligands (Glass, 1994; Kersten and Wahli, 2000). In the nucleus, PPARs form obligate heterodimers with retinoid X receptors (RXR).

Of the three isoforms PPARgamma is the most extensively studied. Two PPARgamma isoforms, PPARgamma1 and PPARgamma2, result from alternative splicing and differential promoter use (Tontonoz et al., 1994). The PPARgamma isoforms are identical except that PPARgamma2 contains an additional 28 amino acids at its N-terminus that convey a 5- to 10-fold more effective ligand-independent transactivation and increased ligand binding affinity to the LBD relative to PPARgamma1 (Werman et al., 1997; Shao et al., 1998; Castillo et al., 1999; Bugge et al., 2009). The expression of PPARgamma1 appears ubiquitous. In contrast, PPARgamma2 is restricted to adipose tissue; however, high-fat diets can induce the expression of PPARgamma2 (Vidal-Puig et al., 1996, 1997).

PPARgamma activation is initiated by ligand binding. The PPARgamma ligand binding pocket is large (1,300 angstroms³), which allows structural promiscuity for a wide variety of endogenous or natural agonists (i.e., unsaturated fatty acids, eicosanoids, oxidized lipids, nitroalkenes), synthetic agonists (e.g., thiazolidinediones or TZDs), and synthetic antagonists (currently there are no known endogenous antagonists) (Itoh et al., 2008; Fang et al., 2010; Kroker and Bruning, 2015; Sauer, 2015). The nature of PPARgamma's large LBD makes it an effective sensor and transducer of environmental nutritional and inflammatory states.

Ligand binding induces a conformational change in the receptor leading to dissociation of corepressors such as NCoR (nuclear receptor corepressor 1) and SMRT (silencing mediator of retinoic acid and thyroid hormone receptor), which normally keep basal levels of PPAR-mediated transcription low. Without corepressor complexes, the PPARgamma-RXR heterodimer is free to bind specific recognition sequences called PPAR-response elements (PPREs) in the promoter regions of target genes. Once corepressors disengage, coactivators are recruited and alter chromatin structure and
recruit transcriptional machinery to promote the commencement of transcription (Figure 20.1b). Coactivators of PPARgamma include CREB binding protein (CBP), SRC1/2/3 (steroid receptor coactivator), and PPARgamma coactivator 1alpha (PGC-1alpha) (Auwerx, 1999; Katsouri et al., 2012; Krocker and Bruning, 2015). The PPARgamma-RXR-corepressor or -coactivator complexes are further regulated by several posttranslational modifications such as phosphorylation, acetylation, SUMOylation, and ubiquitination, which can increase or decrease PPARgamma transcriptional activity (Ahmadian et al., 2013).

PPARgamma controls hundreds of genes, with many involved in lipid and glucose metabolism and homeostasis, insulin-sensitization, fluid homeostasis, anti-inflammation, antioxidant, and mitochondrial health. Its best-known role is as a master regulator of adipogenesis, where it coordinates gene expression necessary for adipocyte formation (Auwerx, 1999; Fong et al., 2010; Ahmadian et al., 2013; Krocker and Bruning, 2015). The insulin-sensitizing effects of TZDs such as pioglitazone and rosiglitazone are clinically useful in the treatment of metabolic disorders such as type II diabetes mellitus. However, these full agonists can lead to weight gain, fluid retention, and bone loss due to PPARgamma activation in a wide range of tissues (Ahmadian et al., 2013). Several strategies to reduce side effects are under investigation, ranging

**FIGURE 20.1** Illustrations of PPARgamma splice variants and activity. (A) PPARgamma has two splice variants that have identical primary sequences except that the N-terminal of PPARgamma 2 has an extension of 28 amino acids. PPARgamma1 and PPARgamma2 have an activation function 1 domain (AF-1) required for ligand-independent activation, a DNA-binding domain required for sequence-specific binding to genomic DNA at a peroxisome proliferator response element (PPRE), a Hinge domain required for receptor dimerization, a ligand-binding domain required for ligand-dependent modulation and an activation function 2 domain (AF-2) within the ligand-binding domain that is required for ligand-dependent activation, ligand-dependent dimerization, coactivator recruitment, and corepressor release. The extra 28 amino acids in the AF-1 of PPARgamma2 confers 5- to 10-fold ligand-independent activation and greater ligand binding affinity relative to PPARgamma1. (B) PPARgamma forms a heterodimer with the retinoic acid receptor (RXR) and in the absence of agonist it is bound to corepressor complexes (e.g., NCoR and SMRT). Once an agonist is bound, the corepressors are released, PPARgamma-RXR recruits coactivators (e.g., CBP/p300, SRCs and PGC-1alpha), and the complex binds to PPREs in the promoter region of target genes leading to initiation of transcription by RNA polymerase II and general transcription factors.
from partial agonism to tissue-specific activation (Suji and Evans, 2011).

Many of the PPARgamma regulated genes overlap with KD-responsive genes. However, determining which genes PPARgamma regulates at any one time is not straightforward. An additional layer of complexity is that the gene sets regulated by PPARgamma depend on tissue-dependent differential expression of types of corepressors and coactivators, the state of biochemical cascades that regulate post-translational modifications, and the nature of the bound agonist (full or partial), which determines the degree of conformational change and ability of PPARgamma to interact with particular corepressors and coactivators (Ahmadian et al., 2013; Krocker and Bruning, 2015; Sauer, 2015). For example, the full agonist TZDs exert beneficial insulin-sensitizing effects as well as detrimental side effects such as weight gain, whereas several partial agonists or so-called selective PPARgamma modulators (SPPARMs) have been shown to retain antidiabetic effects while eliminating weight gain via attenuated and selective gene-regulatory activity in comparison with full agonists (Carmona et al., 2007; Tan et al., 2012). Additionally, phosphorylation of Ser273 in the LBD of PPARgamma dysregulates a group of distinct genes resulting in insulin-insensitivity. TZDs prevent Ser273 phosphorylation, but their effectiveness in producing insulin-sensitization in humans has been found to be inversely related to the degree of PPARgamma phosphorylation (Choi et al., 2010). This implies that PPARgamma activation may differentially regulate gene sets based on the state of the tissue (e.g., normal brain vs. oxidatively stressed and inflamed epileptic brain). This is an intriguing prospect that warrants further investigation.

**PPARGAMMA IN THE BRAIN**

The majority of knowledge concerning PPARgamma has been gained from study of its function in peripheral tissues and diseases as is evident in the previous sections. In the brain, much less is known. PPARgamma has differential regional expression patterns suggesting specialized and complex roles in both cellular and network function. It seems that PPARgamma has roles in maintaining homeostasis and protection against injury. In particular, PPARgamma activation in the brain consistently results in three general effects: prevention of mitochondrial apoptosis signaling pathways, suppression of inflammatory mediators, and limitation of reactive oxygen species. In this section, we will discuss the location and function of PPARgamma in the brain and its potential roles in epilepsy and the KD.

**Where Is PPARgamma in the Brain?**

PPARgamma is regionally distinct with high expression in neurons, astrocytes, and microglia in the cortex and moderate expression in neurons in the hippocampus (Cullingford et al., 1998; Lu et al., 2011; Moreno et al., 2004; Sarruf et al., 2009; Zhao et al., 2009). Moreno et al. (2004) have performed the most extensive study to date, cataloging the expression patterns throughout the brain of not only PPARgamma but also PPARalpha, PPARbeta and the three RXR isoforms. PPARgamma has the most restrictive distribution in the CNS. For example, PPARgamma is absent from neurons in the olfactory bulb, the perirhinal and entorhinal cortices, the temporal and occipital neocortices, the thalamic reticular nucleus, the ventral tegmental area, and the cerebellar Purkinje cells. It is highly expressed in neurons of the basal ganglia, thalamic rhomboid, centromedial and parasubiculum, nuclei of the reticular formation, and cerebellar stellate, basket, and golgi cells. PPARgamma has moderate to weak expression in neurons of the hypothalamic nuclei, septohippocampal nucleus, and the hippocampal formation. Oligodendrocytes do not express PPARgamma, but it is present in some astrocytes (Moreno et al., 2004). These descriptions are in broad agreement with studies using neuron-specific PPARgamma knockout mice (Sarruf et al., 2009; Lu et al., 2011). Sarruf et al. (2009) confirmed moderate expression in the hypothalamus but found expression in the ventral tegmental area in contrast to the previous study. They also reported that neuronal loss of PPARgamma resulted in 90% reduction of PPARgamma mRNA in brain, indicating that most PPARgamma in the brain is expressed in neurons (Sarruf et al., 2009). A subsequent study found that in normal mice, PPARgamma mRNA was greatest in cortex, followed by cerebellum, hippocampus, diencephalon, and hypothalamus (Lu et al., 2011). Knockout of PPARgamma from neurons reduced mRNA in all CNS regions except cerebellum. The reduction was greatest in the hippocampus and least in cortex (Lu et al., 2011). If the residual mRNA is from expression in glia, than we can infer that hippocampal glia express minimal levels of PPARgamma.

Only one study has attempted to discern differential expression of the two PPARgamma isoforms in the hippocampal CA1 region of mice (Gahring et al., 2005). The PPARgamma antibody, which
recognizes both isoforms, stained essentially all cells of the CA1 pyramidal layer and some associated cells including presumed astrocytes. However, using an antibody that recognized an epitope within the extra 28 N-terminal amino acids of PPARgamma2, it was found that only a small subset of neurons associated with the pyramidal cell layer were stained, suggesting that PPARgamma1 is the primary isoform in the hippocampus. The PPARgamma2 positive cells lacked astrocytic morphology, but did colabel with nicotinic acetylcholine receptors alpha4 and beta4 subunits; thus, the authors concluded that PPARgamma2 was exclusively expressed in putative interneurons (Gahring et al., 2005). This intriguing finding warrants confirmation and expansion to other brain regions.

What Is the Function of PPARgamma in the Brain?

The role of PPARgamma in the brain during normal conditions remains largely unknown, but most likely involves minimal activity in order to maintain lipid and glucose homeostasis. PPARgamma may also have a role in neuronal development and neurogenesis. PPARgamma is highly expressed in mouse embryonic brain and neural stem cells (NSCs) compared to the relatively low levels expressed in adult mouse brain (Wada et al., 2006), and may have differential roles in NSC differentiation during development and adulthood. Activation of PPARgamma in embryonic neural stem cells promotes NSC proliferation and inhibits NSC differentiation. In contrast, the ever-interesting compound cannabidiol (CBD) increased adult rat hippocampal neurogenesis via interaction with a PPARgamma pathway (Espisito et al., 2011). PPARgamma has also been implicated in promoting neurite outgrowth and dendritic spine density (Brodbeck et al., 2008; Quintanilla et al., 2013).

PPARgamma’s primary action appears to be neuroprotective in environments that involve inflammation and oxidative stress, such as during Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, amyotrophic lateral sclerosis, multiple sclerosis, and stroke. There are multiple reviews detailing PPARgamma effects in each of these neurodegenerative disorders, to which the reader is referred (Heneka and Landreth, 2007; Collino et al., 2008; Mandrekar-Colucci and Landreth, 2011; Dineley et al., 2014; Johri et al., 2014; Carta and Simuni, 2015; Corona and Duchen, 2015; Shen et al., 2015). Here, an overview of PPARgamma’s major effects that most likely contribute to neuroprotection is briefly presented.

PPARgamma has low expression in the brain and is primarily in neurons, however, in in vitro and in vivo models of various neurodegenerative disorders PPARgamma expression increases in neurons, astrocytes, and microglia (Kitamura et al., 1999; Diab et al., 2002; Victor et al., 2006; Fong et al., 2010; Wang et al., 2012). It is hypothesized that the inflammatory response and increase in oxidative stress results in the generation of fatty acid metabolites such as eicosanoids, oxidized lipids, and nitroalkenes, which are all potent endogenous PPARgamma agonists (Figure 20.2a; Fong et al., 2010). In a positive feed-forward loop, PPARgamma increases expression of itself and coactivators such as PGC-1alpha (Figure 20.2b). Experimental evidence has proven that this endogenous neuroprotective mechanism is important in limiting damage, because when it is absent mice experience significantly more brain damage and oxidative stress in response to an insult such as middle cerebral artery occlusion (Victor et al., 2006; Zhao et al., 2009).

The neuroprotective properties of PPARgamma activation in all of the above-mentioned neurodegenerative disorders have consistently been found to involve three general areas: prevention of mitochondrial apoptosis signaling pathways (Figure 20.2c), suppression of inflammatory mediators (Figure 20.2d), and limitation of reactive oxygen species (ROS; Figure 20.2e). Mitochondrial membrane potential depends on several factors, one important one being the balanced presence of anti-apoptotic Bcl-2 and Bcl-xl and pro-apoptotic Bad and Bax. Upon injury, Bad and Bax translocate to mitochondria, bind Bcl-2 and Bcl-xl, and depolarize mitochondria. Significant depolarization of the mitochondrial membrane potential can lead to the release of pro-apoptotic factors such as cytochrome C, caspase 9, and caspase 3 (Youle and Strasser, 2008). PPARgamma activation increases neurotrophic alpha-1 transcription, which leads to activation of Akt signaling pathways, resulting in increased Bcl-2 expression and phosphorylation of Bad (p-Bad). PPARgamma activation also up-regulates 14-3-3epsilon expression, which sequesters p-Bad and prevents Bad translocation into mitochondria (Wu et al., 2009a; Wu et al., 2009b; Fuenzalida et al., 2007; Thouennon et al., 2015).

PPARgamma activation suppresses pro-inflammatory gene expression by inhibiting the transcription factor NFkappaB. PPARgamma
accomplishes this by increasing the expression of IkappaBalpha and IkappaBbeta, which prevent the nuclear translocation and DNA binding of NFkappaB (Heneka et al., 2003). PPARgamma may also inhibit NFkappaB activity by direct physical interaction, steric inhibition, or cofactor competition (Sauer et al., 2015). The end effect is that PPARgamma decreases apoptosis by increasing the expression of 14-3-3epsilon, which prevents the translocation of p-Bad into the mitochondria and the release of pro-apoptotic factors and it increases mitochondrial biogenesis, which provides neurons increased ATP and additional calcium buffering.

Inflammation is dampened by PPARgamma-mediated inhibition of the pro-inflammatory transcription factor NFkB and oxidative stress is controlled by up-regulating the expression of antioxidants such as superoxide dismutase (SOD), catalase, glutathione (GSH), and uncoupling protein 2 (UCP2).

As mentioned previously, PPARgamma increases PGC-1alpha. PGC-1alpha also acts as a coactivator of the oxidative stress-induced transcription factor Nrf2, which is a master regulator of antioxidant gene sets as well as mitochondrial biogenesis (St. Pierre et al., 2006; Milder et al., 2010; Clark and Simon, 2009). Deficiency in PPARgamma is linked to reduced expression of antioxidants (superoxide dismutase 1, catalase, glutathione S-transferase, uncoupling protein-1, UCP1), lipid metabolism enzymes (lipoprotein lipase), and the transcription factor liver X receptor-alpha (Victor et al., 2006; Zhao et al., 2009).
Ketogenic Diet Regulation of PPARgamma

PPARgamma in Epilepsy

PPARgamma was first cloned from Xenopus laevis and mouse liver in the early 1990s (Dreyer et al., 1992; Zhu et al., 1993; Elbrecht et al., 1996). Since then, thousands of studies have addressed PPARgamma’s structure/function, regulation, pharmacology, expression, and importance in disease primarily in peripheral organs. At the turn of the 21st century, neuroscientists began investigating PPARgamma’s therapeutic potential for neurodegenerative diseases, in which a role for neuroinflammation and oxidative stress in disease processes has long been recognized (Kitamura et al., 1999; Combs et al., 2000) and has been investigated in hundreds of publications.

Interest in the epilepsy community has had a late and slow start. The first research study appeared in 2006, and the total count to date is a little over a dozen (Okada et al., 2006; Luna-Medina et al., 2007; Maurois et al., 2008; Yu et al., 2008; Hong et al., 2008, 2011, 2013; Abdallah, 2010; Han et al., 2011; Jeong et al., 2011; Adabi Mohazab et al., 2012; Chuang et al., 2012; Hughes et al., 2014; Boes et al., 2015; Simeone et al., 2016). These studies span acute seizure models, post-status epilepticus (SE) models, and kindling and genetic models of chronic epilepsy and, for the most part, consistently support beneficial neuroprotective and antiseizure effects of PPARgamma agonists in epilepsy.

Comparable to findings in chronic neurodegenerative disorders and acute stroke models, the expression of brain PPARgamma increases subsequent to SE induced by lithium-pilocarpine, unilateral intrahippocampal kainic acid, intraperitoneal injection of kainic acid, and electrically induced self-sustaining SE (Yu et al., 2008; Hong et al., 2008; Jeong et al., 2011; Chuang et al., 2012; Boes et al., 2015). Concomitantly, the products of the PPARgamma-regulated genes Pgc-1alpha and Ucp2 are increased after lithium-pilocarpine and intrahippocampal kainic acid (Han et al., 2011; Chuang et al., 2012). In a recent study, we examined the nuclear content of both splice variants, PPARgamma1 and gamma2, in the brains of wild-type mice and epileptic littermates that lack the Kv1.1 potassium channel (Kcnal-null). Kcnal-null mice develop severe spontaneous recurrent seizures (SRS) and are a model of temporal lobe epilepsy and sudden unexpected death in epilepsy (SUDEP) (Smart et al., 1998; Wenzel et al., 2007; Fenoglio-Simeone et al., 2009a; Fenoglio-Simeone et al., 2009b; Glasscock et al., 2010; Simeone et al., 2013; Simeone et al., 2014a; Simeone et al., 2014b; Kim et al., 2015; Simeone et al., 2016). Similar to previous reports, we found that PPARgamma1 predominated in wild-type brain. In contrast, in epileptic Kcnal-null brain we found that PPARgamma2 was the dominant form. The total nuclear PPARgamma did not change, but the PPARgamma2/gamma1 ratio increased threefold in Kcnal-null brains (Simeone et al., 2016). Further studies are needed to determine the cause of this flip in isoform nuclear content. Potential causes that preferentially enhance nuclear translocation of PPARgamma2 over PPARgamma1 may be generation of isoform specific ligands, altered expression of isoform-specific cofactors, or post-translational modifications. Alternatively, transcription, translation, or splicing of PPARgamma2 could be increased in epilepsy. Recently, it was found that at the initial stage of adipogenesis another nuclear receptor, glucocorticoid receptor (GR), is transiently recruited along with the transcription factor C/EBPbeta to a complex consisting of PBP/MED1/TRAP220 and p300 to enhancer regions of the Pparg2 isoform. In response to glucocorticoids, this results in a transient increase in H3K9 acetylation and enhances the induction of PPARgamma2, which becomes the principal driver of adipogenesis (Steger et al., 2010). Intriguingly both C/EBPbeta and glucocorticoids increase in the brain with seizures (Lu et al., 2013; Engel et al., 2013; Maguire and Salpekar, 2013). PGC-1alpha activation of PPARgamma also enhances interactions with p300/CBP (Puigserver et al., 1999). Whether PPARgamma2 regulates distinct gene sets is unclear, but it has been shown to up-regulate catalase expression to a greater degree than PPARgamma1 and is important in providing protection against lipotoxicity (Medina-Gomez et al., 2007a; Medina-Gomez et al., 2007b; Yakunin et al., 2014).

Assuming that the seizure/injury-induced changes in PPARgamma expression are part of an endogenous neuroprotective mechanism that limits damage, similar to what has been proposed for other neurodegenerative disorders, then loss of PPARgamma should exacerbate markers of injury and possibly seizures. Chuang et al. (2012) found that pretreatment with bilateral focal injections of the PPARgamma antagonist GW9662 (150 nl of a 12 mM solution or 1.5-2 mg/kg) reduces UCP2; exacerbates intrahippocampal kainic acid SE-induced...
increases in ROS, oxidized proteins, mitochondrial Bax, cytosolic cytochrome C, and DNA fragmentation; and decreases mitochondrial respiratory complex I (MRCI) activity. Unfortunately, the authors failed to report the details of SE, so it is not known whether PPARgamma antagonism worsened the SE (Chuang et al., 2012). We administered GW9662 (1 mg/kg/day in the drinking water for 2 weeks) to Kcnal-null mice and wild-type littermates, however, contrary to expectations, inhibiting PPARgamma did not increase SRS in Kcnal-null mice, nor did it lower seizure threshold in wild-type mice (Simeone et al., 2016). A possible explanation for the lack of effect on Kcnal-null seizures may be that the dose of GW9662 was too low. Supporting this hypothesis, we found that GW9662 had no effect on the nuclear PPARgamma2/gamma1 ratio in nonepileptic wild-type brain. It did reduce the PPARgamma2/gamma1 ratio in epileptic Kcnal-null brain, but only from threefold to twofold of wild-type ratio (Simeone et al., 2016). A greater reduction may be needed to observe an effect on SRS. We also found that seizure thresholds of Ppar-2-null mice and neuron specific-PPARgamma knockout mice were no different than control littermates (Simeone et al., 2016). From these experimental results we can draw the tentative conclusions that PPARgamma itself is somehow involved in increasing nuclear PPARgamma2 and that the seizure-induced increase affords some neuroprotection against injury, but may not raise the seizure threshold per se.

**PPARgamma Agonism in Epilepsy**

The PPARgamma agonists pioglitazone and rosiglitazone have been clinically useful in the management of type II diabetes mellitus since 1999, but as of today there are no published reports, case reports or otherwise, describing epilepsy patients with comorbid type II diabetes that have been prescribed pioglitazone or rosiglitazone. Nevertheless, experimental evidence from animal studies supports that PPARgamma agonists will have antiseizure, neuroprotective, and possibly disease-modifying effects in epilepsy. Even though this field of research is small and there are relatively few studies, there is wide diversity in treatment protocol (agonist, dose, pre/post-treatment) and endpoints (acute seizures, SRS, inflammatory markers, cell death, cognitive comorbidities), and it is worth detailing the similarities and differences to gain a sense of the robustness of PPARgamma effects.

**PPARgamma Agonist Pretreatment Raises Thresholds in Acute Seizure Models**

Abdallah (2010) and Adabi Mohazab et al. (2012) utilized penetrenyltetrazole (PTZ) to induce seizures in mice with two different paradigms. Abdallah (2010) pretreated mice with pioglitazone (10 mg/kg; p.o.) 30 minutes prior to an intraperitoneal (i.p.) injection of PTZ and measured latency to stage 4/5 clonic-tonic seizures. All vehicle controls reached stage 4/5, whereas only 20% of the pioglitazone group reached stage 4/5 and those that did took twice as long as the control group. Adabi Mohazab et al. (2012) injected PTZ intravenously and measured the threshold dose required to induce a whole-body clonic seizure. They found that a 1-h pretreatment with pioglitazone was ineffective, whereas a 4-h pretreatment provided a dose-dependent (10–80 mg/kg; p.o.) increase in the seizure threshold dose. Maurois et al. (2008) also found that the PPARgamma agonist FOMC-L-leucine ((N-[9-fluorenylmethoxycarbonyl])-L-leucine; up to 100 mg/kg; i.p.) was not effective in the 6-Hz seizure test with 1-h pretreatment. We have found similar effects exposing mice to flurothyl gas and measuring latency to clonus and generalized tonic-clonic seizures. A 1-h pretreatment with pioglitazone had inconsistent effects, whereas a 4-h pretreatment dose-dependently (1–80 mg/kg; i.p.) increased the latency to generalized tonic-clonic seizures but had no effect on clonic seizures (Simeone et al., unpublished observations).

**PPARgamma Agonists Raise Thresholds in Seizure Susceptible and Kindling Models**

The EL mice develop susceptibility to stress-induced tonic-clonic seizures beginning 12 weeks postnatally. Providing pioglitazone (20 mg/kg/day) from 5 to 11 weeks postnatally delayed the age of onset and duration of stress-induced seizures (Okada et al., 2006). Maurois et al. (2008) used a dietary-induced magnesium deficiency-dependent audiogenic seizure model. Mice were pretreated with either FOMC-L-leucine or rosiglitazone (up to 100 mg/kg; i.p.) 1 h prior to acoustic stimuli. FOMC-L-leucine dose-dependently reduced the number of mice responding to acoustic stimuli by 50%, whereas rosiglitazone was ineffective. The FOMC-L-leucine effects were blocked by coadministration of the PPARgamma antagonist GW9662 (1 and 2 mg/kg, i.p.) (Maurois et al., 2008). Finally, Abdallah (2010) kindled mice with daily i.p. injections of subconvulsive PTZ (40 mg/kg) until the mice exhibited stage 4/5 clonic-tonic seizures on three consecutive days. Pretreatment with pioglitazone (10 mg/kg; p.o.) 30 minutes prior
to each PTZ injection resulted in only 30% of mice reaching stage 4/5 seizures by day 17 (Abdullah, 2010). However, because pioglitazone was administered before PTZ and most pioglitazone-treated mice did become kindled as defined by the author, it is difficult to determine whether pioglitazone exerted any antiepileptogenic effect in addition to its anticonvulsant effect.

PPARgamma Agonists Reduce SRS and Improve Spatial Learning

Multiple studies have used SE models to later investigate neuropathology during the latent period and after development of SRS (discussed in the next section), however, only three studies have provided information concerning the effect of PPARgamma agonists on either seizures during SE and/or the development of SRS (Luna-Medina et al., 2007; Hong et al., 2013; Boes et al., 2015). Luna-Medina et al. (2007) pretreated rats with a thiazolidine compound, NP031112 (50 mg/kg; p.o.), 1 hour before kainic acid injection (10 mg/kg; i.p.) and monitored the development of behavioral seizures and SE. NP031112 had no effect on SE. Similarly, Hong et al. (2013) found that rosiglitazone had no effect on the development of lithium-pilocarpine SE. Rats were pretreated with rosiglitazone (0.1 mg/kg; i.c.v.) 1 hour before lithium chloride injection (127 mg/kg; i.p.). Twenty-four hours later, rats were again pretreated 1 hour prior to pilocarpine hydrochloride (30 mg/kg; i.p.) administration. Rats then received repeated injections of pilocarpine (10 mg/kg; i.p.) every 30 min until they developed convulsive seizures, which were scored behaviorally according to the Racine scale. Rosiglitazone had no effect on SE. The authors continued daily administration of rosiglitazone and examined development of SRS by performing video-EEG recordings beginning 2 weeks post-SE for 6 weeks. They found that 70% of rosiglitazone-treated rats developed SRS compared with 83% of vehicle-treated rats. Moreover, rosiglitazone nearly doubled the latency to SRS development and decreased the frequency and duration of SRS (Hong et al., 2013). An earlier study by this group demonstrated that the same protocol improved the SE rats’ spatial learning as assessed in the Morris water maze (Hong et al., 2011). In contrast, rosiglitazone (3 mg/kg; i.p.) administered to rats daily immediately following the end of self-sustained SE had no effect on the development and occurrence of SRS; however, the rosiglitazone-treated rats did have improved spatial learning in the Morris water maze (Boes et al., 2015). Finally, we have found that administration of pioglitazone (10 mg/kg; i.p.) for 5 days decreases SRS and seizure severity in epileptic Kcna1-null mice by 75% (Simeone et al., 2016).

PPARgamma Agonists Are Neuroprotective post-SE and during SRS

Within hours to days, intrahippocampal kainic acid-induced SE results in increases in UCP2, ROS, oxidized proteins, mitochondrial Bax, cytosolic cytochrome c, DNA fragmentation, and decreases in MRCI activity in rat hippocampi (Chuang et al., 2012). Pretreatment with bilateral focal injections of rosiglitazone (150 nL of a 4 mM solution or 0.7–0.8 mg/kg) further increased UCP2 and prevented all other changes. In fact, MRCI activity was actually improved compared with the sham controls (Chuang et al., 2012). In a similar study, Luna-Medina et al. (2007) found that intrahippocampal injection of kainic acid resulted in significant cerebral edema, hippocampal cell loss, astrogliosis, and the induction of TNFalpha in astrocytes, microglia, and neurons. Coinjection of NP031112 (5–8 ng/kg) prevented edema, cell loss, and TNFalpha induction and reduced astrogliosis. Furthermore, pretreatment with NP031112 (50 mg/kg; p.o.), 1 hour before kainic acid injection (10 mg/kg; i.p.) also prevented hippocampal neuronal loss as quantified 72 hours post-SE (Luna-Medina et al., 2007).

In the rat lithium-pilocarpine SE model, post-SE daily administration of rosiglitazone has antioxidant and anti-inflammatory effects. Rosiglitazone increases hippocampal neuronal survival, decreases ROS and lipid peroxidation, increases SOD and GSH, and decreases microglia and astrocytic activation, TNF-alpha, and BDNF (Yu et al., 2008; Hong et al., 2008, 2012, 2013). Similarly in EL mice and the PTZ-kindling model, pioglitazone decreased TNF-alpha, IL-1beta, IL-6, IL-10, and caspase3 (Okada et al., 2006; Abdallah, 2010). However, rosiglitazone did not reduce hippocampal cell death in the self-sustained SE model (Boes et al., 2015).

Evidence for Ketogenic Diet Activation of PPARgamma

Although the possibility of a role for PPARgamma in the mechanism of the KD has been briefly speculated in review articles for the past several years (Kobow et al., 2012; Masino and Rho, 2012; Gano et al., 2014), there have only been two studies published exploring this possibility, one using an acute SE model and one using a chronic epilepsy model and acute seizure threshold model (Jeong et al., 2011; Simeone et al., 2016). Jeong
et al. (2011) fed wild-type mice a KD (4:1 ratio of fats to carbohydrate+protein) for 3 weeks and found a threefold increase of PPARgamma protein in cell lysate from hippocampal tissue compared with standard diet (SD)-fed mice. The mice were injected with kainic acid (30 mg/kg) to induce status and tissue collected at 2 and 6 hours post-kainic acid injection. The KD delayed the onset of generalized tonic-clonic seizures, but the overall seizure burden that the mice experienced during the 2 and 6 hours post-kainic acid was not reported. In the SD-fed mice, there was a fourfold and twofold increase of PPARgamma at the 2- and 6-hour time points after injection, respectively. In contrast, the KD-induced elevated PPARgamma levels fell to normal diet control levels by 6 hours post-kainic acid. Hippocampal TNF-alpha followed the same pattern as PPARgamma, whereas the KD blunted the SE-induced increases in NF-kappaB, microglial CD11b, and COX2 expression. From the studies mentioned previously detailing the effect of injury or degeneration and PPARgamma agonists on the expression of PPARgamma, one would expect that PPARgamma would be further increased, not decreased, by SE in the KD-fed mice. The authors do not address this discrepancy (Jeong et al., 2011). Nevertheless, the authors do present evidence that the KD does have effects on PPARgamma and downstream markers of inflammation known to be regulated by PPARgamma.

In a recent study, we aimed to determine whether the KD changes PPARgamma expression in the brains of epileptic and normal mice, and, if so, whether it is necessary for the antiseizure effects of the KD. As described earlier, we have found that in nuclear extracts from wild-type mouse brain, PPARgamma1 is the dominant isoform, whereas PPARgamma2 is dominant in epileptic Kcnal-null brain (Simeone et al., 2016). Treating Kcnal-null mice for 2 weeks with a KD (6:1 ratio) reduced seizure incidence and severity by ~75%, as we have reported previously (Fenoglio-Simeone et al., 2009b; Kim et al. 2015; Simeone et al., 2016). Ketogenic diet treatment increased PPARgamma2 in both genotypes, resulting in PPARgamma2/gamma1 ratios that were twofold and sixfold for wild-type and Kcnal-null brain, respectively, compared with SD-fed wild-type mice.

To determine whether the effect of the KD on PPARgamma was necessary for KD antiseizure efficacy, we performed pharmacologic and genetic experiments. Coadministering the PPARgamma antagonist GW9662 (1 mg/kg/day) in the drinking water for the 2-week KD treatment prevented the increase in nuclear PPARgamma2 and prevented KD-mediated seizure reduction in Kcnal null mice. To further test the importance of PPARgamma in the KD mechanism, we obtained Pparg2 null mice and conditional neuron-specific PPARgamma knockout mice. Previous studies have demonstrated that KD treatment raises the threshold for flurothyl seizures (Rho et al., 1999; Dutton et al., 2011). Similarly, we found that KD treatment effectively raised seizure thresholds of control mice. However, KD treatment was unable to raise the seizure threshold of Pparg2 null mice and conditional neuron-specific PPARgamma knockout mice. Furthermore, the pharmacologic and genetic manipulations of PPARgamma expression/function did not affect the stereotypic KD increase of blood beta-hydroxybutyrate or decrease of glucose (Simeone et al., 2016). Results from this study strongly support that central actions of PPARgamma, more specifically PPARgamma2, play a critical role in the antiseizure mechanism of the KD.

**What Does the KD provide That Could Be Activating PPARgamma?**

The selective increase of nuclear PPARgamma2 over PPARgamma1 may be due to the additional 30 amino acids in the PPARgamma2 AF1 that convey a 5- to 10-fold more effective ligand-independent transactivation and increased ligand binding affinity to the LBD relative to PPARgamma1 (Werman et al., 1997; Shao et al., 1998; Castillo et al., 1999; Bugge et al., 2009). PPARgamma2 is the only PPARgamma isoform regulated at the transcriptional level by nutrition (Medina-Gomez et al., 2007b). The PPARgamma2 expanded AF1 also confers differential interaction with transcriptional cofactors and post-translational modifications that would most likely result in tissue-specific differences in the regulation of gene sets by the PPARgamma splice variants. This is evident in the periphery, where PPARgamma2, but not PPARgamma1, is induced during high-fat diets and initiates adipogenesis, increases lipid-buffering, and reduces lipotoxicity (Medina-Gomez et al., 2007a). Therefore, the most likely mechanisms for this isoform-specificity of the KD probably involve either regulation of signaling cascades responsible for post-translational modifications that contribute to selective nuclear translocation or transcription of PPARgamma2 and/or providing a PPARgamma ligand, possibly selective for PPARgamma2.
The KD provides plenty of fat, of course, and unsaturated fatty acids, such as omega-3 and omega-6 long chain polyunsaturated fatty acids, are notably increased in blood serum of patients (Fraser et al., 2003). Importantly, long chain polyunsaturated fatty acids and their metabolites, eicosanoids, oxidized lipids, and nitroalkenes are all natural ligands for PPARgamma (Yamamoto et al., 2005; Fong et al., 2010). Also, it was recently determined that the saturated fatty acid decanoic acid (a.k.a. capric acid), a primary constituent of the medium chain triglyceride ketogenic diet, is a ligand for PPARgamma at physiologically relevant concentrations (Malapaka et al., 2012).

In vivo treatment suggests that decanoic acid is a selective PPARgamma modulator (i.e., partial agonist) as it improves glucose sensitivity and lipid profiles without weight gain in diabetic mice (Malapaka et al., 2012). In vitro experiments in hippocampal slices found that decanoic acid decreases PTZ-induced epileptiform activity in a concentration-dependent manner (Chang et al., 2013). Furthermore, decanoic acid-induced increases in citrate synthase, catalase, and MRCI activity in SH-SY5Y neuronal cultures were inhibited by a PPARgamma antagonist (Hughes et al., 2014). Alternatively, Jeong et al. (2012) found that treatment of cultured HT22 cells (a hippocampal neuronal cell line) with the ketone body acetoacetate (5 mM) increases PPARgamma over a 12-hour period; however, we have been unable to replicate this finding in primary hippocampal neuronal cultures, and in fact this concentration of acetoacetate results in significant neuronal death within 24 and 48 hours (Simeone et al., unpublished observations). Therefore, at the moment it seems that the unsaturated and saturated fatty acids provided by a ketogenic diet may be the ligands for PPARgamma, and that PPARgamma may be involved in the anti-seizure mechanism of the various formulations of the ketogenic diet regardless of the type of fat content.

**Functional Consequences of PPARgamma Activation Relevant to Epilepsy**

PPARgamma agonists and the KD regulate similar anti-inflammatory, antioxidant and promitochondrial pathways. These include, but are not limited to, up-regulation of IkappaB; inhibition of NFkappaB; reduction of cytokines such as IL-1beta, IL-6, and TNF-alpha; up-regulation of genes encoding mitochondrial enzymes involved in oxidative phosphorylation (e.g., multiple subunits of complexes I, II, IV, and V); induction of mitochondrial biogenesis; and up-regulation of UCP2, catalase, and glutathione (Masino and Rho, 2012; Mandrekar-Colucci et al., 2013; Fong et al., 2010; Bernardo et al., 2006; Heneka and Landreth, 2007; Chuang et al., 2012; Hong et al., 2008, 2012, 2013; Abdallah, 2010; Adabi Mohazab et al., 2012; Bough et al., 2006; Miglio et al., 2009; Sullivan et al., 2004; Yang and Cheng, 2010; Yu et al., 2008). All of these have been suggested as possible disease modifying targets for epilepsy. Mitochondria are of particular interest in temporal lobe epilepsy. In human epilepsies and animal chemoconvulsant seizure models, brain tissue from seizure foci exhibit mitochondrial reactive oxygen species (ROS) and oxidative damage, reduced ATP-producing complex I activity and diminished antioxidant systems (Sullivan et al., 2003; Wallace, 1999; Bruce and Baudry, 1995; DiMauro et al., 1999; Kunz et al., 2000; Mueller et al., 2001; Sudha et al., 2001; Malthankar-Phatak et al., 2006; Vielhaber et al., 2008; Kudin et al., 2009; Ryan et al., 2012; Simeone et al., 2014a; Kim et al., 2015; Simeone et al., unpublished observation). Oxidative stress and mitochondrial overload likely contribute to the neuronal cell loss in severe epilepsy associated with sclerosis. In this circumstance, it is clear that the proposed actions of PPARgamma would be neuroprotective and increase the number of surviving cells. But this illustrates only one outcome of unhealthy mitochondria and does not address the potential consequences that chronic mitochondrial dysfunction has on neuronal hyperexcitability and seizure severity.

Several elegant studies have demonstrated that mitochondria regulate synaptic transmission via three properties: (1) production of ATP and (2) ROS and (3) sequestration of cytosolic calcium (Lee et al., 2007; Lee et al., 2012; Harris et al., 2012). Thus, any perturbation of mitochondrial health will send ripples of dysregulation across synaptic, neuronal, and network activity. Stabilizing synaptic mitochondria could be another mechanism by which PPARgamma and the KD reduce seizure activity.

To test this hypothesis, we turned once again to the Kcnal-null mouse model of epilepsy, because we have found mitochondrial pathology similar to reports from human epilepsies (Simeone et al., 2014a; Kim et al., 2015; Simeone et al., unpublished observations). Mitochondria isolated from cortical and hippocampal tissue from epileptic Kcnal-null mice have reduced ATP-producing MRCI-driven respiration, increased ROS, and decreased UCP2.
(Simeone et al., 2014a). The decrease of UCP2, a mitochondrial protein with downstream antioxidant effects, may contribute to the rise in ROS. The MRCI inhibition appears to be due to post-translational inhibition by elevated ROS because acute addition of antioxidants such as ascorbic acid restores Kcnal-null MRCI-driven respiration to wild-type levels, whereas exogenous H$_2$O$_2$, mimics the MRCI dysfunction of Kcnal-null mitochondria (Simeone et al., 2014a). In addition, the mitochondrial membrane potential is depolarized and mitochondrial calcium sequestration capacity is reduced (Simeone et al., 2014a; Kim et al., 2015; Simeone et al., unpublished observation).

Thus, the three properties of mitochondria that play a role in synaptic transmission (ATP, ROS, and calcium) are dysfunctional in Kcnal-null mitochondria and predict widespread synaptic and network activity. This is exactly what we observed using a multielectrode array to record extracellular potentials from in vitro hippocampal slices from Kcnal-null brains (Simeone et al., 2013). We found that Kcnal-null hippocampi generate spontaneous network activity originating in the CA3 region in the form of sharp waves (SPWs) and ripples (80–200 Hz bandwidth) with an increased incidence and duration compared with slices from wild-type hippocampi. Also present in Kcnal-null hippocampi were epilepsy-associated pathologic high-frequency oscillations in the fast ripple bandwidth (200–600 Hz), which can be viewed as biomarkers of hyperexcitable networks. Furthermore, Kcnal-null CA3 has enhanced coupling of excitatory inputs and population spike generation and CA3 principal cells have reduced spike-timing reliability. Removing the influence of granule cell mossy fiber path inputs by microdissecting the Kcnal-null CA3 region mostly rescued the network oscillatory behavior and improved spike timing. We found that Kcnal-null mossy fibers are hyperexcitable and reduced paired pulse ratios, suggesting increased neurotransmitter release at these terminals (Simeone et al., 2013). Collectively, these data indicate enhanced synaptic release in the Kcnal-null CA3 region reduces spike timing precision of individual neurons leading to disorganization of network oscillatory activity and promotion of the emergence of fast ripples.

This synaptic phenotype is mimicked by acute application of the MRCI inhibitor rotenone to wild-type hippocampal slices. Experimental inhibition of MRCI results in increased SPWs, ripples, and fast ripples (Simeone et al., 2014a; Simeone et al., unpublished observations). Moreover, rotenone exacerbated provoked seizure-like events in vitro, supporting a possible role in worsening in vivo seizures (Simeone et al., 2014a).

We have further demonstrated that in vivo treatment of Kcnal-null mice with either pioglitazone or a KD or a cocktail of ascorbic acid, alpha-tocopherol, and pyruvate designed to target mitochondrial health rescues MRCI respiration, restores mossy fiber neurotransmitter release probabilities, and dampens network hyperexcitability (i.e., reduces incidence of SPWs, ripples, and fast ripples) (Simeone et al., 2014a; Simeone et al., 2014b; Simeone et al., unpublished observations). Therefore, we speculate that in Kcnal-null mice one important antiseizure mechanism resulting from KD modulation of PPARgamma is restoration of MRCI function, possibly by increasing endogenous antioxidant pathways and decreasing ROS. Recently, Chuang et al. (2012) implicated just such a pathway involved in the neuroprotective effects of rosiglitazone in the intrahippocampal kainate model of status epilepticus. Specifically, rosiglitazone increased UCP2, decreased ROS, and restored MRCI function.

**CONCLUDING REMARKS**

The development of epilepsy necessarily originates with a precipitating event (e.g., genetic predisposition, traumatic brain injury, virus, etc.) that lowers the seizure threshold or moves the baseline activity of the brain closer to the seizure threshold so that previously nonsignificant internal or external stimuli are capable of synchronizing large networks into high-frequency oscillatory activity. If the resulting seizure phenotype is severe, than a chronic inflammatory and oxidative state develops with concomitant mitochondrial dysfunction. The result is not only death of vulnerable cell types but also dysregulation of cellular, synaptic, and network excitability, which further lowers the seizure threshold and exacerbates the seizure phenotype (Figure 20.3, left).

Experimental evidence indicates that the KD prevents cell death and hyperexcitability by promoting mitochondrial health and anti-inflammatory and antioxidant pathways. Limited, but strong, evidence that we have reviewed here suggests that the KD regulates PPARgamma to achieve these effects (Figure 20.3, right). This discovery presents multiple opportunities for both researchers and clinicians. The immediate translational potential resides in the availability of the PPARgamma agonists pioglitazone and rosiglitazone that are FDA-approved for therapeutic use in type II diabetes mellitus. The TZDs are not perfect and can
have significant side effects. The good news is that the diabetes academic and industry communities have active research efforts into developing PPARgamma partial agonists (SPPARMs) with improved side-effect profiles. These may also prove useful in epilepsy. Furthermore, additional targets with therapeutic potential in epilepsy may be identified by focusing on the upstream pathways or ligands leading to PPARgamma activation and the downstream effectors that result in increasing the seizure threshold and/or dampening hyperexcitability and neuroprotection.

The identification of PPARgamma as a potential mediator of KD efficacy in epilepsy does not preclude the other mechanisms discussed in this volume. Some may work synergistically with PPARgamma, such as ketone bodies, and others may be downstream effectors of PPARgamma, such as adenosine.

ACKNOWLEDGMENT

This work was supported by an award from Citizens United for Research in Epilepsy Foundation and the NIH (NS085389). I thank Dr. Kristina
A. Simeone for many insightful discussions and comments on this manuscript.

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Ketogenic Diet in a Hippocampal Slice

Models and Mechanisms

MASAHITO KAWAMURA JR., MD, PHD

HIPPOCAMPUS AS A MODEL FOR TEMPORAL LOBE EPILEPSY

The hippocampus is well known as a brain area involved in learning and memory and also as the key region underlying the form of epilepsy known as mesial temporal lobe epilepsy. The temporal lobe refers to the ventrolateral middle part of neocortex, and abnormal neuronal discharge or a lesion affecting this lobe causes seizures (Gastaut, 1973). There are two main types of temporal lobe epilepsy classified by the epileptic focus: mesial and lateral. The focus of mesial temporal lobe epilepsy is the hippocampus, amygdala, or parahippocampal gyrus, and that of lateral temporal lobe epilepsy is in neocortex. Over 80% of patients with temporal lobe epilepsy have the mesial form (Quarato et al., 2005; Schramm et al., 2001). The typical symptom of mesial temporal lobe epilepsy is complex partial seizures, which have a high probability of an accompanying aura. The aura is characteristic, usually presenting as epigastric discomfort sometimes described as nausea or psychiatric symptoms including fear. Complex partial seizures often begin with arrest of motor activities or staring. Autonomic motor behaviors are usually oroalimentary automatisms or complex automatisms. Dys tonic posturing lasting 1–2 minutes often occurs involving the arm contralateral to the ictal discharge (Engel, 2001). In a majority of patients, mesial temporal lobe epilepsies are associated with hippocampal sclerosis (Watson, 2003), which is atrophy with global gliosis and loss of CA1 and/or CA3 pyramidal neurons in the hippocampus (Thom, 2009). The structure of the hippocampus is simple. It includes principal cells (granular cells of dentate gyrus and CA1-4 pyramidal neurons) and surrounding interneurons. The principal cells form an excitatory circuit that is modulated by inhibitory interneurons.

Interestingly, CA3 pyramidal neurons are connected to each other by excitatory recurrent collaterals. Thus, the hippocampal circuit is regulated by a balance between excitation from recurrent collaterals and inhibition from interneurons. When the balance collapses, the hippocampal circuit becomes hyperexcitable and causes seizures easily. Therefore, the atrophy in the hippocampus is thought to be one of the main focuses of mesial temporal lobe epilepsy, and excision of hippocampal sclerosis with selective amygdalohippocampectomy successfully improves ~70% of surgical patients (Paglioli et al., 2006; Wiebe et al., 2001). From these clinical findings, the hippocampus is thought to be a good experimental target for investigating epileptogenesis and therapy of temporal lobe epilepsy.

The ketogenic diet was designed in the 1920s to treat epilepsy by mimicking fasting (Wilder, 1921). Despite almost 100 years of clinical use, the mechanisms underlying the success of ketogenic diet therapy are not well understood. In recent decades, ketogenic diet has increasingly been noted as a useful therapy for medically refractory epilepsy (Hallbook et al., 2007). The patients of temporal lobe epilepsy are well known to be frequently resistant to antiepileptic drugs (Wiebe and Jette, 2012). As mentioned previously, the first choice of treatment for mesial temporal lobe epilepsy associated with hippocampal sclerosis is surgery (anterior temporal lobectomy or selective amygdalohippocampectomy) because of good therapeutic outcomes (Tanriverdi et al., 2008). For temporal lobe epilepsy patients who are not good candidates for surgery, however, ketogenic diet is one of the therapeutic options (Ray and Wyllie, 2005). Thus, a natural question is how the ketogenic diet produces its beneficial effects in temporal lobe epilepsy. In this chapter, we focus on investigating ketogenic diet antiseizure mechanisms with acute...
slice preparations of the hippocampus, reviewing our own and other laboratories' work.

THE IMPORTANCE OF HIPPOCAMPAL SLICE PREPARATIONS FOR STUDYING ANTIEPILEPTIC MECHANISMS OF KETOGENIC DIET

Because epilepsy is caused by abnormal neuronal discharges in the brain, electrophysiological measurements are the most direct and useful approach for researching epilepsy and its treatments. There are two approaches for electrophysiological recordings of any brain region: in vivo and in vitro. In vivo electrophysiological recording of hippocampus is usually done by extracellular recording of electrically evoked activity (Stewart and Reid, 1993), or continuous recording of spontaneous field activity (Li et al., 2008) or multiple unit activity (Lin et al., 2006); these preparations can be acute or chronic. In vitro electrophysiological recording is done using single-cell intracellular sharp electrodes (Abe and Ogata, 1981) or patch-clamp electrodes (Kawamura et al., 2004), or extracellular field recording with single electrodes (Masino and Dunwiddie, 1999) or electrode arrays (Knowles et al., 1987) using acute slices of hippocampus. As compared with in vivo hippocampal recordings, the advantages of hippocampal slices are several-fold: (1) Ease of use: acute brain slices must be maintained by perfusion with oxygenated artificial cerebrospinal fluid (Sakmann et al., 1989). Continuous perfusion allows us to change the extracellular fluid easily. Thus, it is easy to apply and wash out several agonists and/or antagonists of various proteins such as channels, receptors, and transporters, and it is easy to examine the detail of functional mechanisms of neuronal activities; (2) Reduction: we usually make 3–6 brain slices from one rodent and get 3–6 recordings from them, allowing us to reduce the number of animals used; (3) History: a huge number of electrophysiological experiments have been done using hippocampal slice preparations in the last half-century. Several methods for causing seizure-like bursting in vitro have been used in the hippocampal slice preparation, including kindling (Sayin et al., 1999), kainic acid treatment (Congar et al., 2000; Smith and Dudek, 2001), inhibition of GABA receptors (Kohling et al., 2000; Stafstrom et al., 2009), inhibition of potassium ion channels (Stafstrom et al., 2009), and neuronal hyperexcitability by changing extracellular ion concentrations (Congar et al., 2000; Dulla et al., 2005; Kojima et al., 1989; Stafstrom et al., 2009).

All these approaches support the use of hippocampal slice preparations to elucidate epileptic mechanisms. On the other hand, the pitfall of in vitro recording is that the environment of acute brain slice preparations is different from the in vivo condition. We make a slice by cutting brain tissue and that causes traumatic injury: reactive gliosis occurs in the acute hippocampal slice (Takano et al., 2014). Also, artificial cerebrospinal fluid does not exactly reproduce actual cerebrospinal fluid. Thus, results from in vitro hippocampal slice preparations should be confirmed by in vivo electrophysiological recordings or behavioral tests as much as possible. For that reason, both in vivo and in vitro electrophysiological recordings are useful, and both are essential for epilepsy research.

THREE APPROACHES FOR REPRODUCING THE CONDITIONS OF KETOGENIC DIET FEEDING IN HIPPOCAMPAL SLICES

The major difficulty in using acute hippocampal slices for ketogenic diet research is the inability to precisely reproduce or maintain the condition of diet therapy in vitro preparation. In vivo recording clearly does not have this problem, because it uses the whole body of experimental animals and diet-altered metabolism is maintained (Koranda et al., 2011; Masino et al., 2011). Therefore, special strategies must be implemented for examining mechanisms of the ketogenic diet using hippocampal slice preparations; in this section, we review three of these approaches (Figure 21.1).

Direct Application of Ketone Bodies

The ketogenic diet was developed to mimic fasting, which alleviates epileptic seizures (Wilder, 1921). Ketogenic diet increases ketone bodies (β-hydroxybutyrate, acetoacetate, acetone), synthesized from free fatty acids in the liver (Masino and Rho, 2012) and then used for energy in the brain instead of glucose (Masino et al., 2009). Chronic ketosis is one hallmark of the ketogenic diet. Therefore, one approach to reproducing a ketogenic diet in a hippocampal slice is direct application of ketone bodies, revealing whether ketone bodies regulate neuronal activity directly.

In this paradigm, hippocampal slices are taken from control diet–fed animals and dissolved ketone bodies are applied in an extracellular solution such as artificial cerebrospinal fluid (Figure 21.1A1,
Table 21.1. Thio et al. reported that direct application of ketone bodies had no effect on synaptic activity in acute hippocampal slices from Sprague-Dawley (SD) rats. They recorded evoked field excitatory postsynaptic potentials (fEPSP) and population spikes (PS) in the CA1 region stimulated by Schaffer collateral fibers, and applied mixed ketone bodies (1 mM acetoacetate and 2 mM β-hydroxybutyrate) to the slices (Thio et al., 2000). A 20-minute application of ketone bodies did not change either fEPSP slope or PS amplitude. They also recorded potassium channel blocker 4-aminopyridine-induced epileptiform discharge from the dentate granule cell layer and CA3 region, and reported that application for 105 minutes did not change the frequency or duration of these ictal events. Kimura et al. also reported that application of mixed ketone bodies (1 mM each acetoacetate and β-hydroxybutyrate) for 20 minutes did not change the high-frequency tetanic stimulation-induced long-term potentiation (LTP) recorded from the CA1 region in acute hippocampal slices from Wistar rats (Kimura et al., 2012). Similar results were reported that mixed ketone bodies (1 mM acetoacetate and 2 mM β-hydroxybutyrate) had no effect on CA1 region synaptic transmission or theta burst-induced LTP in SD rat acute hippocampal slices (Youssef, 2015).

A unique approach was used by Samoilova et al. (Samoilova et al., 2010). They made organotypic hippocampal slices, which were cultured with low glucose and 10 mM β-hydroxybutyrate medium for at least 3 days. This chronic in vitro ketosis, however, did not alleviate intrinsic or induced epileptiform discharges (but was neuroprotective). All of these studies concluded that ketone bodies do not directly affect synaptic transmission in the rat hippocampus.

Other studies, however, have found positive results. Juge et al. (2010) made acute hippocampal slices from C57BL/6 mice and incubated the
<table>
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<th>Reference</th>
<th>Animal</th>
<th>Manipulation</th>
<th>Recording</th>
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<tbody>
<tr>
<td>Thio et al., 2000</td>
<td>SD rat</td>
<td>1 mM AA and 2 mM βHB for 20 min.</td>
<td>fEPSP and PS recordings</td>
<td>No change</td>
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<tr>
<td>Kimura et al., 2012</td>
<td>Wistar rat</td>
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<td>fEPSP recording</td>
<td>No change</td>
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<tr>
<td>Youssef, 2015</td>
<td>SD rat</td>
<td>1 mM AA and 2 mM βHB</td>
<td>PS recording</td>
<td>No change</td>
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<td>Samoilova et al., 2010</td>
<td>Wistar rat</td>
<td>10 mM βHB for 3 days (slice culture)</td>
<td>Whole-cell patch clamp recording</td>
<td>Reducing frequency and amplitude of mEPSC in CA1 pyramidal neurons</td>
</tr>
<tr>
<td>Juge et al., 2010</td>
<td>C57BL/6 mouse</td>
<td>10 mM AA for 2 hrs</td>
<td>Single channel recording</td>
<td>Increasing open probability of K\textsubscript{ATP} channels in granule cells</td>
</tr>
<tr>
<td>Tanner et al., 2011</td>
<td>C57BL/6 mouse</td>
<td>2 mM βHB for 40 min</td>
<td>Multielectrode recordings</td>
<td>Reducing spontaneous seizure-like events</td>
</tr>
<tr>
<td>Kim et al., 2015</td>
<td>Kcna1-null mouse</td>
<td>5 mM βHB and 1 mM AA for 2 weeks (slice culture)</td>
<td>Multielectrode recordings</td>
<td>Reducing spontaneous seizure-like events</td>
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**Direct Application of Ketone Bodies**

**Controlling glucose and ATP levels with whole-cell patch clamp.**

**Acute hippocampal slices from ketogenic diet-fed animals.**

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<th>Animal</th>
<th>Manipulation</th>
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<tr>
<td>Kawamura et al., 2010</td>
<td>SD rat and C57BL/6 mouse</td>
<td>Increased [ATP], and reduced [glucose]</td>
<td>Whole-cell patch clamp recording</td>
<td>Causing hyperpolarization in CA3 pyramidal neurons</td>
</tr>
<tr>
<td>Stafstrom et al., 1999</td>
<td>SD rat</td>
<td>KD (4.8:1 ratio) for 6-8 weeks</td>
<td>fEPSP and PS recordings</td>
<td>Reducing spontaneous seizure-like events</td>
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<td>Simeone et al., 2015</td>
<td>Kcna1-null mouse</td>
<td>KD (6:1 ratio) for 11-15 days</td>
<td>Multielectrode recordings</td>
<td>Reducing spontaneous seizure-like events</td>
</tr>
<tr>
<td>Bough et al., 2006</td>
<td>SD rat</td>
<td>KD (6:1 ratio) for 20 days</td>
<td>fEPSP recording</td>
<td>Inhibiting reduced glucose-induced depressions</td>
</tr>
<tr>
<td>Kawamura et al., 2014</td>
<td>SD rat and C57BL/6 mouse</td>
<td>KD (6:1 ratio) for 13-18 days</td>
<td>PS recording</td>
<td>Reducing evoked seizure-like bursting</td>
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AA, acetoacetate; βHB, β-hydroxybutyrate; fEPSP, field excitatory postsynaptic potentials; PS, population spikes; mEPSC, miniature excitatory postsynaptic currents; K\textsubscript{ATP} channels, ATP-sensitive potassium channels; [ATP], intracellular ATP concentration; [glucose], extracellular glucose concentration; KD, ketogenic diet.
slices with 10 mM acetoacetate for over 2 hours, after which they recorded EPSPs from CA1 pyramidal neurons using whole-cell patch clamp. Frequency and amplitude of miniature EPSPs (mEPSP) from acetoacetate-incubated slices were significantly reduced compared with control slices. Ketone bodies inhibited valinomycin-evoked glutamate uptake by purified vesicular glutamate transporter (VGLUT), suggesting that ketone bodies inhibit synaptic transmission with reduction of glutamate release via direct ketone body-induced suppression of glutamate uptake into vesicles. Importantly, they also investigated the behavioral effects of ketone bodies. Seizures in Wistar rats induced by intrahippocampal 4-aminopyridine were moderated by intrahippocampal 10 mM acetoacetate, both infused by microdialysis. These results clearly show that direct application of ketone bodies modulates synaptic transmission in hippocampal slices and reduces seizure activity in vivo (Juge et al., 2010). In addition, Tanner et al. (2011) recorded single channel activity from dentate granule neurons after incubating acute hippocampal slices from C57BL/6 mice with 2 mM β-hydroxybutyrate for over 40 minutes. Preincubation with this ketone body increased steady-state and stimulus-elevated open probability of ATP-sensitive potassium (K\textsubscript{ATP}) channels, which contribute to the slow afterhyperpolarization after action potential bursts to modulate spontaneous firing, suggesting that direct ketone body-mediated opening of K\textsubscript{ATP} channels in dentate granule neurons may act as a seizure gate in the hippocampus. Similar results were reported from neurons of the substantia nigra in coronal midbrain slices of rats and mice from same laboratory (Ma et al., 2007). Kim et al. recorded from organotypic hippocampal slices that were cultured with 5 mM β-hydroxybutyrate and 1 mM acetoacetate medium for 2 weeks (Kim et al., 2015). They used Kcnal-null mice (C3HeB/FeJ background) lacking voltage-gated potassium (Kv 1.1) channels, which is thought to be a model for several types of epilepsy including human temporal lobe epilepsy. Extracellular multielectrode array recordings showed spontaneous seizure-like events in organotypic hippocampal slice cultures from Kcnal-null mice. The application of ketone bodies for 2 weeks attenuated the seizure-like events generated by the mutant tissue. They also applied 5 mM β-hydroxybutyrate in vivo using subcutaneously implanted osmotic minipumps to Kcnal-null mice and reported that administration of this ketone body reduced the number of seizures (Kim et al., 2015).

In sum, several studies have used direct application of ketone bodies in hippocampal acute slices or organotypic cultures, and both positive and negative results have been found. Negative and positive studies used rats and mice, respectively, so a simple explanation is that the discrepancy arises from species differences. However, this is unlikely, because ketogenic diet is known to reduce behavioral seizures in both rats (Appleton and DeVivo, 1974; Bough et al., 2002; Bough et al., 2006; Hori et al., 1997; Zhao et al., 2004) and mice (Hartman et al., 2008; Kwon et al., 2008; Noh et al., 2003; Rho et al., 1999; Uhlemann and Neims, 1972). The methods for applying ketone bodies varied in these reports, including concentration of ketone bodies, time for application, and application pathway (perfusion or preincubation), and these might contribute to interstudy variation. Using similar protocols for direct application of ketone bodies would be useful for finding common mechanisms.

### Controlling Glucose and ATP Levels with Whole-Cell Patch Clamp

Another approach mimics the altered metabolism during ketogenic diet treatment using single-cell patch clamp recording. Fasting and ketogenic diet are thought to cause anticonvulsant effect by changing brain metabolism. It is well known that ketogenic diet causes a stable, mild hypoglycemia in humans (Huttenlocher, 1976) and rodents (Bough et al., 2006). Interestingly, plasma glucose level correlates with the antiepileptic effect of the ketogenic diet (Mantis et al., 2004), indicating that extracellular glucose is mechanistically relevant. Intracellular conditions are also thought to be changed by ketogenic diet-altered metabolism, and the final product of brain energy metabolism is ATP. Several studies have reported that ketogenic diet increases brain ATP levels in humans (Pan et al., 1995) and rodents (DeVivo et al., 1978; Nakazawa et al., 1983; Nylen et al., 2009). Therefore, intracellular ATP is a promising target for mimicking the ketogenic diet in vitro. From these reports, reducing and increasing extracellular glucose and intracellular ATP, respectively, might in combination reproduce mechanistically important ketogenic diet conditions in acute hippocampal slices (Figure 21.1A2). As mentioned in section 2, it is easy to change extracellular solution with an in vitro brain slice (including moderately lowering glucose), but trickier to change the intracellular milieu. The whole-cell patch clamp
Chapter 21: Ketogenic Diet in a Hippocampal Slice

The technique is one of the methods for recording a single cell (Kawamura et al., 2004). This technique allows physical exchange between the intracellular fluid and the artificial intracellular solution in the recording pipette, which allows us to modify the intracellular fluid composition of the recorded neuron experimentally (Figure 21.1A2, Table 21.1) including elevating intracellular ATP. We found that increased intracellular ATP and reduced extracellular glucose caused outward current (or hyperpolarization when recording membrane potential) in CA3 pyramidal neurons (Kawamura et al., 2010). We recorded from CA3 pyramidal neurons with the whole-cell patch clamp technique in acute hippocampal slices from control diet–fed SD rats or C57BL/6 mice. Reduced extracellular glucose and increased intracellular ATP caused outward current (or hyperpolarization when recording membrane potential) in CA3 pyramidal neurons. This outward current was dose-dependent for both extracellular glucose and intracellular ATP, and importantly it was found in both rats and mice. Pharmacological and genetic experiments demonstrated that when intracellular ATP was sufficient or increased, reduced extracellular glucose opened pannexin-1 channels and released intracellular ATP to the extracellular space. Released ATP was rapidly hydrolyzed to adenosine, which activated adenosine A1 receptors with subsequent opening of KATP channels. Opening of these potassium channels caused hyperpolarization and reduced excitability. These results indicate that mimicking the ketogenic diet condition with increased ATP and reduced glucose reduces excitability in hippocampal CA3 pyramidal neurons with autocrine modulation via adenosine A1 receptors, and this might be one of the key mechanisms of the anticonvulsant effects of the ketogenic diet in vivo (Figure 21.2). This approach for reproducing ketogenic diet conditions in acute hippocampal slice is useful to elucidate detailed mechanisms within single neurons. However, it mimics only two of the aspects of ketogenic diet feeding. Further examinations using behavioral tests and recordings from in vivo ketogenic diet-fed animals are needed to link the results from this approach to the effects of ketogenic diet feeding.

FIGURE 21.2 Schematic of pannexin-1 channel-adenosine A1 receptor-KATP channel autocrine regulation in CA3 pyramidal neurons and relationship to ketogenic diet. Ketogenic diet increases ketone body levels and reduces glucose levels. Ketone bodies might increase intracellular ATP production, and increased ATP is released to the extracellular space due to reduced glucose-induced opening of pannexin-1 channels (panx). After breakdown to adenosine by nucleotidases, activated adenosine A1 receptors (A1R) open KATP channels (KATP), which hyperpolarizes CA3 pyramidal neurons. This hyperpolarization reduces neuronal hyperexcitability and causes the ketogenic diet's antiseizure effects. TCA cycle, tricarboxylic acid cycle; ETC, electron transport chain.
Acute Hippocampal Slices from Ketogenic Diet–Fed Animals

A third approach is possibly the most direct and useful way for investigating mechanisms of ketogenic diet because it uses acute hippocampal slices from ketogenic diet–fed animals (Figure 21.1b, Table 21.1). The question about this approach is whether or not the intra- and extracellular milieu produced by ketogenic diet feeding can be maintained after making and incubating brain slices. However, four reports show that it can work successfully. Stafstrom et al. reported that ketogenic diet (fat:[protein+carbohydrate] ratio of 4.8:1) induced antiseizure effects in acute hippocampal slices from kainic acid–treated rats (Stafstrom et al., 1999). They recorded fEPSP and PS from area CA1 from SD rats fed a ketogenic diet for 6–8 weeks. Synaptic transmission was not significantly different between slices from control diet–fed and ketogenic diet–fed rats. The frequency of kainic acid–induced spontaneous seizures was significantly lower in slices from ketogenic diet–fed rats than from control diet–fed rats. Similar results were reported by Simeone et al. using extracellular multielectrode array recordings in acute hippocampal slices from ketogenic diet–fed Knca1-null mice (Simeone et al., 2014). The pathologic seizure-like events generated in Knca1-null mice slice were diminished by ketogenic diet (6:1 ratio) treatment for 11–15 days. However, mossy fiber-CA3 dendritic field potential slopes and fiber volley amplitudes of mossy fiber are not significantly different between slices from control diet–fed and ketogenic diet–fed rats. The effects were lost after slices were incubated in 10 mM glucose for over 3.5 hours. They concluded that synaptic transmission in hippocampal slices from ketogenic diet–fed rats were more resistant to reduced glucose than slices from control diet–fed rats (Bough et al., 2006). We also reported that ketogenic diet caused antiseizure effects in acute hippocampal slices of rats and mice (Kawamura et al., 2014). Bough et al. recorded medial perforant pathway-evoked fEPSPs from the dentate molecular layer in acute hippocampal slices from SD rats fed a ketogenic diet (6:1 ratio) for 13–18 days. Excitability and bicuculline-induced bursting were significantly inhibited by reduced extracellular glucose concentration in slices from ketogenic diet–fed rats and mice but were not changed by reduced extracellular glucose in slices from control diet–fed rodents. Ketogenic diet–induced suppression of bicuculline-induced bursting was inhibited by adenosine A1 receptor antagonist and did not occur in slices from adenosine A1 receptor knockout mice. Antagonism of KATP channels or pannexin-1 channels inhibited the ketogenic diet–induced suppression of bicuculline-induced bursting. These results suggest that ketogenic diet causes antiseizure effects through a pannexin-1 channel-adenosine A1 receptor-KATP channel autocrine pathway (Figure 21.2), the same pathway revealed by the whole-cell patch clamp technique for mimicking ketogenic diet conditions as described previously (Kawamura et al., 2010).

These studies used successfully acute hippocampal slices from ketogenic diet–fed rodents to elucidate the changing of neuronal activities underlying this treatment. Interestingly, reducing extracellular glucose concentration is thought be one of the most important points for reproducing the effects of ketogenic diet in this approach. Synaptic transmission in hippocampal slices from ketogenic diet–fed rodents was not different from that in slices from control diet–fed rodents when extracellular glucose concentration was standard in all three reports (however, evidence is mounting that this standard glucose concentration for acute brain slices is higher than physiological brain glucose levels; Kealy et al., 2013; Lowry and Fillenz, 2001; Shram et al., 1997). Reduced glucose reveals the difference between ketogenic diet– and control diet–fed animals in two of these studies (Bough et al., 2006; Kawamura et al., 2014), which parallels the finding that the anticonvulsant effect of the ketogenic diet is correlated with plasma glucose levels (Mantis et al., 2004). Therefore it would be useful to make extracellular glucose concentrations lower than standard to reproduce or maintain effects of the ketogenic diet in acute hippocampal slices.

CONCLUSIONS

Here we illustrate three approaches for researching anticonvulsant mechanisms of ketogenic diets by using electrophysiological recording from hippocampal slices. The usefulness of hippocampal slices is that it is easy to elucidate the details of neuronal modulation by ketogenic diet, as
shown in Figure 21.2. All three approaches have contributed to finding detailed potential mechanisms underlying ketogenic diet effects including VGLUT modulation, \( K_{\text{ATP}} \) opening, activation of adenosine receptors, and ATP release from pannexin channels. It is clear that electrophysiological recording from hippocampal slices is a good tool for ketogenic diet research. However, all three approaches need some steps for reproducing ketogenic diet effects in vitro such as ketone application, reduced extracellular glucose, and increased intracellular ATP. These methods are not direct measurements of the in vivo anticonvulsant effects of ketogenic diet. A combination of both in vivo and in vitro recordings should provide further explanations concerning the key anticonvulsant mechanisms underlying ketogenic diet treatment.

ACKNOWLEDGMENT
We thank Dr. David N. Ruskin for comments on this manuscript. We acknowledge the support of the Jikei University Research Fund to MK.

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Chapter 21: Ketogenic Diet in a Hippocampal Slice


Metabolic Therapy and Pain

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INTRODUCTION
Across all cultures, patients with chronic pain have among the lowest reported quality-of-life scores of any medical condition and have comorbid mood or anxiety disorders more frequently than the normal population (Becker et al., 1997; Gureje et al., 1998). In addition, pain has a notable negative impact on economies, with high direct costs (e.g., physician visits, physiotherapy, medication) and, more strikingly, lowered productivity (e.g., lost work days, reduced performance at work) characterized in numerous countries around the world (Phillips, 2006).

Much chronic pain falls into two distinct categories: inflammatory pain and neuropathic pain. Chronic inflammation is typically accompanied by pain due to the release of prostaglandins and their consequent sensitization of sensory neurons (Mense, 1983). Common forms of inflammatory pain are rheumatoid arthritis, chronic inflammatory bowel disease, pancreatitis, back pain, and some cancer pain. In contrast, neuropathic pain is caused by lesion or dysfunction of nervous tissue; common forms are associated with diabetes, herpes zoster infection (shingles), stroke, multiple sclerosis, cancer chemotherapy, and both HIV infection and antiretroviral HIV treatment. Neuropathic pain can also be idiopathic (Lauria et al., 2014).

Severe pain is typically treated with medication, most commonly cyclooxygenase inhibitors such as nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids. Unfortunately, these drugs can present serious dilemmas for both patients and healthcare providers. Many patients view opioids as undesirable because of their fear of addiction or their experiences with cognitive or other side effects (Welshman, 2005); prescription of opioids is contraindicated specifically in patients with pre-existing substance abuse issues. In addition, given present levels of prescription drug abuse (Allread and Paul, 2014; Manchikanti, 2006), there is an appropriate hesitancy of healthcare providers to prescribe or even fill prescriptions for opioids (Glajchen, 2001; Kim et al., 2000). The NSAIDs, conversely, have little to no addiction liability but are poorly effective against neuropathic pain (Moore et al., 2015) and can have serious cardiovascular and gastrointestinal side effects (Brune and Patrignani, 2015). Thus, effective and nonaddictive treatments with few side effects are needed. With the understanding that chronic pain syndromes likely involve derangement of homeostasis, there is an increased interest in applying treatments that aim to normalize metabolism, namely, metabolic therapies. Many metabolic therapies involve dietary changes. Whereas some dietary metabolic therapies narrowly address specific genetic disorders that include pain as a symptom (Roe et al., 2008), this survey of the literature focuses on more wholesale dietary changes, some long established, and their effects on common types of acute, inflammatory, and neuropathic pain.

FASTING
One type of dietary metabolic therapy with a history of use in pain is fasting, or a total or near total (<500 kcal/d) reduction in calorie intake (Michalsen, 2010). Fasting has been practiced for thousands of years in many cultures as a treatment for various disorders (as well as for religious reasons). Biochemically, fasting results in lowered levels of circulating glucose, as intake stops and glycogen stores are metabolized, and in greatly enhanced circulating levels of molecules known as ketone bodies, which are produced by the liver from fatty acids and can be used as fuel for the tricarboxylic acid cycle in the relative absence of glucose. Subjects report a counterintuitive reduction in hunger after a few days, as use of ketone bodies becomes maximal (Michalsen, 2010). Fasting can be used as a treatment for obesity and metabolic syndrome, and is anticonvulsant in epileptic patients (and so spawned the ketogenic diet, see below). In the context of pain, some of the earliest work concerned rheumatoid arthritis (Eisenberg,
1956). Seven days of fasting significantly alleviated joint pain and stiffness in rheumatoid arthritis sufferers (Kroeker et al., 1984; Michalsen et al., 2005; Sköldstam et al., 1979), apparently through anti-inflammatory actions including modified neutrophil function (Hafström et al., 1988; Udén et al., 1983). Eight fasting days significantly improved pain scores in osteoarthritis, an effect that lasted weeks after the end of fasting (Schmidt et al., 2010). One to 2 weeks of fasting significantly alleviated pain in the pain disorder fibromyalgia (Michalsen et al., 2005; Michalsen et al., 2013). Given the commonness of mood and anxiety disorders in chronic pain (Gureje et al., 2008), fasting has the additional benefit of enhancing mood after several days, an effect not limited to pain sufferers (Michalsen, 2010).

These clinical antinociceptive effects are supported by animal experiments. In a rat model of postischemic pain, allodynia (i.e., the perception of normally nonpainful stimuli as painful) to cold and tactile stimulation in the ischemic paw was significantly reduced if the animal had been fasted for 18 hours prior to ischemia. (Given the higher metabolic rate of rats, this time is comparable to a longer fast in a human.) In addition, the effect of the fast was totally reversed by glucose injection during the ischemic event, highlighting the importance of lowered glucose (Ross-Huot et al., 2011). The anti-inflammatory effects of fasting seem to be mediated by ketosis (a state of elevated ketone bodies) which limits oxidative stress and production of free radicals and reactive oxygen species (Veech, 2014).

Unfortunately, the clinical benefits of prolonged fasting for rheumatoid arthritis are not long lasting (unlike for osteoarthritis; Sköldstam et al., 1979), and fasting is necessarily a time-limited undertaking, especially for subjects who do not have extra adipose tissue to burn through over days to weeks. An alternative procedure is intermittent fasting, usually taking the form of every-other-day (EOD) fasting, which can be implemented for extended periods. Notably, in humans a 24-h fast is sufficient to limit activation of the NLR family, pyrin domain containing 3 (NLRP3) inflammasome activation (Traba et al., 2015), which is associated with hyperalgesia (Bullón et al., 2015); therefore EOD fasting might be beneficial in rheumatoid arthritis. This effect on the NLRP3 inflammasome seems to be mediated by ketone bodies (Youm et al., 2015). Studies in humans (Rothschild et al., 2014) and rodents (Poon et al., 2006; Tajes et al., 2010; Tikoo et al., 2007) have shown positive effects of EOD fasting on various aspects of health, but none have addressed pain. Although EOD fasting generally causes weight loss clinically (Varady et al., 2015), there is minimal body weight change in many rodent studies (Tajes et al., 2010; Tikoo et al., 2007). Therefore, studies of EOD fasting in animals could dissociate weight loss from antinociceptive effects, essentially impossible in clinical studies of prolonged standard fasting. Animal studies suggest that subjects who do not need or wish to lose weight may limit this effect of EOD fasting by exercising (Sakamoto and Grunewald, 1987).

**CALORIE RESTRICTION**

Those who wish for pain alleviation but who cannot tolerate long periods of time without food might consider instead calorie restriction (CR). Typically, this procedure limits total daily calorie intake to 60% of normal (not less than ~900 kcal/d). There is abundant experimental evidence for antinociceptive effects of CR on acute pain (Wideman et al., 1996), inflammatory pain (Hargraves and Hentall, 2005), and heat hyperalgesia in arthritic models (Jurcovicova et al., 2001). Clinically, CR (applied for 12 weeks to 1 year, and typically combined with exercise) in overweight patients reduced back pain (thought to involve inflammation; Roffey et al., 2011), knee pain (White et al., 2015), overall body pain (Landeta-Díaz et al., 2013), osteoarthritic pain (de Luis et al., 2012), and prediabetic neuropathic pain (Smith et al., 2006). All these benefits could be secondary to weight loss, which would lessen physical wear on joints and backs (as suggested in most of these papers), though this explanation does not appear to apply for the positive outcome in diabetic neuropathy, in which pain reduction related to actual reinnervation of the skin (Smith et al., 2006). Testing of CR treatment in fibromyalgia (see below), which is not limited to overweight individuals, could dissociate weight loss from CR-related pain relief.

Apart from possible weight loss effects, it is unclear whether ketosis contributes to this antinociception, as none of these clinical studies measured blood or urinary ketones. However, improved glycemic control might be involved. As already noted, low circulating glucose is necessary for fasting amelioration of postischemic pain (Ross-Huot et al., 2011). A meta-analysis of studies of diabetic neuropathy (including almost 8,000 patients) concluded that more aggressive glucose control was associated with a lower incidence of neuropathic pain and improved electrophysiological measures (Callaghan et al., 2012). In animal studies, glycemic control partially alleviated diabetic neuropathy (Yorek et al., 2014). Also, partial glycolytic inhibition by 2-deoxy-D-glucose...
decreases sensation of acute pain (Bodnar et al., 1979) by central mechanisms (Bodnar et al., 1981). This action does not involve endogenous opioids, but seems to involve the ubiquitous neuromodulator adenosine, which has broad analgesic effects (Sawynok, 2015; Yamaoka et al., 2013) and is released by 2-deoxy-D-glucose or mildly lowered glucose (Kawamura et al., 2014; Zhao et al., 1997).

Notably, fibromyalgia has been associated with disordered glycolysis (Eisinger et al., 1994). One of the regulators of glycolysis is the enzyme adenosine monophosphate-activated protein kinase (AMPK; Carling, 2005), which has also been linked to sensitization of nociceptors in pain states (Tillu et al., 2012). Cells from fibromyalgia sufferers displayed underactive AMPK and a related range of metabolic impairments (Alcocer-Gómez et al., 2015; Bullón et al., 2016). These problems were reversed by incubation with serum from CR-treated mice (Alcocer-Gómez et al., 2015). Pharmacological blockade of AMPK produced hyperalgesia in mice that was reversed by CR (Bullón et al., 2015). In fibromyalgia sufferers, pharmacological activation of AMPK reversed metabolic impairments and significantly improved a range of symptoms including pain (Bullón et al., 2015). Thus CR seems to restore balanced metabolism through an AMPK/glycolysis pathway, one that can be engaged pharmacologically with AMPK activators.

**Ketogenic Diet**

Those who wish for pain alleviation but who can tolerate neither CR nor fasting might consider the ketogenic diet (KD). This diet was based on the hypothesis that the beneficial effects of fasting in epilepsy were due to the elimination of carbohydrates (Wilder, 1921a, 1921b); thus, the KD minimizes carbohydrates and replaces the lost calories with fats, and is in fact strongly anticonvulsant (Dressler et al., 2015; Hallböök et al., 2015; Megaw and Wilmshurst, 2015; Walker and Said, 2015) and mounting evidence suggests that it is antiepileptogenic (Gama et al., 2015; Hu et al., 2011; Jiang et al., 2012; Lusardi et al., 2015; Muller-Schwarze et al., 1999; Neal et al., 2008; Su et al., 2000; Todorova et al., 2000; Xu et al., 2006). The KDs result in ketosis and moderately lowered glucose like fasting or sufficiently strong CR. Our animal studies showed that KDs have a clear antinociceptive effect in acute thermal pain. This effect was present in both male and female rats (Figure 22.1) and was long-lasting (Figure 22.2).

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**FIGURE 22.1** Sprague-Dawley rats on a control diet or a 78% fat KD were challenged with a temperature-response curve for nocifensive behavior. One temperature was tested per day. Top and bottom X-axes illustrate hotplate temperature and days on diet, respectively. Dietary treatments began at 3–3½ weeks of age. Y-axis indicates latency to avoidance behavior, e.g. paw lifting, licking, or fluttering. All tests had a 60-s ceiling. At left, KD treatment in young male rats causes heat hypoalgesia at moderate 49°C, 50°C, and 51°C temperatures. Diet F = 158.4, p < .001; Temperature F = 158.4, p < .001; Diet × Temperature interaction F = 4.0, p < .001; n = 18–20. Neuman-Keuls comparisons at each temperature: *p < .05, **p < .01, ***p < .001. Adapted from Ruskin et al. (2013). At right, KD treatment in young female rats also causes heat hypoalgesia at the same temperatures. Diet F = 28.2, p < .001; Temperature F = 134.2, p < .001; Diet × Temperature interaction F = 9.6, p < .001; n = 12–16. All points are mean ± standard error. Neuman-Keuls comparisons at each temperature: ***p < .001. Unpublished data.
had a slower onset than ketosis and lowered blood glucose (Figure 22.2), both of which were already significant at 2 days of KD feeding (Ruskin et al., 2013), suggesting that these biochemical changes are not directly involved.

Clinically, the KD was used very early as a treatment for pain (Maggioni et al., 2011). For instance, in one study KD feeding improved or completely controlled migraine in 39 of 50 patients (Baborka, 1930). More recent results support its antimigraine efficacy (Di Lorenzo et al., 2013, 2015). The KD treatment was effective in treating inflammatory bowel syndrome, including pain symptoms (Austin et al., 2009). Overall body pain was significantly improved in one study of overweight subjects (Guldbrand et al., 2014) and was very near significance in another (Yancy et al., 2009); quality of life was significantly improved in both studies. One of these studies specifically involved overweight diabetics (Guldbrand et al., 2014), but did not parse the types of pain involved, so it is unknown whether KD feeding alleviated neuropathic pain in these patients. We found no beneficial effect of KD in models of nerve constriction and chemotherapeutic neuropathic pain (Masino and Ruskin, 2013); it remains unclear whether KD is poorly effective against neuropathic pain generally, or rather has type-specific positive effects. Regardless, abundant evidence shows that KDs are clinically beneficial in diabetes (e.g. Adams, 1931; Mobbs et al., 2013).

Antinociceptive effects of the KD might be strongest in inflammatory pain, as this diet is anti-inflammatory (Pérez-Guisado and Muñoz-Serrano, 2011; Ruskin et al., 2009; Sullivan et al., 2004; Tendler et al., 2007), and this effect might be due largely to ketosis, as reviewed earlier for fasting. Such findings might appear contradictory to much published literature showing that high-fat diets promote inflammation (Johnson and Makowski, 2015); however, this literature refers to diets such as the so-called Western diet, high in fat but not low in carbohydrates. The metabolic response to dietary fats differs greatly depending on the presence of carbohydrates: the high-fat-plus-carbohydrate diet promotes fat storage, whereas the high fat, low-carbohydrate diet promotes fat metabolism.

Apart from anti-inflammatory effects, some antinociceptive effects of the KD might relate directly to its high fat content. The receptor GPR40 binds and is activated by free medium- and long-chain fatty acids, and thus is sometimes called free fatty acid receptor 1 (Covington et al., 2006). Although this receptor may be considered a nutrient sensor, it is expressed in the central nervous system and turns out to affect nociception. In animal testing, synthetic GPR40 agonists and natural ligands injected intrathecally or intracerebroventricularly reduce inflammatory and neuropathic pain in numerous models (Harada et al., 2014; Karki et al., 2015; Nakamoto et al., 2013; Nakamoto et al., 2015). There are electrophysiological correlates of these behavioral effects (Karki et al., 2015). Conversely, GPR40 knockout augments the arthritic phenotype in an osteoarthritis model (Monfoulet et al., 2015), although pain behavior was not studied in this report. In “Western” high-fat diets, antinociceptive effects of GPR40 activation might be unable to compete against the pro-inflammatory nature of these diets.

Regarding epilepsy, there is some controversy over whether the KD is more effective when combined with CR (Bough et al., 1999b; Raffo et al., 2008). Certainly, KD plus CR will lead to stronger ketosis and a more pronounced lowering of circulating glucose. If the KD’s antinociceptive effects are primarily mediated by fatty acids, however, the addition of CR should add little benefit to this outcome.
POLYUNSATURATED FATTY ACIDS

Some have suggested that many of the beneficial effects of the KD in epilepsy and general health are due to enhanced dietary levels of polyunsaturated fatty acids (PUFAs; Fraser et al., 2003; Fuehrlein et al., 2004; Paoli et al., 2015; Taha et al., 2005; Xu et al., 2008), based on anticonvulsant effects of PUFAs in some studies (Taha et al., 2009; Voskuyl et al., 1998; Yehuda et al., 1994). These effects are controversial (Dahlín et al., 2007; Dupuis et al., 2015; Willis et al., 2009). Nevertheless, whereas saturated fatty acids bind to GPR40, PUFAs additionally act on other molecular targets that could modulate pain (see below), and PUFAs and their epoxide metabolites indeed help in inflammatory and neuropathic pain (Wagner et al., 2014). In animal studies, for instance, elimination of dietary PUFAs promotes the neuropathic pain phenotype after nerve injury (Martin and Avendaño, 2009). Conversely, addition of fish oil (rich in PUFAs) to the diet reverses diabetic neuropathy including derangement of pain sensation (Coppey et al., 2012; Coppey et al., 2015; Redivo et al., 2015). Clinically, PUFA efficacy is good in inflammatory pain (Goldberg and Katz, 2007; Lee et al., 2012) but not osteoarthritis (Boe and Vangsness, 2015).

Some researchers believe that the various health benefits of PUFA supplements are due to the high ratio of ω-3 to ω-6 PUFAs, two types of PUFA that differ in the location of double bonds in their carbon backbones. Fish oil has a high ω-3/ω-6 ratio. Thus, animal studies have specifically enriched diets with ω-3 PUFAs and found that this reduces acute pain and neuropathic pain after spinal cord contusion (Escudero et al., 2015; Figueroa et al., 2013). An additional benefit was that ω-3 PUFAs reduced the development of tolerance to morphine (Escudero et al., 2015). On the other hand, there is other evidence that ω-3 PUFAs promote neuropathic pain (Pérez et al., 2005). In clinical studies, ω-3 PUFA supplementation improved pain and quality of life in chronic headache sufferers (Ramsden et al., 2013; Ramsden et al., 2015).

How might PUFAs influence pain? PUFAs are agonists of the peroxisome proliferator-activated receptors (PPARs), another type of nutrient sensor that also happens to be involved in nociception (Freitag and Miller, 2014). For instance, activation of PPARγ subtype alleviates inflammatory and neuropathic pain in animal models (Morgenweck et al., 2010; Morgenweck et al., 2013); PPARα agonists given to patients reduce pain in a number of inflammatory and neuropathic conditions (Freitag and Miller, 2014). Additionally, PUFAs augment voltage-activated A-type (K1.4) potassium channels (Tigerholm et al., 2012; Xu et al., 2008), which could reduce hyperexcitability of sensitized neural tissue. Certain PUFAs also block pain due to activation of the heat-sensing transient receptor potential vanilloid subtype I (the “capsaicin receptor”), an inflammation-related receptor expressed by peripheral nociceptive neurons (Matta et al., 2007).

OTHER MECHANISMS

Ketone bodies, augmented in fasting, CR, and KD feeding, might act on pain through multiple mechanisms. As already noted, a ketone body–based metabolism limits inflammation. A ketone body–based metabolism also produces ATP more efficiently than one based on glucose (Veech, 2004). Thus, ketone body treatment leads to elevated levels of ATP and phosphocreatine (for instance, Deng-Bryant et al., 2011; Kim et al., 2010; Tieu et al., 2003), which might allow restoration of energy homeostasis in distressed and painful tissue. Besides effects on energy, ketone body treatment modulates glutamate and its receptors (Chmiel-Perzyńska et al., 2011; Donevan et al., 2003; Juge et al., 2010; Lund et al., 2009; Zarnowsky et al., 2012), γ-aminobutyric acid and its receptors (Erecińska et al., 1996; Yang et al., 2007), potassium channels (Giménez-Cassina et al., 2012; Ma et al., 2007; Tanner et al., 2011), and the receptor PUMA-G (Taggart et al., 2005) in a manner often leading to reduced excitability that could limit activity in sensitized nociceptive systems. Multiple mechanisms acting in concert might be especially effective in normalizing the deranged metabolism related to inflammation and sensitized neural tissue. Future work on ketone bodies will surely be aided by the availability of ketone esters (Kashiwaya et al., 2010; Viggiano et al., 2015), which can be taken orally and are rapidly metabolized to ketone bodies, thus bypassing the need for wholesale dietary changes.

On the other hand, regarding the KD, some have concluded that ketone bodies are not directly involved in its anticonvulsant effect (Bough et al., 1999a, 2000; Harney et al., 2002; Likhodii et al., 2000; Raffo et al., 2005), similar to our interpretation of KD reductions in thermal pain (Ruskin et al., 2013). In the context of pain relief, what might low blood glucose do besides promote ketosis? Such a question is necessarily difficult to address given that the former produces the latter. Fatty acid oxidation, and hence ketone body synthesis, can be blocked pharmacologically in vivo, but such work has
mostly focused on studies of ingestive behavior (Langhans et al., 2011). Fortunately, the ketosis complication is removed in in vitro preparations. With single-neuron recording in hippocampal slices, we investigated a model of the KD in which intracellular ATP was at medium-to-high concentration and extracellular glucose was moderately low. This treatment hyperpolarized the recorded neuron, an effect mediated by ATP released through pannexin channels, metabolized extracellularly to adenosine, which acted on adenosine A_3 receptors to open potassium channels (Kawamura et al., 2010). We suggested that this autocrine inhibitory mechanism is the major contributor to the KD's anticonvulsant effect (Masino et al., 2011). If this same mechanism were recruited in nociceptive brainstem and spinal cord tissue during KD (or fasting or CR) treatment, it would have clear analgesic effects, possibly most strikingly in hyperexcitable sensitized tissue.

Promotion of adenosine transmission by pharmacological means (promoting synthesis, blocking uptake/metabolism, allosterically modulating receptors) ameliorates pain in a wide variety of models (e.g., Hurt and Zylka, 2012; Imlach et al., 2015; Little et al., 2015; Maes et al., 2012; Martins et al., 2013; Street et al., 2011). Even pain treatment by acupuncture and exercise seems to involve enhanced adenosine (Goldman et al., 2010; Martins et al., 2013). The presence of a low-glucose-activated adenosine-based autoinhibitory mechanism remains to be directly shown in brainstem and spinal cord. However, adenosine also has antinociceptive effects in the periphery (Lima et al., 2010; Liu et al., 2013; Poon and Sawynok, 1999; Valério et al., 2009). In addition, immune cells express several types of adenosine receptor that can influence inflammation (Kumar and Sharma, 2009).

There are other nutrient sensors besides GPR40 and the PPARs that could be involved in nociception. GPR41 (free fatty acid receptor 3) is expressed in the peripheral nervous system and is activated by short-chain fatty acids and is yet another molecular target for ketone bodies (Kimura et al., 2011; Won et al., 2013). GPR120 (free fatty acid receptor 4), activated by PUFA's, is known to be anti-inflammatory (Cintra et al., 2012). Studies have yet to investigate any specific involvement in pain for either of these receptors.

Many of the postulated antinociceptive mechanisms of fasting, CR, KD feeding, and PUFA supplementation can be recruited pharmacologically: for example, metformin to control glycemia, selective agonists to activate nutrient sensors, adenosine A_3 receptor agonists to limit neural tissue hyperexcitability. Whereas such shortcuts may be useful in cases where the dietary treatment cannot be followed, they can have their own problems; for instance, the striking bradycardia that occurs after sufficiently strong A_3 receptor activation (Jonzon et al., 1986). Dietary recruitment of these mechanisms will be more physiological. In addition, this review has suggested that some of these dietary treatments are likely to involve multiple mechanisms that might work in synergy—drug treatments would lack this advantage. Multiple mechanisms acting together could be ideal for restoring disordered metabolism. Furthermore, these dietary treatments are all possess a wide range of health benefits, making them attractive for people to optimize their health as well as manage their pain.

**ACKNOWLEDGMENTS**

Supported by National Institutes of Health NS065446 and AT008742.

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Chapter 22: Metabolic Therapy and Pain

205


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Ketogenic Diet, Adenosine, Epigenetics, and Antiepileptogenesis

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INTRODUCTION
Fasting has been known as a means to control epilepsy for thousands of years. Formal studies of fasting in the early years of the 20th century led to the critical observation of elevated ketone bodies excreted during fasting, and diets tailored to elevate ketones were established (Wheless, 2008; Peterman, 1925, 1924). The difficulty of compliance in combination with the availability of antiepileptic drugs diminished enthusiasm for the KD throughout much of the 20th century, but more recent studies in patients refractory to conventional drugs have demonstrated the KD’s clear efficacy for seizure suppression. While the earliest studies suggested efficacy of the KD only in children, several studies show similar therapeutic effects in adults (Barborka, 1930; Sirven et al., 1999; Coppola et al., 2002).

The precise mechanisms by which the KD exerts antiepileptic effects remain unknown. Beneficial effects of the KD can manifest clinically as quickly as several days or as long as 8 months after diet initiation. Diet reversal yields even more perplexing outcomes. In some individuals, seizures return within hours of glucose reintroduction, suggesting an antiseizure mechanism. Others are able to return to a “normal” diet and remain seizure-free, suggesting an antiepileptogenic mechanism. A detailed review of the proposed mechanisms of the KD has been published recently (Masino and Rho, 2012), illustrating that the influences of the diet on metabolic processes are broad. Indeed, this broad range of metabolic influences suggests that the therapeutic benefits of the KD are not limited to epilepsy but might extend into other neurological disorders (Stafstrom & Rho, 2012; Kossof & Hartman, 2012).

The dramatic reduction or elimination of seizures in up to 40% of persons with epilepsy on a KD is a strong argument for continued use of the diet. This is particularly compelling when considering that the individuals in controlled trials of the diet are refractory to conventional antiepileptic drugs and have been suffering from epilepsy for many years at the time the diet is initiated. A more comprehensive understanding of the underlying mechanisms could yield evidence for predictive biomarkers that can identify patients who are most likely to respond well to the KD.

ADENOSINE HOMEOSTASIS AND SEIZURES
The purine ribonucleoside adenosine is a ubiquitous homeostatic regulator of brain activity acting at four G-protein-coupled receptors. Receptors are classified by their second messenger activity (Gi/o: A1R and A3R; or Gs: A2A R and A2B R) and their affinity for adenosine (nM: A1R, A2AR; μM: A2BR, A3R). In the brain, basal adenosine levels are typically in the nM range, regulated largely through a balance between ATP breakdown by ectonucleotidases (CD39, CD73) (Fredholm et al., 2005; Cunha, 2001) and metabolic clearance by astrocytic adenosine kinase (ADK) (Boison, 2013; Lloyd and Fredholm, 1995). In the epileptic brain, this balance is disrupted by overexpression of ADK in astrogliotic cells (Fedele et al., 2005), which results in increased clearance of adenosine and loss of A1R-mediated synaptic inhibition (Boison, 2008).

The role of adenosine in seizure termination has been demonstrated directly (During and Spencer, 1992; Van Gompel et al., 2014). Astrogliosis and ADK up-regulation is well documented in the epileptic brain (Sofroniew and Vinters, 2010; Aronica et al., 2011), and strategies to restore adenosine signaling have been quite successful for seizure suppression. Pharmacologic activation of the A1R blocks seizures (Gouder et al., 2003), as does inhibition of ADK with 5-iodotubercidin (ITU) (Gouder et al., 2004). Transgenic mice have
provided further evidence of the importance of the A_1R in seizure suppression and a causal role for ADK overexpression in seizure susceptibility. A_1R knockout mice are susceptible to lethal status epilepticus (Fedele et al., 2006; Kochanek et al., 2006). Overexpression of ADK in the hippocampus is associated with spontaneous seizures, which can be blocked by ITU (Fedele et al., 2005), and ADK suppression in the hippocampus confers protection against epileptogenesis (Li et al., 2007). The mechanistic rationale and therapeutic efficacy of adenosine homeostasis in seizure suppression is well established; however, the ubiquitous nature of adenosine signaling makes direct clinical translation of these therapies impractical. Chronic systemic treatment has sedative, cardiovascular, and renal side effects that have led to the near abandonment of pharmaceutical drug development efforts. To avoid limitations of systemic adenosine augmentation, focal delivery strategies have been developed. It was shown that focal adenosine supplementation provided similar efficacy to systemic treatment. Cells engineered to release adenosine inhibited kindled seizures in an A_1R dependent manner (Huber et al., 2001) and blocked seizures in a model of drug-resistant epilepsy (Li et al., 2008). Similarly, a silk-based scaffold for controlled focal adenosine release suppressed kindled seizures and epileptogenesis in a kindling model (Wilz et al., 2008; Szybala et al., 2009). Of particular interest, in the clinically relevant kainic acid status epilepticus (KASE) model of epilepsy, silk-based transient adenosine release halted epilepsy progression in epileptic rats with fully generalized spontaneous seizures, with lack of seizure progression lasting at least 3 months after the conclusion of the adenosine supplementation, whereas seizure rate and severity continued to progress in all control animals (Williams-Karnesky et al., 2013). This finding for the first time suggested a potent antiepileptogenic role of adenosine. Maladaptive changes in adenosine metabolism are tightly linked to epileptogenesis, and restoration of adenosine homeostasis therefore presents a potentially powerful means to prevent epilepsy or its progression.

The influence of adenosine on biologic systems is complex. It may be pro- or anti-inflammatory (Hasko et al., 2005) and can inhibit or activate synaptic activity (Sebastiao and Ribeiro, 2009). Adenosine concentration is under strict local control, with both intra- and extracellular enzymes contributing to the maintenance of adenosine “tone.” While our understanding of the local mix of adenosine receptors and their subcellular localization explains some of the apparently contradictory roles proposed for adenosine, a broader view of adenosine as a “retaliatory metabolite” has been proposed (Newby, 1984) to explain the emerging role of adenosine in response to physiologic stress. This concept was applied to the KD, postulating a role for adenosine as the molecular link between cellular metabolism and seizure suppression (Masino et al., 2009). Indeed, subsequent studies have shown that administration of the KD to epileptic rats in the pilocarpine model restores adenosine levels in the hippocampus to normal (nonepileptic) levels, and that returning to a normal-fat/carbohydrate diet results in a return to adenosine deficiency (Lusardi et al., 2015). A causal role for adenosine in the antiseizure effects of the KD has been demonstrated in A_1R knockout mice and ADK overexpressing mice, both of which have spontaneous focal seizures. Spontaneous seizures were eliminated in the ADK overexpressing mice fed with the KD, an effect that was blocked by the A_1R antagonist dipropylcyclopentylxanthine. No reduction in spontaneous seizures was observed in A_1R^+/− mice on the KD, though a partial reduction was measured in A_1R^+/− mice, consistent with reduced A_1R expression levels in the heterozygotic mice (Masino et al., 2011). These results support the hypothesis that the antiseizure efficacy of the KD exerts some of its influence through restoration of local adenosine homeostasis in the epileptic brain.

**EPIGENETICS IN EPILEPSY**

Heritable factors confer a small but appreciable increase in risk of epilepsy development (Kullmann, 2002). Genetic loci have been identified in subpopulations of particular epilepsy syndromes, but only account for a fraction of the incidence, and are not well correlated with seizure severity and comorbidities (Myers and Mefford, 2015). In general, large-scale genomewide studies have not identified broadly applicable genomic variations that would indicate a common genetic risk in epilepsy (Kaspersavicute et al., 2010; Mefford et al., 2010; Cavalleri et al., 2007). However, a recent study suggests that genetic variants of the Adk gene are associated with an increased risk for the development of posttraumatic epilepsy (Diamond et al., 2015). While the role of genetics in epilepsy and epileptogenesis needs further investigation, changes in gene expression and regulation might play an additional role. Studies of gene expression changes following seizures, in the latent phase of epileptogenesis before spontaneous seizures develop, and in the epileptic brain agree that many genes are (dys)regulated in
the epileptogenic brain (Lukasiuk and Pitkanen, 2004). However, the specific genes regulated are not consistent across studies, regardless of species or model specificity (Aronica and Gorter, 2007). Pathway analyses across multiple epilepsy studies have revealed that despite variations in the specific genes regulated, certain functional pathways are commonly identified across studies (Aronica and Gorter, 2007). Thus, expression changes targeting intra- and extracellular signaling, transcription, and protein biosynthesis, and immune responses are all well represented in all time windows evaluated (Aronica and Gorter, 2007). This network view of epilepsy risk and development presents a model that can account for the confounding factors in genomic studies of epilepsy, including phenotypic inconsistencies associated with heritable risk factors, and the variable susceptibility and disease progression in acquired epilepsies.

Epigenetic changes are biochemical alterations of the DNA or the chromatin structure, which do not affect the coding sequence of the DNA, but contribute to the regulation of gene transcription and entire networks. Epigenetic changes include DNA methylation and hydroxymethylation, as well as histone methylation and acetylation (Jaenisch and Bird, 2003; Kiefer, 2007). Importantly, epigenetic changes may affect several genes thought to represent a risk factor for epilepsy simultaneously. In contrast to genetic mutations, epigenetic changes are potentially reversible and may constitute a novel target for therapeutic intervention. DNA methylation was once believed to be stable, but a critical role for the dynamic regulation of DNA methylation has been identified in learning and memory (Roth and Sweatt, 2009; Day and Sweatt, 2010). Environmental disruption of physiologic epigenetic regulation has been implicated in a broad range of diseases, and has been widely studied in the context of exposure to environmental toxins (Pacchierotti and Spano, 2015; Bollati and Baccarelli, 2010). In rodent studies environmental factors such as maternal stimulation and social interaction have been linked to epigenetic changes, which in turn influence stress hormones, brain development, and neuropsychiatric phenotypes (Curley et al., 2011). Clinical evidence supports the conclusions from those rodent studies (McGowan et al., 2009; Zhang et al., 2013). Beyond exposure to overt toxins, diet can influence the epigenome. Naturally occurring compounds in our food have a known influence on epigenetic regulation. Their role has been extensively studied in cancer risk and treatment, but their general mechanisms are likely to influence other disease processes as well (Lim and Song, 2012; Hardy and Tollefsbol, 2011). Food-derived bioactive compounds can act as substrate donors or reaction inhibitors (Choi and Friso, 2010), and dietary imbalances can promote or limit disease progression. Thus, epigenetic regulation of gene expression not only drives the development of our gross anatomy but also shapes our intellectual, emotional, and physiologic phenotype.

**KETOSCIC DIET, ADENOSINE HOMEOSTASIS, AND ANTHEPIEPILEPTOGENESIS IN TEMPORAL LOBE EPILEPSY**

The most common form of focal epilepsy, with about 80% of seizures originating in the hippocampus—a critical structure for consolidation, storage, and retrieval of memories—temporal lobe epilepsy (TLE) is refractory to conventional antiepilepsy drugs in up to 40% of patients, leaving removal of the hippocampus as the antiseizure treatment of last resort for many. The growing field of epigenetic research has led to the discovery that significant epigenetic changes occur in the epileptic hippocampus and may present a unique therapeutic opportunity.

In both clinical and experimental settings the epileptic hippocampus was shown to be characterized by altered DNA methylation when compared with nonepileptic hippocampus. In human TLE samples from resected hippocampus, gene targets with both increased and decreased methylation were identified, with approximately 80% of significantly regulated sites hypermethylated, an equally prominent signature in sclerotic and nonsclerotic hippocampal tissue (Miller-Delaney et al., 2015). In rodent models of TLE, similar patterns of hypermethylation in the epileptic hippocampus have been demonstrated in the KASE model (Williams-Karnesky et al., 2013; Ryley Parrish et al., 2013) and pilocarpine status epilepticus models (Kobow et al., 2013; Lusardi et al., 2015). A receptor-independent role for adenosine in regulating DNA methylation has recently been demonstrated (Williams-Karnesky et al., 2013). Adenosine has a mass effect on biochemical enzyme reactions and is an obligatory end product of the S-adenosylmethionine (SAM) dependent transmethylation pathway (Figure 23.1), necessary for the transfer of methyl groups onto DNA (Boison et al., 2002; Mato et al., 2008). As predicted by this biochemical pathway, exogenous application of either adenosine or its complementary end product homocysteine inhibits the reaction and reduces DNA methylation,
whereas addition of the methyl group donor SAM increases DNA methylation in the naïve rodent brain (Williams-Karnesky et al., 2013). Similar effects can be obtained pharmacologically using an ADK inhibitor (5-iodotubercidin, ITU), which reduced hippocampal DNA methylation by 50%, even in the presence of caffeine, a nonspecific adenosine receptor inhibitor, further demonstrating that the effects of adenosine on methylation are receptor independent. The ADK is up-regulated within epileptogenic brain areas in rodent models of TLE and in human TLE with hippocampal sclerosis (Boison, 2012), resulting in adenosine deficiency, increasing the flux of methyl groups through the transmethylation pathway. Concurrently, adenosine deficiency shifts the equilibrium of the S-adenosylhomocysteine (SAH) hydrolase reaction away from the formation of SAH (Mandaviya et al., 2014), which is also known to block DNA methyltransferase activity by product inhibition (James et al., 2002), thereby reinforcing the increase in DNA methylation in the epileptic brain. Seizures resulting from the proconvulsant L-methionine-dl-sulfoximine, which increases the methylation flux by increasing the SAM/SAH ratio, can be blocked by adenosine and homocysteine (Gill and Schatz, 1985; Schatz et al., 1983; Sellinger et al., 1984). In the KASE model, direct ventricular administration of adenosine for 10 days significantly reduced epilepsy disease progression, including the progressive increase of spontaneous convulsive seizures and additional mossy fiber sprouting, and restored global DNA methylation levels to control levels, lasting well after the conclusion of the adenosine delivery (Williams-Karnesky et al., 2013). These findings show that global DNA methylation levels are under the direct control of adenosine, and that disruption of adenosine homeostasis (due to ADK up-regulation at the epileptogenic focus) affects DNA methylation levels and alters gene expression in the epileptic brain.

As described above, through mechanisms that are not yet well understood, strict adherence to a KD reduces seizures through an adenosine-receptor dependent mechanism (Masino et al., 2011). Using a kindling model of epileptogenesis, the KD also delayed the acquisition of kindling in mice, an effect that persisted even after a return to a standard lab diet, while the conventional antiepilepticogenic drug valproic acid attenuated only the seizures without blocking the kindling.
process. These data demonstrate persistent effects of the KD that are not merely due to seizure suppression (Lusardi et al., 2015). When fed to rats following status epilepticus, the KD not only reduced spontaneous seizure development but also reduced DNA methylation levels both during diet administration and after a return to standard diet (Kobow et al., 2013; Lusardi et al., 2015). Though a direct link between the KD and DNA methylation levels must still be demonstrated, when evaluated together these results show that the lasting effects of the KD may be conferred via adenosine regulation of the DNA methylome, supporting a key mechanism implicated in epilepsy and epileptogenesis.

**CONCLUSION**

With up to 35% of persons with epilepsy considered to be refractory to treatment and no therapies available that prevent epilepsy or its progression, the novel epigenetic functions of KD therapy discussed here might be of therapeutic value to not only relieve the seizure burden in patients with epilepsy but also to modify the development of epilepsy with its sequelae of pharmacoresistance and the development of epilepsy-associated comorbidities. The KD presents a powerful adjunct therapy to existing pharmacologic and surgical approaches to seizure relief. Renewed interest in the KD has led to refinements in the diet formulation and administration (Kossoff et al., 2009; Wibisono et al., 2015) and improved understanding of the potential positive and negative interactions with conventional antiepileptic drugs (Morrison et al., 2009; van der Louw et al., 2015), improving compliance and seizure suppression rates. However, the diet requires close monitoring by physicians and dietitians, and seemingly minor deviations from the ketogenic regimen can negate its beneficial effects. While a “ketogenic diet in a pill” may be unlikely, ongoing studies to understand the biochemical mechanisms of the KD are an essential step in the continued refinement of antiepileptic and antiepileptogenic therapies. The neuroprotective mechanisms of the KD are varied, and diet efficacy may rely on their combined influences. Among the metabolites regulated by the KD, however, adenosine has both a direct relevance to seizure suppression by A1R activation and an indirect influence on epilepsy and epileptogenesis via regulation of DNA methylation. A clearer understanding of how KD therapy affects adenosine metabolism and its epigenetic sequelae may help us understand adenosine dysregulation in epilepsy, and may guide the development of therapies designed to directly restore adenosine homeostasis, with the goal of developing a novel class of antiepileptogenic drugs.

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Ketogenic Diet, Aging, and Neurodegeneration

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OVERVIEW
It has been established for over 50 years that brain can utilize ketone bodies under carbohydrate-(glucose-) sparing conditions (Cahill and Owen, 1967; Owen et al., 1967; Randle et al., 1963). The use of ketogenic diet (KD) to treat intractable epilepsy in children continues to be an option for clinicians (DeVivo et al., 1978; Freeman et al., 1998; Lennox, 1928; Millichap et al., 1964; Tallian et al., 1998; Schwartzkroin, 1999), but the mechanisms of action remain diverse (Bridge, 1931; Jarrett et al., 2008; Lund et al., 2009; Maalouf et al., 2009; Milder and Patel, 2012; Millichap et al., 1964; Nakazawa et al., 1983; Prins and Hovda, 2009; Yudkoff et al., 2007; Masino and Rho, 2012; Maalouf et al., 2007; Stafstrom and Rho, 2012). The mechanistic link of ketosis and neuroprotection also remains unclear and exploratory. The mechanisms appear to be related to the change in the regulation of the cell's stress responses (Milder et al., 2010) and the changes in oxidative (glucose) metabolism (Prins and Hovda, 2009; Puchowicz et al., 2008; Bough and Rho, 2007; Masino and Rho, 2012). Neuroprotection by ketosis is thought to be associated with improved mitochondrial function, decreased reactive oxygen species (ROS) and apoptotic and inflammatory mediators, and increased protective pathways (Veech, 2004; Julio-Amilpas et al., 2015). This review focuses on two potential mechanisms that elucidate the neuroprotective effects of ketosis in the brain. We also concentrate on the applications toward the treatment of the aged brain, and present current data from rodent studies where KD was used to induce a chronic state of ketosis and where neuroprotection was assessed. Our view is that the neuroprotection associated with diet-induced ketosis is mainly through stabilization of glucose metabolism and downstream metabolic pathways such as the citric acid cycle and cytosolic-mitochondrial redox shuttle systems. We also maintain that ketone bodies can act as signaling molecules that target HIF1α-related cellular defenses and regulatory systems. HIF1α activation can result in stabilization of the "redox state" of the cell (Semenza, 2011). We have previously shown neuroprotection by ketosis (in model of middle cerebral artery occlusion, MCAO) in rat. The hypothesis that the mechanistic link between ketosis and neuroprotection is through HIF1α and its downstream effects on metabolic enzymes was established (Puchowicz et al., 2008).

KETONE BODIES ARE ALTERNATE ENERGY SUBSTRATES TO GLUCOSE
Ketone bodies (or the state of ketosis) have been shown to be neuroprotective following oxidative stress and metabolic challenges, such as those associated with stroke, ischemia, injury, Alzheimer's disease, Parkinson's disease, glucose transporter deficiency, and seizures (Maalouf et al., 2009; Prins and Hovda, 2009; Kashiwaya et al., 2000; Cunnane et al., 2011). Ketone bodies are alternate energy substrates to glucose and thus they have the potential to restore energy balance via stabilization of ATP supply. They are thought to be more efficient energy substrates to glucose for utilization by brain (Cahill and Veech, 2003; Kashiwaya et al., 2000; Sato et al., 1995; Veech et al., 2001; Veech, 2004). One application for the use of ketone bodies as an alternative substrate is for those with aging defects in glucose metabolism.

Hypometabolism is a clinical presentation in some Alzheimer's disease patients and is thought to be associated with defects in glucose metabolism (Cunnane et al., 2011). Studies measuring the ratio of $^{13}$C-amino acid / $^{13}$C-lactate in brain showed the fraction of amino acid carbon derived from glucose decreased with ketosis, reflecting the utilization of ketones as carbon precursors for amino acid synthesis (aspartate, glutamine,
glutamate, GABA) (Yudkoff et al., 2007). The neuroprotective role of ketone bodies may be through improvement in metabolic efficiency, by sparing glucose and the degradation of muscle-derived amino acids for substrates.

Ketone body utilization has properties that are considered metabolically favorable over glucose: (1) the direct generation of NADH, FADH₂, and succinate, which makes it readily available (compartmentalized) to the mitochondria for energy production; (2) the direct generation of acetyl-CoA, which enters the citric acid cycle via the citrate synthase reaction, thus relieving oxidative stress induced by pyruvate dehydrogenase inhibition, as well as making available ATP and lactate generated from glycolysis for other functions, such as the shuttling of substrates between neurons and glia; (3) having the potential to reduce mitochondrial ROS production associated with reperfusion injury; and (4) stabilization of HIF1α independent of hypoxia.

**USE OF KETOGENIC DIET TO INDUCE KETOSIS: A MODEL OF STABILIZED CHRONIC KETOSIS**

The metabolic modulations associated with ketone bodies require a state of stable ketosis. The use of a KD is an approach to establish chronic stable ketosis. Diet-induced approaches avoid the constraints (sodium or fluid overloads, pH imbalance, or starvation with chronic fasting) of using alternate modes of inducing ketosis, such as with infusions of ketone bodies or ketone body precursors (sodium salts or acids), or long-term fasting (Desrochers et al., 1995; Puchowicz et al., 2000). It is well described that ketosis can be induced in rodents and humans by fasting or feeding KDs (Gano et al., 2014; Leino et al., 200; Milder et al., 2010; Pan et al., 2000; Puchowicz et al., 2007; Tallian et al., 1998; Veech, 2004; Veldhorst et al., 2010; Yudkoff et al., 2007). We have previously reported that 3 weeks of diet-induced stable ketosis is necessary to induce metabolic adaptations in brain of rats resulting in neuroprotection (Puchowicz et al., 2007; Puchowicz et al., 2008; Zhang et al., 2013; Xu et al., 2012), which is consistent with another study (Milder et al., 2010). This regime can be inferred to as a preconditioning state where blood concentrations of ketone bodies remain stable over time. Adaptation to chronic ketosis includes up-regulation of monocarboxylate transporters (MCT) at the blood-brain barrier (Gjedde and Crone, 1975; Leino et al., 2001; Vannucci and Simpson, 2003) and moderate to high blood levels of ketosis (2–6 mM) (Leino et al., 2001; Prins and Hovda, 2009). More recently, we have established in our rat model of ketosis that metabolic adaptations also include a correlation between cerebral metabolic rate for glucose (CMRglc) and blood ketone levels (Zhang et al., 2013). Using positron emission tomography imaging technology we showed a decrease of almost 10% in CMRglc for each 1-mM increase of total plasma ketone bodies in cortical and cerebellar brain regions. Together with our meta-analysis, these data revealed that the degree and duration of ketosis played a major role in determining the corresponding change in CMRglc with ketosis and that preconditioning with KG diet resulted in significant glucose sparing (Zhang et al., 2013; Zhang et al., 2015).

**OXIDATIVE STRESS INDUCED ALTERED GLUCOSE METABOLISM**

Supply of energy substrate to the mitochondria is critical, especially during conditions of high energy demand where glucose metabolism may be deficient, such as with oxidative injury. The ability of the central nervous system to recover from an ischemic and/or hypoxic event is its capacity to recover from metabolic stress. It is well known that the status of cellular bioenergetics of the neurovascular unit is a major determinate of the pathophysiologic outcome. Stoichiometric analysis would predict that glycolysis cannot sustain NADH demand (energy demand) for more than a few hours, depending on glycogen stores. The dogma of tissue reperfusion for cell survival together with associated oxidative damage, immune responses, and increased risk for cell death remains problematic.

Under pathologic conditions the brain's energy demand and supply are mismatched, resulting in energy inefficiency. For example, survival and recovery of neurologic function after resuscitation following cardiac arrest is limited by the ability of the central nervous system to recover from an ischemic event due to metabolic stress to brain. Following cardiac arrest and resuscitation, increased glycolytic rates are associated with lactic acidosis and downstream metabolic blocks in energetics. After 24 hours of reperfusion there is an "apparent" defect in glucose metabolism, which may continue and occur as a secondary response to oxidative stress-induced injury (Selman et al., 1990). At this stage of oxidative damage there is a decrease in CMRglc (Bentourkia
Mitochondria are among the most susceptible organelles to oxidative stress. Brain mitochondrial function is known to decrease 2 days following ischemia reperfusion (Xu et al., 2008), which more often results in delayed neuronal death after 4 days post recovery (Xu et al., 2006). The degree of neurological deficit is highly dependent on the length of time to restored blood reflow and on the severity of oxidative damage. The generation of ROS and cytotoxic products of lipid peroxidation, such as 4-hydroxy-2-nonenal (HNE) and glutathione (GSH), also play a major role in the cell’s ability to recover. In a previous study we have shown that KD-induced ketosis resulted in significantly higher overall survival rates 4 days post cardiac arrest and resuscitation compared with the standard diet group (86% vs. 55%, \( p < .05 \), Figure 24.1). These data support the idea that diet-induced ketosis is neuroprotective under conditions when glucose utilization is limited (Xu et al., 2012).

Studies, past and present, continue to show evidence that altered energy metabolism is associated with aging and/or oxidative damage to key enzymes that regulate glucose metabolism (Cunnane et al., 2011; Gibson et al., 1998). The imbalance between nonoxidative- and oxidative-derived ATP is recognized but not clearly understood (Hemmila and Drewes, 1993; Hempel et al., 1977; Hoxworth et al., 1999; Xu et al., 2006; Xu et al., 2008). One explanation may be the lack of glucose carbon entry (flux) into the citric acid cycle (i.e., via pyruvate dehydrogenase complex) resulting in a dysregulation or “leakiness” of the citric acid cycle (cataplerosis). These consequences can lead to irreversible neurological deficit. Anaplerosis, a process that balances cataplerosis, ordinarily maintains/supplies intermediates to the citric acid cycle, which becomes vital during increased leakiness associated with ischemia reperfusion injury (Brunengraber and Roe, 2006; Kasumov et al., 2007) and other neuropathologies. The balance between cataplerosis and anaplerosis is essential to maintaining energy balance and, hence, mitochondrial function. In a recent study, we investigated the role of ketosis on the partitioning of glucose and BHB as substrates at the level of the acetyl-CoA and citric acid cycle (Zhang et al., 2015). This study revealed that glucose entry into the citric acid cycle was decreased with ketosis, whereas BHB entry was increased. The increased contribution of BHB to central metabolism suggests that carbon backbones from ketones play a role in the balancing of the citric acid cycle. The possibility of increased pyruvate carboxylation via malic enzyme should also be considered as a mechanism of ketosis in energy stabilization (Hassel, 2000).

**FIGURE 24.1** Overall survival rates 4 days following cardiac arrest and resuscitation. The rats fed with 3 weeks of ketogenic diet (KD) had a significantly higher survival rates compared with the rats fed with standard diet (STD) (86% vs. 55%, \( p < .05 \), Wilcoxon survival analysis) (Xu et al., 2012).

**DIET-INDUCED KETOSIS AS A NEUROPROTECTIVE STRATEGY IN THE AGED: CLINICAL RELEVANCE**

The aging population is at risk for increased morbidity and mortality following ischemic or hypoxic events, such as those related to stroke or other neurodegenerative conditions. Stroke is a leading disease/disability in the United States, as there are few (if any) clinical treatment strategies that can
reverse cellular damage. All brain cells are susceptible to infarction following ischemia-reperfusion (Pundik et al., 2012). Another example of a condition that results in moderate to severe ischemia/reperfusion injury is cardiac arrest and resuscitation, as the brain is exposed to ischemic-hypoxia and reperfusion. In the United States about one million cardiac arrests occur per year; about half of the population (aged or adult) having first-time cardiac arrests will not survive the first few days. Of those who do survive, about 90% will have short- or long-term neurological deficits (Chugh et al., 2004; Goldberger et al., 2008; Safar, 1993; Zheng et al., 2001). These deficits are more often related to oxidative stress induced post-resuscitation mortality and delayed selective neuronal cell loss (Hoxworth et al., 1999; Xu et al., 2006; Xu et al., 2008). In the aged, failure to recover is especially pronounced, as the aged brain is susceptible to oxidative stress damage as result of declines in repair systems and decreased antioxidant capacity. Brain pathophysiology associated with oxidative stress and injury, such as with ischemic reperfusion injury, often results in energy imbalances related to dysregulation of glucose (oxidative) metabolism. It could be that ketones are effective against pathology associated with altered glucose metabolism (Cahill and Veech, 2003; Prins and Hovda, 2009; Sato et al., 1995; Veech et al., 2001; Veech, 2004; Veech et al., 2012). Ketones may also play a role as signaling molecules that directly or indirectly act through the regulation of cell salvation pathways, such as HIF1α. In a recent study, we showed that ketosis was induced in aged rats (18 months old) following 3 weeks of feeding a KD and that diet-induced ketosis was neuroprotective against focal cerebral ischemia (MCAO; Figure 24.2).

**FIGURE 24.2** The total brain infarct volume artery occlusion (MCAO) in 18-month-old rats fed with ketogenic or standard diet. Rats were randomly assigned to two diet groups, fed ad libitum, ketogenic (high fat, no carbohydrate; KG) or standard lab-chow (STD) diet for 3 weeks prior to ischemic stroke. Rats underwent 90 minutes of MCAO, the total infarct volume was evaluated by triphenyltetrazolium chloride (TTC) staining 24 hours after reperfusion. Results: After fed with 3 weeks of ketogenic diet, plasma ketone bodies (mM) were increased 5-fold (3.1 ± 0.8 vs. 0.6 ± 0.1, mean ± SEM, ketogenic diet vs. Standard diet, n = 4 each). After 24 hours of reperfusion following MCAO, the infarct volumes were 5-fold lower in the KG diet group compared with the STD diet group (22 ± 10 vs. 124 ± 26 mm3, mean ± SEM, n = 4 each).
The role of HIF1α as a key regulator of oxygen homeostasis during hypoxia is well described (Agani et al., 2002; Chavez et al., 2000; Chavez et al., 2006; Semenza, 2007, 2011; Semenza et al., 2000; Sharp et al., 2001). However, the metabolic role of HIF1α on neuroprotection remains unclear.

HIF1α is a nuclear factor associated with neuroprotection via regulation of energy metabolism and is a key regulator of oxygen homeostasis during hypoxia, but may also play a role in neuroprotection following ischemia through gene regulation (Shi, 2009; Guo et al., 2008). HIF1 activation can also promote cell survival (Bergeron et al., 2000). The translational impact on understanding the role of ketone bodies as metabolic regulators can lead to a more definitive approach on treatment strategies that target defects in energy metabolism associated with neuropathologies and degenerative disorders. Since the aged population has an increased risk for ischemic events, such as those associated with transient global or focal stroke, the development of treatment strategies that incorporate ketosis may be wise.

The mechanism of HIF1α stabilization through ketosis has been proposed (Puchowicz et al., 2008) to be most likely through changes in cytoplasmic/mitochondrial redox state (Guo et al., 2008). This is consistent with others reporting stabilization of HIF1α through impairment of proteasome function, as a result of very low ratios of alpha-ketoglutarate / fumarate (Serra-Perez et al., 2010). A mechanism that explains diet-induced HIF1α stabilization (via KD) is through the feedback inhibition of the prolyl-hydroxylase (PHD) reaction by succinate, an intermediate of energy metabolism (see scheme below). Elevated cellular levels of succinate and fumarate compete with alpha-ketoglutarate / fumarate (Serra-Perez et al., 2010). The mechanisms of ketosis that are relevant to neuroprotection and stabilization of HIF1α (via succinate), need to be further studied. We propose that PHD inhibition occurs as a result of ketone body utilization by the cell via the activation of acetoacetate to acetoacetyl-CoA (via 3-ketoacyl-CoA transferase), which involves the generation of succinate in the mitochondria. Once activated, HIF1 modulates energy metabolism through downstream regulation of metabolism and related target genes. HIF1α target genes include those related to angiogenesis, erythropoiesis, glucose metabolism, and cell survival (Agani et al., 2002; Baranova et al., 2007; Chavez et al., 2006; Chen et al., 2015; Semenza, 2001; Sharp et al., 2001; Puchowicz et al., 2008; Helton et al., 2005).

We have reported that HIF1α response to hypoxia is blunted in the aged brain due to increased PHD levels (Ndubuizu et al., 2010). The lack of HIF1α induction following an oxidative insult, such as with reperfusion injury and/or aging process, increases vulnerability to oxidative damage that can result in poor recovery or cell death (Xu et al., 2007; Xu et al., 2008). These findings are consistent with other reports on mice with neuron-specific knockdown of HIF1α and subjected to transient focal cerebral ischemia; the results showed increased tissue damage and reduced cell survival (Baranova et al., 2007).

Neuroprotection by HIF target genes such as erythropoietin (Epo) has been described (Chong et al., 2003a; Ghezzi et al., 2010). As a neuroprotective agent Epo has many functions: antagonizing glutamate cytotoxic action, enhancing antioxidant enzyme expression, reducing free radical production rate, and affecting neurotransmitter release (Bartesaghi et al., 2005; Leist et al., 2004). It exerts its neuroprotective effect indirectly through restoration of blood flow or directly by activating transmitter molecules in neurons that also play a role in erythropoiesis. Although the mechanism is unclear, it is apparent that Epo has anti-apoptotic action after central and peripheral nerve injury (Chong et al., 2003b). Although apoptosis is not reversible, early intervention with neuroprotective therapeutic procedures such as Epo administration or preconditioning with KD may reduce the number of neurons that undergo apoptosis and thus improve outcome.

An additional target gene of HIF is the transcription factor vascular endothelial growth factor (VEGF). The VEGF is an endothelial cell-specific growth factor (Storkebaum et al., 2004), but recent evidence indicates that VEGF also has direct effects on neuronal and glial cell types (Silverman et al., 1999). A neuroprotective mechanism of VEGF is thought to be through increased perfusion of the penumbra via vasodilation and angiogenesis, such as with ischemic brain insults (Zhang et al., 2002). We have previously reported that preconditioning with KD results in an angiogenic response similar to what we have found with mild hypoxic exposure (Puchowicz et al., 2007). However, we found no changes in cerebral regional blood flow with KD-induced ketosis in adult rats. In another study we reported that VEGF expression was decreased in cerebral cortex of aged mice (Benderro and LaManna, 2011). Taken all together, KD might be a therapeutic strategy that targets neuroprotective
mechanisms through HIF-regulated VEGF, independent of modifications in blood perfusion (Chong et al., 2003b).

**DISCUSSION**

The mechanism of HIF1 regulation under various metabolic conditions, such as with stroke, inflammation, and altered glucose metabolism, continues to be explored. We describe two mechanisms that explain neuroprotection through diet-induced ketosis. First, ketone bodies are alternate energy substrates to glucose, thus reducing oxidative stress by relieving metabolic blocks downstream of glycolysis at the level of the citric acid cycle and, hence, enabling mitochondrial electron transfer and generation of ATP (Semenza, 2011; Prins, 2008; Julio-Amilpas et al., 2015; Sato et al., 1995; Veech, 2004). Second, ketosis induces the stabilization of HIF1α and its downstream target genes.

One possible link between ketosis and the stabilization of HIF1α is through feedback product inhibition of PHD via elevated tissue succinate (Semenza, 2007), as a result of a change in the metabolic redox state (see Scheme figure 24.3) (Guo et al., 2008; Serra-Perez et al., 2010; Mills and O’Neill, 2014). This is consistent with a study where mitochondrial redox in hippocampal tissue was improved by KD via the activation of NF E2-related factor 2(Nrf2) transcription factor, a primary responder to cellular stress (Milder et al., 2010). One known mechanism of Nrf2 is to up-regulate GSH biosynthesis. On the other hand,

**FIGURE 24.3** Scheme: Proposed mechanism of neuroprotection by diet-induced ketosis.

Our scheme emphasizes that cytosolic generated succinate (via PHD-coupled activity with alpha-ketoglutarate), must be balanced by mitochondrial redox of the citric acid cycle (shown in gray text). An accumulation of succinate would result in an imbalance of cytosolic redox; HIF1α would then be stabilized through feedback inhibition of PHD (text shown in black, Puchowicz et al., 2008). Ketone body utilization also results in the generation of succinate. **Steps 1–5 (circled):** HIF1α stabilization is shown to be through cellular redox, which is balanced by malate-aspartate shuttle. A link to the stabilization HIF1α is through feedback product inhibition of prolyl-hydroxylase (PHD) via elevated tissue succinate (Semenza, 2007) or reduced α-ketoglutarate (McFate et al., 2008) **(steps 3,4)**. Thus, an accumulation of succinate (or reduction in α-ketoglutarate) would result in an altered in redox state (malate aspartate shuttle, **step 4**) and stabilization of HIF1α (via inhibition of PHD) **(step 5)**. Other intermediates of energy metabolism acting on HIF1α regulation have been recently described (Chen et al., 2015; Mills and O’Neill, 2014; Tannahill et al., 2013). The potential mechanism by which ketosis stabilizes HIF1α is through a shift in “redox state” of the cell via changes in carbon flux into / out of the malate-aspartate shuttle (Semenza, 2011); a target would include changes in succinate-to-α-ketoglutarate ratio.
oxidizing environments (high levels of ROS) may suppress stabilization of HIF1α (Guo et al., 2008). These findings are consistent with a study showing that HIF1α played a role in N-acetylcyesteine-mediated neuroprotection in rats subjected to MCAO (Zhang et al., 2014).

Our view is that there is a feedback regulation on HIF1α activation through shifts in metabolic redox, such as with KD or fasting which is independent of hypoxia-induced stabilization of HIF1α (Mills and O’Neill, 2014). Thus, HIF1α acts as a metabolic sensor through a continued feedback loop directly coupled to oxidative metabolism via the citric acid cycle and malate aspartate shuttle systems (Figure 24.3). It has been suggested that HIF1α may also play a role promoting apoptosis (Helton et al., 2005; Serra-Perez et al., 2010), however there is little known about this mechanism.

Neuroprotection by HIF1α may be cell specific. It has been reported that neuron-specific inactivation of the HIF1α results in increased brain injury, as studied in a mouse model of transient focal cerebral ischemia (Baranova et al., 2007). The significance of these findings suggests that HIF1α and target genes are regulated distinctly, especially under pathological conditions. Although HIF1α is expressed in neurons and glia, the neuroprotective efficacy of a KD diet (via HIF1α) may be cell specific and conditional (Bergeron et al., 2000; Chavez and LaManna, 2002). Previously, we have reported that HIF1α response to hypoxia is suppressed in the aged and proposed that this was an explanation to why the aged are at greater risk for morbidity and mortality following an oxidative insult (Ndubuizu et al., 2010). Additionally, the potential role of KD relative to HIF2α should also be explored (Chavez et al., 2006). Our approach to understanding the central role of ketosis in the regulation of HIF1α as a signaling molecule in brain under pathophysiological conditions will help to delineate the role of HIF1α in neuroprotection.

CONCLUSION AND FUTURE

Over the past decade our research has consistently shown that ketosis is neuroprotective against ischemic insults in rats (Puchowicz et al., 2008) and, more recently, in mice (unpublished observation). Studies in ketogenic rats showed significantly decreased infarct volumes (via MCAO model) and improved survival and recovery after cardiac arrest and resuscitation (Xu et al., 2012). We purport that the mechanisms of ketosis include glucose sparing and ketone body utilization in brain as well as a shift in metabolic redox, which plays a role in energy balance and the downstream innate signaling of HIF1α. We view that the mechanism of neuroprotection by KD-induced HIF1α is through the regulation and activation of genes associated with salvation pathways and stabilization of energy metabolism. The neuroprotection by ketosis may also be through pathways independent of HIF1α regulation, such as AKT-modulation of NRF2 and nuclear factor kappa B (NF-kB), and these mechanisms remain to be elucidated.

REFERENCES


SECTION III: KETONIC DIET IN THE LABORATORY


Endocrine and Reproductive Effects of Ketogenic Diets

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INTRODUCTION

The impacts of maternal nutrition during gestation on reproductive success have been appreciated for centuries (Wu et al., 2004). The fact that mothers provide nutrients, oxygen, and the means to eliminate metabolic waste products from the developing offspring via the placenta links the ultimate health of both individuals to the resources available to the mother and her ability to distribute them effectively. It is becoming increasingly clear that the metabolic status of the mother impacts the phenotype and metabolic disease susceptibility of the offspring (Rando and Simmons, 2015). The global epidemic of metabolic syndrome has increased the concern over the effects of parental nutrition (lifestyle and environment) on offspring growth, development, cognitive function, and metabolic health (Hartil et al., 2009). With both extremes (starvation and obesity) prevalent across the world, a better understanding of the impacts of diet and dietary therapies on health is critical to having any hope of improving quality of life worldwide (Fontana and Partridge, 2015).

HIGH-FAT/KETOGENIC DIETS

The complex hormonal and metabolic mechanisms that control and are impacted by substrate availability drive changes in behavior that can be uncovered through manipulation of diet. A high-fat or ketogenic diet (KD) has been in use as a treatment for intractable epilepsy in children for close to 100 years (Vining et al., 1998; Neal et al., 2008; Stafstrom and Rho, 2012). The neuroprotective effects of the diet are compelling, and in recent years the potential therapeutic uses of the diet in humans (Kossoff et al., 2012; Kossoff and Hartman, 2013) has been extended through animal studies alone into thermal pain management (Ruskin et al., 2013), neural degeneration produced by a variety of insults/pathologies including cerebral ischemia (Yin et al., 2015), traumatic brain injury (Prins, 2008, 2012; Prins and Matsumoto, 2014), amyotrophic lateral sclerosis (Zhao et al., 2006), metastatic (Poff et al., 2015) and brain (Maroon et al., 2015) cancers, and in Alzheimers (Brownlow et al., 2013), Huntington’s (Ruskin et al., 2011), and Parkinson’s disease (Cheng et al., 2009), mouse models. In each case investigators assessed the effectiveness of the diet, or ketones themselves, on limiting the states of injury, disorder, or dysfunction. The ketone body acetone has anticonvulsant effects, and it could play a role in seizure control. Multiple mechanisms have been implicated in the neuroprotective effects of a KD (Schwartzkroin, 1999; Hartman et al., 2007; Ruskin and Masino, 2012; Stafstrom and Rho, 2012; Danial et al., 2013; Giordan et al., 2014; Mantis et al., 2014; Meidenbauer and Roberts, 2014; Rho, 2015; Rogawski et al., 2016) and while some effects may be temporary and/or reversible there is mounting evidence that long-term use of the diet can impact gene expression and behavioral phenotype on a more permanent basis.

ENDOCRINE EFFECTS OF HIGH-FAT/KETOGENIC DIETS

Regulation of nutrient intake, metabolism, energy generation, storage, distribution, and utilization is mediated in an endocrine manner and is dependent on the nature of the foodstuff (carbohydrate, protein, or fat) and the present energy needs of the organism. From an evolutionary perspective there is nothing more important than the consistent provision of food/energy to allow for growth and successful reproduction.
Hormones involved in regulation of food intake and energy expenditure come from the gastrointestinal tract (GI), adipose tissue, pancreas, adrenal gland, and peripheral sensory receptors and converge on the central nervous system. The drive to eat comes, in part, from the hypothalamus, where the “hunger/satiety” center of the brain is influenced by nutrients, hormones, and other signaling factors. Specific signals convey information regarding overall body metabolism, dietary composition, and the status of carbohydrate and lipid stores, in muscle and liver (carbohydrate) and adipose (lipid) tissue, respectively. During emergencies the sympathetic division of the autonomic nervous system is activated, resulting in the “fight or flight” response that diminishes any drive for food intake until the situation is resolved. Similarly, elevated stress levels result in elevated glucocorticoids (corticosterone in animals, cortisol in humans), which also decrease GI tract activity and the drive for food intake.

During nonemergencies the parasympathetic division of the autonomic nervous system predominates and eating, digestion, absorption, and transport occur without interruption. The hormones involved include insulin, amylin, glucagon, and pancreatic peptide (PP) from the endocrine pancreas; neurotransmitters neuropeptide Y (NPY), agouti gene-related protein (AGRP), and pro-opiomelanocortin (POMC) in the CNS; and ghrelin, peptide YY (PYY), somatostatin, secretin, and cholecystokinin (CCK) from the GI tract. Normal physiology involves integration of these signals that converge on the brain and regulate food intake and energy metabolism (Paoli et al., 2015).

During and immediately after a meal is the absorptive stage, when mechanical and chemical digestion are complete and amino acids, triglycerides, and glucose elevation stimulate insulin release from the beta cells of the endocrine pancreas. Insulin stimulates uptake of glucose and glycogenesis by liver and muscle cells, uptake and immediate use of glucose for energy by all cells except skeletal muscle, uptake of amino acids and protein synthesis by all tissues, and uptake of lipid by skeletal muscle for ATP and by adipose tissue for storage as triglycerides. The postabsorptive state is the time between meals when glucocorticoids generally rise and work in concert with glucagon and epinephrine to tap into energy reserves that were stored during the absorptive state. Glycogen and triglycerides are broken down via glycogenolysis and lipolysis, respectively, increasing circulating levels of glucose and fatty acids and glycerol. The fatty acids can be converted to acetyl-CoA, moved into the citric acid cycle, and used as energy to produce ATP. The use of fatty acids by systemic tissues reduces the need for glucose and thus helps to maintain homeostasis. In order for glucose levels to be sufficient for the brain, glycogen breakdown, glycerol conversion to glucose, and gluconeogenesis (production of glucose from noncarbohydrate sources) by the liver must be maintained during the postabsorptive stage. In addition to gluconeogenesis, the liver also carries out ketogenesis (production of ketone bodies from lipid and protein breakdown), resulting in the generation of acetoacetate, β-hydroxybutyrate, and acetone. Ketone bodies produced can be used by the entire body, including the brain, for energy. The efficiency of energy conversion is actually higher for ketones than it is for glucose (Schwartzkroin, 1999). The production of ketone bodies serves as the signal for fasting, or starvation, to the organism, as most diets in the wild are not “ketogenic.”

Paoli et al. (2012) published blood levels of glucose, insulin, ketone bodies, and blood pH (see Table 25.1) in response to normal diets, KDs, and the development of diabetic ketoacidosis. The elevation in blood ketones and the decrease in blood glucose, at least in the short term, are well established. Of particular interest is the lower range in concentrations of insulin in response to KDs versus normal diets. Dysfunctional insulin signaling, possibly due to hypoinsulinemia, may be central to a link reported between type II diabetes and the development of Alzheimer’s disease (Gotz et al., 2009; Bandaru et al., 2010). Further investigations into insulin signaling in the brain are imperative and ongoing.

There are two modes of energy regulation, short-term, regulated by satiety signals, for single-meal control, and long-term, regulated by

### TABLE 25.1 BLOOD LEVELS DURING A NORMAL DIET, KETOGENIC DIET, AND DIABETIC KETOACIDOSIS

<table>
<thead>
<tr>
<th>Blood levels</th>
<th>Normal diet</th>
<th>Ketogenic diet</th>
<th>Diabetic ketoacidosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>80–120</td>
<td>65–80</td>
<td>&gt;300</td>
</tr>
<tr>
<td>Insulin (µU/L)</td>
<td>6–23</td>
<td>6.6–9.4</td>
<td>≥ 0</td>
</tr>
<tr>
<td>Ketone bodies (mmol/L)</td>
<td>0.1</td>
<td>7–8</td>
<td>&gt;25</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
<td>7.4</td>
<td>&lt;7.3</td>
</tr>
</tbody>
</table>

Source: Paoli et al. (2012). Published with permission from author (open access).
adiposity signals, which are a reflection of energy availability in storage, especially body fat. While the body can only store enough glycogen in liver and muscle for one day’s worth of energy demand, it can store lipid capable of supporting weeks of energy demand. The arcuate nucleus in the hypothalamus responds with signals that either stimulate (orexigenic) or inhibit (anorexigenic) food intake. It is here where the effects of ketones can be either orexigenic or anorexigenic (Paoli et al., 2015; see Figure 25.1), the resultant behavioral effects being essentially opposite.

Adiponectin (released from adipose tissue), ghrelin (from the GI tract), and insulin and glucagon (from the pancreas) are all impacted by ketosis, resulting in mixed endocrine profiles and resultant behaviors of increased or decreased food intake. The biochemical make-up of that food will dictate the metabolic response. Predicting the endocrine and behavioral effects of KDs is going to require step-by-step analysis of the impact of changing both the lipid and protein profiles under different physiological scenarios and assessing the effects on ketosis and other metabolic endocrine signals (Bielohuby et al., 2011).

Ellenbroek and colleagues (2014) reported that long-term KD treatment (22 weeks) resulted in glucose intolerance, reduced beta cell cluster size and mass (see Figure 25.2), and the establishment of plasma markers associated with dyslipidemia and inflammation in mice (see Table 25.2). Reduced beta cell mass and subsequent inadequate insulin secretory responses result in glucose intolerance and type II diabetes in humans (Kahn et al., 2006; Nolan et al., 2011). The regulatory mechanism was also compromised with respect to glucagon as alpha cell mass loss was even greater than beta cell mass loss (Ellenbroek et al., 2014). Glucagon is critical for counter-regulatory control of blood glucose, and if insufficient the individual is subject to hypoglycemic episodes resulting in HAAF (hypoglycemic associated autonomic failure). This occurs in both type I and II diabetes and
section III: Ketogenic Diet in the Laboratory

FIGURE 25.2 Beta cell mass in control and KD-fed mice after 22 weeks. (A) representative image of beta cells (brown) in control mice; (B) representative image of beta cells (brown) from KD-fed mice; (C) beta cell mass (n = 8–10 mice); (D) islet density (n = 8–10 mice); (E) median beta cell cluster size (n = 8–10 mice); (F) beta cell cluster distribution (n = 8–10 mice). *p < .05; **p < .01 vs. control.


TABLE 25.2 PLASMA MARKERS OF THE METABOLIC SYNDROME IN CONTROL MICE AND MICE FED A KETOGENIC DIET FOR 22 WEEKS

<table>
<thead>
<tr>
<th>Plasma markers</th>
<th>Control</th>
<th>KD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>92.7 ± 3.1</td>
<td>141.3 ± 9.5b</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>42.0 ± 1.5</td>
<td>64.9 ± 6.8b</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>0.63 ± 0.37</td>
<td>2.35 ± 0.99a</td>
</tr>
<tr>
<td>MCP-1, pg/mL</td>
<td>6.20 ± 0.73</td>
<td>12.65 ± 1.40c</td>
</tr>
<tr>
<td>IL-1β, pg/mL</td>
<td>1.19 ± 0.31</td>
<td>3.86 ± 1.17a</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>3.20 ± 2.11</td>
<td>19.84 ± 8.02c</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>31.3 ± 0.9</td>
<td>80.6 ± 15.8b</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>89.0 ± 10.7</td>
<td>155.2 ± 31.4a</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8–10 mice. MCP, monocyte chemoattractant protein; IL, interleukin; ALT, alanine aminotransferase; AST, aspartate aminotransferase. *p < .05, **p < .01, *p < .001.


contributes to long-term complications associated with low energy, fatigue, and potential for loss of consciousness due to insufficient counter-regulatory mechanisms to raise glucose (Dagogo-Jack, 2015).

Collectively, results are consistent with the notion that long-term KD treatment, while manageable and not life-threatening from a ketoacidotic standpoint, is not ideal for long-term metabolic health. Enhanced and extended inflammatory responses are counter to organismal well-being and long-term survival. For the KD to be an effective, long-term therapy, its deleterious side effects of dyslipidemia and inflammation will have to be addressed. Whether the beneficial effects are mediated through ketone bodies (Kim et al., 2015), specific fatty acids, altered glucose, altered insulin and glucagon, or a combination of the above, an understanding of the mechanism(s) of action of the various constituents is necessary.
BEHAVIORAL EFFECTS OF HIGH-FAT/KETOGENIC DIETS

Initial animal studies investigating the neuroprotective effects of KDs on seizure susceptibility in rodent models were relatively short-term feeding regimens (3 weeks) followed by quantitative (time to onset; time of total seizure) and qualitative (tonic, clonic, or grand mal) assessment of seizure severity (Hori et al., 1997; Bough and Eagles, 1999; Su et al., 2000; Harney et al., 2002; Loscher, 2011). The success of the KD in the treatment of pediatric epilepsy (Lefevre and Aronson, 2000; Vasconcelos et al., 2004; Neal et al., 2008; Suo et al., 2013) and the relative ease with which a dietary therapy can be administered led to the natural hope that the KD could impact positively numerous other neurological or metabolic disorders (Kossoff et al., 2012; Stafstrom and Rho, 2012). The therapeutic use of KDs has been primarily limited to children (Lefevre and Aronson, 2000) but studies indicate that they may be effective in adult patients suffering from pharmacoresistant epilepsy (Coppola et al., 2002).

Behaviors reportedly impacted by KDs include epilepsy, sleep, cognition (Hallbook et al., 2012), and motor performance, but not cognition, in two mouse models of Alzheimer’s pathology (Brownlow et al., 2013). In addition, high-fat (nonketogenic) diets alone have reportedly impacted hippocampal-dependent spatial learning and memory in rats (Alzoubi et al., 2009) that was exacerbated further in combination with chronic stress. Maternal high-fat diets (HFD) themselves can act as a stressor, increasing maternal glucocorticoids and disrupting maternal behavior and brain activation (measured as increased c-Fos) in C57BL/6J mice (Bellisario et al., 2015). Changes in mouse brain gene expression in response to both ketogenic and nonketogenic HFDs (Selfridge et al., 2015) suggest that the specific lipid components of the diet likely dictate the ultimate biological effects, including alterations in the brain’s aero- bic infrastructure and mitochondrial DNA transcriptional efficiency. Greenwood and Winocur (1996) reported that the degree of cognitive impairment is highly associated with the level of saturated fatty acids fed and independent of the mono- or polyunsaturated fatty acids in the HFD. Davidson and coworkers (2013) reported that cognitive impairment induced by western diets (high in saturated fats and sugar) may be diminished by ketones. Collectively, present results suggest that specific dietary components and not simply the level of ketone production, ultimately determine the levels and changes in gene expression that result in behavioral responses of the organisms.

Central to the development of type II diabetes is the ineffectiveness of the hormone insulin to lower blood glucose by stimulating uptake in peripheral tissues. The brain has been considered to be insulin independent for glucose uptake; however, there are insulin receptors located in several brain regions including the hippocampus, hypothalamus, and cerebellum (Kahn, 1994; Ho et al., 2004, Plum et al., 2005; Banks and Kastin, 1998). Over the past decade a potential link between type II diabetes and Alzheimer’s disease has been established and a dysfunctional insulin signaling pathway has been proposed to be involved (Gotz et al., 2009; Bandaru et al., 2010). The cognitive decline reported with long-term KD treatment in this study and others (Su et al., 2000; Zhao et al., 2004; Snead 2004; Behassan and Jan, 2006) might be the result of dysfunctional insulin signaling, a likely outcome of long-term KD treatment and its metabolic side effects.

Despite the potential metabolic side effects, individuals may choose any available means to experience relief from their primary ailment, especially epileptic seizures that can occur at any time. As such, it is imperative that the impacts of KDs on normal physiology, including reproduction, be investigated. The timing and duration of exposure to dietary treatments will likely dictate whether effects are temporal or more long-lasting (epigenetic modifications), as would be expected during embryonic, fetal, or neonatal development (Rando and Simmons, 2015).

EFFECTS OF LONG-TERM KETOGENIC DIET CONSUMPTION ON PUBERTY AND REPRODUCTION

If children are going to be consuming KDs growing up, what is the impact going to be on normal development? Our laboratory has begun to investigate the effects of a KD on growth, onset of normal female development including day of vaginal opening, onset of nonnormal cycles, and onset of normal 4-day cycles in rodents. Three KDs containing 8%, 14%, or 18% protein, with ketogenic ratios (fat/carb + protein) of 6.2, 4.2, and 3.2, respectively (Bio-Serv, NJ), or rodent chow (RC) were administered to 48 female Long-Evans rats starting on Day 23. Dates of vaginal opening were recorded, and only the KD 8% group was significantly delayed (see Figure 25.3), this effect likely the result of the more shallow growth curve (see Figure 25.4).
FIGURE 25.3 The average date of vaginal opening in female Long-Evans rats maintained on rodent chow (control), KD8%, KD14%, or KD18%. The KD8% were significantly delayed compared with controls (*; \( p < .05 \)). Statistical analyses included one-way ANOVA and post hoc Fisher’s least significant difference (LSD).

![Graph of vaginal opening dates](image1.png)

FIGURE 25.4 The average weights of female Long-Evans rats fed rodent chow (control) or KD8%, KD14%, or KD18% from weaning (day 22) to 180 days. KD8% were significantly lower \( (p < .05) \) at days 43 and 73, but not 93. Statistical analyses included one-way ANOVA and post hoc Fisher’s least significant difference (LSD).

![Graph of weight measurements](image2.png)

**TABLE 25.3** AVERAGE AGES AND WEIGHTS OF CONTROL RODENT CHOW- AND KD-FED FEMALE LONG-EVANS RATS AT THE FIRST REGULAR 4-DAY ESTROUS CYCLE AND ON COMPLETION OF THREE CONSECUTIVE 4-DAY ESTROUS CYCLES

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Age of first regular 4-day cycle (days)</th>
<th>Weight at first regular cycle (g)</th>
<th>n</th>
<th>Age after three consecutive 4-day estrous cycles (days)</th>
<th>Mean Weight on repeatable cycling (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>45.1 ± 2.4</td>
<td>219.6 ± 15.3</td>
<td>12</td>
<td>65.2 ± 5.4</td>
<td>249.5 ± 18.6</td>
</tr>
<tr>
<td>KD8%</td>
<td>11</td>
<td>51.4 ± 6.8</td>
<td>123 ± 18.6</td>
<td>9</td>
<td>81.7 ± 5.7</td>
<td>272.3 ± 22.6</td>
</tr>
<tr>
<td>KD14%</td>
<td>12</td>
<td>46.8 ± 2.9</td>
<td>168.4 ± 18.9</td>
<td>11</td>
<td>65.2 ± 4.5</td>
<td>256.3 ± 17.4</td>
</tr>
<tr>
<td>KD18%</td>
<td>12</td>
<td>45.6 ± 3.1</td>
<td>172.3 ± 24.1</td>
<td>12</td>
<td>64.7 ± 3.7</td>
<td>262.7 ± 10.8</td>
</tr>
</tbody>
</table>
The time till completion of three consecutive 4-day (normal) estrous cycles was similarly only significantly delayed in the KD8% group (see Table 25.3), once again likely delayed in concert with the delayed growth. The KD8% rats were the lightest of the groups at first regular estrous cycle despite the additional 16 days of eating.

Blood glucose levels were not significantly different between any of the groups after 47 days on the diet (see Figure 25.5). This result is consistent with long-term KD treatment in our laboratory.

Blood ketones were significantly different, reflecting the elevated ketogenic ratio of the diets as total protein plus carbohydrates drops and total fat increases (see Figure 25.6).

With the establishment of multiple KD groups (varying in circulating ketones), investigations continued into effects of maternal KD administration prior to and throughout gestation.

Pregnancy establishment and maintenance requires signaling between the developing conceptus (embryo and extraembryonic membranes)
and the mother. Prior to development of a functional placenta, commonly referred to as the peri-implantation period, the conceptus relies on uterine secretions or histotroph for support (Bazer et al., 2013; Harney et al., 1990; Harney et al., 1993; Harney et al., 1994). The components of histotroph can be impacted by the maternal diet (Wu et al., 2004) as supplementation of the diet with the amino acid arginine enhances fetal-placental development in rodents, swine, and humans (Bazer at al., 2013; Bazer et al., 2015). Arginine is a nutritionally essential amino acid for embryonic survival and fetal and neonatal growth (Wu et al., 2009). The hormonal milieu (levels of estrogen and progesterone) combined with diet and overall metabolic and energy status of the mother will play a key role in establishing the components of uterine histotroph and the substrates available to the developing conceptus. Continued successful development requires a functional placenta, delivery of necessary nutrients and oxygen, and removal of metabolic waste and carbon dioxide (Bazer et al., 2015).

Just as the quality of the environment impacts health in adults, the quality of the intrauterine (IU) and early postnatal environment impacts the development of offspring. An adverse IU can result in intrauterine growth restriction (IUGR). The health of offspring later in life is impacted by the occurrence of IUGR, or complications in the mother, including diabetes or obesity, that affect nutrient availability (Hales and Barker, 1992, 2001; Ravelli et al., 1976; Ravelli et al., 1998; Valdez et al., 1994). Models used to examine IUGR include dietary calorie or protein restriction, glucocorticoid administration, and uteroplacental insufficiency induced by ligation of the uterine artery. All conditions induced a stressor into the maternal system during gestation and examined an impact on offspring glucose metabolism into adulthood (Fowden and Forhead, 2004; McMillen and Robinson, 2005). Any nutritional deficiency will impact energy availability, metabolism, and biochemical substrate availability. In studies where nutritional overabundance is the standard (obesity and type 2 diabetes), results show significant increases in establishment of the same chronic diseases in offspring (Rich-Edwards et al., 1999). Collectively, these results suggest that the IU environment is likely impacted by any significant changes in maternal nutrition or behavior (especially stress-induced), which, in turn, can have any number of phenotypic effects on the offspring. The mechanism(s) by which maternal diet influences the phenotype of offspring have yet to be elucidated; however, epigenetic (external modifications to DNA) changes appear to be involved (Masuyama and Hiramatsu, 2012; Rando and Simmons, 2015).

**EFFECTS OF LONG-TERM KETOGENIC DIET CONSUMPTION BEFORE AND DURING GESTATION**

There have been limited studies focused on KD administration throughout gestation and its effects on maternal and offspring-phenotypes; however, findings suggest, as we have always appreciated, that maternal diet does indeed impact pregnancy outcome. Sussman and coworkers examined the effects of KD administration during pregnancy on embryonic growth (Sussman et al., 2013b), offspring physiological growth and brain structure (Sussman et al., 2013a), and brain structure and susceptibility to depression and anxiety in the adult mouse offspring (Sussman et al., 2015). Dams fed a KD (67% fat; 15% protein) prior to and during gestation exhibited a significant reduction in maternal fertility and litter size, which the authors indicated could be due to protein deficiency. However, others have reported contrary results (Derrickson, 2007), and the 15% protein should have been sufficient for normal growth. In addition, since the dams were prone to fatal ketoacidosis by midlactation (Sussman et al., 2013a), pups were fostered by dams on standard chow, allowing examination of the effects of KD exposure during gestation alone. The results from their studies in mice indicate that KD exposure during all of gestation and through weaning is not conducive to normal anatomical, behavioral, and physiological development of the offspring. They report delayed growth and significant relative decreases bilaterally in the cortex, fimbria, hippocampus, corpus callosum, and lateral ventricle. The hypothalamus and medulla experienced volumetric enlargements as well. Differences were detected as early as postnatal day 11.5 and were greater at postnatal day 21.5 (Sussman et al., 2013a). In an earlier report, Sussman and colleagues (2013b) demonstrated that anatomical comparisons of embryos at day 13.5 detected larger volumetric size of embryos and their hearts from dams on a KD but smaller brains, pharynx, cervical spinal cord, hypothalamus, midbrain, and pons. By day 17.5 embryos from KD dams were volumetrically smaller with smaller hearts and thymus glands but enlarged cervical spine, thalamus, midbrain, and pons (Sussman et al., 2013b). The most recent study by Sussman’s group examines...
both brain structure and susceptibility to depression and anxiety in adult mouse offspring from KD-fed dams throughout gestation. Eight-week-old adult CD-1 mice exhibited reduced susceptibility to anxiety and depression and increased physical activity compared with standard chow controls. Our studies in adult rats on KDs have generated similar findings with decreased depression appearing as early as 14 days after onset of treatment and continuing through 6 months (A. Patel, unpublished results). In addition, recent evidence suggests that maternal high-fat diets might make offspring susceptible to behavioral disorder responses to stressful challenges. Collectively these results suggest that KD exposure throughout gestation and weaning has significant anatomical and developmental implications on the offspring. Likely, changes in gene expression, which may be mediated epigenetically (Masuyama and Hiramatsu, 2012; Rando and Simmons, 2015), ultimately impact anatomy and the resultant physiology and behavior into adulthood.

Our lab has conducted experiments in Long Evans rats (see Figure 25.7; Gudsnuk unpublished), where females were administered rodent chow (RC) or KDs with 8%, 14%, or 18% protein from day of weaning (day 22) through puberty, breeding, gestation, and out to 180 days postpartum and postweaning of their pups. Twelve female offspring per group were isolated and maintained on rodent chow till day 70, when MWM was performed.

![Figure 25.7](image-url)

**FIGURE 25.7** Treatment groups of Long-Evans dams maintained on control rodent chow or KD8%, KD14%, or KD18% from weaning (day 22) through puberty, breeding, gestation, and out to 180 days postpartum and postweaning of their pups. Twelve female offspring per group were isolated and maintained on rodent chow till day 70, when MWM was performed.

<table>
<thead>
<tr>
<th>Group 1: 12 female offspring</th>
<th>Group 2: 12 female offspring</th>
<th>Group 3: 12 female offspring</th>
<th>Group 4: 12 female offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Mothers (n = 12)</td>
<td>Group 2 8% KD Mothers (n = 12)</td>
<td>Group 3 14% KD Mothers (n = 12)</td>
<td>Group 4 18% KD Mothers (n = 12)</td>
</tr>
<tr>
<td>29 offspring</td>
<td>45 offspring</td>
<td>65 offspring</td>
<td>67 offspring</td>
</tr>
</tbody>
</table>

**TABLE 25.4 EFFECTS OF DIETS ON PREGNANCY, LITTER SIZE, PUPS DELIVERED, AND PUPS WEANED**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>% Pregnant Rats</th>
<th>% of Pregnant rats to have pups</th>
<th># Rats died during labor</th>
<th>Average litter size</th>
<th>Total live pups</th>
<th>Total pups weaned</th>
<th>% of Pups weaned</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>83.3</td>
<td>83.3</td>
<td>0</td>
<td>10.2</td>
<td>51</td>
<td>29</td>
<td>56.8</td>
</tr>
<tr>
<td>KD8%</td>
<td>6</td>
<td>100</td>
<td>100</td>
<td>1</td>
<td>8</td>
<td>48</td>
<td>45</td>
<td>93.7</td>
</tr>
<tr>
<td>KD14%</td>
<td>6</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>12.7</td>
<td>76</td>
<td>65</td>
<td>83.5</td>
</tr>
<tr>
<td>KD18%</td>
<td>6</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>12.5</td>
<td>75</td>
<td>67</td>
<td>89.3</td>
</tr>
</tbody>
</table>
female pups were weaned at day 21, put on standard RC, and maintained till day 70, when their spatial memory was assessed by the MWM.

KD dams showed no significant difference in blood glucose at day 70 (see Figure 25.5) but had significantly elevated β-hydroxybutyrate (see Figure 25.6), prior to breeding compared with controls.

Two breeding trials were conducted (n = 6/group/trial) and the results from the second trial are presented above. The KD rats all successfully produced litters and actually weaned a greater percentage of pups than the controls (Table 25.4). Average litter sizes were smaller for the KD8% than the other groups. This was not surprising, as the low protein delayed onset of puberty and likely has other deleterious effects on the gonads.

The results from the MWM of the pups of KD- or RC-fed dams tend to follow the reverse relationship, where elevated ketones are coincident with less time spent in the correct quadrant and thus worse spatial memory. The KD8% group performed significantly worse (p < .02) than the RC-fed controls. The KD14% and KD18% tended to perform better than the KD8%, but not as well as the controls. Statistical analyses included one-way ANOVA and post hoc Fisher’s least significant difference (LSD).

**FIGURE 25.8** Performance of female Long-Evans pups during diestrous (day 70–72), 47 to 49 days postweaning from control rodent chow- or KD-fed dams. Offspring of the KD-fed dams spent less time (s) in the correct quadrant of the water maze (where the hidden platform is located) than controls. KD8% again performed significantly worse (p < .02) than the controls. The KD14% and KD18% also performed better than the KD8%, but not as well as the controls. Statistical analyses included one-way ANOVA and post hoc Fisher’s least significant difference (LSD).

The long-term use of KDs by women during gestation may result in diminished cognitive function and other phenotypic effects in the offspring (Hartil et al., 2009). Bellisario and coworkers (2015) reported changes in dams’ behavior including increased aggression and decreased locomotor activity and decreased enzymatic activity (11β-dehydrogenase-2) in the placenta, which is designed to protect the fetus from maternal glucocorticoids. Increased exposure to glucocorticoids may have detrimental consequences to the developing fetus.

**SUMMARY**

The increased interest in KDs is not surprising, as the accumulated data for the successful treatment of epileptic seizures, especially, makes giving it at least a try a “no-brainer.” The known potential side effects of KDs, including hypoglycemia and its associated symptoms, especially in the short term; elevated lipids and cholesterol; and an elevated risk of kidney stones, may be tolerable to patients whose options are limited (Schwartzkoirin, 1999).

Dietary changes, especially radical ones, bring about metabolic changes throughout the body as the substrates for reactions change and the by-products of those metabolic changes impact homeostasis. It is certainly clear that HFDs and KDs can vary in percentage of lipid, source of lipid, and saturation state of lipid, thus inducing potential orexigenic or anorexigenic effects that will ultimately change the behavior
of the organism (Paoli et al., 2015). The possibility of deleterious consequences to offspring's metabolic regulatory system as well as cognitive function means it is imperative that investigations continue into the specific effects of HFD, both ketogenic and nonketogenic to uncover the components (saturated and nonsaturated fatty acids, protein content and source) necessary for safe therapies in relation to the endocrinology, development, and neuroendocrinology of animals and ultimately humans.

ACKNOWLEDGMENTS
The authors thank Dr. William Neace for assistance with statistical analyses. This research was supported by the Neuroscience Graduate Program and the College of Arts and Sciences Dean's Research Fund at the University of Hartford.

REFERENCES


Ketogenic Diet in the Laboratory


Alzheimer's Disease

Causes and Treatment

RICHARD L. VEECH, MD, PHD, DPHIL AND M. TODD KING

INCIDENCE
The prevalence of Alzheimer's disease is strongly correlated with age. In a general community, 3% of those between 65 and 74 years of age had probable Alzheimer's disease, compared with 18.7% of those between 75 and 84 years. In those over 85 the prevalence rose to 47.2% (Evans et al., 1989). In the United States it was estimated that in 2013 there were 5.2 million patients with Alzheimer's disease, costing $203 billion in direct medical care and another $216 billion in care from unpaid caregivers (Thies & Bleiler, 2013).

PREDISPOSING FACTORS
The major predisposing factor for Alzheimer's disease is age. Women are more likely to be affected than men. Diabetes, obesity and lack of exercise predispose to the disease. The genetic environmental, nutritional and metabolic risk factors are discussed below.

Genetic
Only about 10% of the cases of Alzheimer's disease are familial; about 90% are classed as "sporadic," with age being the major risk factor. A primary cause of the aging-associated damage is free radical damage (Harman, 1956). Factors other than aging can predispose individuals to the onset of Alzheimer's disease, the major genetic factor being the apolipoprotein E genotype (Strittmatter and Roses, 1995). Apolipoprotein E (ApoE) is the lipoprotein involved in transport of lipids and cholesterol in the circulatory system. There are three major alleles, ApoE-2, ApoE-3, and ApoE-4. In one large kindred group of the ApoE-4 genotype, the onset of clinical symptoms was between 55 and 78 years of age (Martin et al., 1997), demonstrating that this genetic factor predisposes individuals to a "late-onset" form of the disease.

Early-onset Alzheimer's disease is associated with three genes that increase the production or deposition of β-amyloid protein. These include the precursor of amyloid protein, found in Alzheimer's disease and in Down syndrome. Missense mutations in the presenilin 1 and 2 genes, which code for several secretases, involved in the catabolism of amyloid, lead to the early onset of an aggressive familial form of Alzheimer's disease. Mutations in these genes result in the onset of Alzheimer's disease, often in the 40s or 50s, but occasionally in the 30s. The increased incidence of early Alzheimer's disease with genetic abnormalities in amyloid suggests that amyloid can exacerbate the inhibition of pyruvate dehydrogenase (PDH) (Hoshi et al., 1996). It follows that inhibition of cerebral PDH could be the primary cause of Alzheimer's disease and the primary factor to be overcome in its treatment and prevention (Kashiwaya et al., 2000).

Environmental
Radiation is known to induce production of free radicals, or reactive oxygen species (ROS), and induce oxidant damage (Alexander and Stacey, 1959; Riley, 1994; Szilard, 1959). Low levels of radiation, under 5 Gy, can induce both cognitive dysfunction and the pathological changes of neurodegeneration seen in both Parkinson's and Alzheimer's disease (Kempf et al., 2013). In cultured neurons, radiation also induced tau phosphorylation (Li et al., 2014), characteristic of both Alzheimer's disease and frontotemporal dementia. These effects have application to cancer therapy, prolonged space flight, and exposure to nuclear radiation (Li et al., 2014).

Traumatic brain injury (TBI) prematurely causes pathology similar to both Alzheimer's and Parkinson's disease (Dekosky et al., 2013; DeKosky et al., 2010). In addition to the
accumulation of amyloid and phosphorylated tau, patients with TBI can develop both cognitive impairment and Parkinsonian symptoms. In contrast to the damaging effects of brain trauma, physical exercise can retard neurodegeneration and decrease inflammation in brain (Cotman et al., 2007).

**Nutritional and Metabolic**

Type II diabetes is strongly associated with both vascular dementia and Alzheimer’s dementia (Ott et al., 1996; Xu et al., 2009). Diabetes has been shown to accompany an increase in dementia in twin studies (Kuusisto et al., 1997). Conversely, caloric restriction or intermittent fasting ameliorates amyloid accumulation (Mouton et al., 2009) and the cognitive deficits in the triple transgenic mouse models of Alzheimer’s disease (Halogappa et al., 2007).

**PATHOPHYSIOLOGY**

The original clinical and pathological description of neurofibrillary tangles, amyloid plaques, paranoid ideation, and memory loss was given by Alois Alzheimer in 1907 (Alzheimer, 1907). Transgenic mouse models based on mutations in amyloid precursor protein and its processing show accumulation of amyloid and phosphorylated tau protein in their brains (Johnson-Wood et al., 1997). The most prevalent hypothesis to explain the pathophysiology of Alzheimer’s disease derives from the pathological findings described by Alzheimer of increased amyloid plaques and phosphorylated tau tangles. The theory that Alzheimer’s disease results from amyloid accumulation in the brain has been called the amyloid hypothesis (Selkoe, 1997). Alternatively, it is possible that an inhibition of brain PDH due to insulin resistance in the brain results in a deficiency in substrate availability in the tricarboxylic acid (TCA) cycle. Thus brain energy deficit and mild cognitive impairment can precede amyloid accumulation (Biesels and Reagan, 2015; Hoyer, 1991, 1996; Kuusisto et al., 1997; Talbot et al., 2012; Willette et al., 2015). The accumulation of amyloid and phosphorylated tau protein exacerbates the preexisting inhibition of PDH (Hoshi et al., 1997a; Hoshi et al., 1996). Mutations in amyloid precursor protein (APP) are found in comparatively rare early-onset forms of the disease. Amyloid accumulates in brains of subjects with Alzheimer’s disease, but its accumulation does not correlate, either quantitatively or temporally, with the cognitive impairment or loss of brain volume (Josephs et al., 2008).

Alterations in the alleles of the ApoE-4 lipoprotein (Roses, 2006) involved in cholesterol and lipid transport, are associated with predisposition to the more common late-onset form of the disease. While the association of the ApoE-4 allele with late-onset Alzheimer’s has been confirmed by exhaustive genomewide association studies (GWAS), no other major genetic links to Alzheimer’s disease were found (Naj et al., 2014). The majority of cases of late-onset Alzheimer’s disease are not associated with any identified genetic factor but are simply correlated with increased age. Alzheimer’s disease is, however, strongly correlated with insulin resistance both epidemiologically and pathophysiologically (Kuusisto et al., 1997; Ott et al., 1996; Reaven et al., 1990; Xu et al., 2009). The incidence of the disease can be increased by free radical damage, radiation, TBI, immune dysfunction, and indolence (Selkoe, 2001), all of which are also associated with insulin resistance (Zhai et al., 2011).

Evidence suggests that amyloid β-1-42 fragments can stimulate the phosphorylation and hence the inhibition of PDH. This amyloid fragment can exert toxic effects on hippocampal neurons in culture (Kashiwaya et al., 2000), which is compatible with the report that amyloid can inhibit PDH (Hoshi et al., 1997b; Hoshi et al., 1996). Triple transgenic mouse models of Alzheimer’s disease, in addition to showing increased amyloid and phosphorylated tau deposits, also exhibit decreased fluorodeoxyglucose (FDG) uptake in brain (Nicholson et al., 2010). These findings are also compatible with the ability of ketone body metabolisms to bypass the inhibition of PDH and produce large amounts of acetyl CoA required to supply the Krebs cycle (Sato et al., 1995). These and other reports have led to the proposal that the amyloid cascade hypothesis be rejected (Herrup, 2015), although defenders of the amyloid hypothesis remain and propose that amyloid accumulation is a necessary accompaniment of the disease (Musiek and Holtzman, 2015).

Central to Alzheimer’s disease is the decrease in cerebral glucose use. Decreases in cerebral glucose metabolism occur well before the clinical or pathological changes of Alzheimer’s disease (Blum-Degen et al., 1995; Cunnane et al., 2011; Hoyer et al., 1991). Inhibition of brain PDH has long been reported in the autopsy specimens from brains of patients with Alzheimer’s disease (Gibson et al., 1998). Indeed, intracerebral injection of streptozotocin, a drug that inhibits insulin secretion, leads to a decrease in insulin synthesis in brain (Grunblatt et al., 2007). Decreases in
brain oxidative metabolism lead to altered processing of amyloid precursor protein, increased amyloid accumulation (Hoyer, 1996), and to neuronal death. These changes in brain nutrient metabolism, which precede either the clinical or pathological changes of the disease, suggest that decreased brain insulin sensitivity (Talbot et al., 2012; Willette et al., 2015) and decreased brain energy production is an important factor in understanding the etiology of Alzheimer’s disease (Ding et al., 2013; Morgen and Frolich, 2015). Indeed, a deficit in brain energy metabolism alters the processing of brain APP (Gabuzda et al., 1994), suggesting the possibility that the accumulation of amyloid in the brains of patients with Alzheimer’s disease results from a deficit in cerebral energy metabolism. The accumulation of protein is not unique to Alzheimer’s disease, but occurs in other neurodegenerative conditions as reflected in the accumulation of α-synuclein in Parkinson’s disease and huntingtin in Huntington’s disease. These observations suggest that the accumulation of amyloid in the brains of patients with Alzheimer’s disease is secondary to a primary defect in cerebral energy metabolism resulting from PDH inhibition due to a decrease in cerebral insulin sensitivity. There is mounting evidence suggesting that brain insulin resistance is the major factor in the etiology of Alzheimer’s disease (Carro and Torres-Aleman, 2004; Craft et al., 2012; de la Monte, 2012; Bomfim et al., 2012; Kleinridders et al., 2014; Talbot et al., 2012). A recent study of patients with mild Alzheimer’s dementia were compared with cognitively normal age-matched controls and were found to have a decrease in cerebral 18F-fluorodeoxyglucose uptake but no decrease in cerebral metabolism of 11C-acetoacetate (Castellano et al., 2015). These results suggest that in patients with mild cognitive impairment, perfusion with a ketone body can overcome the metabolic defect resulting from the brain’s inability to use glucose. Indeed Alzheimer’s disease has been called type 3 diabetes (Steen et al., 2005) due to the decrease in insulin and insulin-like growth factor signaling mechanisms in autopsy specimens of patients with Alzheimer’s disease.

The metabolism of ketone bodies can, therefore, correct this insulin deficiency in brain because the metabolism of ketone bodies mimics the metabolic effects of insulin by increasing the production of acetyl CoA for use in the Krebs cycle in the absence of normal PDH activity (Kashiwaya et al., 1997; Sato et al., 1995). This property of ketone body metabolism renders the administration of ketone bodies a rational method for the therapy of this disease (Figure 26.1).

In addition to insulin resistance, patients with Alzheimer’s disease also show multiple physiological signs indicative of free radical damage (Wang et al., 2013). The metabolism of ketone bodies can reduce free radical damage by reducing the free cytosolic [NADP⁺]/[NADPH] ratio making it the lowest redox potential in the cell (Krebs, 1969). NADPH is the terminal destroyer of oxygen free radicals. Additionally, the β-hydroxybutyrate molecule itself inhibits histone deacetylase (HDAC). This inhibition of HDAC allows the acetyl groups on histones to remain in place so that the tightly

![FIGURE 26.1 D-β-hydroxybutyrate metabolism produces acetyl CoA, which enters the Krebs, tricarboxylic acid cycle by an alternative pathway that does not require the usual path through pyruvate dehydrogenase (PDH).](image-url)
packed structure can be relaxed and available to the FOXO3 transcription factors which leads to the transcription of the enzymes responsible for the destruction of ROS (Offermanns, 2006; Shimazu et al., 2013). The actual removal of the ROS is powered by NADPH of the free cytosolic [NADP+/NADPH] ratio (Krebs and Veech, 1969).

TREATMENT

The hypothesis that amyloid accumulation is the central etiological factor leading to Alzheimer’s disease has led multiple pharmaceutical companies to develop monoclonal antibodies to remove amyloid. Active amyloid vaccine trials were suspended due to the development of meningoencephalitis (Schenk, 2002). Passive immunotherapeutics with two antibodies have failed to significantly improve cognitive function or prevent progression but are now being tested in the early stage of the disease (Panza et al., 2014). Newer antibodies are currently being tested in asymptomatic individuals at risk of developing the disease, based on the amyloid β cascade hypothesis, in the hope that this approach will retard the development of clinically observable disease. It is fair to say that, to date, the results have been very disappointing (Prins and Scheltens, 2013).

An alternative treatment for Alzheimer’s disease has been developed based on the hypothesis that the disease results from a primary failure of the brain to develop both phosphorylation and redox energy. The therapy has been based on the observations that during starvation ketone bodies can replace glucose as the major metabolic fuel in brain (Cahill and Aoki, 1980; Owen et al., 1967). The detailed effects of the metabolism of ketone bodies were not well understood. A study was undertaken to explore the metabolic effects of ketone body metabolism in the working perfused rat heart (Kashiwaya et al., 1994; Sato et al., 1995). The working perfused heart provides the complete metabolic pathway, which results in the aerobic production of ATP by the complete oxidation of glucose. In brain, glucose is essentially the only energy-producing substrate under fed conditions. The entry of glucose into the Krebs cycle occurs through the production of acetyl CoA from pyruvate in a reaction catalyzed by PDH. The speed of that essential reaction is increased by insulin and decreased by loss of sensitivity to insulin. The variation in the speed of this reaction strongly suggests the loss of sensitivity of the brain’s PDH to insulin is the root cause of Alzheimer’s disease.

More remarkably, the addition and metabolism of ketone bodies, like addition of insulin, increased the joules of hydraulic work put out by the heart per mole of O₂ consumed (Kashiwaya et al., 1994), demonstrating that either ketone bodies or insulin could improve the metabolic efficiency of the working heart. Moreover, ketone body metabolism can mimic the metabolic effects of insulin (Kashiwaya et al., 1994). This increase in the efficiency of hydraulic work of the heart induced by either ketone bodies or insulin indicated an increase in the efficiency of mitochondrial energy generation. That increase in mitochondrial energy generation could later be translated into an increased physiological performance in elite athletes or in disease states where metabolic energy generation is deficient.

A detailed examination of the metabolites of the Krebs tricarboxylic acid cycle showed that addition of insulin to the working glucose-perfused heart increased acetyl CoA content ninefold, while addition of 4 mM ketone bodies increased acetyl CoA 15-fold. This very large increase in acetyl CoA in the case of insulin addition was caused by that hormone’s increased PDH activity, the enzymatic gateway to the Krebs cycle (Coore et al., 1971; Taylor and Jungas, 1974; Taylor et al., 1975). The even larger increase in acetyl CoA caused by the metabolism of ketone bodies demonstrated that metabolism of ketone bodies could bypass the major metabolic block in insulin sensitivity. The metabolism of ketone bodies mimics the effect of insulin’s activation of PDH by producing acetyl CoA from the ketone body acetoacetate via the succinyl CoA transferase reaction (Sato et al., 1995). The metabolism of ketone bodies mimics a major metabolic effect of insulin (Kashiwaya et al., 1997) and could therefore overcome insulin resistance, which is a common factor in many disease (Schuemann-Freestone et al., 2004; Zhai et al., 2011) or injury states (Li and Messina, 2009), including the brain (Talbot et al., 2012) of Alzheimer’s disease patients.

The Krebs cycle is the major energy-producing metabolic pathway, which results in the aerobic production of ATP by the complete oxidation of glucose. In brain, glucose is essentially the only energy-producing substrate under fed conditions. The entry of glucose into the Krebs cycle occurs through the production of acetyl CoA from pyruvate in a reaction catalyzed by PDH. The speed of that essential reaction is increased by insulin and decreased by loss of sensitivity to insulin. The variation in the speed of this reaction strongly suggests the loss of sensitivity of the brain’s PDH to insulin is the root cause of Alzheimer’s disease.

Other metabolic changes of importance also occur with addition of ketone bodies or insulin. The ratio of [isocitrate]/[α-ketoglutarate] is increased by both, indicating a reduction of the free NADP system (Krebs and Veech, 1969). The free mitochondrial [NAD⁺]/[NADH] ratio is also.
reduced 3- to 10-fold by the addition of either ketone bodies or insulin. Also of major significance is the increase in the ratio of [fumarate]/[succinate], indicating an oxidation of Q as seen in the ratio of [Coenzyme Q]/[Coenzyme QH₂] (Bergman et al., 2010). The increase in the redox span between the free mitochondrial NAD and Q couples indicates an increase in the ΔG of ATP.

FIGURE 26.2 The study of perfused rat hearts treated with glucose, glucose + insulin, glucose + ketone (D-β-hydroxybutyrate), or glucose + insulin + ketone (D-β-hydroxybutyrate). The relevant pathways are shown along with concentration changes relative to just glucose. Images are modified from Sato 1995 (Sato et al., 1995) originally drawn by Y. Kashiwaya.
FIGURE 26.3 The study of perfused rat hearts treated with glucose, glucose + insulin, glucose + ketone (D-β-hydroxybutyrate), or glucose + insulin + ketones (D-β-hydroxybutyrate). The relevant pathways are shown along with the relevant measures of ΔG, Gibbs free energy calculated for the nonstandard concentrations, E, reduction potentials based on actual concentrations, VO2 is oxygen consumption in mL/kg/min. Images are modified from Sato 1995 (Sato et al., 1995) originally drawn by Y. Kashiwaya.
hydrolysis from $\sim -53$ to $\sim -60 \text{ kJ/mole}$ in spite of a decrease in $O_2$ consumption from 18.5 to 16–17 $\mu$mol/min. An increase in the energy of ATP hydrolysis per $O_2$ consumed was observed with the addition of either ketone bodies or insulin (see Figures 26.2 and 26.3) (Sato et al., 1995).

**WHAT FORMS OF KETONE BODIES ARE AVAILABLE?**

Following the realization of the widespread effects of ketone body metabolism on the redox and phosphorylation states (Sato et al., 1995) and metabolic efficiency, it was recognized that therapeutic effects could result from the administration of ketone bodies (Cahill and Veech, 2003; Veech et al., 2001). During prolonged fasting (3 days) blood ketone body levels reach 5–7 mM (Cahill and Aoki, 1980). This blood level of ketone bodies results from the endogenous production of about 150 g of ketone bodies per day (Reichard et al., 1974). Therefore, to mimic the effects of the ketosis of starvation, about 150 g of ketone bodies, or about 1.5 moles, would be required to mimic the effects of starvation while maintaining safe levels (5–7 mM) of ketones. It is important to note that the minimum effective dose of ketone bodies in various disease states has not been established and may be much less than the 150 g per day produced during prolonged starvation.

Administration of ketone bodies as a simple acid or salt would present an unsafe load of either counter ion. Accordingly, examination of a variety of alcohols was undertaken. $R,1,3$ butanediol was the alcohol chosen, because it was converted in the liver to ketone bodies (Mehlman and Veech, 1972). Thus, a monoester of $R,\beta$-hydroxybutyrate $-R,1,3$ butanediol monoester presents no other metabolite load than the ketone body.

Additionally, the oxidation state of the ketone body being administered needs to be considered. Ketone bodies are readily interconverted between the oxidized form, acetoacetate, and the reduced form, $\beta$-hydroxybutyrate.

$$\beta$-hydroxybutyrate + $\text{NAD}^+ \leftrightarrow \text{acetoacetate} + \text{NADH} + \text{H}^+$$

While both forms of the ketone body can be metabolized, the energetic effects are quite different. Acetoacetate can readily be used in the Langendorf perfused heart, which is a simpler model than the working perfused heart (Williamson and Krebs, 1961). In the working perfused rat heart, $\beta$-hydroxybutyrate can be used to power the beating heart effectively. When acetoacetate is substituted, the heart fails (Taegtmeyer et al., 1980), showing that the excessive oxidation of the mitochondria NAD couple produced by acetoacetate impairs cardiac energy production. Although acetoacetate is attractive because of its low cost, clearly it should be avoided as a supplement and the more expensive chiral molecule $\beta$-hydroxybutyrate should be considered instead.

$R,1,3$ butanediol is converted to $\beta$-hydroxybutyrate, the physiological form of the ketone body, which is first oxidized to acetoacetate and then activated by succinyl CoA transferase to acetoacetyl CoA and hence to 2 acetyl CoA s by thiolase (see Figure 26.1). In contrast, $S,1,3$ butanediol is converted to $\beta$-hydroxybutyrate, which is activated by ATP and CoA to form $\beta$-hydroxybutyryl CoA and further metabolized by the $\beta$ fatty acid oxidation pathway (Lehninger & Greville, 1953). The metabolic fates of the $R$ versus the $S$ form of $\beta$-hydroxybutyrate are quite different, as shown in Figure 26.4. Metabolism of one molecule of the physiological enantiomer $\beta$-hydroxybutyrate consumes one molecule each of NAD$^+$, succinyl-CoA, CoASH, and CoQ and forms two molecules of acetyl-CoA, one molecule of fumarate, and one molecule each of the reduced forms of NAD$^+$ and coenzyme Q (QH$_2$). On the other hand, metabolism of the nonphysiological enantiomer, $\beta$-hydroxybutyrate consumes two molecules of CoA-SH, one molecule of NAD$^+$, and one molecule of ATP and forms two molecules of acetyl-CoA and one molecule each of AMP and pyrophosphate as well as one molecule of the reduced form of NAD$^+$. A second molecule of ATP is also consumed to convert the AMP formed to ADP.

In metabolizing mid-chain triglycerides (see Figure 26.5), a reduced flavoprotein is produced in the $\beta$ oxidation pathway, which results in a reduction of mitochondrial coenzyme Q, narrowing the redox span between mitochondrial NAD and Q and hence decreasing the energy available for ATP synthesis. This is in contrast to the increase in redox span that accompanies the metabolism of the physiological form of the ketone body, $\beta$-hydroxybutyrate (Sato et al., 1995).

It follows from the above, that the oxidized form of ketone body, acetoacetate, should not be included in esters for administration for the purpose of elevating ketone bodies because such solutions would oxidize the mitochondrial NAD couple and lower the energy available for the formation of ATP. Similarly, the use of racemic $R, S,1,3$ butanediol should not be used because the unphysiological $S$ form undergoes $\beta$ oxidation,
producing mitochondrial flavoprotein, thus decreasing the oxidation of the Q couple, which results from the metabolism of the physiological R-3-hydroxybutyrate. Several studies using the acetoacetate and racemic 1,3 butanediol ester have appeared (D’Agostino, 2013; Desrochers et al., 1995). The effects of these ketone ester formulations would be quite different from those produced by the D-β-hydroxybutyrate–R-1, 3 butanediol monoester.

Evidence presented here suggests that Alzheimer’s disease results from a deficit in cerebral energy generation, primarily due to a loss of insulin sensitivity in brain, and that amyloid accumulation results from this decrease in cerebral metabolic energy. The primary therapeutic target would therefore become not the removal of amyloid but the overcoming of the block of cerebral metabolic energy production caused by cerebral insulin resistance. This could be achieved by (1) feeding a therapeutic amount of D-β-hydroxybutyrate–R-1, 3 butanediol monoester, producing acetyl CoA without requiring the activity of brain PDH (Kashiwaya et al.,

FIGURE 26.4 The metabolism and sum reactions for both D and L β-hydroxybutyrate.

Metabolism of the L form of β-hydroxybutyrate (A) uses two molecules of CoASH and one molecule each of ATP and NAD+ and forms 2 acetyl-CoAs and one molecule each of AMP, PPi, and reduced NAD+ (NADH, H+). Metabolism of the D form of β-hydroxybutyrate (B) on the other hand uses one molecule each of NAD+, succ-CoA, CoASH, and CoQ and produces two molecules of acetyl-CoA and one molecule each of fumarate and the reduced forms of NAD+ (NADH, H+), and CoQ (CoQH2). Sum reactions for each isomer are given in C and D.
Figure 26.5 The beta-oxidation pathway of metabolizing fatty acids. The β fatty acid oxidation pathway produces 1 NADH and 1 flavoprotein compared with the two NADHs produced by the oxidation of the physiologically normal D-β-hydroxybutyrate. Once you cycle down to the last two carbon atoms the terminal L-β-hydroxyacyl-CoA is L-β-hydroxybutyryl-CoA, not the D form of β-hydroxybutyrate.

2013; Newport et al., 2015). This approach has decreased amyloid and phosphorylated tau in the brains of triple transgenic mice and improved cognitive performance. In a single human subject, behavioral improvement on feeding ketone has been noted.

Alternatively, increasing the brain’s concentration of insulin by introducing insulin into brain through intranasal administration of insulin has been reported. This therapy also has proven successful in treating Alzheimer’s disease in a limited number of cases. Cerebrospinal fluid insulin levels were shown to be significantly lower in patients with Alzheimer’s than normal controls (Craft et al., 1998). The olfactory lobe contains the highest concentration of insulin receptors of any brain area (Kleinridders et al., 2014). Intranasal administration of large doses of insulin improve cognitive performance in patients with early cognitive impairment due to Alzheimer’s disease (Reger et al., 2008).

Ketone bodies mimic the metabolic effects of insulin. They increase the energy of ATP hydrolysis, decrease free radical damage, and increase the transcription of antioxidant enzymes by inhibiting histone deacetylase (Shimazu et al., 2013; Veech, 2014). In the genetic mouse model of early-onset Alzheimer’s disease, feeding mice ketone esters resulted in a decrease in brain amyloid and phosphorylated tau accumulation as well as improvement in cognitive function (Kashiwaya et al., 2013). Feeding ketone ester to a patient with advanced Alzheimer’s disease improved his behavior and cognitive function (Newport et al., 2015). These results support the need for larger clinical trials of ketone esters in the treatment of Alzheimer’s disease and other diseases where insulin resistance is a major etiological factor.

References


kinase 3beta in brain. Proc Natl Acad Sci USA 93, 2719–2723.


Mitigation of Damage from Reactive Oxygen Species and Ionizing Radiation by Ketone Body Esters

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INTRODUCTION
Free radicals play an important role in a number of chronic degenerative diseases including autoimmune disorders, aging (Harman, 1956), cataracts, rheumatoid arthritis, Parkinson’s disease (Kashiwaya et al., 2000), cardiovascular disease, and other neurodegenerative diseases (Pham-Huy et al., 2008). However, the pathophysiology of these diseases can be complex. The simplest form of ROS-induced disease is radiation sickness.

ORIGINS OF REACTIVE OXYGEN AND NITROGEN SPECIES
Reactive oxygen species (ROS) and reactive nitrogen species (RNS) arise from numerous sources including cellular metabolism, ionizing radiation, and various enzymes. Once created, individual ROS and RNS participate in a cascade of interconversions (Figure 27.1) both enzymatic and not, ultimately being detoxified by being converted into inert molecules or by damaging various biomolecules.

Basal production of ROS occurs in the mitochondria as a byproduct of normal metabolism by the spontaneous oxidation of coenzyme Q semiquinone by oxygen, creating superoxide (O$_2^•−$) (Chance et al., 1979).

\[ \text{Q}^{•−} + \text{O}_2 \rightarrow \text{O}_2^{•−} + \text{Q} \]

Another common origin of ROS is radiation; ionizing radiation with energy ($h\nu$) above 33eV can ionize water, which makes up the vast majority of human cell volume. This process occurs with many radiation types including particles (e.g., α or β), electromagnetic (e.g., γ or X), and neutrons. Radiation of sufficient energy will split water into a hydroxyl radical (HO•), H', and a free electron, e−. These three species interact with a variety of other molecules to form the cascade of RNS and ROS shown in Figure 27.1.

Superoxide can also be generated without radiation by NADPH oxidases (NOXs) in white blood cells in response to pathogens (Bedard and Krause, 2007) or as an inducible response to inflammation and cellular stress. Enzymes producing ROS are also induced after exposure to radiation, particularly the NOX family members DUOX 1 and 2, which continue producing superoxide for a number of days post radiation (Ameziane-El-Hassani et al., 2015). This prolonged ROS production leaves a therapeutic window for post-radiation treatment that targets these molecules.

Numerous other enzymes produce other types of ROS as part of their normal function, such as monoamine oxidase (MAO) (EC 1.4.3.4), an enzyme integral in monoaminergic neurotransmission and xenobiotic metabolism. This enzyme produces hydrogen peroxide as part of its breakdown of monoamines, such as dopamine in this reaction: Dopamine + H$_2$O + O$_2$ \rightarrow Dopaldehyde + NH$_3$ + H$_2$O$_2$. This is an important issue in the treatment of Parkinsonian patients, who are often given high doses of L-dopa, which is known to increase ROS load (Acquier et al., 2013) and can lead to further loss of dopaminergic neurons, often seen in patients on high doses of L-dopa (Yamato et al., 2010).

Figure 27.1 provides a roadmap showing the cascade of ROS and RNS. Superoxide plays a central role in the cascade. In the case of radiation, superoxide results from the free electron combining with oxygen. The enzyme superoxide dismutase (SOD) catalyzes a reaction where two superoxide ions

* Authors contributed equally.
react to produce oxygen and hydrogen peroxide. The hydrogen peroxide can decompose to hydroxyl radical and hydroxide in the presence of iron and other metals via the Fenton reaction. Superoxide reacts with nitric oxide (NO) to produce peroxynitrite and begins the cascade of RNS. The reaction of NO with superoxide is diffusion limited and non-enzymatic. This is especially problematic as NO is uncharged, enabling it to pass quickly through cellular membranes, and possesses a relatively long half-life. The kinetics of the reaction of NO with superoxide make the production of peroxynitrite inevitable whenever NO is produced within a few cell diameters of superoxide (Pacher et al., 2007). Peroxynitrite in turn can either react directly with CO₂ to form nitrosoperoxycarbonate, which dissociates to form other radical species, or be protonated to form peroxynitrous acid, which dissociates into hydroxyl radical and nitrogen dioxide radical. The formation of hydroxyl radical from peroxynitrite may contribute more to the formation of free radicals than the iron catalyzed Fenton reaction (Pacher et al., 2007).

Oxygen-containing free radicals and other ROS or their nitrogenous counterparts perform useful functions in cell signaling and physiology (Pham-Huy et al., 2008). While the causes of excessive ROS and RNS in diseases may have complex or indeterminate origins, the exposure to radiation provides a model with a single known initiator. Generation of ROS/RNS is a component of many common diseases including cataracts, rheumatoid arthritis, neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and Parkinson’s (Kashiwaya et al., 2000) and Alzheimer’s disease, cancer (Pham-Huy et al., 2008), and aging (Harman, 1956). Table 27.1 summarizes reactions that generate or destroy RNS and ROS.

**DAMAGE CAUSED BY REACTIVE OXYGEN AND NITROGEN SPECIES**

Free radicals attack nucleic acids, proteins, and lipids indiscriminately. Nucleic acids are attacked either at the sugar (ribose or deoxyribose) or the...
base that is responsible for pairing. If hydroxyl reacts with the sugar, it results in strand breaks, recombination, chromosomal changes and mutations. If hydroxyl radical reacts with the base, it adds alcohol groups, OH, to the base where none belong and interferes with the hydrogen bonding and fit of the matching base pair, leading to point mutations. Modified guanine interferes with the telomerase binding proteins TRF1 and TRF2 and causes a loss of telomere function, which requires these binding proteins for both the protective shelterin complex and for telomerase to interact with shelterin and extend the telomere (Sfeir, 2012). See for example Figure 27.2.

Proteins have various reactions depending on the amino acid. One that is vulnerable is cysteine. Oxidative stress can cause cysteine on two proteins or within one protein to form a disulfide bridge and change the folding of the protein. This can be reversed as a protein disulfide bond is reduced by thioredoxin reduction reaction shown in Figure 27.4. However, further oxidation leads to SOH, SO₂H and SO₃H, which makes the protein permanently damaged.

Damage to lipids is primarily to polyunsaturated fatty acids (PUFAs). There are three stages, initiation, propagation, and termination. Initiation causes the PUFA to become a free radical. Propagation generates other free radicals, oxygen is often involved. Oxygen is a dual free radical, and one might draw it as •O₂ to show the two unpaired electrons. Being a dual radical makes •O₂ especially reactive with transition metals that are free radicals and also other free radicals. Having the two unpaired electrons allows •O₂ to propagate damage as in R• + O₂ → ROO• → further reactions. NO• being a single free radical would result in a terminating reaction with other free radicals as RS• + NO• → RS–NO.

Peroxynitrite (ONOO⁻) is very reactive but it is not a free radical, therefore it tends to modify proteins on specific residues and motifs such as methionine, tyrosine, zinc fingers, and heme (see Figure 27.1). THE ROLE OF REACTIVE OXYGEN SPECIES IN AGING

In 1956, Denham Harmon, working in the Donner Biophysics Lab at Berkeley, wrote a paper postulating that the process of aging was the result of free radicals produced either endogenously or by environmental sources (Harman, 1956). In 1961 Hayflick and Moorman showed that cultured cells could undergo only a finite number of replications for reasons that were unclear. In 1973 Olovnikov postulated that the end replication of DNA would result in the shortening of that strand at every replication (Olovnikov, 1973). In 1978, Elizabeth Blackburn published a paper describing a special form of DNA at the end of chromosomes, which is the telomere (Blackburn

### TABLE 27.1 THE GENERATION AND DESTRUCTION OF RNS AND ROS

<table>
<thead>
<tr>
<th>ROS/RNS formation reactions</th>
<th>ROS/RNS destruction reactions</th>
<th>Catalyst</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O + hν → HO• + H⁺ + e⁻</td>
<td>NOX, xanthine oxidase</td>
<td>spontaneous</td>
</tr>
<tr>
<td>O₂ + e⁻ → O₂•⁻</td>
<td>Superoxide dismutase</td>
<td>spontaneous</td>
</tr>
<tr>
<td>Fe²⁺ + H₂O₂ → Fe³⁺ + H₂O</td>
<td>Catalase</td>
<td>spontaneous</td>
</tr>
<tr>
<td>H₂O₂ + O₂ → H₂O + O₂•⁻</td>
<td>Glutathione peroxidase</td>
<td>spontaneous</td>
</tr>
</tbody>
</table>

FIGURE 27.2 Guanine reacts with hydroxyl radical to produce 8-OH-G. There are three guanines in the telomere repeat sequence, TTAGGG, making it vulnerable to oxidative damage. R is deoxyribose.
Cell senescence can be triggered by a number of mechanisms including telomere uncapping, DNA damage, oxidative stress, oncogene activity, lack of nutrients, and other factors via various signaling pathways (Ben-Porath and Weinberg, 2005). A number of studies reported that oxidative stress shortened telomeres (von Zglinicki, 2002). The formation of 8-oxo-guanine lesions in telomeric tandem repeats by ROS-damaged telomeric protein binding to telomeres (Opresko et al., 2005) impaired their functioning, causing replication to cease.

In the absence of disease, ROS damage depends on the rate of metabolism and with it, the rate of ROS production by mitochondria, which roughly correlate with the life span (Speakman, 2005), a phenomenon known as scaling. From these and other observations it was thought that the rate of metabolism inversely correlated with life span and slowing it by measures such as caloric restriction would lengthen life span (Sohal and Weindruch, 1996), in part by limiting mitochondrial ROS generation and hence ROS damage. Studies also appeared which reported that overexpression of the antioxidant enzyme catalase increased life span in transgenic mice (Schriner et al., 2005). Other antioxidant enzymes also increased life span in Drosophila (Orr et al., 2013).

The most important system providing antioxidants and defending against ROS is the NADPH defense system described later in this chapter. The preceding reports show that ROS can accelerate telomere shortening, damage DNA directly, induce senescence, and hasten the aging process (Correia-Melo et al., 2014). Suppressing ROS may slow the shortening of telomeres and decrease activation of other mechanisms implicated in the pathology of aging (Harman, 1956, 1981). We postulate that using ketone body esters to suppress ROS would be effective in retarding the aging process itself.

Telomere shortening occurs during normal aging, leading to uncapping, activating senescence and apoptotic programs in tissues with high cell turnover. Rapidly dividing cells—gut, skin, hematopoietic, and immune cells—replicate constantly during life, and aging is visible to anybody in skin as it changes from thick and smooth in youth to thin and wrinkled in the aged. The effects of nuclear radiation are likewise amplified in these tissues, exposure in the range of 2 to 5 Gy suppresses replication of blood and gut cells through generation of ROS, causing death in weeks. In these rapidly dividing cell types, a telomeric process as described by Hayflick could play a more dominant role.

There is, however, another large group of cells that undergo almost no replication after embryonic life. They include heart, skeletal muscle, and brain cells. The death of these slowly dividing cells does not have a unitary mechanism. The gradual onset of senescence that characterizes normal aging may in part be due to the accumulation of genetic damage over a lifetime (Szilard, 1959).

Mice lacking the gene for dystrophin (MDX) demonstrate little cardiac abnormalities. However, mice in which the mRNA component of telomerase, mTR, was deleted show progressive shortening of telomeres with age. Crossing these two strains to form the mdx/mTRko mouse with deleted dystrophin and TERC produced a mouse with heart abnormalities, mitochondrial dysfunction, and oxidative stress with reduced levels of the mitochondrial regulators peroxisome proliferator activated receptor gamma coactivator 1 α and 1 β (PGC1-α and PGC1-β), both reduced by 80%. The pathological changes in hearts of these animals were ameliorated by administration of antioxidants (Mourkioti et al., 2013). These findings show that both mitochondrial ROS production and telomere shortening play a role in the death and dysfunction of non-rapidly dividing cells such as heart cells and that these functional abnormalities induced in mdx/mTRko can be reduced by administration of antioxidants.

The toxicity from radiation is therefore both acute and chronic. Acute death occurs from effects on the central nervous system (CNS) at doses of 20 Gy, or slow, long-term accumulated DNA damage leading to cell death from multiple hits (Szilard, 1959). An earlier speculation by Erwin Schrodinger attributed life to the maintenance of negative entropy by homeostatic processes (Schrodinger, 1944). In this view, aging and death would be the result of an increase in entropy. This general statement without a biochemical or physiological mechanism is of limited practical use, whereas applications of thermodynamics as exemplified by the energetics of the various redox states and the process of oxidative phosphorylation have heuristic value.

**HOW ANTIOXIDANTS WORK**

There is a basic pattern to how antioxidant small molecules work. They follow the pattern of reaction shown in Figure 27.3.
The antioxidant is oxidized and the free radical is reduced. Why then is it not a problem that the antioxidant has become a free radical? The answer is that the unpaired electron in the now antioxidant radical is delocalized and thus less reactive. This property makes ascorbate and tocopherol distinct from other cellular reducing agents, as they remain stable compounds after a single electron transfer. This is in contrast to glutathione, a powerful and important reducing agent, but one that must transfer two electrons at once to remain stable, as the glutathione radical is highly reactive. Tocopherol is able to transfer a single electron but only a single electron, ascorbate is able to transfer two—and, importantly, two ascorbate radicals are able to react, forming a single molecule of dihydroascorbate and one molecule of ascorbate. Dihydroascorbate in turn is able to accept two electrons from glutathione and be regenerated, bridging single electron redox couples with two electron couples. Glutathione itself is reduced by the free [NAD$^+$]/[NADH] redox couple. It is important to understand factors controlling the cellular redox states to understand how the antioxidants get recharged (reduced).

The redox state of the cytoplasmic NAD-couple in vivo was first defined in 1955 by Lynen and his coworkers (Holzer et al., 1956) in yeast by measurement of the ratio of the metabolites [ethanol]/[acetalddehyde] in a reaction brought to near-equilibrium by the very active cytoplasmic enzyme alcohol dehydrogenase. Soon thereafter it was shown by Bucher and Klinenberg (1958) that the free [NAD$^+$]/[NADH] in rat liver cytoplasm could be estimated by measurement of either the [lactate]/[pyruvate] or [dihydroxyacetone-P]/[3-glycerophosphate] ratio. Because of the rapidity with which these cellular redox state estimates change with hypoxia, accurate determinations were facilitated by the development of freeze clamping (Wollenberger et al., 1960). That ratio of [lactate]/[pyruvate] was found to be between 5 and 10, giving a free cytosolic [NAD$^+$]/[NADH] calculated from the measured Keq of the reaction to be about 1,000 in the fed liver and 250 in a starved liver. Following these principles, in 1967 Krebs and his coworkers (Williamson 1967) showed that the free [NAD$^+$]/[NADH] ratio in mitochondria could be estimated by measurement of ether the [acetoacetate]/[β-D-hydroxybutyrate] or [α-ketoglutarate]×[NH$_4^+$]/[glutamate] ratio and the ratio of free mitochondrial [NAD$^+$]/[NADH] was found to be between 2 to 10. Following a question to Krebs by George Cahill, Krebs assigned Richard Veech to determine the free cytoplasmic [NAD$^+$]/[NADPH] ratio, which was known be used in the regenerative synthesis of fats. This ratio was found to be about 0.01 to 0.02 and could be estimated by
measurement of $[\alpha$-ketoglutarate]×[CO$_2$/[isocitrate] or several other couples (see Table 27.2). The standard potential of the NAD- couple is about $-0.32$ V, but these couples have three potentials in the cell depending on the intracellular location of the enzymes involved. The three redox couples have redox potentials appropriate to the metabolic pathways they drive.

First, in cytoplasm, the $\Delta E$ of the free NAD- couple is $-0.19$ V, this smaller potential poising it to receive reducing power from glycolysis. Second, for NAD in mitochondria, it is $-0.28$ V, allowing for more energy to be available for the electron transport pathway to convert ADP to ATP. Third, the NADP- couple in the cytosol was estimated to be about $-0.42$ V, the lowest reduction potential in the cell (Krebs & Veech, 1969). This strongly reducing potential allows this couple not only to synthesize fats but also to favor the inactivation of oxygen free radicals through the two major intracellular antioxidants, glutathione (GSH) and ascorbic acid (Vitamin C). As these antioxidants are in near equilibrium with the free cytoplasmic [NADP$^+$]/[NADPH] couple, whose large reducing potential favors the reduced, active forms of GSH and Vitamin C (see Table 27.2 for a list of these redox couples and Figure 27.4 for a diagram of the ROS-quenching reactions driven by the NADP couple).

These major cellular redox states are related to one another through common intermediary metabolites (Bergman et al., 2010; Krebs, 1969) and also to the cellular phosphorylation potential or ATP/ADP ratio keeping the energy of ATP hydrolysis in the range of $-53$ to $-57$ kJ/mole (Veech et al., 1979). It can be seen that these intracellular redox potentials cannot be inferred by study to the standard potential of redox half reactions, which have led to much confusion in writing about cellular redox potentials (Winterbourn, 1979).

**TABLE 27.2 THE ACTIVITY OF NADP- LINKED ENZYMES IN RAT LIVER**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activity in µmol/min/g</th>
<th>$K_{eq}$ = Metabolite couple</th>
<th>$K_{eq}$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isocitrate DH EC 1.1.1.41</td>
<td>22</td>
<td>$K_{eq} = \frac{[\alpha$-KG$^+][\text{CO}_2][\text{NADPH}]}{[\text{isocitrate}^+][\text{NADP}^+]$}</td>
<td>1.17 M</td>
</tr>
<tr>
<td>Glutathione Reductase EC 1.6.4.2</td>
<td>7</td>
<td>$K_{eq} = \frac{[\text{GSSG}][\text{NADPH}][\text{H}^+]}{[\text{GSH}][\text{NADP}^+]$}</td>
<td>$100 \times 10^{-7}$ (Scott et al., 1963)</td>
</tr>
<tr>
<td>6 P Gluconate DH EC 1.1.1.44</td>
<td>2.8</td>
<td>$K_{eq} = \frac{[\text{Ribulose 5P}^+][\text{CO}_2][\text{NADPH}]}{[6\text{P Gluconate}^+][\text{NADP}^+]$}</td>
<td>1.72 $\times 10^{-1}$ M (Villet and Dalziel, 1969)</td>
</tr>
<tr>
<td>Glucose 6-P DH EC 1.1.1.49</td>
<td>1.4</td>
<td>Not applicable because of lactonase</td>
<td></td>
</tr>
<tr>
<td>Malic Enzyme EC 1.1.1.39</td>
<td>1.27</td>
<td>$K_{eq} = \frac{[\text{pyruvate}][\text{CO}_2][\text{NADPH}]}{[\text{Malate}^+][\text{NADP}^+]$}</td>
<td>$3.44 \times 10^{-2}$ M</td>
</tr>
</tbody>
</table>

**Source:** Veech et al. (1969)

**FIGURE 27.4** Thioredoxin is a family of three proteins in mammals—TrxR1 (cytosolic), TrxR2 (mitochondrial), TrxR3 (testis specific)—that can reduce protein disulphide bonds formed by ROS/RNS damage. Abbreviations: TrxR—thioredoxin, FAD—flavin adenine dinucleotide, S2—disulphide, SH2—two sulphydryl moieties.
Measurement of each of the metabolite couples listed in Table 27.2 yields the same value of the free cytosolic [NADP\(^+\)]/[NADPH] ratio with a redox potential of about \(-0.4\, \text{V}\), the most negative of any intracellular potential (Krebs, 1969). With small refinements of the Keq, CO\(_2\) concentrations and intracellular metabolite concentrations in the intervening years, the potential of the NADP system is more likely to be in the range of \(-0.36\) to \(-0.38\, \text{V}\), but is still the most negative potential in the cell.

The formalism to calculate the redox potential in the cell estimated from measured intracellular metabolites and the measured Keq of the reactions involved is as follows. The standard redox potential is derived from the equilibrium constant of the reaction. The standard potential of both the NAD and NADP couples, \(E^0\), is, within experimental error, \(-0.32\, \text{V}\) (Burton, 1974).

The free cytosolic [NAD\(^+\)]/[NADH] calculated from the lactate dehydrogenase reaction:

\[
\text{[lactate]}^- + \text{[NAD]}^+ \rightarrow \text{[pyruvate]}^- + \text{[NADH]} + \text{[H]}^+
\]

At pH 7

\[
\frac{[\text{Pyr}^-]}{[\text{Lac}^-]} = K_{LDH} = 1.11 \times 10^{-4}
\]

Rearranged and substituted into equation 1 above

\[
E_{\text{NAD/NADH}} = E^0_{\text{NAD/NADH}} + \frac{RT}{nF} \ln \left( \frac{[\text{Pyr}^-]}{[\text{Lac}^-]} \times \frac{1}{K_{LDH}} \right)
\]

For [pyruvate]/[lactate] = 1:1

\[
E_{\text{NAD/NADH}} = -0.32\, \text{V} + 0.122\, \text{V} = -0.198\, \text{V}
\]

The free cytosolic [NADP\(^+\)]/[NADPH] redox potential calculated from isocitrate dehydrogenase reaction and from the malic enzyme agree well and are the most negative potential in the cell, although with improved metabolite measurements are slightly above the \(-0.42\, \text{V}\) estimated in the original Krebs and Veech paper (Krebs, 1969).

The redox potential from isocitrate dehydrogenase reaction:

\[
E_{\text{NADP/NADPH}} = E^0_{\text{NADP/NADPH}} + \frac{RT}{nF} \ln \left( \frac{[\text{NADP}^+]}{[\text{NADPH}]} \right)
\]

\[
[\text{Isocitrate}^2-] + [\text{NADP}^+] \rightarrow [\alpha\text{-Ketoglutarate}^2-] + [\text{NADPH}] + [\text{CO}_2]
\]

\[
\frac{[\alpha\text{-Ketoglutarate}^2-] \times [\text{CO}_2]}{[\text{Isocitrate}^2-]} = 1
\]

\[
E_{\text{NADP/NADPH}} = E^0_{\text{NADP/NADPH}} + \frac{RT}{nF} \ln \left( \frac{[\alpha\text{-Ketoglutarate}^2-] \times [\text{CO}_2] \times 1}{[\text{Isocitrate}^2-] \times K_{ICDH}} \right)
\]

For [\alpha\text{-Ketoglutarate}]/[Isocitrate] = 7:1 (Liver) and [CO\(_2\)] = 1.985mM

\[
E_{\text{NADP/NADPH}} = -0.32\, \text{V} - 0.044\, \text{V} = -0.364\, \text{V}
\]

The redox potential from from the malic enzyme reaction:

\[
\frac{[\text{NADP}^+]}{[\text{NADPH}]} = \frac{[\text{Pyr}^-] \times [\text{CO}_2]}{[\text{Mal}^2-]} \times \frac{1}{K_{MalEnz}}
\]

where [pyruvate]/[malate] = 1:3 in liver

\[
E_{\text{NADP/NADPH}} = -0.32\, \text{V} - 0.055\, \text{V} = -0.375\, \text{V}
\]

Because the glutathione couple reacts with the NADP- couple (see Table 27.3), the potential of the NADP- couple determines the redox potential of the glutathione redox couple [GSH]\(^2-\)/[GSSG)] with which it is in near-equilibrium at \(-0.37\, \text{V}\), not the standard potential of \(-0.32\, \text{V}\) at pH 7.0. The terminal reduction of the oxygen free radicals involves
glutathione, which constitutes the most abundant intracellular antioxidant at 5 mM (Krebs, 1969).

Ascorbate, at 2–3 mM, is the second most abundant reducing agent and antioxidant, and is also the cofactor for a number of metal-dependent oxygenases and dioxygenases, such as collagen prolyl and lysyl hydroxylases (Linster and Van Schaftingen, 2007). Ascorbate, when acting as a reducing agent, loses an electron and a proton to form semidehydroascorbate (SDA), which upon losing another electron forms dehydroascorbate (DHA). Regeneration of ascorbate by glutathione occurs by the reaction: $\text{DHA} + 2\text{GSH} \leftrightarrow \text{ascorbate} + \text{GSSG}$, which can occur spontaneously (Linster and Van Schaftingen, 2007) but can also be catalyzed by the enzyme glutathione dehydrogenase (EC 1.8.5.1), an enzyme with an activity in rat liver of about 3 µmol/min/g wet weight (Carlberg and Mannervik, 1975). These reactions (Table 27.3) ensure that the redox potential of ascorbic acid and the glutathione couple in vivo assume the redox potential of the cytosolic NADP− couple of −0.37 V and not the standard potential—this is in sharp contrast to ascorbate’s standard reduction potential of −0.06 V (Table 27.4) or the standard potential of the glutathione couple at −0.23 V (Clark, 1960).

The semidehydroascorbate (SDA) is also reduced to ascorbate by thioredoxin reductase (EC 1.6.4.5) (Linster and Van Schaftingen, 2007), an NADP-linked enzyme that is the major cellular protein disulfide reductase (Arner and Holmgren, 2000). This enzyme catalyzes the reaction: $\text{NADPH} + \text{thioredoxin}_{\text{red}} \rightarrow \text{NADP}^+ + \text{thioredoxin}_{\text{ox}}$. This enzyme is critical in signaling as part of thiol redox control, and regulates the activity of a number of transcription factors. The potential of the thioredoxin system is likewise set by the potential of the free cytosolic [NADP⁺]/[NADPH] couple.

Thus, the potential of the ascorbate couple and the glutathione couple are the same as that of the NADP− couple. Likewise, disulfide (SS) groups in protein are reduced to −SH by the thioredoxin system.

### THE REDOX STATE OF OXYGEN FREE RADICALS AND OTHER RELEVANT REACTANTS

A list of relevant redox potentials is listed in Table 27.4 below. The values for the redox potentials given are taken from Bergman et al. (2010), Clark (1960), Dawes (1971), and Krebs (1969).

This table shows the oxidizing potential of ROS compounds. However, it should be pointed out that these half reactions do not represent the redox potential inside of cells, which are set by the NAD and NADP couples which carry the dominant metabolic flux during metabolism.

### HOW D-ß-ßHB ALTERS CELLULAR REDOX STATES

The effects of insulin, glucose, and D-ß-hydroxybutyrate (D-ßHB) on a perfused rat heart gave insights into the changes in relative concentrations of key metabolites involved in the Krebs cycle and oxidative phosphorylation and the free [NADP⁺]/[NADPH] ratio. Prior work on the effects of hearts perfused with D-ßHB showed that the [isocitrate]/[α-ketoglutarate] (Sato et al., 1995) couple was increased. This change indicated a reduction of the NADP− couple (Kashiwaya et al., 1997). Figure 27.5 shows the increased ratio of [NADPH]/[NADP⁺] found in the perfused rat heart studies, which supports a highly significant increase in the capacity for cellular ROS/RNS detoxification. (We are intentionally breaking a
<table>
<thead>
<tr>
<th>Half reaction</th>
<th>Redox Potential of half reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{H}^+ + e^- \rightarrow \frac{1}{2} \text{H}_2$</td>
<td>-0.41</td>
</tr>
<tr>
<td>Acetyl-CoA + 2H$^+$ + 2e$^-\rightarrow$ Acetaldehyde + CoA</td>
<td>-0.42</td>
</tr>
<tr>
<td>Acetoacetate$^-$ + 2H$^+$ 2e$^-\rightarrow$ β-hydroxybutyrate$^-$</td>
<td>-0.36</td>
</tr>
<tr>
<td>NAD$^+$ + 2H$^+$ + 2e$^-\rightarrow$ NADH + H$^+$</td>
<td>-0.32</td>
</tr>
<tr>
<td>NADP$^+$ + 2H$^+$ + 2e$^-\rightarrow$ NADPH + H$^+$</td>
<td>-0.32</td>
</tr>
<tr>
<td>$\alpha$ ketoglutarate$^{2-}$ + CO$_3^+$ 2H$^+$ + 2e$^-\rightarrow$ isocitrate$^{3-}$</td>
<td>-0.318</td>
</tr>
<tr>
<td>Pyruvate + 2H$^+$ + 2e$^-\rightarrow$ Lactate</td>
<td>-0.19</td>
</tr>
<tr>
<td>FAD + 2H$^+$ + 2e$^-\rightarrow$ FADH$_2$</td>
<td>-0.06</td>
</tr>
<tr>
<td>Fumarate + 2H$^+$ + 2e$^-\rightarrow$ Succinate</td>
<td>+0.031</td>
</tr>
<tr>
<td>Dehydroascorbate + 2H$^+$ + 2e$^-\rightarrow$ Ascorbate</td>
<td>+0.06</td>
</tr>
<tr>
<td>$\alpha$-ketoglutarate$^{2-}$ + CO$_3$ + isocitrate$^{3-}$</td>
<td>+0.002</td>
</tr>
<tr>
<td>Ubiquinone + 2H$^+$ + 2e$^-\rightarrow$ Dihydroubiquinone</td>
<td>+0.10</td>
</tr>
<tr>
<td>$\frac{1}{2}$O$_2$ + H$_2$O + 2e$^-\rightarrow$ H$_2$O$_2$</td>
<td>+0.30</td>
</tr>
<tr>
<td>O$_2$ + 2H$^+$ + 2e$^-\rightarrow$ H$_2$O$_2$</td>
<td>+0.605</td>
</tr>
<tr>
<td>Fe$^{3+}$ + e$^-\rightarrow$ Fe$^{2+}$</td>
<td>+0.77</td>
</tr>
<tr>
<td>$\frac{1}{2}$O$_2$ + H$^+$ + 2e$^-\rightarrow$ H$_2$O</td>
<td>+0.816</td>
</tr>
<tr>
<td>O$_2$ + 2H$^+$ → H$_2$O$_2$</td>
<td>+0.89</td>
</tr>
<tr>
<td>•OH + e$^-\rightarrow$ OH$^-$</td>
<td>+1.89</td>
</tr>
</tbody>
</table>

**FIGURE 27.5** Studies of a perfused rat heart demonstrated that the ketone body D-βOHB raised the ratio of [NADPH]/[NADP+]. Modified presentation of data published in Kashiwaya et al. 1997.
KETOSIS AS A MEANS OF PROTECTION AGAINST IONIZING RADIATION

At present there are very few approved treatments for exposure to nuclear accidents or rogue nuclear bombs (Buddemeier, 2011). Experiments have been conducted at the Armed Forces Radiobiology Research Institute (AFRRI). The study investigating D-βHB protection against acute ROS injury utilized in vitro radiation lethality on HOS (human osteoblast) cells exposed to several doses and types of radiation. For the studies described below, a novel ester of D-β-hydroxybutyrate and R-1,3-butane diol referred to as ketone ester (KE), was used. This compound has been shown to safely increase levels of blood D-βHB to 5–7 mM (Clarke et al., 2012a; Clarke et al., 2012b). The ester is hydrolyzed in the gut and blood by esterases, and the resulting R-1,3 butane diol is converted to D-βHB in the liver (Figure 27.7).

THE REMOVAL OF FREE RADICALS IN VIVO

Clinical trials of administration of antioxidants have often been disappointing (Winterbourn, 2008). Most placebo trials of antioxidants have failed (Steinhubl, 2008), most notably in the long-advocated use of high-dose ascorbic acid. The use of vitamin antioxidants has not objectively been proven helpful in the absence of vitamin deficiency and may even be harmful (Howes, 2011). More recently, high doses of Vitamin E are reported to increase melanoma metastases in mice (LeGal, 2015).

While it is true that, quantitatively, glutathione at about 5 mM and ascorbic acid at about 2–3 mM
in most cells are the predominant intracellular antioxidants, one cannot change the redox potential of an antioxidant in vivo by simply adding more of the reduced form of the couple.

**REMOVAL OF REACTIVE OXYGEN SPECIES BY GENERATION OF NADPH**

We have discussed earlier the failure to remove ROS by addition of antioxidants such as ascorbic acid (Steinhubl, 2008). It is likewise not possible to add the reduced component of most NADPH producing metabolite couples (see Table 27.2 above), since these multiply-charged compounds do not readily enter the interior of cells. It is, however, possible to introduce the reduced ketone body, d-βHB, which readily enters cells on the monocarboxylate transporter, MCT. While not an NADP- coupled metabolite, d-βHB produces acetyl CoA and, via the Krebs cycle, increased concentrations of citrate and isocitrate, but causes little change in α-ketoglutarate (Sato et al., 1995). This increase in the [isocitrate]/[α-ketoglutarate] ratio causes a reduction of the NADP- system (Kashiwaya et al., 1997) generated by the metabolism involving the most active NADP linked dehydrogenase, isocitrate dehydrogenase. The cellular metabolic processes thus produce an increased reducing power of the NADP system, which is regenerated by the cellular metabolism.

**THE ENZYMES DESTROYING REACTIVE OXYGEN SPECIES**

Superoxide dismutase also can combine two superoxide radicals, along with two protons to form the less toxic hydrogen peroxide.

\[
O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2
\]

Hydrogen peroxide can react with catalase (EC 1.11.1.6) in cytoplasm, but not mitochondria, to form water and O₂:

\[
2H_2O_2 \rightarrow O_2 + 2H_2O
\]

Alternatively, H₂O₂ can react with glutathione in a reaction catalyzed by glutathione peroxidase (EC 1.11. 1.9) to form oxidized glutathione and water.

\[
2GSH + H_2O_2 \rightarrow GSSG + 2H_2O
\]

**THE EFFECTS OF d-βHB ON TRANSCRIPTION**

In addition to the ability of d-βHB metabolism to generate NADPH, the metabolite d-βHB is the natural inhibitor of class I and II histone deacetylases (Shimazu et al., 2013; Veech, 2014) that were formerly identified by their ability to bind nicotinic acid (Offermanns, 2006; Tunaru et al., 2003). The inhibition of histone deacetylase increases transcription of the enzymes of the antioxidant pathway controlled by the FOXO3A and Mt1 transcription factors, thus increasing the activity of Mn superoxide dismutase and catalase (see Table 27.3). d-βHB thus increases the reductive capacity of the pathways used to destroy free radicals by increasing the enzymes in the pathway used for free radical destruction. Recent studies have shown that HDAC inhibitors may have a significant benefit in radiation protections (Brown et al., 2008; Miller et al., 2011). Therefore, in vitro studies were initiated to test whether KE could improve radiation survival and decrease radiation-induced damage.

**IN VITRO STUDIES**

Human osteoblast cells (HOS) were chosen as the model system to study KE’s potential as a radiation countermeasure. The HOS cells have been
previously used to evaluate potential radiation countermeasures (Miller et al., 1998; Miller et al., 2011). The HOS cells were incubated with KE (50 µM, 15 min to 1 h) either before (Figure 27.8A) or immediately after (Figure 27.8B) exposure to gamma radiation (60Co, 0.66 cGy/min). Following radiation, HOS cells were plated in 100-mm petri dishes to assess survival of colony-forming cells; at 10 days post radiation, colonies were fixed, stained, and counted to determine the survival fraction. The survival curves show that KE increased survival when delivered either immediately before or immediately after radiation. These data strongly suggest that KE would have benefit to human health both as a protectant delivered to populations at risk of exposure or as a mitigant after radiation. The ability of KE to improve survival even when given 24 hours after radiation is compatible with the persistent production of superoxide from NADPH oxidase s, DUOX 1 and 2, for several days after radiation exposure (Ameziane-El-Hassani et al., 2015).

Proton radiation was used to determine the ability of the KE to protect against particle radiation, which produces less oxidative damage than gamma radiation but is more likely to induce double-stranded DNA breaks (Lomax et al., 2013). Cells were exposed to KE before proton radiation exposure, similar to that performed above in Figure 27.8A with gamma radiation. The KE was able to confer significant protection against proton radiation in this model (Figure 28.8C). Overall cell death was higher for this type of radiation, which is to be expected, based on previous studies. Protection against proton radiation is of particular interest to NASA, as astronauts have a substantially greater likelihood of exposure to proton radiation than other populations.

Genomic effects of ROS begin as discrete events such as a break in the phosphate backbone in DNA, nitrogenous base oxidation, or dimerization. Breaks in the phosphate bonds occur as either single, clustered, or double-stranded breaks, depending on the level of the ROS or radiation dose and type. As the frequency of breaks increases, macro effects such as abnormal chromosomes, mutagenesis, and inhibited cell division become more frequent (Lomax et al., 2013). In general, single-stranded breaks are quickly repaired, have low cytotoxicity and occur at high levels even without exogenous ROS or radiation (Cadet et al., 2008). Single-stranded breaks are induced at levels four- to eightfold more than double-stranded breaks with exposure to electromagnetic radiation such as gamma or x-rays (Lomax et al., 2013). Double-stranded or clustered single-stranded breaks occur with particle radiation, especially α and proton, or when ROS is focused in a confined area (Lomax et al., 2013). A double-stranded break is defined as two breaks within 10 bases (Lomax et al., 2013). These breaks are much more difficult to repair and can persist for more than 24 hours when large numbers are generated (Schmid et al., 2010). These breaks are repaired by either homologous recombination or by nonhomologous end joining (Lomax et al., 2013), with the latter being the predominant mechanism in most phases of the cell cycle. It is possible to observe these breaks by examining some of the hallmarks of their repair, by observing different types of aberrant chromosomes, or by observing cell cycle arrest. Indeed, when cultured cells were treated with D-βHB before an exposure

![FIGURE 27.8 Survival curves for human osteoblasts (HOS) in culture exposed to either gamma (60Co, 66 gCy/min) or proton radiation (4MeV). KE-treated cell cultures received 50 µM of ketone ester (KE) in the cell media 30 minutes prior to or for 30 minutes after radiation exposure.](image-url)
A novel approach for stimulating repair of radiation or ROS-induced DNA damage utilizes HDAC inhibitors. Inhibitors of HDAC activate important DNA repair pathways such as the Nrf2 and Rad51 and decrease markers of damage, even if the inhibitor is given after radiation exposure (Brown et al., 2008; Miller et al., 2011). Histone deacetylase (HDAC) inhibitors have broad activity, their primary function is to increase the acetylation of histones by preventing their deacetylation, in turn making the genome less tightly packed and more transcriptionally active (Verdin and Ott, 2015). The deacetylating activity of HDACs can also extend to various other cellular proteins, modulating activity of enzymes and protein-protein binding (Verdin and Ott, 2015). The deacetylating activity of HDACs can also extend to various other cellular proteins, modulating activity of enzymes and protein-protein binding (Verdin and Ott, 2015). With huge numbers of potential effects resulting from inhibiting HDACs, it is difficult to determine what the final effectors of the protection are, but studies have demonstrated that a series of chemically unrelated compounds that all target HDACs seem to confer similar protection and enhanced repair after exposure (Brown et al., 2008; Miller et al., 2011; Shimazu et al., 2013).

The effects of persistent DNA damage are most quickly seen in tissues with high cell turnover that rely on actively dividing stem cells such as skin, the gastrointestinal system, and blood. Damage in these systems produces some of the rapid and most visible radiation effects, such as hair loss, nausea/vomiting, bone marrow suppression, and skin lesions. Damage to DNA is also responsible for much of the increased risk of cancer that results from radiation exposure.

**FIGURE 27.9** The percentage of chromosomal anomalies (aberrant metaphases, fragments, breaks, and rings and dicentrics) occurring from γ-radiation-exposed human osteoblast cells (HOS) with or without KE. The 50-µM KE was added to the cell culture media 30 minutes prior to radiation exposure and removed immediately prior to radiation exposure. Cells were scored using a light microscope.

**EFFECTS OF KETONE ESTERS AFTER EXPOSURE TO RADIATION IN VIVO**

A project is underway to evaluate the ability of KE to increase murine survival after gamma radiation exposure. Previous studies have shown that exposure of the DBA2 inbred mouse strain to 6 Gy of γ-radiation results in reproducible bone...
Chapter 27: Mitigation of Damage from Reactive Oxygen Species

Marlow chromosomal damage in 85-day-old mice (Grahn, 1958). In our study we have tested a single KE administration of 750 mg/kg by gavage 24 hours after radiation exposure. This postradiation treatment led to a consistent 50% decrease in numerous types of radiation-induced chromosomal malformations and abnormal cell divisions as observed by microscopy 24 hours after KE administration (Figure 27.10). The importance of decreasing these indicators of DNA damage and genomic instability is underscored by the cytotoxicity of this type of damage and the potential for neoplastic transformation, ultimately resulting in cancer. This dose of KE also blunted the radiation-induced increase in incidence of micronuclei, which arise from improper chromatid separation during anaphase, in both reticulocytes and erythrocytes, which were counted using the methods of Schmid (1975).

In order to explore KE effects on radiation bone marrow suppression and impact on hematopoiesis, an important mechanism for radiation-induced mortality at the 6 Gy level, the ratio between total reticulocytes and erythrocytes in bone marrow was determined. Bone marrow was extracted as before, 24 hours after KE or saline gavage, a total of 48 hours after either 0.5 or 6 Gy γ-radiation. This ratio, which is approximately 1 in bone marrow under normal circumstances, was significantly decreased by both 0.5 and 6 Gy doses of γ-radiation (Figure 27.11) in control animals. This decrease was significantly attenuated by 750mg/kg KE 24 hours after radiation. Further experiments to determine KE effects on radiation lethality are ongoing.

FIGURE 27.10 The effect of KE on the incidence rate of chromosome anomalies and micronuclei in mouse bone marrow 48 h after 6 Gy γ-radiation. KE (750 mg/kg) or saline were delivered by gavage 24 hours after radiation exposure. (A) Chromosomal malformation incidence per 100 cells, all cells in bone marrow were examined and malformations were scored using a light microscope. For metaphases approximately 1,000 total were counted, 200/animal. (B) Micronuclei were counted only in reticulocytes and erythrocytes, 10,000 total were counted per animal. Data presented as average ± SEM, N = 5 animals, *, p < .01 by t-test with a Sidack-Bonferroni multiple comparison correction.

FIGURE 27.11 The effect of KE on the relative abundance of polychromatic and normochromatic erythrocytes in mouse bone marrow 48 h after 0, 0.5, or 6 Gy γ-radiation. KE (750 mg/kg) or saline were delivered by gavage 24 hours after radiation exposure. 10,000 erythrocytes were counted per animal. Data presented as average ± SEM, N = 5 animals. *, p < .01 significant difference between KE and control, +, p < 0.05 significant difference versus no radiation, by t-test with a Sidack-Bonferroni multiple comparison correction.
CONCLUSION

Reactive oxygen species play a central role in both radiation damage and aging. It is proposed that both processes can be ameliorated by the administration of KE to increase the amount of antioxidant, enzymes, and the reducing power of the NADP- system.

REFERENCES


Chapter 27: Mitigation of Damage from Reactive Oxygen Species


Metabolic Seizure Resistance via BAD and $K_{\text{ATP}}$ Channels

JUAN RAMÓN MARTÍNEZ-FRANÇOIS, PHD, NIKA N. DANIAL, PHD, AND GARY YELLEN, PHD

INTRODUCTION
The mechanisms of the ketogenic diet are poorly understood, but it has been used for almost a century and is arguably the most effective single treatment for epilepsy (Hartman et al., 2007; Neal et al., 2009; Thiele, 2003). This diet, when adhered to strictly, may reduce seizures in children by up to 50%, with 10%–15% of children becoming seizure-free. Unfortunately, maintaining the ketogenic diet's strict regimen is challenging. Thus, it would be very valuable to understand the ketogenic diet's mechanisms and harness them to create better epilepsy therapies.

On a ketogenic diet the liver produces the ketone bodies β-hydroxybutyrate and acetoacetate from fatty acids, elevating ketone bodies to micromolar concentrations in the blood. Under these conditions, ketone bodies provide an alternative fuel source to tissues, including the brain, which otherwise would primarily utilize glucose (DeVivo et al., 1978; Mergenthaler et al., 2013; Owen et al., 1967; Zielke et al., 2009). The remarkable antiepileptic effect of increased ketone body metabolism points to a link between fuel utilization and neuronal excitability.

However, the molecular mechanisms of this link are poorly understood (Danial et al., 2013; Hartman et al., 2007; Lutas and Yellen, 2013). Some of the mechanisms that have been proposed include increased adenosine signaling through A1 purinergic receptors (Masino et al., 2011), changes in gene expression following glycolysis reduction (Garriga-Canut et al., 2006), suppression of glutamate release (Juge et al., 2010), and changes in excitability mediated by lactate dehydrogenase activity (Sada et al., 2015).

A mouse model with altered metabolism similar to the ketogenic diet produces similar antiseizure effects, and this nondietary manipulation provides a better opportunity to dissect the mechanisms involved in metabolic seizure resistance (Giménez-Cassina et al., 2012). This model involves metabolic changes in brain cells mediated by the protein BAD (BCL-2-associated agonist of cell death). Alteration of BAD's function reduces the capacity of cells to utilize glucose and increases the capacity to use ketone bodies. The key metabolic change involved in seizure protection appears to be the switch away from glucose as the preferred energy source toward utilization of other fuels, rather than elevation of circulating ketone bodies. In addition, BAD alteration leads to increased activity of metabolically sensitive ATP-sensitive potassium ($K_{\text{ATP}}$) channels in the brain. Modulation of $K_{\text{ATP}}$ channel activity may also play an important role in the seizure-protective actions of the ketogenic diet.

BAD MODULATES GLUCOSE AND KETONE BODY METABOLISM
BAD is best-known as a pro-apoptotic protein that is a member of the BCL-2 family of proteins (Czabotar et al., 2014; Danial and Korsmeyer, 2004; Moldoveanu et al., 2014). Besides its role in apoptosis, BAD modulates glucose metabolism in multiple cell types, including hepatocytes, pancreatic β-cells, and fibroblasts (Giménez-Cassina and Danial, 2015). The switch between BAD's apoptotic and metabolic roles is mediated through phosphorylation of its serine 155, located within an alpha helical segment known as the BH-3 domain (Danial et al., 2008; Datta et al., 2000). In hepatocytes and pancreatic β-cells, serine 155 (equivalent to serine 118 in human BAD) phosphorylation promotes mitochondrial metabolism of glucose. In the presence of apoptotic signals, dephosphorylated BAD promotes apoptosis.
The reduction in glucose metabolism provoked by BAD modification is reminiscent of metabolic changes associated with the ketogenic diet, that is, reduced glycolysis and increased ketone body metabolism. Thus, modifying BAD may be a productive avenue to study the mechanisms that underlie the seizure protective effects of changes in metabolism. Given the systemic, nonspecific effects of dietary manipulation, the study of BAD alteration enables the study of metabolic seizure resistance mechanisms in a more specific manner and without the use of dietary interventions.

**METABOLIC CHANGES IN BAD-ALTERED PRIMARY NEURONS AND ASTROCYTES**

Two key mouse models have been used to study the effects of BAD on neurons and astrocytes: a Bad knockout and a Bad knockin. The knockin mouse expresses a mutant of BAD that contains a nonphosphorylatable residue, alanine, at position 155 (BAD^{S155A}). In knockin animals, BAD's metabolic role is altered while leaving its apoptotic role intact, as BAD^{S155A} cannot be phosphorylated (Danial et al., 2008; Giménez-Cassina et al., 2014). Importantly, these mutant mice display neither gross neuroanatomical nor neurobehavioral abnormalities. Therefore, it is unlikely that genetic modification in these mouse models substantially impairs normal brain function.

To elucidate whether BAD has similar effects on metabolism in the brain as it does in other tissues, Giménez-Cassina et al. (2012) measured the mitochondrial oxygen consumption rates in primary cultures of neurons and astrocytes using mitochondrial respirometry. Bad^{-}-ablated cells exhibited reduced glucose metabolism and elevated capacity to metabolize ketone bodies compared with wild-type cells. Similar changes in brain metabolism are produced by the presence of ketone bodies. In hippocampal brain slices, \( \beta \)-hydroxybutyrate competes with glucose, lactate, and pyruvate for the generation of acetyl-CoA, inhibiting total glycolytic flux upstream of pyruvate kinase (Valente-Silva et al., 2015). Additionally, in humans, glucose flux in the brain is inversely correlated with the degree of ketosis, allowing ketone bodies to partially replace glucose in cerebral metabolism (Haymond et al., 1983; Owen et al., 1967). These observations cement the idea that disrupting BAD function may mimic brain metabolic changes produced by the ketogenic diet and is a useful model to study the cellular mechanisms linking metabolism to excitability.

To corroborate that the effects of Bad disruption in the metabolism of cultured neurons and astrocytes were due to the metabolic, and not the apoptotic role of BAD, mitochondrial respirometry was also performed in Bad^{S155A} cells (Giménez-Cassina et al., 2012). Indeed, Bad^{S155A} neurons and astrocytes also preferentially utilize \( \beta \)-hydroxybutyrate over glucose, like the BAD knockout. Conversely, BAD phosphorylation on serine 155 inhibits ketone body utilization. This confirms that BAD, through its metabolic role, changes the ability of brain cells to metabolize different fuels.

**ALTERATION OF BAD FUNCTION PRODUCES SEIZURE RESISTANCE**

Alteration of BAD's metabolic role by either knockout of the protein or knockin of a phosphorylation-resistant mutant variant leads to altered metabolism without dietary manipulations. This metabolic switch from brain glucose to ketone body utilization also influences excitability and produces seizure resistance in vivo (Giménez-Cassina et al., 2012). The seizure resistance has been studied in two acute chemoconvulsant models of seizure, involving intraperitoneal injection of kainate (cf. Ben-Ari et al., 1980) or subcutaneous injection of pentylenetetrazole (PTZ) (cf. Ferraro et al., 1999).

When injected with kainate, wild-type mice display a series of seizures of increasing severity that peak 1–2 hours after kainate injection and then slowly decay (Giménez-Cassina et al., 2012; Figure 28.1A). Most wild-type mice experience status epilepticus with very severe tonic-clonic seizures, and many die. In contrast, seizure severity in Bad^{-/-} mice is much milder than in wild-type mice, and mutant mice rarely go into status epilepticus or die (Figures 28.1 and 28.3). Electrographic seizures are also substantially milder (Figure 28.3). The in vivo seizure protection is due to BAD's metabolic, not apoptotic role, because Bad^{S155A} mice were similarly resistant to kainate-induced seizures. Thus, disruption of the metabolic function of BAD alleviates both forebrain seizure activity and generalized behavioral seizures during status epilepticus.

BAD deletion is similarly protective for PTZ-induced seizures (Figure 28.1C); PTZ produces seizures by a different mechanism than kainate, by inhibiting GABA action rather than by stimulating glutamate receptors (Bough and Eagles, 1999; Ferraro et al., 1999). It remains to be seen whether BAD mutations can also be protective in chronic epilepsy models.
Chapter 28: Metabolic Seizure Resistance via BAD and $K_{\text{ATP}}$ Channels

BAD EFFECTS ON SEIZURE ARE UNLIKE EFFECTS OF OTHER BCL-2 FAMILY PROTEINS

Due to their apoptotic roles, certain BCL-2 family proteins such as BIM and PUMA have been implicated in protection against neuronal loss and seizure damage after prolonged periods of status epilepticus (Engel et al., 2010, 2011; Murphy et al., 2010). In a chronic seizure model (intra-amygdala microinjection of kainate), neuronal death in the hippocampus 24 hours after seizure initiation is decreased in $\text{Bim}^{-/-}$ or $\text{Puma}^{-/-}$ mice, demonstrating a role for these pro-apoptotic proteins in cell death after chronic seizures. In contrast, loss of BIM or PUMA is not seizure protective to seizures immediately after kainate injection (Engel et al., 2010; Murphy et al., 2010). Similarly, knockout of BIM or another BH3-only protein, BID, does not produce resistance to acute seizures elicited by intraperitoneal kainate injection (Giménez-Cassina et al., 2012). Unlike BAD, neither of these proteins affects glucose metabolism (Giménez-Cassina and Danial, 2015). It appears that certain BCL-2 family members may play a role in epileptogenesis by promoting hippocampal neuron death, but that this is distinct from the role of BAD in altering acute seizure susceptibility, which involves a switch in fuel preference rather than apoptosis.

BAD has also been implicated in regulating synaptic transmission. The BAD-BAX-caspase-3 cascade can induce long-term depression in CA1 hippocampal neurons (Jiao and Li, 2011). Additionally, ABT-737, an inhibitor of BCL-2, BCL-X$_L$, and BCL-w survival proteins, slows the recovery of neurotransmission after intense synaptic activity and decreases hypoxia-triggered damage to synaptic function (Hickman et al., 2008). This effect of ABT-737 could result from BAD function, since this compound is likely acting on downstream signaling partners of BAD. However, ABT-737 also blocks binding of other BH3-only proteins to BCL-2 and BCL-X$_L$, which involves the same binding pocket used by BAD. Thus, disruption of these protein interactions by ABT-737 is not indicative of specific actions of BAD in synaptic transmission. In any case, these synaptic changes are unlikely to be part of the anticonvulsant action of BAD deletion. For these synaptic effects, $\text{Bad}^{-/-}$ and $\text{Bad}^{S155A}$ mice would have opposite phenotypes, as these alterations are dependent on the interaction of nonphosphorylated BAD with the downstream BCL-2 proteins; but in fact, both genotypes produce seizure protection. These observations highlight the varied and complex roles of BAD in neuronal physiology, and uniquely distinguish the switch in fuel preference as a potent mechanism to produce seizure protection.

FIGURE 28.1 $\text{Bad}^{-/-}$ mice are resistant to kainate- or pentylenetetrazole-induced acute seizures. (A) Raw seizure scores in $\text{Bad}^{-/-}$ 8- to 10-week-old male mice compared with wild-type (WT) mice over a 4-hour period after a single intraperitoneal injection of kainate (30 mg/kg). (B) Kainate-induced seizure severity calculated as $\sum$(all scores of a given mouse)/ (time of experiment) for wild-type (WT) or $\text{Bad}^{-/-}$ mice. The mean of the seizure severity values from WT mice was assigned a value of 100%. This value was then used to normalize the severity of the other tested genotypes and/or conditions within the same scale. WT, $n = 42$; $\text{Bad}^{-/-}$, $n = 13$. (C) Integrated seizure severity (calculated as in B) in $\text{Bad}^{-/-}$ and WT mice ($n = 16$) subjected to a single subcutaneous injection of pentylenetetrazole (PTZ) at 80 mg/kg monitored over a 70-minute period. Data in A–C are presented as mean ± SEM. *** $p < .001$; two-tailed Student’s t-test. (Adapted from Giménez-Cassina et al., 2012)

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THE METABOLIC SEIZURE RESISTANCE OF BAD−/− MICE LIKELY OCCURS IN THE BRAIN

Upon BAD modification, the capacity to metabolize glucose is decreased. This recapitulates changes in brain fuel utilization during fasting or on the ketogenic diet. In contrast, the switch in fuel choice produced by BAD alteration occurs without any dietary alterations. As opposed to dietary manipulations that affect the whole organism, seizure protection arising from BAD modification seems to specifically originate from metabolic changes in the brain. Knockdown of BAD by adenosovirus-produced shRNA in the liver, the major source of ketone body production, does not protect against kainate-induced seizures, though it does produce the same metabolic effects observed in the liver of Bad−/− animals (Giménez-Cassina et al., 2012; Giménez-Cassina et al., 2014).

Interestingly, circulating levels of ketone bodies are similar in Bad−/− and wild-type mice, but β-hydroxybutyrate levels are increased in whole-brain tissue derived from Bad−/−. This suggests that in the knockout animals, ketogenesis is being increased specifically in the brain; it also argues that changes in brain metabolism upon BAD alteration are unlikely to be generated by systemic modifications in metabolism. The precise changes in metabolism responsible for BAD’s seizure-protective effect remain to be established. Given that the ketogenic diet’s antiseizure effect can be reversed in patients promptly after carbohydrate consumption (Huttenlocher, 1976; Pfeifer et al., 2008), it seems likely that the transition in fuel type utilization is the crucial factor. Metabolomic studies comparing wild-type to Bad−/−-altered genotypes could shed light on the metabolic pathways implicated in BAD’s effect.

\[ K_{ATP} \] CHANNLES LINK METABOLISM WITH EXCITABILITY

One likely candidate for linking altered brain metabolism with altered excitability, in the ketogenic diet and in BAD-altered animals, is the ATP-sensitive potassium (\(K_{ATP}\)) channel. \(K_{ATP}\) channels are inhibited by intracellular ATP, activated by intracellular ADP, and are widely expressed throughout the body, including the brain (Liss and Roeper, 2001; Nichols, 2006; Proks and Ashcroft, 2009). Their role linking metabolism with cell excitability has been best studied in control of insulin release by pancreatic β-cells (Ashcroft, 2005; Ashcroft et al., 1984). In these cells, the intracellular ATP:ADP ratio determines \(K_{ATP}\) channel activity. In basal conditions, \(K_{ATP}\) channels are spontaneously active and maintain the cell’s resting membrane potential near the potassium equilibrium potential, that is, hyperpolarized. Upon an increase in extracellular glucose concentration, and consequently an increase in the intracellular ATP concentration, \(K_{ATP}\) channels are inhibited. This decrease in \(K_{ATP}\) channel activity depolarizes the cell and leads to insulin release. This exemplifies how \(K_{ATP}\) channels can link metabolism to cell excitability, but the effect is likely not unique to glucosensing cells like the β-cells.

Two brain regions that are particularly enriched in \(K_{ATP}\) channels are the hippocampus and the substantia nigra pars reticulata (SNr) (Dunn-Meynell et al., 1998; Karschin et al., 1997; Ma et al., 2007; Tanner et al., 2011; Yamada et al., 2001; Zawar et al., 1999). \(K_{ATP}\) channels present in these two areas are octamers comprising two types of subunits: four Kir6.2 pore-forming subunits and four SUR1 regulatory subunits. Both of these brain regions are involved in the generation of seizure activity (Depaulis et al., 1994; Heinemann et al., 1992; Hsu, 2007; Iadarola and Gale, 1982; Krook-Magnuson et al., 2015; Lothman et al., 1992; McNamara et al., 1984), and excitability of neurons in these areas can be modulated by \(K_{ATP}\) channels (Lutas et al., 2014; Stanford and Lacey, 1996; Tanner et al., 2011; Yamada et al., 2001; Zawar et al., 1999).

Neuronal \(K_{ATP}\) channel activity appears generally to be low, implying that the intracellular ATP concentration in neurons is often sufficiently high to inhibit these channels (Giménez-Cassina et al., 2012; Haller et al., 2001; Pelletier et al., 2000; Schwanstecher and Panten, 1993; Tanner et al., 2011; Yamada et al., 2001). Substantial metabolic insults to the brain are known to be capable of activating the channels; for instance in brain ischemia, intracellular ATP and ATP/ADP markedly decrease after ~2 minutes (Katsura et al., 1992). In the CA1 area of the hippocampus, a brain region particularly susceptible to ischemic insult (Davolio and Greenamyre, 1995; Schmidt-Kastner and Freund, 1991; Smith et al., 1984), hypoxia triggers neuronal hyperpolarization by activating potassium conductances, including \(K_{ATP}\) channel currents (Yamada and Inagaki, 2005; Yamada et al., 2001). Consistent with this, \(K_{ATP}\) channel inhibitors, such as the sulfonamide drugs glibenclamide or tolbutamide, can suppress the hypoxia-induced hyperpolarization. Furthermore, mice lacking either Kir6.2 or SUR1 exhibit a markedly reduced
threshold for generalized seizures upon a hypoxic insult, that is, they are hypersensitive to hypoxia-induced seizures (Hernández-Sánchez et al., 2001; Yamada et al., 2001). Interestingly, similar to Bad-altered mice, mice overexpressing SUR1 display a decrease in kainate-induced seizure susceptibility. These observations indicate that $K_{ATP}$ channels play a crucial role in mediating seizure protective effects provoked by metabolic insufficiency or hypoxia.

Besides ischemic/hypoxic insult, other metabolic changes in brain regions implicated in seizure activity can also modulate $K_{ATP}$ channel activity. Poisoning of GABAergic neurons in the SNr or hippocampal CA1 neurons with cyanide activates $K_{ATP}$ channels (Matsumoto et al., 2002; Schwanstecher and Panten, 1993). In addition, in dentate granule neurons (DGNs) of the hippocampus, a much more subtle energetic challenge can lead to $K_{ATP}$ channel opening: neuronal firing elicited by antidromic stimulation increases single $K_{ATP}$ channel activity (Tanner et al., 2011). Firing of action potentials produces metabolic changes by activating the pumps, for example the Na/K ATPase, that maintain intracellular ionic concentrations (Ivannikov et al., 2010; Mercer and Dunham, 1981). Activation of these pumps triggers ATP consumption, leading to an opening of $K_{ATP}$ channels (Haller et al., 2001; Tanner et al., 2011). It has also been shown that $K_{ATP}$ channel open probability in DGNs increases by extracellular application of β-hydroxybutyrate (Tanner et al., 2011). These observations are consistent with the idea that $K_{ATP}$ channels could mediate the anticonvulsant effect of the ketogenic diet. First, $K_{ATP}$ channels might open in conditions of hyperexcitability, such as during a seizure. Second, elevated levels of circulating ketone bodies could also increase $K_{ATP}$ channel activity. These effects are synergistic, and the resulting increase in $K_{ATP}$ channel current would reduce excitability and produce seizure resistance.

**BAD DISRUPTION INCREASES $K_{ATP}$ CHANNEL ACTIVITY**

As previously stated, $K_{ATP}$ channels link metabolism to excitability and are activated by both ketone bodies and electrical activity in the brain. This raises the question of whether these channels mediate the seizure resistance elicited by BAD disruption. In single-channel, cell-attached experiments in DGNs in hippocampal brain slices, the basal $K_{ATP}$ channel open probability is dramatically increased in Bad$^{-/-}$ mouse DGNs, from ~0.5% in wild-type to ~20% in Bad$^{-/-}$ (Giménez-Cassina et al., 2012). Confirming this increase in basal $K_{ATP}$ channel activity in DGNs with disrupted BAD function, whole-cell $K_{ATP}$ current is also inhibited by dialyzing the intracellular medium of either Bad$^{-/-}$ or Bad$^{R155A}$ DGNs with high ATP (4 mM)—a phenomenon denominated “washdown” (Figure 28.2A). On the other hand, washdown does not occur in wild-type DGNs (as $K_{ATP}$ channels are mostly closed in this case) or in DGNs lacking both BAD and Kir6.2.

These whole-cell results corroborate the fact that basal $K_{ATP}$ channel open probability is increased in Bad$^{-/-}$ DGNs. Additionally, the number of functional $K_{ATP}$ channels in DGNs is not affected by altering BAD's metabolic role. The maximal $K_{ATP}$ conductance, observed in whole-cell recordings dialyzing the neuron’s intracellular medium with a low concentration of ATP (0.3 mM), is unchanged between wild-type and Bad$^{-/-}$ or Bad$^{R155A}$ DGNs.

The increased $K_{ATP}$ channel activity observed in Bad-altered DGNs suggests that $K_{ATP}$ channels might lead to decreased neuronal excitability. Even though further studies are needed to demonstrate such a scenario, consistent with this idea, studies using a hippocampal neuron cell line have shown that $K_{ATP}$ channels can mediate changes in hippocampal neuron excitability (Huang et al., 2006, 2007). Thus, a decrease in excitability mediated by $K_{ATP}$ channels could be a central mechanism in the ketogenic diet’s seizure protective effects.

**$K_{ATP}$ CHANNELS MEDIATE SEIZURE PROTECTION ELICITED BY BAD DISRUPTION**

Lack of BAD leads to a remarkable increase in $K_{ATP}$ channel activity and also produces seizure protection. It appears that these two effects are causally related: eliminating Kir6.2 (Kcnj11) in a Bad$^{-/-}$ background nearly abolishes the seizure resistance produced by Bad ablation (Figure 28.2B). This substantial attenuation of seizure resistance is not due to an increase in seizure severity produced by lack of Kir6.2; deletion of Kir6.2 alone does not increase mouse seizure sensitivity compared with wild-type animals. These results constitute genetic evidence that $K_{ATP}$ channels are necessary in mediating BAD’s effect on seizure protection.

$K_{ATP}$ channels are well-known mediators of changes in metabolism with neuronal excitability. Indeed, either glucose deprivation or ketone body
metabolism can increase \( K_{\text{ATP}} \) channel activity. Furthermore, these effects can be potentiated by neuronal firing, for example, during seizure activity. The mechanism by which a metabolic fuel switch could increase \( K_{\text{ATP}} \) channel activity is unknown. Given that ATP and ADP directly control the open probability of \( K_{\text{ATP}} \) channels, changes in ATP/ADP local to \( K_{\text{ATP}} \) channels might be different in the presence or absence of BAD’s metabolic effects.

Why would ATP/ADP change in the presence of abundant alternative fuels? One possibility is that \( K_{\text{ATP}} \) channel activity is controlled by local glycolysis. Glycolysis is bypassed and inhibited by mitochondrial metabolism of ketone bodies (DeVivo et al., 1978; Haymond et al., 1983; Valente-Silva et al., 2015). This metabolic change would increase ATP produced by mitochondria and decrease ATP produced by glycolysis.

Indeed, \( K_{\text{ATP}} \) channels and glycolytic enzymes can be found associated in large complexes in the plasma membrane (Dhar-Chowdhury et al., 2007; Dubinsky et al., 1998; Hong et al., 2011). Evidence suggests that these glycolytic enzyme complexes may produce ATP compartmentation in the cell (Chu et al., 2012; Hoffman et al., 2009; Proverbio and Hoffman, 1977). Furthermore, glycolysis inhibition can trigger the opening of \( K_{\text{ATP}} \) channels (Matsumoto et al., 2002; Schwanstecher and Panten, 1993; Tantama et al., 2013). Another possibility is that glucose metabolism is special in its ability to respond rapidly to acute energy challenges, as might occur in the elevated brain activity leading to seizures. Thus, BAD alteration, by switching metabolism away from glucose consumption, may produce a decrease in the ATP concentration at the plasma membrane, which then could trigger \( K_{\text{ATP}} \) channel opening and reduced neuronal excitability.

Even though modulation of \( K_{\text{ATP}} \) channel activity by ATP/ADP is a straightforward explanation, \( K_{\text{ATP}} \) channel regulation is complex, and other processes, such as PIP\(_2\) binding or phosphorylation, can also alter \( K_{\text{ATP}} \) channel activity (Nichols, 2006; Proks and Ashcroft, 2009). In addition, \( \beta \)-hydroxybutyrate levels are increased in \( \text{Bad}^{-/-} \) compared with wild-type brains, and, as mentioned above, this can induce \( K_{\text{ATP}} \) channel opening. While it is unlikely that the effects of ketone bodies on \( K_{\text{ATP}} \) channel activity explain the totality of the observed \( K_{\text{ATP}} \) channel activation, these metabolites may constitute part of multiple actions needed to exert the full effect observed upon BAD alteration.

How does \( K_{\text{ATP}} \) channel activation in certain brain cells elicit seizure protection? A simple explanation is that elevated activity, which would ordinarily lead to a seizure in a susceptible individual, produces increased \( K_{\text{ATP}} \) channel current,
which then reduces subsequent neuronal activity by hyperpolarizing neurons. It will be important to reveal the relevant brain regions associated with this seizure protective effect. \(K_{\text{ATP}}\) channels are present in two brain regions associated with seizure activity: the DGNs in the hippocampus and the GABAergic neurons of the SNr. It has been shown that reducing neuronal firing in either of these two brain areas inhibits seizure activity (Akman et al., 2015; Coulter and Carlson, 2007; Krook-Magnuson et al., 2013; Paz et al., 2007; Vercueil et al., 1998). Studies on \(K_{\text{ATP}}\) channel activity or in vivo seizure models in conditional Kir6.2 (\(Kcnj11\)) knockout lines could be used to define the brain regions and brain cell types implicated in seizure resistance.

**CONCLUSION**

In summary (Figure 28.3), certain manipulations of BAD's metabolic role produce a metabolic change in brain cells, switching away from glucose utilization toward consumption of alternative fuels. The metabolic changes triggered by BAD modification are reminiscent of those elicited by a ketogenic diet. Remarkably, BAD disruption produces robust seizure protection in the absence of dietary treatments. At the cellular level, it appears that \(K_{\text{ATP}}\) channels mediate BAD's anticonvulsant effect. Disruption of BAD function increases \(K_{\text{ATP}}\) channel activity in neurons in the dentate gyrus, a brain region that is likely a gate for seizure activity. The mechanisms by which BAD modulates brain metabolism and
how these metabolic changes affect excitability have just started to be revealed. These seizure-protective mechanisms will hopefully serve as the basis for the development of more effective therapies for epilepsy.

ACKNOWLEDGMENTS
We thank members of the Yellen lab for critical review and discussion of our manuscript. This work was supported by grants from the National Institutes of Health (R01 NS083844 to G.Y. and N.N.D. and R01 NS055031 to G.Y.)

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THE KETOGENIC DIET FOR DRUG-RESISTANT EPILEPSY

Epilepsy is a neurological disorder that is characterized by the hyperexcitation of electrical activities in the brain. Approximately 1% of the world’s population has epilepsy, and one-third of epileptic patients are resistant to currently available antiepileptic drugs (Kwan and Brodie, 2000). Therefore, it is important to develop new antiepileptic drugs that can treat the drug-resistant epilepsy. As a step toward achieving this goal, “endogenous proteins” that have the ability to suppress epileptic seizures need to be identified, and will become target molecules for the development of antiepileptic drugs. “Chemical compounds” that act on these target molecules and suppress epileptic seizures must then be identified, and will become prototypes of new antiepileptic drugs. Thus, the search for new target molecules in basic neuroscience will ultimately lead to drug development for drug-resistant epilepsy.

Since epileptic seizures are caused by the hyperexcitation of electrical activities in the brain, antiepileptic drugs also need to be designed in order to suppress the neuronal hyperexcitation. Electrical activities in the brain are generated by ion channels, synaptic receptors, and neurotransmitter transporters. Therefore, currently available antiepileptic drugs have been designed to act on the molecules generating electrical currents (Meldrum and Rogawski, 2007). However, these antiepileptic drugs are ineffective for one-third of epileptic patients (Kwan and Brodie, 2000); that is, other molecular targets need to be identified. The ketogenic diet is known to be effective for some patients with drug-resistant epilepsy (Neal et al., 2008), but there are no antiepileptic drugs that mimic the ketogenic diet. Based on this background, neuroscientists have recently focused on the molecular mechanisms underlying the antiepileptic effects of the ketogenic diet.

MECHANISMS RESPONSIBLE FOR THE ANTIEPILEPTIC EFFECTS OF THE KETOGENIC DIET

The ketogenic diet mainly elicits two metabolic changes in the body; increases in ketone bodies and decreases in glucose (Bough et al., 2006). The neural inhibition and antiepileptic effects elicited by these two metabolic changes have been actively studied (Lutas and Yellen, 2013). The following rodent studies have demonstrated the antiepileptic effects of ketone bodies. Ketone bodies consist of acetoacetate, \( \beta \)-hydroxybutyrate, and acetone. The ketogenic diet was previously shown to increase \( \beta \)-hydroxybutyrate levels up to ~8 mM in the blood plasma of rodents (Bough et al., 1999). An intraperitoneal injection of ketone bodies directly suppresses seizures in vivo in audiogenic seizure-susceptible mice (Rho et al., 2002). At the molecular and cellular levels, ketone bodies decrease the firing rate of neurons in the substantia nigra via adenosine 5’-triphosphate (ATP)-sensitive K\( ^+ \) channels (K\( _{\text{ATP}} \) channels) (Ma et al., 2007). Single channel recordings have revealed that ketone bodies open K\( _{\text{ATP}} \) channels in the dentate granule cells of the hippocampus (Tanner et al., 2011). Acetoacetate is also known to be an inhibitor of vesicular glutamate transporters (VGLUTs) (Juge et al., 2010). The role of the VGLUTs is to fill glutamate into synaptic vesicles and regulate excitatory synaptic transmission in the brain, and thus, ace- toacetate reduces miniature excitatory postsynaptic currents (miniature EPSCs) and suppresses 4-aminopyridine-induced acute seizures in vivo (Juge et al., 2010).

In terms of decreases in glucose, seizures are suppressed by 2-deoxy-D-glucose, a glycolytic
inhibitor (Garriga-Canet et al., 2006). Glucose metabolism is reduced in BAD (BCL-2-associated agonist of cell death) knockout mice, which opens metabolically sensitive K_{ATP} channels and leads to resistance to convulsants such as kainic acid and pentylenetetrazol (Giménez-Cassina et al., 2012).

The ketogenic diet has also been shown to suppress seizures in mice by increasing the activation of adenosine A_1 receptors (Masino et al., 2011). In vitro recordings from hippocampal slices have revealed that reductions in glucose hyperpolarize pyramidal neurons, and this is mediated by adenosine A_1 receptors and K_{ATP} channels (Kawamura et al., 2010). Consistent with these findings, the direct activation of adenosine A_1 receptors has been shown to suppress chronic seizures in a mouse model of mesial temporal lobe epilepsy (Gouder et al., 2003).

These extensive studies have uncovered the mechanisms underlying the antiepileptic effects of the ketogenic diet. However, all of the molecules revealed by these studies are ion channels (K_{ATP} channels), synaptic receptors (adenosine A_1 receptors), and neurotransmitter transporters (VGLUTs); namely, molecules regulating electrical currents. Since the ketogenic diet changes energy metabolites (glucose and ketone bodies), it is hypothesized that the brain has “metabolic pathways” that play key roles in the antiepileptic effects of the ketogenic diet. In other words, there are assumed to be “metabolic enzymes” that regulate electrical activities in neurons and suppress seizures in vivo in the same manner with the ketogenic diet. In order to explore these metabolic pathways and enzymes, we focused on the astrocyte-neuron lactate shuttle in the brain, because this lactate shuttle was known to be a metabolic pathway involved in the regulation of electrical activities in the brain (Rouach et al., 2008, Parsons and Hirasawa, 2010).

**ASTROCYTE-NEURON LACTATE SHUTTLE**

Glucose is the main energy source in the brain, and produces ATP in neurons and glial cells. The concentration of glucose in vivo is approximately 2.5 mM in the brain (Silver and Erecińska, 1994), and is maintained at a lower concentration than that in the blood. Glucose is directly transported into neurons and then converted to pyruvate by glycolysis in neurons, which produces ATP in the TCA cycle (Figure 29.1). The role of astrocytes (a type of glial cell) as the energy supplier to neurons has recently received increasing attention (Belanger et al., 2011). As shown in Figure 29.1, glucose is initially transported into astrocytes, and converted to lactate by lactate dehydrogenase (LDH). This lactate is then released to extracellular spaces, transported into neurons, and converted to pyruvate by LDH in glycolysis, which produces ATP in the TCA cycle (Figure 29.1). This metabolic communication from astrocytes to neurons is called the astrocyte-neuron lactate shuttle (Belanger et al., 2011).

Several lines of evidence support that this astrocyte-neuron lactate shuttle is used in the supply of energy to neurons. First, astrocytes show lower rates of oxidative metabolism than neurons, and consequently release a large amount of lactate to the extracellular spaces (Itoh et al., 2003; Bouzier-Sore et al., 2006). Second, lactate appears to be used as an energy source preferred over glucose in the brain (Larrabee, 1995; Smith et al., 2003). Third, although glucose uptakes into neurons and astrocytes are similar in the resting state of the brain (Nehlig et al., 2004; Chuquet et al., 2010), increases in neuronal activities elicit the higher glucose uptakes in astrocytes (Chuquet et al., 2010) and also elicit sustained increases in extracellular lactate (Hu and Wilson, 1997). Thus, the energy supply to neurons is achieved by not only the direct glucose pathway to neurons but also the astrocyte-neuron lactate shuttle (see Figure 29.1).

There is also growing evidence to show that the astrocyte-neuron lactate shuttle regulates electrical activities in neurons. First of all, the following studies have demonstrated that lactate regulates electrical activities in neurons. The inhibition of glycolysis reduces synaptic transmission in the hippocampus, and this is rescued by the presence of lactate (Schurr et al., 1988), which demonstrates that the metabolism of lactate to pyruvate in neurons contributes to the maintenance of synaptic transmission. Consistent with this finding, the inhibition of monocarboxylate transporters that transport lactate into neurons also reduces synaptic transmission in the hippocampus (Izumi et al., 1997). Furthermore, orexin-containing neurons in the hypothalamus are hyperpolarized by the inhibition of monocarboxylate transporters (Parsons and Hirasawa, 2010). Importantly, they are also hyperpolarized by fluoroacetate, a glial toxin, and this hyperpolarization is recovered by the application of lactate (Parsons and Hirasawa, 2010). These findings suggest that lactate released from astrocytes regulates membrane potentials in neurons.

Rouach and colleagues provided more direct evidence to show that electrical activities in neurons are regulated by the metabolic pathway from
astrocytes (Rouach et al., 2008). Astrocytes in the hippocampus are connected to one another by gap junctions, and thus, small molecules injected into single astrocytes using patch pipettes diffuse into many neighboring astrocytes (D’Ambrosio et al., 1998). By using this property, a group of nearby astrocytes can be controlled by selective filling of active small molecules into single astrocytes using patch pipettes (Rouach et al., 2010). Rouach and colleagues made filling of glucose into single astrocytes in hippocampal slices, and then examined the effects of the deprivation of extracellular glucose on hippocampal synaptic transmission. They demonstrated that, although glucose deprivation reduced synaptic transmission, these reductions were rescued by the selective filling of glucose into astrocytes (Rouach et al., 2008), showing that hippocampal synaptic transmission is regulated by metabolic communication from astrocytes. Rouach and colleagues also showed that epileptiform activities in hippocampal slices were regulated by the selective filling of glucose into astrocytes (Rouach et al., 2008).

**LACTATE DEHYDROGENASE: A METABOLIC TARGET FOR EPILEPSY**

The findings of these studies prompted us to investigate electrical regulation by the astrocyte-neuron lactate shuttle, and we recently reported that this lactate shuttle is involved in the antiepileptic effects of the ketogenic diet (Sada et al., 2015). The ketogenic diet is known to increase ketone bodies and decrease glucose in the body (Bough et al., 2006). Therefore, we made patch clamp recordings from neurons in the subthalamic nucleus in mouse brain slices, and switched glucose in the extracellular perfusate (artificial cerebrospinal fluid; ACSF) to ketone bodies. The switch from glucose to ketone bodies hyperpolarized subthalamic neurons; and this hyperpolarization was recovered by the addition of lactate in ACSF (Sada et al., 2015). Based on the metabolic pathways shown in Figure 29.1, it is likely that the hyperpolarization is elicited by the decrease in extracellular lactate derived from the switch of glucose to ketone bodies, and recovered by the addition of lactate to ACSF. Thus, the astrocyte-neuron lactate shuttle is likely to regulate membrane potentials in neurons.

In order to confirm this idea, we focused on LDH, a metabolic enzyme located in the astrocyte-neuron lactate shuttle (Figure 29.1). Thus, this lactate shuttle can be suppressed by inhibiting LDH. We used oxamate, an LDH inhibitor that has been used to inhibit LDH in the brain (Lam et al., 2005), and examined the effects of the LDH inhibitor on membrane potentials in neurons (Sada et al., 2015; see Figure 29.2). The inhibition of LDH hyperpolarized neurons in the subthalamic nucleus, and also hyperpolarized pyramidal cells in the hippocampus (Figure 29.2A). Furthermore, the hyperpolarization elicited by the LDH inhibition was not recovered by the addition of lactate, but was recovered by the addition of pyruvate (Figure 29.2B). Lactate is an upstream metabolite of LDH in neurons, whereas pyruvate is a downstream metabolite in neurons (see Figure 29.1). Thus, neuronal LDH in the lactate shuttle regulates membrane potentials in neurons (Figure 29.2C).

We also obtained direct evidence to show that membrane potentials in neurons are regulated by lactate released from astrocytes (Sada et al., 2015; see Figure 29.3). Patch-clamp recordings were obtained from neighboring pairs of pyramidal cells and astrocytes in hippocampal slices, and oxamate was selectively applied to recorded astrocytes.
through the patch pipette (Figure 29.3A). The selective inhibition of LDH in astrocytes elicited hyperpolarization in neighboring pyramidal cells (Figure 29.3B), which demonstrated that LDH in astrocytes regulates membrane potentials in neurons. In order to further confirm that this hyperpolarization in pyramidal cells was actually due to reductions in the release of lactate from astrocytes (see Figure 29.3A), we performed the same experiments under the extracellular perfusion of lactate. The hyperpolarization induced by filling oxamate into astrocytes (see Figure 29.3B) was not observed when lactate was present in the extracellular perfusate (Figure 29.3C). Thus, astrocytic LDH in this lactate shuttle also regulates membrane potentials in neurons (Figure 29.3D).

These in vitro studies revealed that the release of lactate from astrocytes to neurons regulates membrane potentials in neurons. We also obtained evidence to show that this lactate release is actually weakened by the ketogenic diet (Sada et al., 2015; see Figure 29.4). Mice were fed a ketogenic diet for about 3 weeks, and the lactate concentration in the hippocampus was measured and compared with that in the hippocampus of age-matched mice fed a standard diet. The measurement of lactate concentration revealed that the hippocampal lactate was lower in mice fed the ketogenic diet than in those fed the standard diet (Figure 29.4A). Thus, the release of lactate in this lactate shuttle is lowered by the ingestion of the ketogenic diet (Figure 29.4B).

Taken together, these findings show that the astrocyte-neuron lactate shuttle is regulated by the ketogenic diet, and LDH in this lactate shuttle is a key enzyme that regulates membrane potentials in neurons. We also obtained evidence to show that this lactate release is actually weakened by the ketogenic diet (Sada et al., 2015; see Figure 29.4). Mice were fed a ketogenic diet for about 3 weeks, and the lactate concentration in the hippocampus was measured and compared with that in the hippocampus of age-matched mice fed a standard diet. The measurement of lactate concentration revealed that the hippocampal lactate was lower in mice fed the ketogenic diet than in those fed the standard diet (Figure 29.4A). Thus, the release of lactate in this lactate shuttle is lowered by the ingestion of the ketogenic diet (Figure 29.4B).

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FIGURE 29.3 Electrical regulation by the astrocyte-neuron lactate shuttle. (A) The experimental design used for simultaneous recordings from pyramidal cells and astrocytes in the hippocampus. LDH in astrocytes are selectively inhibited by the intracellular application of oxamate via patch pipettes. (B) Pyramidal cells are hyperpolarized by the LDH inhibition in astrocytes. Neurons are recorded in the whole-cell mode using normal intracellular solution, whereas astrocytes are recorded in the cell-attached mode using intracellular solution including oxamate. Oxamate is selectively applied by rupturing the patch membranes in astrocytes. (C) Pyramidal cells are not hyperpolarized by the LDH inhibition in astrocytes when lactate is present extracellularly. Thus, lactate is a mediator for this astrocyte-induced electrical regulation in neurons. (D) The summary scheme showing the neuronal inhibition by inhibiting LDH in astrocytes. Reproduced from Sada et al. (2015).

FIGURE 29.4 Decrease in hippocampal lactate by the ketogenic diet. (A) Lactate concentrations in the hippocampus are lower in mice fed a ketogenic diet than in those fed a standard diet. **p < .01 (unpaired t-test; n = 11 in each group). (B) The summary scheme showing the reduction in the release of lactate elicited by the ketogenic diet. Reproduced from Sada et al. (2015).
neurons. We further reported that seizures in vivo were suppressed by the inhibition of LDH (Sada et al., 2015). Microinjection of kainic acid into the hippocampus is known to produce a mouse model of mesial temporal lobe epilepsy, which exhibits spontaneous paroxysmal discharges with abnormal morphology in the hippocampus (Riban et al., 2002; Gouder et al., 2003; Heinrich et al., 2006). The LDH inhibitor oxamate suppressed the paroxysmal discharges in the hippocampus of this chronic seizure model (Sada et al., 2015). The in vivo knockdown of lactate dehydrogenase A (LDHA), a subunit of LDH, by an antisense oligodeoxynucleotide also exerted antiepileptic actions in this seizure model. Thus, the inhibition of LDH suppresses seizures in vivo.

**FUTURE DIRECTIONS TOWARD DRUG DEVELOPMENT**

The findings of our recent study (Sada et al., 2015) indicate that LDH is a metabolic target for epilepsy, and also that the inhibition of LDH mimics the antiepileptic effects of the ketogenic diet. This metabolic target is clearly different from molecules that have already been identified in previous studies about the ketogenic diet: K_{ATP} channels (Ma et al., 2007; Tanner et al., 2011), adenosine A_1 receptors (Masino et al., 2011), and VGLUTs (Juge et al., 2010) are molecules that directly regulate electrical currents in neurons. Lactate dehydrogenase as a metabolic target against epilepsy will be useful in the development of new antiepileptic drugs. The next step toward drug development is to identify chemical compounds that act on LDH. Although many antiepileptic drugs are now clinically used, none of antiepileptic drugs have been designed to act on LDH. Antiepileptic drugs to act on LDH are also not known. Therefore, we explored LDH inhibitors from clinically used antiepileptic drugs, and found that stiripentol, a clinical drug for Dravet syndrome (Chiron et al., 2000), is an LDH inhibitor (Sada et al., 2015). By modifying its chemical structure, we further identified a stiripentol analog that inhibits LDH and strongly suppresses seizures in vivo (Sada et al., 2015). Thus, new antiepileptic drugs to mimic the ketogenic diet can be developed in the future by targeting LDH enzymes with stiripentol derivatives.

In order to develop new antiepileptic drugs, it will be important to create more powerful and selective LDH inhibitors. The design and synthesis of selective inhibitors for each LDH subunit (LDHA or LDHB) and/or for the unidirectional conversion of LDH activities (pyruvate-to-lactate or lactate-to-pyruvate conversion) will be useful because these are expected to act on more specific components and functions in the brain. To date, LDH inhibitors have been synthesized to create antimalarial compounds (Deck et al., 1999; Cameron et al., 2004). More importantly, LDHA is known to be involved in antitumor actions based on the Warburg effect (Fantin et al., 2006). Also, LDH inhibitors have already been synthesized and studied as antitumor reagents (Le et al., 2010; Granchi et al., 2011; Farabegoli et al., 2012). Drug development for epilepsy by targeting LDH enzymes, which is based on the antiepileptic mechanism of the ketogenic diet, may also lead to new antitumor drugs based on the Warburg effect.

**REFERENCES**


Chapter 29: Lactate Dehydrogenase


THE BLOOD-BRAIN BARRIER

The blood-brain barrier (BBB) is a selectively permeable cellular boundary that protects the mammalian brain from systemic factors (Daneman and Prat, 2015). Even though the microvessel endothelial cells (ECs) have a primary role in the formation of the BBB, several other cells are equally important to maintain BBB integrity. Endothelial cells, pericytes, neurons, and other glial cells form a "neurovascular unit" (Figure 30.1; Abbott et al., 2006; Grant and Janigro, 2010; Neuwelt et al., 2011). In addition to the layer of capillary ECs, other layers exist between blood and brain. These include a basement membrane (BM), consisting of type IV collagen, fibronectin, and laminin that cover capillaries completely; pericytes embedded in the BM; and astrocytes whose processes surround the BM (Figure 30.1). Each of these layers has potential to restrict the movement of solutes (Hawkins et al., 2006). Thus, the microenvironment of the BBB acts in concert with tight junctions to maintain the selective permeability of the neurovascular unit barrier.

Simply stated, BBB integrity is an essential player involved in the maintenance of brain homeostasis. Disruption of the BBB occurs in various conditions such as neoplasia, trauma, epilepsy, Alzheimer’s disease, infections (e.g., meningitis), and sterile inflammation (e.g., multiple sclerosis) (Huber et al., 2001; Zlokovic, 2008). Thus, in these conditions it is highly desirable to reverse the disruption of the BBB. However, a highly restricted BBB is a huge challenge for the delivery of drugs into the diseased brain (Gloor et al., 2001). This dual nature of the BBB has been recently reviewed by Saunders et al. (2014).

CELLULAR ASSOCIATIONS AT THE BBB

The BBB maintains the proper ionic composition of the brain interstitial fluid, which is essential for optimum neuronal function. It achieves a function of “transport barrier” by facilitating the uptake of necessary nutrients, while at the same time preventing the uptake or actively effluxing other molecules. Additionally, the BBB functions as a “metabolic barrier,” possessing intracellular (e.g., cytochrome P450) and extracellular (e.g., peptidases and nucleotidases) enzymes (Abbott et al., 2010; Ghosh et al., 2013; Ghosh et al., 2011a; Ghosh et al., 2011b). Functions of individual cells in brain microvessel are discussed in detail in this section.

Endothelial Cells

Endothelial cells line the interior of blood vessels. The membranes of the capillary ECs are divided into two distinct sides: luminal (blood side) and abluminal (brain side) (Daneman and Prat, 2015). The ECs form blood vessels, and mural cells sit on the abluminal side of the EC. These brain ECs are less “leaky” than those of the peripheral vessels. However, it has been shown that brain ECs become “leaky” when they are allowed to vascularize peripheral tissue, while peripheral ECs form tight junctions resembling the BBB when allowed to vascularize the brain parenchyma (Rubin et al., 1991). Central nervous system (CNS) microvascular ECs are 39% thinner than muscle ECs (Coomer and Stewart, 1985).

The type and amount of membrane lipids and proteins vary between luminal and abluminal sides of the blood vessel (Tewes and Galla, 2001). Thus, nutrients must transverse two sheaths of membrane; their combined characteristics determine which particles cross the barrier, and how fast. The EC transporters are divided into two major categories: efflux transporters and nutrient transporters. Efflux transporters are polarized to the luminal surface and transport a broad variety of lipophilic molecules (Cordon-Cardo et al., 1989; Thiebaut
Nutrient transporters are specific for the transport of nutrients into, and the removal of waste products from, the CNS (Mittapalli et al., 2010). The ECs of the BBB contain high numbers of mitochondria, which are important to generate a large amount of adenosine triphosphate (ATP) to maintain ion gradients critical for the function of transporters. In addition, CNS ECs express low levels of leukocyte adhesion molecules, which ultimately limits the number of immune cells that can enter the CNS (Daneman et al., 2010). Moreover, these ECs generate a barrier by altering the physical properties of molecules, which then can modulate their solubility, reactivity, and transport properties. These metabolic properties are further regulated by the flow (Cucullo et al., 2011; Desai et al., 2002). The combination of a physical barrier (e.g., tight junctions and low transcytosis), a molecular barrier (e.g., low leukocyte adhesion molecules, efflux transporters, and specific metabolism), and

**FIGURE 30.1** Schematic diagram of a brain capillary. A. Cell-to-cell interaction at the BBB. The endothelial cells (E) are joined together by tight junctions (TJ) and surrounded by a basement membrane (BM). Pericytes (P) reside within the BM and are distributed discontinuously along the length on cerebral capillaries. The cerebral capillaries are surrounded by astrocytic foot processes or end-feet from astrocytes (A). Axonal projections from neurons (N) containing vasoactive neurotransmitters and peptide onto arteriolar smooth muscle cells (S). BBB permeability may be regulated by the molecules released from cells associated with endothelium (e.g., microglia, astrocytes). B. Schematic representation of structural and transport components at the BBB. GLUT1, glucose transporter 1; MCT, monocarboxylate transporter; LAT1, L-type amino acid transporter 1; Tfr, transferrin receptor; JAM, junctional adhesion molecules; LAM, leukocyte adhesion molecules; ZO-1/2/3, zonula occludin-1/2/3; JACOP, junction-associated coiled-coil protein; Ocln, Occludin; CD31, cluster of differentiation 31; Pgp, p-glycoprotein; BCRP, breast cancer resistant protein; MRP, multidrug resistance-associated protein.
presence of specific nutrient transporters (e.g., Glut1; McAllister et al., 2001) allows CNS ECs to firmly regulate CNS homeostasis (Daneman and Prat, 2015; Ghosh et al., 2010). The specific nutrient transporters are probably topographically organized for the development or function of brain-specific region or particular subclass of neurons. A major unanswered question regarding the BBB and ECs is whether they possess unique properties in different brain regions.

The following features distinguish BBB ECs from peripheral vasculature (Arcangeli et al., 1999; Dalvi et al., 2014; Gloor et al., 2001; Hawkins and Davis, 2005; Rubin et al., 1991):

1. Presence of a large number of tight junctions to limit the paracellular movement of macromolecules (Figures 30.1 and 30.2).
2. Controlled fluid-phase endocytosis rate to limit transcellular passage of macromolecules.
3. Presence of highly specific transporters and carrier molecules.
4. Absence of fenestrations.
5. Few cytoplasmic vesicles.
6. Increased mitochondrial content.

**Astrocytes**

Astrocytes are important glial cells that help in conditioning and developing the brain microvesSEL ECs. The interactions of ECs and astrocytes are known to regulate the phenotype of the BBB under (patho)physiological conditions (Prat et al., 2001). Astrocytes are known to alter the properties of ECs in multiple ways (Grant et al., 1998; Haseloff et al., 2005; Janzer and Raff, 1987; Lee et al., 2007; Stanness et al., 1996; Stanness et al., 1997):

1. Tightening the BBB, as evidenced by the decreased paracellular permeability of sucrose.
2. Elevating transendothelial electrical resistance.
3. Increasing the activity of barrier-related marker enzymes such as alkaline phosphatase and γ-glutamyl transpeptidase.
4. Enhancing the number, length, and complexity of tight junctions.
5. Increasing the expression of glucose transporters.
6. Secreting angiogenic factors like vascular endothelial growth factor (VEGF),

**FIGURE 30.2** Schematic diagram of transport pathways in cerebrovascular endothelial cells. The transport of diverse molecules from the circulation across the BBB may include (1) transcellular lipophilic pathway, (2) paracellular aqueous pathway across tight junctions, (3) transport proteins, (4) receptor-mediated transcytosis, (5) adsorptive transcytosis, and (6) efflux transporters.
which is essential for the formation and remodeling of embryonic blood vessels, and decreasing vascular stability in adult blood vessels.

Angiotensin-converting enzyme-I produced by astrocytes converts angiotensin I into angiotensin II, which in turn acts on type I angiotensin receptors expressed by brain ECs. Angiotensin II promotes the recruitment of junctional proteins into the lipid raft, restricting BBB permeability (Wosik et al., 2007). Furthermore, retinoic acid (RA) secreted by radial glial cells acts on RA-receptor β expressed in the developing vasculature, which increases transendothelial electrical resistance by enhancing the expression of vascular endothelial-cadherin, p-glycoprotein, and zonula occludin-1 (Figure 30.1; Mizee et al., 2013). Other factors such as transforming growth factor-β, glial cell-derived neurotrophic factor, and src-suppressed C-kinase substrate are postulated to play a role in maintaining tightness of the BBB (Haseloff et al., 2005).

Pericytes
Pericytes, the mural cells of blood microvessels, are specialized cells of mesenchymal lineage that surround the surface of the vascular tube. They are on the abluminal side of the microvascular endothelial tube and are embedded on the vascular basement membrane (Abbott et al., 2010). The CNS microvasculatures have a higher pericyte-to-endothelial ratio (1:3 to 1:1) than muscle (1:100) (Shepro and Morel, 1993). Pericytes in the BBB have several major functions (Armulik et al., 2011; Hall et al., 2014; Lai and Kuo, 2005):

1. Formation and maintenance of tight junctions.
2. Autoregulation of cerebrovascular blood flow.
3. Secretion of angiopoietin-1 and brain angiogenesis.
4. Initiation of extrinsic blood coagulation pathway after cerebrovascular injury.
5. Regulation of inflammation by the secretion of cytokines (e.g., IL-1β and IL-6), leukocyte transmigration, antigen presentation, and T-cell activation.
6. Contraction of capillary diameter by contractile proteins in pericytes.

The lack of a pericyte-specific marker, however, often leads to misidentification of pericytes as other cells occupying the perivascular space.

All blood vessels, including those of the brain, are ensheathed by extracellular matrix layers called basement membranes (BMs). The inner vascular BM and outer parenchymal BM are two BMs that surround the vascular tube (Daneman and Prat, 2015; Sorokin, 2010). The BM is an extracellular matrix secreted specially by ECs and pericytes in vascular BM, and by astrocytic processes in parenchymal BM. Type IV collagens, nidogen, heparin sulfate proteoglycans, and laminin are some molecules present in BMs. These membranes anchor many signaling processes and provide an additional barrier that molecules and cells must cross prior to accessing neural tissue. The degradation of BMs by matrix metalloproteinases is observed in different neurological diseases, allowing the infiltration of leukocytes (Daneman and Prat, 2015; Sorokin, 2010).

MOLECULES AT THE BLOOD-BRAIN BARRIER
The structure and function of the BBB are highly specialized by the presence of typical components that are discussed in this section.

Tight Junctions
The tight junction (TJ) consists of membrane and cytoplasmic proteins (Figure 30.1). Identification of molecules expressed by CNS ECs led us to understand the structural and transport components of the BBB (Ohtsuki et al., 2014). Claudins, occludin, and junctional adhesion molecules (JAM) are the integral membrane proteins. Cingulin, zonula occludin proteins (ZO-1, -2, -3), AF6, 7H6 antigen, and symplekin are the cytoplasmic accessory proteins that form plaque and function as adaptor proteins. The tight junctional complexes are dynamic entities that can “bend without breaking,” thereby sustaining structural integrity (Huber et al., 2001).

Claudins are the largest family of transmembrane phosphoproteins; so far 24 members (claudins 1–24) have been characterized (Ballabh et al., 2004). Among these, claudins 1, 3, 5, and 12 have a role in tight-junction formation at the BBB. Claudin-1 is an integral component of tight junctions, whose absence is associated with several pathological conditions, such as stroke, inflammatory diseases, and tumors (Liebner et al., 2000).

Occludin is another transmembrane phosphoprotein, and its subcellular localization parallels that of claudin. In adult brain microvessel ECs, occludin expression is higher than the peripheral ECs that interact with claudins. Jointly, they form
channels to regulate the paracellular flow of ions and other hydrophilic molecules (Hirase et al., 1997; Huber et al., 2001; Morita et al., 1999).

The JAMs are members of the immunoglobulin superfamily that form homotypic interactions at the endothelial and epithelial cells TJ, which are known to regulate leukocyte extravasation and paracellular permeability (Aurrand-Lions et al., 2001).

Several cytoplasmic proteins are also essential components of the TJs. Actin is the cytoskeleton protein that plays a major role in maintenance of the TJ. Actin-degrading molecules (e.g., cytochalasin-D and phalloidin) can disrupt the actin cytoskeleton, compromising the TJ (Huber et al., 2001). Zonula occludin proteins (ZO-1, -2, -3) are also important. These proteins make direct contact with claudins, occludins, and JAMs on one side and the actin cytoskeleton on the other (Ballabh et al., 2004). Cingulin is another protein that links the TJ accessory proteins with the cytoskeleton (Huber et al., 2001). Several intracellular processes involving calcium-signaling, phosphorylation, G-proteins, proteases, and inflammatory cytokine secretion can modulate TJ proteins (Huber et al., 2001; Kniesel and Wolburg, 2000).

**Leukocyte Adhesion Molecules**

The CNS of healthy individuals has an extremely low immune surveillance, lacking neutrophils and lymphocytes in the parenchyma (Galea et al., 2007). Entry of leukocytes into the brain parenchyma requires multiple steps, including rolling adhesion, firm adhesion, and extravasation. This requires several leukocyte adhesion molecules (LAMs) that include selectins (E-, P- selectin) for rolling adhesion and immunoglobulin family members for firm adhesion (Huang et al., 2006). In a normal brain, the expression of these adhesion molecules is extremely lower in CNS ECs than in peripheral ECs. However, LAMs are elevated in neuroinflammatory conditions like stroke and multiple sclerosis (Daneman et al., 2010; Huang et al., 2006).

**TRANSPORTERS**

The paracellular junctions, “having low permeability,” control the transport of molecules and ions between the blood and brain. The ECs of the CNS are highly polarized and have distinct luminal and abluminal compartments. As mentioned earlier, there are two major transporter types that are expressed in CNS ECs: efflux transporters and nutrient transporters (Figures 30.1–30.4).

Efflux transporters (e.g., multidrug resistance protein 1, MDR1; breast cancer resistance protein, BCRP; multidrug resistance proteins, MRPs) hydrolyze ATP to transport substrates against their concentration gradient (Ha et al., 2007). Many efflux transporters are localized on the luminal surface in order to transport substrates into the blood compartment. These transporters facilitate the movement of a diverse range of substrates, and provide a barrier to many small lipophilic molecules, which would otherwise passively diffuse through the membrane of ECs. MDR1, also called p-glycoprotein (Pgp), is a well-studied transporter, which is associated with drug-resistant epilepsy (Dombrowski et al., 2001; Marchi et al., 2004) and tumors (Abbott et al., 2001). Endogenous substrates of efflux transporters are still not well studied.

Nutrient transporters facilitate the movement of nutrients according to their concentration gradients. A wide variety of such transporters are expressed in CNS ECs to deliver specific nutrients into the brain parenchyma. Most of these nutrient transporters are in the solute carrier class of facilitated transporters (slc2a1 transports glucose, slc16a1 transports lactate and pyruvate, SCL7A1 transports cationic amino acids, slc7a5 transports neutral amino acids and L-Dopa) (Zlokovic, 2008). Slc2a1, also called glucose transporter 1 (Glut1), is a well-studied nutrient transporter that provides glucose to the CNS (Figure 30.4). It is more frequently expressed by brain ECs than nonbrain ECs (Cornford et al., 1994). In humans, Glut1 deficiency leads to an epileptic syndrome, which can be treated with a high ketone diet (De Vivo et al., 2002; De Vivo et al., 1991). Most of the transporters in the BBB-ECs provide nutrients to the brain, however, in mild cognitive impairment and Alzheimer’s disease a receptor (receptor for advanced glycation end products) is thought to assist in transporting waste (amyloid β) from blood to brain (Daneman and Prat, 2015).

**OTHER COMPONENTS**

The rate of transcytosis is lower in brain ECs; however, it is up-regulated upon BBB dysfunction during brain injury and disease. In ECs, transcytosis (Figure 30.2) is mediated via caveoline-based vesicle trafficking (Gu et al., 2012). Additionally, the plasmalemma vesicle-associated protein number is lower in the ECs of a healthy BBB than the ECs of a BBB following traumatic brain injury (Shue et al., 2008). The significance of these changes in the context of disease etiology is unknown.
FUNCTIONS OF THE BLOOD-BRAIN BARRIER

Regulation of Transit across the Blood-Brain Barrier

The BBB provides a suitable environment for neural functions because it regulates ionic composition in the brain. The combination of ion-specific channels and transporters at the BBB regulates the composition and quantity of ions in the CNS. The concentration of potassium in mammalian brain cerebrospinal fluid (CSF) and interstitial fluid (ISF) is maintained at around 2.5–2.9 mM, though it is approximately 4.5 mM in plasma. Even though plasma K+ concentration changes following exercise or meals, it remains within 2.5–2.9 mM range in the brain (see Janigro, 1999, 2012). In addition, Ca2+, Mg2+, and pH are actively regulated in the BBB (Abbott et al., 2010; Jeong et al., 2006; Nischwitz et al., 2008; Somjen, 2004).

The BBB acts as a shield to prevent the entry of most macromolecules into the brain. The total protein content in plasma is much higher than in CSF, and the individual protein composition is distinctly different (Abbott et al., 2010). Proteins that are markedly high in plasma (e.g., albumin, prothrombin, and plasminogen) are detrimental to nervous tissue, as they can trigger cell apoptosis (Gingrich and Traynelis, 2000). Factor Xa and tissue plasminogen activator are widely expressed in the CNS, and can convert prothrombin and plasminogen to thrombin and plasmin, respectively. If present, thrombin and plasmin can initiate cascades resulting in seizures, glial activation, glial cell division, and cell death (Abbott et al., 2010). Hence, the ingress of these macromolecules upon BBB disruption can have serious pathological consequences.

After taking meals, blood levels of a neuroexcitatory amino acid, glutamate, are elevated. If glutamate is freely transposed into the brain ISF, it can be detrimental. For example, in an ischemic stroke patient, uncontrolled glutamate release into the brain can cause permanent neuroexcitatory damage of neural tissue. The peripheral and central nervous systems share many of the same neurotransmitters; the BBB, however, keeps them separate and minimizes the cross-talk (Abbott et al., 2010; Abbott et al., 2006; Bernacki et al., 2008).

The BBB is a protective barricade that shields the brain from neurotoxic substances circulating in the blood (e.g., endogenous metabolites or proteins, and xenobiotics ingested in the diet or acquired from the environment). The ECs in the CNS express a number of energy-dependent efflux transporters (Figures 30.1 and 30.2) that actively extrude neurotoxins out of the brain (Abbott et al., 2010). These transporters also prevent neuronal cell death where neurons are exposed to toxins (Marchi et al., 2004).

Transport of Nutrients to the Brain

Most of the essential water-soluble nutrients and metabolites required for neuronal survival and differentiation are transported into the brain by specific transport systems (Figures 30.2–30.4) at the BBB. The endothelium begins to differentiate into a barrier layer from the embryonic angiogenesis stage, and is maintained in adults by its association with other cell types, especially the endfeet of astroglial cells. Astrocytic glial cells promote the up-regulation of tight junction proteins and the differential expression of luminal and abluminal membrane-specific transporters (Abbott et al., 2006; Wolburg et al., 2009). Other cell types, namely pericytes, microglia, and nerve terminals play supporting roles in barrier induction, maintenance, and function (Abbott et al., 2006; Nakagawa et al., 2009; Shimizu et al., 2008).

Together with oxygen, glucose is the obligatory nutrient for the human brain. The BBB regulates the transport of glucose in the CNS and prevents equalization of plasma levels (~ 5 mM) to the CSF and ISF levels (< 2.5 mM) (McAllister et al., 2001). It is used for ATP production, which is primarily used for ion transport and the maintenance of the ion gradient (Magistretti and Pellerin, 1995). However, a small fraction is also used for biosynthetic processes. The diffusion of this vital nutrient across the BBB is facilitated by the glucose transporter type 1 (Glut1). In the brain, Glut1 interacts with other Glut1 isoforms that mediate glucose transport into neurons and astrocytes (Klepper, 2008).

Brain glucose levels in a healthy and normally active person are kept adequate by a complex regulatory mechanism to ensure an appropriate supply to neurons, in spite of massive drops in plasma levels. Glucose is controlled by intracellular enzymes (e.g., hexokinase) and Glut1 proteins that are expressed asymmetrically in the ECs (lower concentration in abluminal membrane than in luminal membrane) (McAllister et al., 2001). Thus, glucose levels in the brain parenchyma and CSF ultimately depend on the “barrier” nature of the brain endothelium.

Under normal conditions, asymmetrically distributed Glut1 in the BBB transports blood glucose into the brain, in which barrier integrity
is maintained by tight junctions, preventing potassium leakage in the brain (Figure 30.5). However, under pathological conditions GLUT1 expression is altered (Cornford et al., 1998a; Cornford et al., 1998c). Cerebral blood flow also fails to meet the brain metabolic demand when the BBB is damaged (Bruehl et al., 1998), and in such cases, potassium leaks across the open tight junctions in the brain (Cornford et al., 1998a; Cornford et al., 1998b; Cornford et al., 1998c). The elevated extracellular [K+] further increases the metabolic demand due to exaggerated neuronal firing and lost homeostasis by voltage-dependent channels.

However, increased plasma ketone levels produced due to starvation, exercise, or ketogenic diet (KD) ingestion provide an alternative energy source to fulfill the energy demand in BBB-damaged conditions (Figures 30.3 and 30.5). In addition, ketone bodies (KBs) directly enhance Glut1 activity (Janigro, 1999).

**KETOSIS**

The KD is a strict high-fat, low-carbohydrate, and low-protein diet. As a result of this diet, the body receives a minimal dietary source of glucose, which is required for all metabolic needs (Freeman et al., 2006). Fatty acids become an obligatory source of cellular energy production, however, they do not readily cross the BBB due to the presence of tight junctions (Mitchell et al., 2009). Their transport into the brain by diffusion or via specific protein-mediated transport is still debated and controversial (Abumrad et al., 1998; Hamilton, 1998). The KD is characterized by elevated serum levels of circulating KBs: acetacetate (AcAc), β-hydroxybutyrate (BHB), and acetone (Ac), which are mainly produced by the liver. The plasma concentration of KBs increases to three- to four- times its basal levels (AcAc: 100 µM, BHB: 200 µM) (Musa-Veloso et al., 2002). The KBs are utilized by extrahepatic tissues as an energy source. However, Ac is not an energy source and is exhaled or excreted as a waste. In the absence of glucose, KBs are the preferred source of brain energy. They are transported into the brain by simple (Ac) or facilitated diffusion (AcAc and BHB) mediated by the monocarboxylic acid transporter (MCT) (Figures 30.3–30.5) (Cremer, 1982; Klepper, 2008). These MCTs are expressed in the plasma membrane of choroid plexus and BBB cells (endothelial and epithelial), glia, and neurons, which ultimately oxidize ketones in the mitochondrial matrix, releasing acetyl-CoA that enters into the tricarboxylic acid (TCA) or Krebs cycle (Morris, 2005) (Figures 30.3 and 30.4).

Once KBs cross the BBB, brain cells take up KBs via diffusion or a carrier-mediated process to support cellular energy requirements. Even though neurons and glial cells express MCTs, since KBs must transverse the BBB first, their role appears more significant at the BBB.

**Regulation of Cerebral Ketone Uptake**

In the absence of glucose, monocarboxylic acids (e.g., ketones, lactate, and pyruvate) generate a substantial amount of energy for the brain (Pierre and Pellerin, 2005). Prolonged fasting elevates normal circulating KB concentrations from ~5.8 mM to 9 mM (White and Venkatesh, 2011). The plasma concentration of KBs is a major factor regulating the rate of cerebral uptake. Since KBs are hydrophilic compounds in the blood, specific transporters are required to facilitate KB diffusion across the BBB and maintain proper levels of these metabolic products in the brain (Pierre and Pellerin, 2005). Monocarboxylic acid transporters (e.g., MCT1 and MCT2) involved in transporting monocarboxylic acids (e.g., lactic acid and pyruvate) are thought to facilitate the transport of KBs. The first monocarboxylic acid transporter identified in brain ECs was MCT1. Additionally, small amounts of MCT1 were found in astrocytic endfeet surrounding the capillaries. Interestingly, MCT2 was found only in BBB ECs and neurons, but not in astrocytes. On the other hand, MCT4 was present exclusively in the astrocytes and glial cells. Glucose and monocarboxylate transporters expressed in the major brain cells are summarized in a schematic diagram (Figure 30.4). This distribution of MCT is associated with the distinctive functional characteristics. Brain MCT1 and MCT4 facilitate the release of monocarboxylates into the extracellular space and allow cells to maintain a high glycolytic rate (Pierre and Pellerin, 2005). These monocarboxylates produced and released by astrocytes in the extracellular space are an energy substrate for active neurons; monocarboxylates are taken up by MCT2 expressed in the dendrites and axons of neurons (Pierre and Pellerin, 2005; Takimoto and Hamada, 2014).

At a given arterial concentration, AcAc uptake is double that of BHB; however, it is not yet understood how the expression of MCT is regulated (White and Venkatesh, 2011). Prolonged fasting increases BBB uptake of KBs by increasing the expression of MCT1 as high as eightfold in mice (Leino et al., 2001). Similarly, another report in human subjects showed a 13-fold increase in cerebral BHB uptake following several days of fasting (Hasselbalch et al., 1994). Interestingly, a
FIGURE 30.3 Schematic diagram of ketogenic diet metabolism and brain uptake. The ketogenic diet is deficient in carbohydrates, but provides large amounts of long chain free fatty acids (FFA). The acetyl-CoA produced by fatty acid oxidation goes into the Krebs/tricarboxylic acid (TCA) cycle or is converted to ketone body (KB) acetoacetate (AcAc), which spontaneously degrades to acetone (Ac). AcAc is further converted to β-hydroxybutyrate (BHB) in a reversible reaction. These KBs represent alternative energy substrates for the brain. GLUT1, glucose transporter 1; MCT, monocarboxylate transporter.

FIGURE 30.4 Schematic diagram of glucose and monocarboxylate transporters in the brain. Multiple types of glucose transporters (GLUTs) and monocarboxylate transporters (MCTs) are responsible to transport glucose and monocarboxylic acids (ketone bodies, lactate, and pyruvate), respectively.
rapid increase in plasma ketone levels, following an intravenous infusion of BHB, does not induce cerebral uptake as significantly as prolonged fasting; this suggests that MCT up-regulation is partially dependent on the length of exposure to increased plasma KBs (Pan et al., 2000; Pan et al., 2001). Thus, a prolonged high-fat diet increases blood ketone concentrations, leading to an elevated expression of MCT and cerebral uptake of KBs (Figure 30.5).

### Anti-Inflammatory Effects of Ketone Bodies

Neuroinflammation is defined as inflammation of CNS that occurs upon invasion of pathogens, trauma, and/or neurodegeneration. Cells such as macrophages, astrocytes, and oligodendrocytes and molecules such as cytokines and complement and pattern-recognition components are the known contributors to neuroinflammation (Banjara, 2014; de Vries et al., 2012; Glass et al., 2010; Marchi et al., 2014). Proinflammatory molecules are either generated locally within the CNS or transported from the peripheral system upon BBB disruption. Moreover, inflammatory mediators have been shown to influence tight junctions and to activate astrocytes and microglia (David et al., 2009; Ivens et al., 2007; Tomkins et al., 2008), ultimately compromising the BBB integrity. An optimum amount of neuroinflammation is considered neuroprotective, and this generally occurs for a short period of time. Chronic neuroinflammation is often detrimental, inducing further cell and tissue damage. However, the opposing mechanisms of this ostensibly paradoxical exacerbation or amelioration of parenchymal brain injury are largely unknown (Cederberg and Siesjo, 2010; Finnie, 2013).

The therapeutic efficacy of large spectrum anti-inflammatory drugs is limited by side effects after even transient immune suppression. Recent studies suggest that the KD could be useful, particularly in patients with recent worsening of epilepsy and inflammation (Janigro, 1999; Marchi et al., 2012; Nabbout et al., 2011). The KD has been used as an effective treatment in patients with inflammation-induced epileptic encephalopathies (e.g., fever-induced refractory epileptic encephalopathy in school-aged children) (Nabbout et al., 2011). Additionally, polysaturated fatty acids, mostly eicosapentaenoic acid and docosahexaenoic acid, are thought to have anti-inflammatory actions, mediated typically by their hydroxylated metabolites (Grimble, 1998; Porta et al., 2009).

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**FIGURE 30.5** Schematic diagram of cellular and molecular events in normal and BBB damaged tissue. The ketogenic diet potentially has a role to reestablish physiologic metabolic supply and repair damaged BBB by inducing the expression levels of monocarboxylate transporter (MCT) and connexin 43 (Cx43), and enhancing glucose transporter 1 (GLUT1) activity.
Furthermore, KD attenuated thermal nociception and decreased peripheral edema, indicating an anti-inflammatory effect (Ruskin et al., 2009).

A systemic review published by Gibson et al., which analyzed 16 independent studies containing a total of 733 rodent studies, demonstrated a significant protective (both pathological and functional) effect of KD or exogenous administration of KBs ($p < .001$) on outcomes following cerebral ischemia. Moreover, regardless of dietary intervention or administration-induced ketogenic state, ketones were found to have beneficial/anti-inflammatory effects on functional/behavioral tests, lesion volume, and brain water content (Gibson et al., 2012).

In traumatic brain injury (TBI) models, concussion or mild TBI models, and spinal cord injury, KD intervention (pretreatment or administration to postinjury animals) revealed improved structural and functional outcomes (Prins, 2008; Prins and Matsumoto, 2014). Within minutes after injury, the ionic equilibrium across the neuronal membrane is disrupted, which requires cellular energy to reestablish homeostasis. Thus, the cerebral glucose uptake is increased in both rodent and human within 30 min after fluid percussion and within 8 days after TBI, respectively (Bergsneider et al., 1997). However, this transient “hyperglycolysis” is followed by a prolonged period of reduction in glucose metabolism. Hence, utilizing brain ketone metabolism after TBI as a therapeutic approach is appealing as it can bypass the early glucose metabolic derangements and also offers multiple consequences that are beneficial to the brain (Prins and Matsumoto, 2014). Ketone metabolism is particularly advantageous to TBI patients because it decreases the production of free radicals in mitochondria and cytosol (Sullivan et al., 2004; Ziegler et al., 2003). In addition, animals on the KD produced less Bcl-2 associated protein, ultimately decreasing cellular apoptosis and brain swelling. Furthermore, KD-induced decrease in mitochondrial release of the cytochrome c into the cytosol inhibits the stimulation of apoptotic signaling cascade (Hu et al., 2009a; Hu et al., 2009b).

More recently, it has been shown that KD can modulate lipopolysaccharide-induced fever by decreasing peripheral inflammation and CNS IL-1β expression (Dupuis et al., 2015). These properties potentially contribute to the beneficial effect of KD in neuroinflammatory conditions (e.g., epilepsy, Alzheimer’s disease, Parkinson’s disease, multiple sclerosis) and/or brain trauma.

The NOD-like receptor P3 (NLRP3) inflammasome is a multiprotein complex of the innate immune system, which is primarily responsible for IL-1β derived autoinflammatory disorders such as gout, obesity, atherosclerosis, and neurodegenerative diseases (Levy et al., 2015). And BHB, but not structurally similar AcAc or butyrate, was shown to limit the cytokine production by inhibiting the activation of these NLRP3 inflammasome in human monocytes in vitro (Yasui et al., 2011). Since NLRP3 is a common player of sterile neuroinflammation, the identification of BHB as an NLRP3 inhibitor provides a rationale to further investigate the effectiveness of KD for the treatment of neuroinflammatory diseases.

**Neuroprotective Effects of Ketone Bodies**

Ketones, an alternative energy source for normal or injured brain, may also have neuroprotective effects (Prins, 2008). Ketones are considered neuroprotective due to the following:

1. BHB is a more efficient energy source than glucose. It can also stimulate mitochondrial biogenesis by up-regulating mitochondrial enzymes and genes that stimulate energy metabolism (Veech et al., 2001).

2. KBs protect cells against glutamate-mediated apoptosis and necrosis by attenuating the formation of reactive oxygen species (Ziegler et al., 2003). They also attenuate apoptosis by reducing the activation of caspase-3 (Gasior et al., 2006).

3. KBs enhance the conversion of glutamate to gamma-aminobutyric acid (GABA) and boost the GABA-mediated inhibition (Gasior et al., 2006).

4. Cerebral blood flow elevated by ketone metabolism is often considered as an effect of neuroprotective therapy (Hasselbalch et al., 1996).

Even though studies so far demonstrated beneficial neuroprotective effects of KD, further research is necessary to clarify issues such as dosing, timing, and the route and duration of administration.

**Effects of Ketone Bodies on the Blood-Brain Barrier**

Data on the effects of the KD and/or KBs on the BBB are based largely on animal studies. There is evidence of rapid changes in cerebral blood flow and cellular transporters (decreased Glut1 expression) that favor KBs’ uptake and metabolism in several neurological conditions such as...
TBI, hemorrhagic shock, ischemia, and hypoxia (Gasior et al., 2006; Gibson et al., 2012; Prins and Matsumoto, 2014; White and Venkatesh, 2011).

Prins et al. demonstrated an increase in expression of MCT (MCT1 and MCT2) levels and ketone transport following TBI in rats (Prins and Giza, 2006). Additionally, ketone metabolizing enzyme, β-hydroxybutyrate dehydrogenase, is elevated following cerebral injury that converts BHB to AcAc, which is scarce in the adult brain (Tieu et al., 2003). These evidences suggest brain's improved ability to utilize exogenous KBs. Thus, taking advantage of improved transport and cellular metabolism of ketones, brain's reliance for energy may shift from glucose to KBs in injured brain. It is beneficial for the brain, particularly as hyperglycemia is deleterious to the injured brain (Salim et al., 2009).

Astrocyte endfeet lacking the gap junction protein connexin 43 (Cx43) are inefficient in transmembrane receptor anchoring. This weakens the BBB, which further fails upon increased hydrostatic vascular pressure and shear stress (Ezan et al., 2012). Interestingly, higher concentrations of KBs, either alone or in combination, significantly up-regulated the expression of the Cx43 at both mRNA and protein levels (Ho et al., 2013) (Figure 30.5). Further, the up-regulation of Cx43 protein in ECs and glial cells by KBs accelerated cell migration (Bates et al., 2007).

In a separate report, BHB (0.5 mM) has been shown to promote human endothelial cell proliferation (Cheng et al., 2006). These studies suggest that the KD plays a role in repairing damaged BBB and restoring normal brain function. In contrast, Freeman et al. demonstrated AcAc-induced inhibition of EC proliferation by elevation of oxidative stress (Freeman et al., 2006; Freeman et al., 1998). Hence, the response of cell types to different KBs might vary depending on cell-specific characteristics.

In severely uncontrolled diabetes, KBs are produced in massive quantities, which is described as ketoacidosis; this causes high concentrations of protons and overwhelms the buffering system of the body by activating a pH sensitive sodium-proton pump (Bohn and Daneman, 2002). In contrast, during high-fat/low-carbohydrate intake, ketone bodies are produced in a regulated manner causing a harmless physiological state called dietary ketosis. There is minimal data on the adverse effects of ketogenic diet on the BBB. Depending on concentrations, acute administration of KBs alters the pH, sodium level, lipids, and glucose in the blood. The acute intravenous infusion of BHB also significantly increases the pH and sodium concentrations in the brain (Hiraide et al., 1991). Thus, hypoglycemia and dehydration are two predicted side effects of continuous ketone consumption. The long-term consequences of reduction in glucose cerebral metabolism are thought to increase cerebral blood flow, increase expression levels of MCT, and enhance KBs uptake and metabolism on the BBB, however details are not yet known. In contrast, a report showed that the prolonged intake of KD can significantly raise mean blood cholesterol levels, leading to lipid deposition in blood vessels (Freeman et al., 1998).

CONCLUDING REMARKS

It has long been recognized that increased concentration of KBs associated with the KD is efficient fuel for the brain. Dramatic effects of such high-fat dietary treatment were shown in patients with multiple neurological disorders and Glut1 deficiency syndrome. Studies suggest that the ketogenic diet potentially has the ability to re-establish physiologic metabolic supply by activating glucose transporters, enhancing the expression of ketone transporters, and repairing damaged BBB by inducing the expression levels of a gap junction proteins. However, additional studies are essential to validate these initial observations and explore further.

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The ketone bodies β-hydroxybutyrate (BHB) and acetoacetate (AcAc) are produced from fatty acids in the liver and serve as alternative energy sources for the brain, heart, skeletal muscle, and peripheral tissues during prolonged fasting, calorie restriction, strenuous exercise, or adherence to a high-fat, low-carbohydrate ketogenic diet (KD) (Cahill and Veech, 2003). Ketones have historically been labeled as abnormal metabolic byproducts of a pathological state (VanItallie and Nufert, 2003), particularly due to their association with diabetic ketoacidosis (DKA) in uncontrolled type 1 diabetics, but emerging interest has focused on nutritional ketosis as a powerful metabolic therapy for general health and a growing number of medical conditions in addition to drug-resistant epilepsy, where its use is well established (Stafstrom and Rho, 2012). Nutritional ketosis produces a nonpathological hyperketonemia resulting from decreased glucose availability, lower insulin, and increased fat oxidation; however, long-term maintenance of the KD can be difficult for some and requires strict adherence. The restrictive nature of the KD has limited the clinical applicability of therapeutic ketosis due to practical considerations. Emerging data suggests that many of the benefits of the KD are mechanistically attributable to the ketone bodies or specific medium chain triglycerides (MCTs), and this has motivated investigators to develop strategies to further augment the efficacy of the KD or use metabolic-based supplements to circumvent the need for dietary restriction to improve compliance and the maintenance of this therapeutic state (Newman and Verdin, 2014; Veech, 2004).

This section, “Ketone-Based Metabolism: General Health and Metabolic Alternatives” includes chapters that discuss the expanding medical and performance applications of nutritional ketosis and the emerging science of ketones and other related metabolites as alternative fuels and potent signaling molecules.

Induction of hyperketonemia through fasting or the KD is known to produce acute and chronic changes in metabolic physiology and molecular signaling pathways that provide therapeutic effects in varied disease states. Metabolic-based mechanisms of ketone therapies include an elevation of blood ketones and associated anaplerosis with simultaneous suppression of blood glucose, increased insulin sensitivity, improved mitochondrial efficiency, and suppressed oxidative stress and inflammation. The reported benefits of hyperketonemia and similar metabolic alternatives have generated significant interest in the science and application of implementing strategies for inducing and sustaining blood levels of specific metabolites (Kesl et al., 2016). Most exogenous ketone supplements and engineered anapleurotic agents are currently under investigation for understanding their potential benefits for both healthy and diseased individuals alike. It is likely that most, if not all, of the conditions that are known to benefit from the KD would receive some benefit from exogenous ketone supplementation by elevating blood ketones and lowering blood glucose (Cahill and Veech, 2003). Currently the most studied type of exogenous ketone would be ketone esters, which induce a dose-dependent hyperketonemia (1–7mM) in rats, mice, dogs, pigs, and humans (Birkhahn and Border, 1978; Clarke et al., 2012; Desrochers et al., 1995; Pascual et al., 2014). A recent case study of Alzheimer’s disease highlights its application in humans (Newport et al., 2015). There are a growing number of promising metabolic alternatives to ketone esters, and many of these agents and formulas are being evaluated for their therapeutic efficacy, practical application and potential synergy with the KD (Borges and Sonnewald, 2012; Kesl et al., 2016). Importantly,
ketone supplementation and metabolic alternatives provide a tool for achieving ketosis in patients who are unable, unwilling, or uninterested in consuming a low-carbohydrate or ketogenic diet.

Included in this section are chapters that discuss conditions for which ketones and metabolic alternatives have the most potential, including seizure disorders, glucose transporter type 1 deficiency syndrome (GLUT1DS), Alzheimer's disease (AD), brain and metastatic cancer, insulin resistance and type 2 diabetes mellitus (T2DM), weight loss, and performance. GLUT1DS is a rare genetic disorder caused by a mutation in the SLC2A1 gene, which encodes the glucose transporter protein type 1. Patients with GLUT1DS have impaired glucose transport into the brain and other tissues, and thus alternative energy substrates in the form of ketones or other metabolic alternatives offer a means for the metabolic management of this disorder (Pascual et al., 2014). A known hallmark of AD is impaired brain glucose metabolism, which is associated with neurodegeneration and rapid progression (Cunnane et al., 2016). Preclinical data demonstrate that ketones protect against AD development and slow its progression (Kashiwaya et al., 2013). Cancer is another disorder potentially treated with nutritional ketosis due a metabolic phenotype that is highly glycolytic, has abnormal mitochondrial structure and function, and has a deficiency in many ketolytic enzymes (Seyfried and Shelton, 2010). Indeed, numerous studies have shown that cancer cells lack the capacity to metabolize BHB for energy, and thus glucose restriction can be lethal (Maurer et al., 2011). This section discusses the research showing that ketone ester administration simultaneously decreases blood glucose and insulin concentrations, suggesting that exogenous ketone supplementation could be used for insulin resistance and T2DM (Kashiwaya et al., 1997). The beneficial effects of ketones in T2DM may be via direct regulation of HDAC-dependent glucose metabolism and by inducing resistance to oxidative stress, which helps restore insulin sensitivity.

Included in this section is a chapter by Poff et al. (Chapter 32) that describes the development and testing of exogenous ketone supplements, the diseases they are being investigated for use in, and the potential mechanisms of action of their therapeutic efficacy. The chapter by Walker and Williams (Chapter 33) gives an overview of the acute in vitro and in vivo studies of the MCT, decanoic acid, which imparts specific signaling effects by directly inhibiting AMPA receptors. Thus, this data demonstrates a novel metabolic-independent mechanism of MCTs, which were previously thought only to be nutritional support enhancing ketogenesis. Also included in this section is a chapter by Borges (Chapter 34) on triheptanoin, the triglyceride of heptanoate (C7 fatty acid), a metabolic alternative to the KD that is being used to treat patients with rare genetic metabolic disorders (Pascual et al., 2014). The compelling animal work associated with triheptanoin shows that metabolic alterations found in brains of rodent seizure models can be restored by this compound, which appears to cross the blood-brain barrier (Borges and Sonnewald, 2012). Preclinical and clinical studies indicate that triheptanoin is beneficial in numerous neurological and neuro-muscular disorders, making it a promising treatment for a variety of clinical conditions (Borges and Sonnewald, 2012).

Very little attention has been given to the potential function of protein and specific amino acids in the ketogenic diet. Hartman (Chapter 35) focuses on this topic by discussing the antiseizure potential of select amino acids and the cellular and molecular mechanisms associated with their effects. The chapter by Stafstrom et al. (Chapter 36) discusses inhibition of glycolysis as one way that the ketogenic diet suppresses seizures and how 2-deoxy-D-glucose (2DG) is an anti-glycolytic tool that has anti-seizure effects in numerous seizure models. Westman et al. (Chapter 37) review the rationale and recent clinical research supporting the use of a low-carbohydrate, ketogenic diet in individuals with obesity and T2DM. The last chapter in this section, Hyde et al. (Chapter 38) discuss the many health benefits of nutritional ketosis that are relevant to athletes, including enhanced fat oxidation, reduced inflammation, reduction in blood lipids, and suppression of oxidative stress. Zupec-Kania et al. (Chapter 39), reviews the gen esis and ongoing efforts of the Charlie Foundation and Matthew’s Friends in promoting ketogenic therapies for epilepsy and more recently as an adjuvant for the metabolic management of cancer and other neurological and neurodegenerative disorders. We hope the chapters in this section will help the reader gain an appreciation for the science and emerging applications of nutritional ketosis, ketone supplementation, and other metabolic alternatives that will inevitably be utilized for the treatment and prevention for a broad range of disease states.

REFERENCES
INDUCING THERAPEUTIC KETOSIS WITH KETONE SUPPLEMENTATION

Ketones are typically produced in the liver only under certain physiological conditions associated with the suppression of the hormone insulin: starvation, fasting, calorie restriction, prolonged exercise, or during the consumption of a high-fat, low-carbohydrate ketogenic diet (KD). The restrictive nature of these states has limited the clinical applicability of therapeutic ketosis due to practical considerations. In an effort to circumvent this dilemma, researchers have recently developed a number of exogenous ketogenic supplements—ketogenic precursors that are metabolized to produce a dose-dependent elevation of β-hydroxybutyrate (βHB) and acetoacetate (AcAc) in the blood (Clarke et al., 2012b; D’Agostino et al., 2013; Kesl et al., 2015a; Kesl et al., 2016). Most of the developed ketone supplements are currently under investigation for safety and efficacy in a number of disease states.

DEVELOPMENT AND TESTING OF KETONE SUPPLEMENTS

There are numerous sources of ketones and ketogenic precursors being developed and tested, including medium chain triglycerides, diols, salts, and esters that have been shown to elevate blood ketone levels independent of caloric or carbohydrate restriction. The investigation of natural and synthetically derived ketogenic precursors to establish nutritional ketosis without the need for dietary restriction has revealed that each formulation has distinct properties in terms of extent and duration of ketosis as well as metabolic signaling properties, including anticonvulsant effects (D’Agostino et al., 2013; Kesl et al., 2015a; Kesl et al., 2016). Most of the developed ketone supplements are currently under investigation for safety and efficacy in a number of disease states.

Medium Chain Triglycerides

Medium chain triglycerides (MCTs) contain a glycerol backbone esterified to medium-chain fatty acids (MCFAs), which are fatty acids with hydrocarbon side chains 6 to 12 carbons in length. The MCFAs include caproic acid (C6:0, hexanoic acid), caprylic acid (C8:0, octanoic acid), capric acid (C10:0, decanoic acid), and lauric acid (C12:0, dodecanoic acid). The MCTs are naturally found in coconut oil (~60%), palm kernel oil (~30%), cheese (7.3%), whole milk (6.9%), butter (6.8%), and full-fat yogurt (6.6%) (Karen and Welma, 2015; Koji and Truyoshi, 2010). Compared to long-chain fatty acids (LCFAs), MCFAs have a lower melting point, smaller molecule size, and are less calorically dense (8.3 calories per gram versus 9.2). These distinct physiochemical properties allow MCTs to be absorbed directly into the bloodstream through the hepatic portal vein without the need for bile or pancreatic enzymes for degradation. Additionally, MCTs do not require carnitine to enter the mitochondria, but rather quickly cross the mitochondrial matrix, where they are metabolized to acetyl co-A and subsequently to ketone bodies. Thus, they are easily and rapidly digested, transferred to the liver, and used for energy rather than stored as fat. In comparison, LCFAs require
re-esterification in the small intestine, transport by chylomicrons via the lymphatic and vascular systems, and oxidation in the liver for energy or storage. The MCTs are metabolized as rapidly as glucose but have roughly twice the energy density (Bell et al., 1997). In the early 1980s, Dr. Vigen K. Babayan of the Nutrition Laboratory at Harvard University developed a method to produce MCTs in large quantities (Bach and Babayan, 1982). These commercialized MCTs are acquired through lipid fractionation from natural fats such as coconut oil and milk and are predominantly composed of C:8 and C:10 MCTs (Babayan, 1987; Hashim and Tantibhedyangkul, 1987).

Since the 1970s, MCTs have been used in a modification of the classical KD as an alternative fat source (Huttenlocher et al., 1971). The ketogenic properties of MCTs allow patients to eat less total fat in their diet and include more carbohydrate and protein without sacrificing their nutritional ketosis. Although MCTs have the potential to be an efficient and beneficial ketogenic fat, they are currently limited in clinical usage due to gastrointestinal (GI) side effects stimulated by the large dose needed to induce ketonemia (typically greater than 40 g/day) (Bell et al., 1997). The original modified KD with MCT allowed 60% of its energy to be derived from MCTs; however, this caused GI distress in some children (Huttenlocher, 1976; Mak et al., 1999; Sills et al., 1986; Trauner, 1985). For this reason, an additional modified KD with MCT was developed using only 30% of its energy from MCTs; however, it induced much lower levels of ketosis (Elizabeth et al., 2009; Ruby et al., 1989). In a recent study, juvenile Sprague-Dawley rats were given a daily intragastric bolus of MCT oil (10 g/kg) which rapidly elevated blood βHB levels (4mM βHB, <30 min) and remained significantly elevated for up to 12 hours (Kesl et al., 2016). In addition to their metabolic effects, emerging data suggests that octanoic acid (C8) has specific antiinflammatory and neuroprotective properties independent of ketones (Chang et al., 2015). Research and development is currently being conducted to formulate MCT oil into a powder with soluble fiber compounds, which may delay gastric emptying and enhance absorption to improve tolerability and ketogenic potential.

1,3-Butanediol
1,3-Butanediol (BD; also known as 1,3-butylene glycol) is an FDA-approved organic di-alcohol (diol), naturally present in some species of pepper (Capsicum annuum), and is used as a food flavoring solvent, an intermediate in the manufacture of certain polyester plasticizers, and a humectant for cosmetics, and has been considered as a synthetic food for long-duration space missions (Budavari, 1989; Dymzsa, 1975; Falbe et al., 1985). When ingested orally, BD is metabolized by the liver via alcohol dehydrogenase (ADH) to β-hydroxybutyraldehyde, which is rapidly oxidized to βHB by aldehyde dehydrogenase (Tate et al., 1971). Further, BD contributes approximately 6 kcal/g of energy and can produce dose-dependent millimolar concentrations of ketones in the blood in a ratio of 6:1 of βHB to AcAc (D'Agostino et al., 2013; Desrochers et al., 1992; Drackley et al., 1990; Tobin et al., 1972). Extensive toxicology studies have concluded that BD is safe with very few adverse health effects in humans or animals (Dymzsa, 1975; Hess et al., 1981; Opitz, 1958; Scala and Paynter, 1967).

In a 28-day study, a daily 5-g/kg dose of BD administered via intragastric gavage in Sprague-Dawley rats showed a significant elevation of blood ketones (1mM βHB, <30 min) without an effect on blood glucose, triglyceride, or lipoprotein levels (Kesl et al., 2016). Recently, BD has been investigated as a backbone of ketone mono- and diesters, which are discussed later in this chapter.

**Ketone Salts**
Originally, researchers attempted to administer βHB or AcAc in their free acid forms; however, this was shown to be too expensive and ineffective at producing sustained ketosis. Subsequently, it was suggested to buffer the free acid of βHB with sodium, but this causes potentially harmful sodium overload and mineral imbalance at therapeutic levels of ketosis, and it is has been shown that βHB alone is largely ineffective at preventing seizures in animal models (Bough and Rho, 2007). A study showed that oral administration of Na’/βHB in doses from 80 to 900 mg/kg/day elevated blood ketone levels to 0.19–0.36 mM, which was therapeutic in children with acyl CoA dehydrogenase deficiency (Hove et al, 2003). However, for a 70-kg man to achieve these levels of ketosis would require ingesting between 5.6 and 6.3 g/day. Considering the potential effects of such a large sodium load, the costs of the administration of Na’/βHB salts to achieve ketosis made this approach unrealistic (Veech, 2004).

More recently, ketone salts with a balanced electrolyte formulation to prevent sodium overload have been developed and tested and can include potassium, calcium, magnesium, lithium, arginine, lysine, histidine, ornithine, creatine, agmatine, or citrulline. Maintaining a balanced
electrolyte ratio should help offset any potential adverse effects of sodium on blood pressure. It is speculated that this formulation will be especially beneficial for elderly patients most susceptible to sodium-induced hypertension (Veech, 2004). Furthermore, when first attempting to follow a KD, many people experience headaches and lethargy during the initial stages of “keto-adaptation,” a term that describes the adaptive changes in metabolic physiology associated with transitioning more toward fat and ketone metabolism. The symptoms are largely a result of reduced glucose availability to the brain and a transient depletion of minerals, especially sodium, potassium, and magnesium in the plasma (Zhang et al., 2013b). These symptoms can be attenuated or reversed with sufficient supplementation of sodium, potassium, calcium, and magnesium, and thus a ketone supplement that delivers ketones with these electrolytes would be favorable.

Several mineral ketone salt combinations are currently being tested for safety and efficacy. In a recent study, neither a 5-g/kg nor a 10-g/kg intragastric gavage of Na+/K+ βHB salts significantly elevated blood ketone levels or reduced blood glucose levels in juvenile Sprague-Dawley rats (Kesl et al., 2016). However, there appears to be interspecies variability in absorption, as in a recent case study a 100-kg male subject was administered a 4% Na+/K+ βHB salt solution (containing 11 grams of sodium and 7.1 grams βHB) and his plasma levels of βHB were significantly elevated at 30–60 minutes after administration. After receiving the same dose for 3 days, the patient had sustained elevated blood ketone levels from 15 to 120 minutes after administration. Similar results were seen in a 70-kg male who fasted 3 days prior to administration of the ketone salt supplement (D’Agostino et al., 2014). In a 15-week study, Sprague-Dawley rats were administered Na+/Ca++ ketone salt 20% by weight (~25 g/kg/day) in their food ad libitum. The Na+/Ca++ βHB-supplemented rats exhibited sustained elevated blood ketone levels (1 mM βHB) at 1, 4, 8, 10, and 13 weeks of chronic feeding, which did not affect blood glucose levels compared with controls (Poff et al., 2016).

**Combination βHB Mineral Salt and Medium Chain Triglyceride**

Considering the variety of ketogenic precursors available, researchers are investigating unique combinations of the individual supplements in hopes of optimizing their benefits. A combination of βHB mineral salts (BMS) and MCT oil has been administered in ratios of 1:1 to 1:2 mixtures. Formulating in this way allows for rapid and sustained elevation of ketosis by delivering exogenous ketones while simultaneously stimulating endogenous ketogenesis with MCTs. In addition, the combination formulation allows for a lower dosing of the components as compared to administering the individual compounds, thus reducing potential for side effects (gastric hyperosmolality) and resulting in a distinct blood ketone profile that is sustained over a longer period of time (D’Agostino et al., 2014).

In a 28-day study, the combination of a 50% Na+/K+ βHB salt mixed in a 1:1 solution with MCT oil (BMS+MCT) significantly elevated and sustained blood ketone levels and reduced blood glucose levels in a dose-dependent manner (Kesl et al., 2016). Additionally, the study demonstrated a significant correlation between elevated blood ketone levels and reduced blood glucose levels post intragastric gavage administration with no effect on blood triglyceride or lipoprotein levels. A noteworthy observation from the study revealed that in rats, MCT caused a rise in ketones (>3mM) that exceeds what is possible in humans due to gastrointestinal intolerance. This is likely due to the fact that rats have a higher rates of absorption for MCT and higher rates of hepatic fat metabolism, which stimulates greater ketone production. Considering these results it is important to take into account interspecies variability in the metabolic response to ketone supplements, which will need to be further characterized to fully understand how we could extrapolate findings and translate into human dosing equivalents. In the rats, the BMS+MCT supplement elevated blood ketones similar to that of MCT alone; however, the gastric side effects were not observed, suggesting a potential method for avoiding this unwanted adverse effect (Kesl et al., 2016). In a case study, a 100-kg male was administered a combination of a 4% Na+/K+ βHB salt solution (containing 11 grams of sodium and 7.1 grams βHB) + 20 mL MCT oil. This combination demonstrated higher elevation of blood ketone levels than either βHB salts or MCT oil alone, starting at 15 minutes post consumption and lasting for 4 hours. Similar results were observed in a 70-kg male who fasted 3 days prior to administration; however elevated blood ketone levels were observed sooner after supplementation and were sustained for a considerably longer time after administration (8 hours) (D’Agostino et al., 2015). This effect was accompanied by a reduction in blood glucose, and a lowering starting blood glucose concentration on each subsequent day of supplementation (D’Agostino et al., 2014). In a 15-week
study, Sprague-Dawley rats were administered a 1:1 mixture of Na’/Ca’ ketone salt + MCT oil (20% by weight, ~25 g/kg/day) in their food fed ad libitum. The combination-supplemented rats had significantly sustained and elevated blood ketone levels at weeks 3, 4, 8, 10, and 13 without significantly affecting blood glucose levels during the study (Kesl et al., 2014).

Ketone Esters

Researchers have developed and investigated several synthetic ketone mono- and di-esters to induce a nutritional ketosis independent of dietary calorie or carbohydrate restriction. When ketone esters are administered, gastric esterases liberate ketones (βHB and AcAc) as a free acid from a backbone molecule, which varies depending on the specific formulation, but is favorably a ketogenic precursor such as BD. As previously discussed, BD is subsequently metabolized by the liver to produce βHB (D’Agostino et al., 2013). Thus, the ketone esters currently available are unique among the aforementioned ketone supplements in that they can directly elevate ketones and supply ketogenic precursors that can further sustain ketogenesis. Additionally, synthetically derived ketone esters are currently the most potent form of exogenous ketones available, but their potency also necessitates a thorough investigation of their long-term safety and toxicity.

In the late 1970s, Birkhahn et al. were the first to synthesize a monoester of glycerol and AcAc (monoacetoacetin) for parenteral nutrition. These studies demonstrated that monoacetoacetin induced hyperketonemia comparable to fasted rats at a dose of 50 g/kg per day (Birkhahn and Border, 1978; Birkhahn et al., 1977; Birkhahn et al., 1979). In attempts to increase the caloric density of monoacetoacetin, they synthesized both a monoester and triester of glycerol and βHB. These esters are hydrolyzed to release free βHB in a way that elevates and sustains blood ketones. Later, Desrochers and colleagues synthesized mono- and diesters of AcAc with BD that elevated both AcAc and βHB (Desrochers et al., 1995b). These and other ketone esters developed by or in collaboration with Henri Brunengraber and Richard Veech have demonstrated an ability to induce a dose-dependent hyperketonemia (1–7 mM) in rats, mice, dogs, pigs, and humans (Brunengraber, 1997; Ciriaolo et al., 1995; Desrochers et al., 1995a; Puchowicz et al., 2000; Srivastava et al., 2012; Sylvain et al., 1995). Clarke and colleagues demonstrated the safety of a ketone ester in rats and humans that has also been documented in a recent case study of Alzheimer’s disease (AD; Clarke et al., 2012a; Clarke et al., 2012b; Newport et al., 2015). Recently, the ketone ester R,S,1,3-butanediol acetoacetate diester (BD-AcAc2), given as an intragastric gavage, elevated both AcAc and βHB blood levels to >3mM in rats (D’Agostino et al., 2013). The induction of therapeutic ketosis was rapid (within 30 minutes) and sustained at high levels for over 4 hours. In a 28-day study, a daily intragastric gavage (5 g/kg body weight) of BD-AcAc2 induced significantly elevated blood ketone levels and significantly reduced blood glucose levels without significantly altering blood triglyceride or lipoprotein levels (Kesl et al., 2016). In a 15-week chronic feeding study, the BD-AcAc2 was administered to Sprague-Dawley rats in a low dose (5% food weight, 10 g/kg/day) (LKE) and a high dose (20% food weight, 25 g/kg/day) (HKE) ad libitum. Both doses significantly elevated blood ketone levels without reducing blood glucose levels. Serum clinical chemistry of both LKE and HKE did not reveal any alterations in markers of kidney and liver function compared with rats fed standard chow (Poff et al., 2016).

Potential Therapeutic Mechanisms of Ketone Supplementation

Induction of hyperketonemia produces acute and chronic changes in metabolic physiology and molecular signaling pathways that provide therapeutic effects in varied disease states. Metabolic-based mechanisms of ketone therapies include an elevation of blood ketones and associated anaplerosis with simultaneous suppression of blood glucose, enhancement of insulin sensitivity, mitochondrial efficiency, suppression of specific inflammatory mediators and inhibition of oxidative stress and preservation of mitochondrial health and function.

Suppression of Blood Glucose and Enhancement of Insulin Sensitivity

Hyperglycemia and hyperinsulinemia are pathologically linked to numerous disorders, including cancer, cardiovascular disease, obesity, type 2 diabetes, impaired wound healing, and neurodegenerative diseases, among others (Laakso and Kuusisto, 2014; Ryu et al., 2014). These states are associated with chronic systemic inflammation, oxidative stress, impairment of the immune system, and vascular and metabolic dysfunction (Bornfeldt and Tabas, 2011; de Carvalho Vidigal et al., 2012; Turina et al., 2005). Exogenous ketone supplements may provide therapeutic benefits
in diseases characterized by hyperglycemia or hyperinsulinemia as reports have demonstrated that ketone administration lowers blood glucose by increasing insulin sensitivity. To demonstrate this, male rats were fed a standard diet with 30% of calories replaced with the R-3-hydroxybutyrate-R-1,3-butanediol monoester for 14 days. The ketone ester-supplemented diet induced nutritional ketosis (3.5 mM BHβHB), and both plasma glucose and insulin were decreased by approximately 50% (Srivastava et al., 2012). Glucose was decreased from 5 mM to 2.8 mM, and insulin was decreased from 0.54 ng/mL to 0.26 ng/mL. In a similar study by the same group, mice receiving a KE diet exhibited a 73% increase in the Quantitative Insulin-Sensitivity Check Index (QUICKI), a surrogate marker of insulin sensitivity, compared with control, calorie-matched mice (Srivastava et al., 2012). Fasting plasma glucose levels were not altered in these mice, but fasting plasma insulin levels were reduced by approximately 85% in the KE-fed mice compared with controls, demonstrating that exogenous ketones enhance insulin sensitivity.

In a study examining the potential use of ketogenic supplements as a cancer therapy, mice consuming a standard high carbohydrate diet mixed with the R,S-1,3-butanediol diacetoacetate ester (BD-AcAc2) at 20% by weight had approximately 30% lower blood glucose than control animals (Poff et al., 2014). The administration of BD did not significantly elevate blood ketones in this study, nor did it decrease blood glucose. This result is likely due in part to the comparatively small dose of BD being consumed at any point in time according to the route of administration, as BD in higher doses is known to elevate ketones. Insulin levels were not investigated in this study. In a study designed to assess the dose-dependent effects of exogenous ketone supplements on blood glucose, ketones, and lipids, healthy male rats were administered one of five ketogenic agents daily via intragastric gavage (Kesl et al., 2016). The ketogenic agents investigated included: BD, BD-AcAc2, a sodium/potassium β-hydroxybutyrate mineral salt (BMS), medium chain triglyceride oil (MCT oil), BMS+MCT 1:1 mixture (BMS+MCT), and the R,S-1,3 butanediol diacetoacetate ester (BD-AcAc2). BD-AcAc2, BMS, MCT oil, and BMS+MCT-treated rats demonstrated a decrease in blood glucose following ketone supplement administration given as an acute bolus. Similarly to the previously mentioned mouse study, BD did not lower blood glucose in these animals, although it is known to be a hypoglycemic agent. The duration of the reduction in blood glucose varied between supplements and lasted anywhere from 30 minutes to 12 hours, suggesting that ketone supplementation could potentially provide a novel method of glycemic control.

Enhanced Metabolic Efficiency
The superior metabolic efficiency of ketone bodies has been known since the 1940s, when Henry Lardy compared the energetic efficiency of 16 major carbohydrate, lipid, and intermediary metabolites (Lardy and Phillips, 1945). He demonstrated that βHB and AcAc were unique among the panel of metabolites tested in their ability to increase bull sperm mobility while simultaneously decreasing oxygen consumption. Nearly 50 years later, Richard Veech and colleagues confirmed Lardy’s observation and elucidated the molecular mechanisms underlying the phenomenon in the working perfused rat heart (Kashiwaya et al., 1994). They demonstrated that supplementation of 5 mM ketones (4 mM BHβHB, 1 mM AcAc) to glucose-containing perfusate (10 mM glucose) increased cardiac hydraulic work by approximately 25% while simultaneously reducing oxygen consumption (Kashiwaya et al., 1994). Their study revealed that this effect was mediated by a reduction of the mitochondrial NAD couple and an oxidation of the coenzyme Q couple, which increases the energy of the redox span between these sites. This results in an increase in energy released by electrons in the ETC, causing more protons to be pumped into the inner mitochondrial space, thus enhancing the electrochemical gradient established there and increasing the energy of ATP hydrolysis. Indeed, thermodynamic tables for heat of combustion, calculated with bomb calorimeter experiments, show that βHB produces more energy than glucose per 2-carbon moiety (Cahill and Veech, 2003). These results are supported by human studies that demonstrated a reduction in blood flow and oxygen consumption in the brains of fasted obese subjects in ketosis (McHenry, 1966). Thus, ketones appear to be a superior fuel for ATP production per unit oxygen compared to glucose, and can be considered among the most metabolically efficient energy metabolites (Figure 32.1).

Anti-Inflammatory Effects
The physiological state of ketosis has known anti-inflammatory properties, as several studies have demonstrated that the KD reduces circulating inflammatory markers in animals and in humans (Cassandra et al., 2007; Sharman and Volek, 2004; Torres-Gonzalez et al., 2008). Recent evidence suggests that this effect is, at least in
part, directly mediated by the ketone bodies, indicating that exogenous ketone supplementation could be used to suppress inflammation. Dixit and colleagues reported that βHB inhibits the NLRP3 inflammasome, an important component of the innate immune system that controls activation of caspase-1 and production of the pro-inflammatory cytokines IL-1β and IL-18 by macrophages (Youm et al., 2015). This effect was mediated specifically by βHB, as neither AcAc nor the structurally related fatty acids butyrate and acetate elicited this response. The βHB-induced inhibition of the NLRP3 inflammasome occurs without its being oxidized in the TCA cycle, and is thus independent of its function as an energy metabolite. Furthermore, the anti-inflammatory changes associated with βHB were not dependent on alterations in AMPK, reactive oxygen species (ROS), glycolytic inhibition, UCP, or SIRT2 signaling, further validating its function as a signaling metabolite. To investigate the in vivo translatability of this finding, researchers administered the BD-AcAc<sub>2</sub> to a familial cold anti-inflammatory syndrome (FCAS) mouse model with an induced missense mutation in NLRP3. The ketone ester protected the mice from neutrophilia and hyperglycemia, and did not affect infiltration of peritoneal macrophages or overall frequency of splenic T cells, macrophages, or neutrophils, leading the authors to conclude that elevating blood ketones could be a therapeutic option for patients with NLRP3-mediated chronic inflammatory diseases. Indeed, other research has shown that inhibition of the NLRP3 inflammasome mitigates the severity of numerous inflammatory diseases, including atherosclerosis, type 2 diabetes, AD, and gout.
In recent studies rats were fed one of three exogenous ketone supplements (BD-AcAc, BMS, or a 1:1 mixture of BMS:MCT mixed into standard rodent chow at 18% by weight for 15 weeks. Inflammatory profiling was performed on serum collected from the animals at the end of the chronic feeding study and revealed decreases in several pro-inflammatory cytokines including IL-1β, IL-6, IFN-γ, MCP-1, and RANTES (Poff et al., 2016).

**Inhibition of Oxidative Stress**

The ketogenic diet has been reported to reduce oxidative stress in vivo in a number of preclinical and clinical reports (Jarrett et al., 2008). Studies suggest that this effect is mediated by the ketone bodies themselves, and therefore the effect would likely be recapitulated with exogenous ketone supplementation. Some important molecular mechanisms underlying the effects of ketone metabolism on oxidative stress were delineated by studies investigating the bioenergetic efficiency and mitochondrial respiration in the working perfused rat heart following administering of a glucose-containing perfusate supplemented with exogenous 5 mM βHB (Kashiwaya et al., 1994). As described previously, ketone metabolism increases the oxidation of ubiquinol (Q) in the electron transport chain, reducing semiquinone radical (Q−), an intermediate in the reduction of ubiquinone that is sensitive to oxidation by molecular oxygen to produce superoxide anion (O2−). O2− is an important precursor for the generation of many ROS; therefore, ketone metabolism suppresses mitochondrial ROS production (Kashiwaya et al., 1994). Simultaneously, ketone metabolism suppresses oxidative stress by enhancing endogenous antioxidant capacity. Ketone metabolism induces reduction of the mitochondrial NAD and cytoplasmic NADP couples. And NADH and NADPH are necessary for the regeneration of reduced glutathione (GSH), which is required for the neutralization of ROS by glutathione peroxidase, an important endogenous antioxidant enzyme. These molecular effects are observed in vivo, as the KD has been shown to increase the ratio of reduced to oxidized mitochondrial glutathione (GSH:GSSG) in rat brains (Jarrett et al., 2008). These effects appear to be ubiquitous in various tissues; however, the brain has been the most well characterized in this regard. For example, in vitro treatment with βHB and AcAc decreases neuronal ROS production following glutamate exposure (Maalouf et al., 2007) and inhibits apoptosis in cortical slices exposed to hydrogen peroxide (H2O2) (Kim do et al., 2007).

Eric Verdin and colleagues recently demonstrated that βHB functions as an endogenous histone deacetylase inhibitor (HDACI) in vitro and in vivo at physiologic concentrations easily achievable with exogenous ketone supplementation (Shimazu et al., 2013). Fasting, calorie restriction, and exogenous βHB administration all increased global histone acetylation in mice and induced the transcriptional activation of the oxidative stress resistance factors FOXO3A and MT2. This effect was found to be mediated directly by inhibition of class 1 and 2 HDACs. Furthermore, the authors demonstrated that exogenous ketone supplementation could prevent oxidative stress. Mice were administered βHB via a subcutaneous pump for 24 hours prior to receiving an injection of paraquat, which induces the production and accumulation of ROS. Protein carbonylation was suppressed by 54%, and lipid peroxidation was completely suppressed, in the kidneys of βHB pretreated mice. Immunoblotting of kidney tissue from these animals revealed a significant increase in the mitochondrial superoxide dismutase (MnSOD) and catalase (CAT) endogenous antioxidant systems. This study strongly supports the feasibility and applicability of exogenous ketone supplements for the prevention of oxidative stress.

**Preservation of Mitochondrial Health and Function**

Ketone metabolism is generally recognized to support or enhance mitochondrial health (Veech, 2004). As previously described, ketones suppress mitochondrial ROS production and enhance endogenous antioxidant systems. The resultant reduction in oxidative stress protects the mitochondrial DNA and membranes from damage that would impair respiratory and mitochondrial function. Interestingly, exogenous ketone supplementation with a ketone ester has been shown to induce mitochondrial biogenesis (Srivastava et al., 2012). Mice in this study were fed a diet from which approximately 30% of calories were derived from the ketone ester D-β-hydroxybutyrate-R-1,3-butanediol monoester for one month. The mitochondrial content and expression of electron transport chain proteins were significantly increased in the intrascapular brown adipose tissue as compared with control mice, although calorie intake was matched between the two groups.
Conditions Where Ketone Supplementation Is Likely Beneficial

Because of the previously described therapeutic mechanisms of ketone metabolism, exogenous ketone supplementation is being investigated as a potential treatment for a number of disorders. Here we discuss some conditions for which ketone supplements have been most well demonstrated to be useful, including epilepsy and seizure disorders, glucose transporter type 1 deficiency syndrome, AD, cancer, insulin resistance and type 2 diabetes mellitus, and weight loss.

Epilepsy/Seizure Disorders

The KD is a proven, effective therapy for epilepsy in children and adults (Klein et al., 2014; Li et al., 2013). In many cases, it is more effective than anti-epileptic drugs (AEDs) and is therefore routinely used as a front-line treatment for children with retractable (drug-resistant) epilepsy (Levy et al., 2011). Although the mechanisms of KD therapy are largely unknown, achieving and sustaining therapeutic ketonemia (>1 mM blood ketones) or ketonuria (>40 mg/dL) is generally necessary for antiseizure efficacy. Despite its success, the dietary restrictions of the KD can be unpalatable for some patients and difficult for caregivers, a contributing factor for cessation of treatment (Klein et al., 2014; Levy et al., 2011). Exogenous ketogenic supplementation mimics the metabolic and physiologic effects of the KD, including enhancing mitochondrial biogenesis, anaplerosis, suppression of glycolysis, and increasing ATP and adenosine production, all thought to mediate the therapeutic effects of KD in epilepsy (Kesl et al., 2014; Kesl et al., 2016; Kovac et al., 2013; Masino and Geiger, 2009; Srivastava et al., 2012; Stafstrom et al., 2009; Stafstrom et al., 2008).

One of the earliest reports of the antiseizure efficacy of exogenous ketogenic supplementation was performed in a unique seizure model that uses hyperbaric hyperoxia (HBO) to reliably induce epileptic-like (tonic-clonic) seizures in wild-type rats, a condition known as central nervous system oxygen toxicity (CNS-OT). A single oral dose of the ketone ester BD-AcAc2 induced rapid (within 30 minutes) and sustained (>4 hours) ketosis (>3mM βHB and >3mM AcAc) and prolonged the latency to seizure by 574% (D’Agostino et al., 2013). An elevation in AcAc and acetone appear to be required for the anticonvulsant effects of keto-sis. BD elevated blood βHB (>5mM) but did not elevate AcAc or acetone nor did it affect latency to seizure. This encouraging response prompted preliminary investigation into preventing or delaying seizures with ketogenic supplements in a variety of transgenic rodent and chemical-induced seizure models. Pentylenetetrazole (PTZ) is a GABA antagonist and epileptogenic agent that is used to induce seizures in rodents for preclinical analysis of anticonvulsant therapies. In a recent study by Coppola and colleagues, the dosage threshold for seizure induction of PTZ was assessed in control (water) and ketone ester-treated rats (Viggiano et al., 2015). A single oral dose (4 g/kg body weight) of BD-AcAc2 elevated blood βHB (2.7 mM) and increased the threshold of PTZ seizure from 122±6 mg/kg to 140±11 mg/kg. Unpublished data from preliminary studies also suggest an anticonvulsant effect of ketone ester treatment in the WAG/Rij rat model of absent epilepsy, the Ube3a m-/p+ mouse model of Angelman syndrome, and the kainic acid–induced mouse seizure model.

Glucose Transporter Type 1 Deficiency Syndrome

Glucose transporter type 1 deficiency syndrome (GLUT1 DS) is a rare genetic disorder caused by a mutation in the SLC2A1 gene, which encodes the glucose transporter protein type 1 (GLUT1). This mutation results in a glucose deficiency in the brain, which causes seizures as well as cognitive and physical developmental delay. In a seminal study in 1967, Cahill and colleagues discovered that ketones replace glucose as the predominant energy substrate for the brain during prolonged fasting and starvation (Cahill, 2006). GLUT1 DS is treated with the KD, which circumvents the metabolic blockade by inducing ketosis and is effective at suppressing seizures and enhancing cognitive and motor development in most patients. Maintaining therapeutic levels of ketosis is critical to support the development of children with this disorder. Thus, it is clear how an exogenous ketone supplement could be useful in this patient population. Triheptanoin is a triglyceride containing three heptanoates, a 7-carbon fatty acid whose metabolism produces the 5-carbon ketone bodies β-ketopentanoate and β-hydroxybutyrate. Because it possesses odd carbon FAs, heptanoate is metabolized through β-oxidation to produce propionyl-CoA, which can be subsequently carboxylated to succinyl-CoA, replenishing the TCA cycle via anaplerosis (Borges and Sonnewald, 2012). Triheptanoin has shown therapeutic efficacy in children and adults with GLUT1 DS, reducing seizure activity and improving neuropsychological performance and cerebral metabolic rate (Pascual...
et al., 2014). There are ongoing studies investigating the therapeutic effects of ketone salts and esters in a GLUT1 DS mouse model and may provide an alternative or adjunctive treatment to the KD.

**Alzheimer’s Disease**

In the early stages of AD, the brain exhibits a deficiency in glucose metabolism, which contributes to the neurodegeneration and progression of the disorder (Chu and Jiao, 2015; de la Monte, 2012). Patients with preclinical and clinical AD have decreased cerebral glucose metabolism as visualized by fluorodeoxyglucose positron emission tomography (FDG-PET) (Mosconi et al., 2011). It is thought that this decrease in glucose metabolism is associated with brain insulin resistance (Talbot et al., 2012). As ketones are the principal alternative fuel for the brain during fasting or starvation, elevating blood ketone levels in AD patients would theoretically bypass the deficiencies in glucose metabolism and provide energy to the starving neurons. Data to support this hypothesis was recently reported by Cunnane and colleagues, who demonstrated that while brain glucose uptake is impaired in AD, ketone uptake remains unaffected. Indeed, the authors conclude that supplying ketones to the brains of AD patients could restore the brain fuel supply to serve as a potential therapeutic (Cunnane et al., 2016). Thus, exogenous ketone supplementation could be a useful tool for supporting cerebral energy metabolism in this regard.

There is preclinical data to suggest that ketones could protect against AD development or slow its progression. The ability of a βHB- and BD-containing ketone ester to suppress AD progression was evaluated in a triple transgenic AD mouse model (3xTgAD) (Kashiwaya et al., 2013). Presymptomatic mice were fed a diet with approximately 20% kcal from the ketone ester and compared with isocaloric standard diet-fed control animals. Ketone ester-treated animals exhibited less anxiety and improved performance on learning and memory tests at 4 and 7 months after initiation of the diet. Immunohistochemical analysis of the brain revealed that the ketone ester-fed mice had less Aβ and hyperphosphorylated tau deposition in the cortex, hippocampus, and amygdala. In a similar study, 3-hydroxybutyrate methyl ester (HBME), a derivative of βHB, was assessed for its therapeutic efficacy in a double transgenic mouse model of AD (Zhang et al., 2013a). The HBME-treated mice (40 mg/kg/d via intragastric gavage) exhibited improved spatial learning and working memory and decreased anxiety compared with control mice. Also, MRI analysis revealed that HBME prevented the development of asymmetrical ventricle morphology, which was observed in the untreated AD mice. Following 2.5 months of treatment, the brains of the animals were analyzed via immunohistochemistry. The authors also performed in vitro studies to further investigate mechanism of protection. The HBME-treated mice exhibited reduced Aβ plaque deposition in the cortex and hippocampus. The authors reported that HBME supported neuronal survival following glucose deprivation and prevented NaN3-induced mitochondrial dysfunction by rescuing expression of the respiratory complexes, reducing ROS production, and maintaining mitochondrial membrane potential.

In an encouraging case report by Newport and Vech, supplementation with medium chain fatty acids (MCFA) and (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester (KME) was investigated as a potential therapy for AD (Newport et al., 2015). The patient described was an APOE4-positive, 63-year-old Caucasian male with early-onset, sporadic AD diagnosed 12 years prior to the publication of the report. His disease rapidly progressed prior to initiation of ketosis therapy in 2008, characterized by increasingly severe memory loss and an inability to carry out normal activities of daily living. His Mini-Mental State Examination (MMSE) score declined from 23 to 12 between 2004 and 2008, and his MRI scans revealed diffuse involutional changes of his frontal and parietal lobes with atrophy of the amygdala and hippocampus. The patient initially began ketosis treatment in May 2008 by consuming sources of MCFA—MCT oil and coconut oil (CO). His dose and regimen of administration was optimized, and eventually reached 165 mL of a 4:3 mixture of MCT:CO divided into 3–4 servings over the course of the day. Within 75 days of treatment, the patient’s MMSE score improved from 12 to 20. The patient continued MCFA treatment for 20 months, and during that time he exhibited remarkable cognitive and physical improvements. His Alzheimer’s Disease Assessment Scale-Cognitive (ADAS-Cog) rose 6 points and his activities of daily living (ADLs) score rose 14 points over that time period. Further, MRI scans revealed no changes in brain atrophy from June 2008 to April 2010, suggesting stabilization of the disease process. In 2010, the patient also began taking the KME (28.7 g KME, three times daily). The patient exhibited many improvements following KME treatment. Within a few days of initiating KME therapy, the patient regained the ability to
recite and write out the alphabet, and dress himself. He began improving in a number of ADLs such as showering, shaving, and putting away dishes, and demonstrated improvements in abstract thinking, insight, and sense of humor. The patient himself reported feeling happier and more energetic after beginning treatment with KME. Over time, he exhibited significant improvements in memory retrieval and regained the ability to perform complex tasks such as vacuuming and yard work. Both MCFA and KME treatment significantly elevated blood ketones, even while the patient continued to consume a normal diet.

The KME was particularly effective at inducing ketosis, elevating blood ketones up to 7 mM within 1 hour of administration (Figure 32.2), a level approximately 5–10 times greater than is possible with the classical ketogenic diet or MCFA consumption. The patient’s caregiver, a physician, noted that his improved cognitive and physical performance seemed to track his plasma ketone concentrations, with the greatest improvements seen during peak elevation in blood ketones. Importantly, there were no adverse effects observed in the patient over this 2-year study, suggesting that prolonged hyperketonemia is likely safe. The authors note that not all patients may respond to such therapy in a similar manner, but that appropriately designed trials should be conducted to evaluate the percentage of Alzheimer’s patients responsive to ketone ester therapy.

An oral ketogenic compound and prescription medical food called AC-1202 (trade name Axona) was developed by Accera, Inc., as an AD therapy. AC-1202 elevates blood ketone levels, as it contains MCFAs, which are natural ketogenic precursors. AC-1202 was evaluated in a randomized, double-blind, placebo-controlled, multicenter trial in patients with mild to moderate AD (Henderson et al., 2009). AC-1202 induced a mild level of ketosis in patients (up to 0.3–0.4 mM), which was significantly higher than placebo controls. AC-1202 treatment induced small improvements in ADAS-Cog, MMSE, and ADCS-CGIC (AD Cooperative Study—Clinical Global Impression of Change) scores compared with placebo in some subgroups of AD patients tested. In many of the patient subgroups, AC-1202 did not affect

![Figure 32.2](image_url)

**FIGURE 32.2** β-Hydroxybutyrate (βHB) concentrations rose to 3 to 7 mM 1 hour after ingestion of ketone monoester (KME) in three different doses, 25, 35, and 50 g, taken on separate days. The peak levels measured are in the range of those obtained during adherence to the classical ketogenic diet and are about 10-fold the concentrations achievable by MCFA administration. The findings suggest that therapeutic ketosis can be maintained throughout the day if KME is taken every 3 to 4 hours. Precision Xtra Glucose and Ketone Monitoring System (Abbott Diabetes Care, Inc., Alameda, CA, USA) was used to measure βHB levels in capillary blood samples. Acetoacetate (AcAc) was not measured. This figure was reprinted with permission from Newport et al. (2015).
performance of APOE4 negative patients on these tests. The modest improvements observed in this study may potentially be due to the comparatively low level of ketosis induced by AC-1202, considering what is attainable with KME or similar ketogenic supplements. Furthermore, the lack of effect observed in APOE4 positive patients could also be a result of the mild level of ketosis induced, or alternatively because the study may have lacked the statistical power necessary to reveal a significant effect in this subpopulation (Newport et al., 2015). Indeed, the case report by Newport and colleagues suggests that ketosis can be an effective therapy for AD in some APOE4 positive patients.

Taken together, these reports clearly demonstrate the potential utility of exogenous ketone supplements to confer the therapeutic benefits of ketosis in a population of patients for which severe dietary restrictions would be extremely difficult, if not impossible.

Cancer
Unlike healthy tissues, many cancers do not appear capable of efficiently metabolizing ketone bodies for energy. Cancer cells often lack expression of the ketone utilization enzymes, like succinyl-coenzyme A:3-oxoacid coenzyme A transferase (SCOT) (Chang et al., 2013; Sawai et al., 2004; Skinner et al., 2009; Tisdale and Brennan, 1983). Abnormal mitochondrial function and impaired OXPHOS capacity is another ubiquitous feature of cancers which likely limits the use of ketones, which are metabolized exclusively within the mitochondria, as a fuel for cancer. Indeed, a study on five different brain cancer cell lines concluded that glioma cells lack the capacity to metabolize βHB during glucose restriction, unlike healthy brain cells (Fredericks, and Ramsey, 1978; Maurer et al., 2011).

The ketogenic diet, fasting, and calorie restriction are dietary regimens that have been shown to inhibit cancer progression in both preclinical and clinical studies (Fine et al., 2012; Freedland et al., 2008; Mavropoulos et al., 2009; Nebeling et al., 1995; Poff et al., 2013; Rossifanelli et al., 1991; Shelton et al., 2010; Wheatley et al., 2008; Zucconi et al., 2010). Until recently, the generally accepted mechanism of action of these therapies was decreasing glucose availability to the tumor and suppressing the insulin and IGF signaling pathways. However, all three of these therapies also elevate blood ketones, and recent evidence suggests that ketones themselves may possess inherent anticancer properties. One small clinical trial studied the effects of a 28-day KD in patients with late-stage, metastatic cancer (Fine et al., 2012). Prior to beginning the dietary intervention, all patients exhibited progressive disease. Following 1 month of treatment, over 50% of patients showed stable disease or partial remission. Interestingly, there was no significant drop in blood glucose in the patients over the course of the diet, but rather, patient response was most strongly correlated with degree of ketosis relative to baseline. In vitro and preclinical studies have confirmed the hypothesis that ketones are damaging to cancer. In 1979, Magee et al. demonstrated that βHB inhibited proliferation in a dose-dependent manner up to 20 mM in multiple cancer cell lines of varied origin (Magee et al., 1979). Similarly, both AcAc and βHB inhibited viability and induced apoptosis in neuroblastoma cells, but had no effect on control fibroblasts (Skinner et al., 2009). Another study demonstrated that 5 mM βHB slows proliferation and decreases viability in VM-M3 glioma cells, even in the presence of excess (25 mM) glucose. Exogenous ketone supplementation with BD or the BD-AcAc elicited potent anticancer effects in the VM-M3 model of metastatic cancer, slowing tumor growth and prolonging survival by 51% and 69%, respectively (Poff et al., 2014). These observations strongly suggest that exogenous ketone supplements could be used as an effective adjuvant therapy for cancer.

There are multiple mechanisms by which ketones may be damaging to cancer cells: (1) Cancer is particularly reliant on the glycolytic pathway for energy production and biosynthesis (Gillies et al., 2008), and βHB inhibits the first and third enzymatic reactions of glycolysis (Randle et al., 1964). (2) Excess fermentation in cancer cells causes lactate production and a subsequent acidification of the tumor microenvironment, which promotes malignancy. Both lactate and the ketone bodies are transported across the plasma membrane by the monocarboxylic transporters family of transporters (Halestrap and Price, 1999). And βHB has been shown to inhibit lactate export from isolated rat hepatocytes in vitro (Metcalfe et al., 1986). It is possible that ketones may damage cancer cells by inhibiting lactate export through competitive inhibition of monocarboxylic transporters, subsequently inducing intracellular acidification and preventing the tumor-promoting effects of lactate in the tumor microenvironment. (3) Inflammation and oxidative stress are both known to promote cancer development and progression (Coussens and Werb, 2002; Wang and Yi, 2008); thus, the inhibitory effects of ketone metabolism on both
of these pathways could contribute to its anticancer efficacy. (4) Cancers exhibit widespread differences in such epigenetic patterns compared to their normal tissue counterparts, allowing them to increase expression of oncogenes and inhibit the expression of tumor suppressor genes (Hassler and Egger, 2012). Histone deacetylase inhibitors (HDACI) are being investigated for their use as antineoplastic agents, and have been shown to elicit a plethora of anticancer effects in vitro including activation of apoptosis, induction of ROS generation and DNA damage, and inhibition of DNA repair (Bose et al., 2014). As mentioned, βHB functions as an endogenous HDACI, a mechanism that may underlie its potential use as a cancer treatment. (5) Finally, mitochondrial transfer studies have demonstrated that healthy mitochondria act as a tumor suppressor (Seyfried, 2012). The potential for ketone metabolism to support or enhance mitochondrial health could also account for its therapeutic effects and its potential for preventing carcinogenesis.

**Insulin Resistance/ Type 2 Diabetes Mellitus**

As previously described, ketone ester administration simultaneously decreases blood glucose and blood insulin concentrations (Srivastava et al., 2012). Thus, less insulin is required to promote peripheral glucose uptake, exemplifying an enhancement in insulin sensitivity. These results suggest a potential therapeutic use of exogenous ketone supplementation for insulin resistance and type 2 diabetes mellitus (T2DM). Indeed, exogenous ketones qualitatively mimic the acute metabolic effects of insulin (Kashiwaya et al., 1997). It is known that insulin activates pyruvate dehydrogenase (PDH) to increase the production of acetyl CoA. The administration of 5 mM ketones mimicked this effect, increasing acetyl CoA production 15-fold in the glucose-perfused isolated rat heart (Kashiwaya et al., 1994). Furthermore, in this model, ketones and insulin increased cardiac hydraulic efficiency to a similar degree, approximately 25%–35% (Kashiwaya et al., 1994). In another study by Srivastava et al., mice that were fed a diet formulated with ketone ester (30% kcal) had a 73% increase in the Quantitative Insulin-Sensitivity Check Index (QUICKI), a surrogate marker of insulin sensitivity, compared with controls (Srivastava et al., 2012). Fasting plasma glucose levels were not altered in these mice, but fasting plasma insulin levels were reduced by approximately 85% in the KE-fed mice compared with controls. The authors therefore hypothesized that ketones could be therapeutic by correcting metabolic defects of acute insulin deficiency or in the insulin-resistant state (Kashiwaya et al., 1997).

The HDACI activity of βHB could also be beneficial in T2DM by altering the direct regulation of HDAC-dependent glucose metabolism and by inducing resistance to oxidative stress. The HDACs regulate the expression of genes encoding many metabolic enzymes, and HDAC3 knockout animals exhibit reduced glucose and insulin. Suberoylanilide hydroxamic acid (SAHA), a class I HDAC inhibitor, has been shown to improve insulin sensitivity and increase oxidative metabolism and metabolic rate in a mouse model of diabetes (Gallozzi et al., 2013). Butyrate, a short-chain fatty acid that is structurally similar to βHB and also acts as a HDACI, lowers blood glucose and insulin levels and improves glucose tolerance and respiratory efficiency (Gao et al., 2009). The vascular dysfunction in T2DM is thought to be caused by oxidative stress (Giacco and Brownlee, 2010). And HDAC inhibition prevents renal damage in mouse models of diabetic nephropathy through modulation of redox mechanisms (Advani et al., 2011). Therefore, βHB suppression of oxidative stress through HDAC inhibition may help restore insulin sensitivity and manage complications of diabetes.

**Weight Loss**

The KD is hypothesized to induce weight loss by reducing appetite through the satiety effect of protein and ketone bodies, reducing de novo lipogenesis and increasing lipolysis, and enhancing metabolic efficiency with fat and ketone metabolism (Paoli, 2014). However, the KD can be difficult to maintain long-term for many individuals, and many people regain weight rapidly upon returning to a standard diet (Paoli, 2014). Recently, preliminary studies have shown that exogenous ketone supplementation can also induce weight loss. It should be noted that ketone supplements are sources of calories, with each ketone supplement providing on average 5–8 kcal/gram ingested; therefore, patients would need to decrease dietary caloric intake in order to prevent weight gain. The reported satiating effect of ketosis may aid in this adjustment.

The administration of both βHB and BD has been shown to decrease food intake in rats and pigmy goats (Arase et al., 1988; Carpenter and Grossman, 1983; Davis et al., 1981; Langhans et al., 1983; Rossi et al., 2000). Similarly, it is suggested that MCTs increase satiety, resulting in
CONCLUSION

Exogenous ketone supplements are being developed as an alternative or adjuvant method of inducing therapeutic ketosis aside from the classic ketogenic diet. Emerging evidence has demonstrated that these novel compounds have the potential to offer benefits for both healthy and diseased individuals alike. It is likely that most, if not all, of the conditions that are known to benefit from the KD would receive some benefit from exogenous ketone supplementation by elevating blood ketones and lowering blood glucose. Importantly, ketone supplementation provides a tool for achieving ketosis in patients who are unable, unwilling, or uninterested in consuming a low carbohydrate or ketogenic diet. It may also help circumvent some of the difficulties associated with KD therapy, as it allows for a rapid dose-dependent induction of ketosis, which can be sustained with prolonged consumption and monitored precisely with commercially available technologies (e.g., blood ketone meters). Simultaneously, it could provide patients with the opportunity to reap the benefits of ketosis without the practical and social difficulties of a highly restrictive diet. Further research is needed to fully investigate the clinical utility and feasibility of exogenous ketone supplements as a method of inducing therapeutic ketosis.

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IDENTIFYING THE MOLECULAR MECHANISM OF THE MEDIUM CHAIN TRIGLYCERIDE (KETOGENIC) DIET

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INTRODUCTION

The medium chain triglyceride (MCT) ketogenic diet was first introduced as a more palatable alternative to the classical ketogenic diet for the treatment of refractory epilepsy by Huttenlocher et al. (1971). It now provides a key therapeutic approach for the treatment of children with drug-resistant epilepsy (Levy et al., 2012; Liu, 2008; Neal and Cross, 2010; Neal et al., 2009). The diet involves a stringent reduction in carbohydrate intake, with an elevated consumption of medium chain fatty acids within a triglyceride backbone. Typically, the fats in this diet provide 65% to 75% of total daily energy requirement. These fats comprise two medium, straight chain fats: octanoic acid (comprising eight carbons) and decanoic acid (comprising ten carbons); 60%–80% of the MCT intake is octanoic acid and the remainder is mostly decanoic acid (Sills et al., 1986b). Both fats are rapidly hydrolyzed off the triglyceride backbone in the gut, and absorbed as free fatty acids (Bach and Babayan, 1982) and metabolized into ketones (β-hydroxybutyrate, acetone, and acetoacetate) and carbon dioxide, or catabolized into long chain fatty acids. This diet results in an elevation of the concentration of both fatty acids in peripheral blood: octanoic acid to 104–859 µM and averaging around 306 µM; and decanoic acid to 87–552 µM with an average of 157 µM (Dean et al., 1989; Haidukewych et al., 1982; Sills et al., 1986a). In animal models, decanoic acid has been found to penetrate the blood-brain barrier and to be present in brain at 60%–80% of serum levels (Wlaz et al., 2012).

The dietary restrictions and high fat intake of the MCT ketogenic diet can cause a variety of gastrointestinal-related side effects, such as cramps, bloating, diarrhea, and vomiting (Liu, 2008). Use of the diet has also been limited by poor tolerability, especially in adults, resulting in a high attrition rate (Levy et al., 2012). Due to these adverse effects, and a desire to increase the efficacy of the diet, many studies have sought to identify the therapeutic mechanism of the diet. As the diet was based on the classical ketogenic diet, it was considered to act through the generation of ketones (Bough and Rho, 2007; Rho and Stafstrom, 2011). However, the presence of these ketones poorly correlates with anticonvulsant efficacy, and this ketone-based mechanism has not been widely supported in animal model studies (Likhodii et al., 2000; Thavendiranathan et al., 2000). Furthermore, ketones do not directly alter hippocampal synaptic transmission (Thio et al., 2000) nor do they affect epileptiform activity induced by 4-aminopyridine in ex vivo seizure models (Thio et al., 2000). Thus the therapeutic mechanism of the MCT ketogenic diet has, until recently, been unclear.

MEDIUM CHAIN FATTY ACIDS IN SEIZURE CONTROL

A clinical role for medium chain fatty acids in direct seizure control was proposed over 30 years ago (Dean et al., 1989; Haidukewych et al., 1982; Sills et al., 1986a), but due to limited study sizes, a direct correlation between plasma concentrations and seizure control was not established. Recently, however, an unbiased screen for medium and short chain fatty acids related to valproate (Chang et al., 2012) in a simple cellular model identified a number of fatty acids as potential seizure-control treatments. In this study, a simple non-animal model, in which valproate has been shown to regulate phosphoinositide turnover (Xu et al., 2007), was used to identify potential anti-seizure compounds (Cunliffe et al., 2015). In this study, over 60 compounds were screened for an inhibitory effect on rapid phosphoinositide turnover. Interestingly, this inhibitory effect has since been confirmed as a therapeutic mechanism for valproate in in vitro
and in vivo animal seizure models (Chang et al., 2014). Thus, using this phosphoinositide screen, Chang et al. (2012) were able to investigate a wide range of fatty acids and related compounds, with some structures producing little effect, and some structures showing enhanced activity over valproate. These potent compounds included decanoic acid, nonanoic acid, and the branched 8-carbon backbone 4-methyloctanoic acid. However, these compounds did not act through regulating inositol levels in this simple model that was previously suggested as a mechanism of valproate (Shaltiel et al., 2007; Vaden et al., 2001; Williams et al., 2002; Williams, 2005). These compounds were further tested in a well-established ex vivo mammalian model for drug-resistant epilepsy, the low magnesium hippocampal-entorhinal cortex model (Chang et al., 2012). Here, brain slices were kept “alive” in a bath perfused with oxygenated artificial cerebrospinal fluid, and seizure-like activity was induced by reducing magnesium levels, so enhancing NMDA receptor currents. In these experiments, equimolar concentrations of a range of fatty acids were more potent than valproate, within 10 minutes of compound addition (the time for the compounds to perfuse the bath and penetrate the slice), thus significant levels of ketosis in these experiments is unlikely. These experiments therefore suggested a direct role for medium chain fatty acids in seizure control.

Further studies pursued the analysis of the activity of these medium chain fatty acids in seizure control and related effects (Chang et al., 2013). Here, decanoic acid and nonanoic acid were shown to provide protection against an ex vivo model of epileptiform activity generated by decreasing GABAergic inhibition (pentylentetrazol, PTZ; Figure 33.1). In these studies, decanoic acid completely blocked seizure activity 35 minutes post addition. Interestingly, octanoic acid, also prescribed in the MCT ketogenic diet, did not reduce seizure activity in this model. However, branched derivatives of octanoic acid showed variable efficacy, with some compounds providing strong seizure control (e.g., 4-methyloctanoic acid) and some showing no activity (3,7-dimethyloctanoic acid). These results confirmed earlier studies (Chang et al., 2012) but extended these findings to establish a wide chemical space showing potential efficacy in seizure control. This study also examined these chemicals for inhibition of histone deacetylase activity (HDAC), an effect associated with teratogenicity (Gottlicher et al., 2001; Gurvich et al., 2004; Phiel et al., 2001), and thus limiting their use during pregnancy (Jentink et al., 2010; Koren et al., 2006). Of the compounds analyzed in this study, only valproate and 2-propyloctanoic acid caused significant inhibition of HDAC activity at 1 mM, a concentration considerably higher than that of medium chain fatty acids found during MCT ketogenic diet treatment (Sills et al., 1986a). This suggests that medium chain fatty acid are unlikely to exhibit the teratogenic

![FIGURE 33.1](image-url) Structurally specific medium chain fatty acids strongly reduce frequency of in vitro epileptiform activity. In these experiments, an ex vivo hippocampal slice model was used, with seizure-like activity induced by application of PTZ. Compounds were added (gray box) at 1 mM, and removed after 40 minutes. The frequency of epileptiform activity is plotted against time following control (DMSO). Straight medium chain fatty acids decanoic (10 carbon) and nonanoic acid (9 carbon), two branched chain derivatives (4-methyloctanoic acid and 4-ethylloctanoic acid), and a cyclic congener (trans-4-butylocyclohexylcarboxylic acid; 4-BCCA) were able to strongly reduce seizure-like activity. In contrast, octanoic acid showed no inhibitory activity. The widely used, established epilepsy treatment, valproate, showed weak activity in this seizure model. Data derived from Chang et al. (2014), Chang et al. (2015), and Chang et al. (2016).
effects found following valproate treatment. This study also showed that medium chain fatty acids (4-methylloctanoic acid and nonanoic acid) provide control against self-sustaining status epilepticus (SSSE), induced by perforant pathway stimulation, in an in vivo rat model (Walker and Williams, 2015). Analysis of neuronal cell death in these animals as a result of SSSE showed that 2 months after seizure induction, nonanoic acid significantly reduced cell death in the hilus of the hippocampus, suggesting a neuroprotective effect. This therapeutic effect is also unlikely to be related to ketosis, since these compounds rapidly reduced seizure activity within 10 minutes of treatment. For nonanoic acid, these effects were also unlikely to occur due to sedation, since sedative effects were not shown even at high concentrations (600 mg/kg). Together these studies provide strong evidence for the direct activity of a range of medium chain fatty acids in seizure control and neuroprotection.

One further study has been reported, investigating the breadth of chemical space for medium chain fatty acids in seizure control (Chang et al., 2015). This study specifically investigated octanoic acid–related compounds, including a systematic analysis of methylloctanoic acid derivatives. Using an ex vivo model of seizure activity, induced in rat hippocampal slices by the application of PTZ, a clear structure activity relationship was seen in seizure control, provided by the position of branching on the octanoic acid backbone. The most potent compounds branched around the fifth carbon. This study also examined these compounds for protection against excitotoxic cell death, an effect that is similar to that resulting from status epilepticus (DeLorenzo et al., 2005). In these experiments, exposure of hippocampal neurons in culture to low magnesium for 4 hours triggered cell death (Deshpande et al., 2008), and consistent with the seizure control experiments, neuroprotection was seen with the addition of compounds with methyl branching from the fourth to the seventh carbon (Chang et al., 2015). Based on these results, three related compounds were then examined: 4-ethylloctanoic acid, containing a longer side chain; 4-methylnonanoic acid, containing a longer backbone, and trans-4-butycyclohexane carboxylic acid (4-BCCA), a related cyclic compound. All three compounds also showed strong seizure control in the PTZ model (Figure 33.1), and were not active against HDAC activity. These compounds were further screened in a range of in vivo seizure models (Table 33.1), providing promising activity for trans-4-butylocyclohexane carboxylic acid in multiple models. In a related study, mice treated with decanoic acid by gastric gavage (30 mmol/kg) also showed seizure control in the 6-Hz seizure and maximal electroshock seizure threshold (MEST) models (Wlaz et al., 2012). These data provide evidence that medium chain fatty acids, of defined structure, show activity in a range of in vivo seizure models.

### Table 33.1 In Vivo Seizure Control Data for Active Compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Species</th>
<th>Seizure model</th>
<th>Dose (mg/kg)</th>
<th>Animals (protected/tested)</th>
<th>Animals (Toxic/tested)</th>
<th>ED50 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-EOAa</td>
<td>Mice</td>
<td>6Hz</td>
<td>150</td>
<td>7/8</td>
<td>—</td>
<td>110</td>
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<tr>
<td></td>
<td>Rat</td>
<td>MES</td>
<td>125</td>
<td>12/16</td>
<td>4/15†</td>
<td>100</td>
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<tr>
<td></td>
<td>Mice</td>
<td>scMET</td>
<td>200</td>
<td>8/8</td>
<td>4/8*</td>
<td>142</td>
</tr>
<tr>
<td></td>
<td>Mice</td>
<td>CKM</td>
<td>110</td>
<td>6/8</td>
<td>0/8</td>
<td>71</td>
</tr>
<tr>
<td>4-BCCAa</td>
<td>Mice</td>
<td>6Hz</td>
<td>100</td>
<td>3/4</td>
<td>0/4</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>MES</td>
<td>100</td>
<td>4/8</td>
<td>1/8†</td>
<td>~100†</td>
</tr>
<tr>
<td></td>
<td>Mice</td>
<td>scMET</td>
<td>150</td>
<td>4/8</td>
<td>0/8</td>
<td>~150†</td>
</tr>
<tr>
<td></td>
<td>Mice</td>
<td>CKM</td>
<td>80</td>
<td>8/8</td>
<td>0/8</td>
<td>44</td>
</tr>
<tr>
<td>VPA</td>
<td>Mice</td>
<td>6Hz</td>
<td></td>
<td></td>
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<td>MES</td>
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<tr>
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<td>Mice</td>
<td>CKM</td>
<td></td>
<td></td>
<td></td>
<td>174e</td>
</tr>
</tbody>
</table>

Summary of in vivo seizure control in multiple models, with data provided by collaborative research with (a) NINDS, or previously determined by (b) Barton et al. (2001), (c) Loscher (1999), (d) Rowley and White (2010), or (e) NINDS.

* based on single dose data; — not determined; † unable to grasp rotorod; ‡ ataxia/loss of righting reflex

Source: Partially reproduced from Chang et al. (2015), with permission.
MEDIUM CHAIN FATTY ACIDS ARE AMPA RECEPTOR AGONISTS

The molecular mechanism of decanoic acid in direct seizure control has recently been established (Chang et al., 2016). In this study, decanoic acid (1 mM) was shown to abolish seizure activity in vitro in both the PTZ- and low-magnesium-induced drug-resistant seizure models. As previously described, decanoic acid blocks seizure activity within 15 minutes of addition to each model, strongly suggesting that the effect is directly related to the fatty acid, not to ketone generation. Moreover acetone and β-hydroxybutyrate (two ketones) have no effect in either model at high concentrations (10 mM). The mechanism of decanoic acid for this effect was then shown using whole-cell patch clamp recordings of evoked AMPA receptor–mediated excitatory postsynaptic currents (EPSCs) from CA1 pyramidal neurons, where decanoic acid reduced EPSC amplitude by 17.0 ± 5.6% at a concentration comparable to its steady state level in children on the MCT diet (100 µM) (Sills et al., 1986a). These data, for this first time, provided evidence of a direct mechanism of the MCT ketogenic diet in seizure control through inhibition of AMPA receptor currents.

To investigate a direct effect of decanoic acid on AMPA receptor activity, a range of experiments were carried out using a Xenopus heterologous expression system (Chang et al., 2016). Here, AMPA receptor subunits (GluA1-3) were expressed in oocytes individually or in pairs to produce homotetramers (GluA1) or heterotetramers (GluA1/2 and GluA2/3), and glutamate was applied, resulting in inward currents, on which direct application of decanoic acid and related compounds was tested. This approach allowed the quantification of the direct inhibition of AMPA receptor–mediated currents by medium chain fatty acids (Table 33.2). From these experiments, decanoic acid was shown to be an AMPA receptor antagonist, with efficacy across all subunit composition, and greatest potency against GluA2/3 heterotetramers (IC_{50} = 0.52 mM). Decreasing the length of the backbone reduced potency, such that nonanoic acid and octanoic acid were increasingly less potent (IC_{50} = 1.48 and 3.82 mM, respectively), although addition of a side chain to octanoic acid to produce 4-methyloctanoic acid enhanced potency (IC_{50} = 0.84 mM). These data indicate a direct action of decanoic acid and specific related structures on inhibition of AMPA receptors to regulate neuronal function.

This study also investigated some kinetic aspects of medium chain fatty acid-dependent AMPA receptor inhibition (Chang et al., 2016). First, since during seizure activity, membrane depolarization occurs, increasing excitability, decanoic acid was analyzed at varying membrane potentials. These studies identified that under depolarized membrane potentials, decanoic acid showed enhanced inhibitory activity, thus potentially providing more potent inhibition during seizure activity. Second, since AMPA receptors are activated by glutamate, and glutamate levels increase during seizure activity (Van Den Pol et al., 1996), competition assays were used to explore whether altered glutamate levels modulate decanoic acid-dependent AMPA receptor inhibition. These experiments indicate that decanoic acid is a noncompetitive AMPA receptor antagonist (Table 33.2), thus its inhibitory activity is independent of glutamate concentrations.

These studies have suggested that medium chain fatty acids function in seizure control through inhibition of AMPA receptors. However, it remained possible that these compounds could give rise to seizure control through alternative mechanisms. To address this, Chang et al. (2016) employed the hippocampal/PTZ seizure model, treated with the well-characterized AMPA receptor antagonist GYKI 52466 (Arai, 2001), to provide a similar level of AMPA receptor inhibition as decanoic acid (1 mM). Under these conditions, this study also investigated some kinetic aspects of medium chain fatty acid-dependent AMPA receptor inhibition (Chang et al., 2016). First, since during seizure activity, membrane depolarization occurs, increasing excitability, decanoic acid was analyzed at varying membrane potentials. These studies identified that under depolarized membrane potentials, decanoic acid showed enhanced inhibitory activity, thus potentially providing more potent inhibition during seizure activity. Second, since AMPA receptors are activated by glutamate, and glutamate levels increase during seizure activity (Van Den Pol et al., 1996), competition assays were used to explore whether altered glutamate levels modulate decanoic acid-dependent AMPA receptor inhibition. These experiments indicate that decanoic acid is a noncompetitive AMPA receptor antagonist (Table 33.2), thus its inhibitory activity is independent of glutamate concentrations.

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GYKI 52466 blocked seizure activity. These results indicate that direct AMPA inhibition is sufficient to explain decanoic acid’s antiseizure effect in in vitro seizure models. Moreover, AMPA receptors are widely recognized as targets for seizure control (Meldrum and Rogawski, 2007; Russo et al., 2012; Szenasi et al., 2008). Perampanel, a selective AMPA receptor antagonist, has also recently been marketed for the treatment of refractory partial epilepsy (Rektor, 2013), confirming the relevance of this mechanism to patient treatment. Chang et al. (2016) also showed that inhibition of AMPA receptors by decanoic acid at therapeutically relevant concentrations provided similar inhibitory effects as Perampanel at levels found during clinical use (Rogawski and Hanada, 2013; Ceolin et al., 2012). It is interesting to note however, that the use of Perampanel is limited by neuropsychiatric side effects, in particular aggression (Rugg-Gunn, 2014; Steinhoff et al., 2014). This effect is not seen during the MCT ketogenic diet, suggesting that these side effects may be peculiar to Perampanel rather than a class effect of drugs that act at AMPA receptors; thus the development of pharmaceutical reagents based on the mechanisms of decanoic acid will hopefully not cause this side effect.

Finally, the binding site for decanoic acid on AMPA receptors was investigated in in silico modeling (Chang et al., 2016). Using the transmembrane domains of GluA2, a putative decanoic acid–binding region was identified in the M3 helix, thought to be involved in regulating and gating inward currents. This site is distinct from that reported for Perampanel (Szenasi et al., 2008), suggesting potential differences in the effects of decanoic acid and Perampanel on AMPA receptor function. This binding site is consistent with results from electrophysiology experiments described here, although further studies will need to confirm this site.

IS THERE STILL A ROLE FOR KETONES IN SEIZURE CONTROL?
Over the last 30 years, many studies have suggested a role for ketones in seizure control and neuroprotection (Bough and Rho, 2007; Rho and Stafstrom, 2011). In some types of epilepsy, ketones may provide an alternative energy source for the brain (Kim et al., 2015; Masino et al., 2011), for example in Glut1 deficiency when glucose is poorly transported across the blood-brain barrier. Ketones have also been demonstrated to alter transcriptional regulation relevant to a putative disease-modifying role (Kim et al., 2015; Masino et al., 2011), and to have indirect effects on ion channels (Sada et al., 2015). Thus ketones may still provide some beneficial effects related to specific causes of epilepsy and in protection against long-term epileptogenic changes.

IMPLICATIONS
Understanding the mechanism of action of the ketogenic diet is essential for the development of diets or treatments that are less restrictive, and that do not produce the same diet- associated side effects. The recent data outlined here provide a strong case for the antiseizure effects of the MCT ketogenic diet resulting from increases in plasma fatty acid concentrations rather than through the production of ketones. Thus, due to the burgeoning evidence that fatty acids play a primary role in the diet’s therapeutic effect and despite the potential beneficial roles of ketones, it would perhaps be more appropriate to rename the diet, the MCT diet.

Decanoic acid may have beneficial effects beyond its action at AMPA receptors. A recent study has identified a role for decanoic acid in regulating mitochondrial proliferation (Hughes et al., 2014). Here, decanoic acid acts through regulation of a fatty acid receptor, PPARy (Malapaka et al., 2012; Zuckermann et al., 2015), at therapeutically relevant concentrations (250 μM) over a 6-day period in a neuronal cell line and in human fibroblasts. Interestingly, this effect was not shown by octanoic acid. The consequent increase in mitochondrial load has been suggested to protect against seizure induction (Bough et al., 2006), and to protect against mitochondrial dysfunction in epilepsy that has been clearly demonstrated in animal models of status epilepticus (Cock et al., 2002) and in patients (Kunz et al., 2000).

Medium chain fatty acids are rapidly metabolized in vivo (Bach and Babayan, 1982). It remains, therefore, a key consideration in the design and management of seizure-control treatments based on decanoic acid, to ensure maintenance of therapeutic fatty acid levels. Early studies of medium chain fatty acids show considerable variation in plasma fatty acid levels over a 24-hour period (Sills et al., 1986b). It remains unclear if a reduced carbohydrate load with the diet is necessary to maintain decanoic acid levels at an effective level, and further studies will need to address this in greater detail. However, a corollary of this is that clinicians using the MCT diet should focus on monitoring blood fatty acids rather than just ketone levels.
The discovery of a molecular target for the MCT diet also opens the possibility of a pharmacuetic approach to replace the diet. Several considerations are necessary here. Due to the rapid metabolism of decanoic acid, a chemical that provides similar inhibitory activity against AMPA receptors but is resistant to metabolic degradation may overcome the stringent dietary regime. Developing novel chemical structures that show enhanced binding to the target site on the receptor may also reduce the necessary dose of the compound. This would additionally help to reduce the side effects associated with high fatty acid intake, in particular gastric irritation. Studies outlined here have provided some evidence for the efficacy of decanoic acid congeners in seizure control and in direct inhibition of AMPA receptors. Further studies will be necessary to identify suitable candidates for clinical testing, but may result in the “diet in a pill.”

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INTRODUCTION TO ANAPLEROSIS AND TRIHEPTANOIN

Glucose, under nonfasting conditions, is usually the main fuel for the CNS. Glycolysis, which occurs in cytosol, is the main pathway of metabolic breakdown of glucose into pyruvate. When there is limited oxygen, glycolysis produces lactate, otherwise acetyl-CoA is produced via the pyruvate dehydrogenase pathway for further oxidation in the tricarboxylic acid (TCA) cycle in mitochondria (Figure 34.2). In aerobic metabolism, after citrate synthase transfers the acetyl-group onto oxaloacetate forming citrate, the TCA cycle oxidizes acetyl-CoA to two carbon dioxide molecules in a series of chemical reactions. At the same time, the TCA cycle provides the electron transport chain with reducing equivalents, which then produces the majority of ATP during aerobic conditions (Figure 34.1). The TCA cycle is also part of many other key metabolic pathways. The TCA cycle intermediates containing four or five carbons not only are part of the cycle involved in energy production but also are used for other metabolic pathways, for example, synthesis of various amino acids and neurotransmitters, such as glutamate and γ-aminobutyric acid (GABA). It is therefore important that C4 intermediates of the TCA cycle get “refilled” (anaplerosis) (Brunengraber and Roe, 2006; Hassel, 2000; Kornberg, 1966). Anaplerosis is required for TCA cycling to occur, as it enhances acetyl-CoA entry into the TCA cycle by providing oxaloacetate, thus increasing TCA cycling and ATP production. Moreover with increased amounts of TCA cycle metabolites, more substrate for complex II can be produced and reduction of NADH will be enhanced, which again will contribute to ATP production. Please note that the TCA cycle can also be short-circuited and still produce ATP, for example with substrate entry as 2-oxoglutarate or succinate. In many different models of disease there is evidence of reduced amounts of TCA cycle metabolites, for example, epilepsy (Alvestad et al., 2008; Melo et al., 2005; Smeland et al., 2013; Willis et al., 2010), stroke (Haberg et al., 2009), and amyotrophic lateral sclerosis (ALS) (Niessen et al., 2007). This is expected to lead to low oxaloacetate levels, which then can reduce the binding of acetyl-CoA by citrate synthase, its oxidation, and ultimately decrease ATP production.

Various enzymes partake in the refilling of the TCA cycle. In the CNS, carboxylation of pyruvate to oxaloacetate (number 1 in Figure 34.2) is the main refilling pathway, thought to be taking place in astrocytes (Patel, 1974). Also, several enzymes facilitate the formation of 2-oxoglutarate (also called α-ketoglutarate) from glutamate. This includes glutamate dehydrogenase, which forms 2-oxoglutarate and ammonia from glutamate and vice versa (2 in Figure 34.2). The reaction catalyzed by glutamic pyruvic transaminases (alanine aminotransferases), pyruvate + glutamate ⇔ 2-oxoglutarate + alanine refills the cycle with 2-oxoglutarate (3 in Figure 34.2). In addition, aspartate transaminase (also called glutamic oxaloacetic transaminase) can bypass part of the TCA cycle to produce oxaloacetate from 2-oxoglutarate in the reaction: Asp + 2-OG ⇔ OAA + Glu (4 in Figure 34.2).

Another anaplerotic pathway, the propionyl-CoA carboxylation pathway (5 in Figure 34.2), has largely been studied in peripheral tissues (Deng et al., 2009; Martini et al., 2003; Nuutinen et al., 1981; Owen et al., 2002; Reszko et al., 2003). It involves the carboxylation of propionyl-CoA to methylmalonyl-CoA by propionyl-CoA carboxylase (EC 6.4.1.3). Methylmalonyl-CoA epimerase (EC 5.1.99.1) and methylmalonyl-CoA mutase (EC 5.4.99.2) then produce succinyl-CoA. The branched chain amino acids, isoleucine and valine, as well as uneven fatty acids are anaplerotic via this pathway, all providing propionyl-CoA (Figure 34.1). Treatment with triheptanoin, the triglyceride of heptanoate (C7 fatty acid), appears to currently be
the least toxic treatment when fueling this pathway to a large extent. Unlike the branched chain amino acids, triheptanoin does not overload the body with nitrogen. In addition, providing the uneven medium chain fat as a triglyceride avoids excessive levels of sodium or acid, which otherwise could challenge physiological homeostasis.

Triheptanoin is a tasteless oil and can be mixed with various foods or made into an emulsion. As a medium chain triglyceride, it is hydrolyzed in the gastrointestinal tract, and due to its lipophilicity the free medium chain heptanoate is thought to diffuse directly into blood and mitochondria of all tissues. This is unlike long chain fatty acids, which are much more slowly metabolized, because they first enter the lymph and require various transport proteins in the blood and for final transport into mitochondria for β-oxidation. Similar to even medium chain fats, heptanoate is converted by the liver to “ketones.” While even chain fats octanoate and decanoate (C8 and C10) are turned into “C4 ketones,” β-hydroxybutyrate and acetoacetate, heptanoate is metabolized to “C5 ketones,” β-hydroxypentanoate and β-ketopentanoate (Bruenengraber and Roe, 2006; Gu et al., 2010; Kinman et al., 2006; Roe and Mochel, 2006; Roe et al., 2002). After release into the blood, C4 and C5 ketones are taken up into cells by monocarboxylate transporters. Regarding the crossing of the blood-brain barrier, monocarboxylate transporters of the MCT1 type (Broer et al., 1998; Meredith and Christian, 2008) are thought to transport C5 ketones into the brain, while heptanoate is more likely to enter the brain via diffusion (Oldendorf, 1973). Once in cells, both heptanoate and C5 ketone molecules are metabolized to their CoA adducts via medium chain acyl-CoA synthetases or 3-oxoacid CoA transferase, respectively, and then converted into the main TCA cycle fuel acetyl-CoA and propionyl-CoA. After carboxylation the latter can refill the TCA cycle with succinyl-CoA (see above) and thus promote acetyl-CoA oxidation and ATP production (Figure 34.2).

**ENERGY METABOLISM IN EPILEPSY**

An epileptic seizure or hypersynchronous activation of neuronal networks can be initiated by imbalances between excitation and inhibition. In addition, impairments in energy metabolism can also cause seizures and/or contribute to epilepsy. Numerous studies have attempted to shed light on energy metabolism in brains of patients with epilepsy and rodent epilepsy models. Assessments of metabolic functions or metabolite levels have revealed dysfunction in energy metabolism in patients with temporal lobe and extra-temporal lobe epilepsy (reviewed in Li et al., 2000; Pan et al., 2005; Pan et al., 2008). Similar changes have been found in rodent epilepsy models (Alvestad et al., 2008; Melo et al., 2010; Melo et al., 2005; Smeland et al., 2013; Willis et al., 2010) (Figure 34.3).

Furthermore, mitochondrial dysfunction and mutations within mitochondrial constituents have been described (Kann and Kovacs, 2007; Kudin et al., 2009; Waldbaum and Patel, 2010), which can also contribute to energetic imbalances contributing to seizures.

Many studies in the literature discuss interictal glucose hypometabolism in patients with temporal...
FIGURE 34.2 The TCA cycle and anaplerotic pathways. The numbers indicate five different anaplerotic pathways. 1 Pyruvate carboxylase forms oxaloacetate by carboxylation of pyruvate. 2 denotes the activity of glutamate dehydrogenase, which metabolizes glutamate into 2-oxoglutarate and ammonia and vice versa. 3 shows the reversible reaction catalyzed by glutamic pyruvic transaminases (also called alanine aminotransferases): pyruvate + glutamate $\leftrightarrow$ 2-oxoglutarate + alanine. 4 aspartate transaminase (also called glutamic oxaloacetic transaminase) produces oxaloacetate from aspartate, while transferring the amino group onto 2-oxoglutarate forming glutamate and vice versa. 5 denotes the activity of the propionyl-CoA carboxylation pathway forming succinyl-CoA. 2-OG—2-oxoglutarate.

(a) MEST test
(b) 2nd hit PTZ

FIGURE 34.3 Anticonvulsant effects of oral triheptanoin in three models. (a) Triheptanoin increased the critical current at which 50% of mice seize (CC50) in the maximal electroshock threshold test (Thomas et al., 2012). (b) It also increased the threshold to PTZ-induced tonic seizures in chronically epileptic mice (Willis et al., 2010) and (c) decreased spike wave discharges in a mouse model for absence seizures (Kim et al., 2013).
lobe epilepsy (TLE), which is a common epilepsy type in adults that is largely treatment resistant. In TLE, results from positron emission tomography with $^{18}$F-labeled fluorodeoxyglucose ($^{18}$FDG) have been interpreted to show interictal glucose hypometabolism (Arnold et al., 1996; Henry et al., 1993; Henry et al., 1990). However, care needs to be taken when evaluating these studies, as results only revealed decreased $^{18}$FDG amounts in certain brain areas, which reflects enhanced uptake of $^{18}$FDG. Levels of $^{18}$FDG cannot be used to assess glucose metabolism, because after it is metabolized by hexokinase $^{18}$FDG cannot be further metabolized via glycolysis. Similar to these studies in epilepsy patients, decreased uptake of $^{14}$C-deoxyglucose was found in the chronic phase of the lithium-pilocarpine adult rat model of TLE (Dube et al., 2001). Higher total glucose amounts were found in extracts from brain areas involved in seizure activity compared to tissue from control rats (Melo et al., 2005). However, total glucose amounts by themselves are difficult to interpret, as they depend on uptake and rates of metabolism and no alterations in total glucose amounts were found in the chronic stage of the mouse pilocarpine model (Smeland et al., 2013). In summary, decreased glucose uptake appears to be common in some “epileptic” tissues, but there is little knowledge about the metabolism of this major fuel. Given the anticonvulsant efficacy of dietary treatments, such as the ketogenic and modified Atkins diets, there is a critical need for more knowledge about glycolysis in epilepsy.

Intermediates of the TCA cycle, such as 2-oxoglutarate and oxaloacetate, are precursors of the amino acids and neurotransmitters glutamate, GABA, and aspartate. Decreased levels of TCA intermediates and amino acids have been found in the chronic seizure stage of rat and mouse epilepsy models, which display recurrent spontaneous seizures (Alvestad et al., 2008; Melo et al., 2005; Smeland et al., 2013; Willis et al., 2010). For example, in the mouse pilocarpine epilepsy model, we found lower forebrain levels of malate, aspartate, and acetyl- and propionyl-CoA during the chronic epileptic stage compared with mice without seizures (Willis et al., 2010). While several other TCA cycle intermediates were not quantified, TCA intermediate levels seemed to be decreased overall, which can result in decreased oxidative phosphorylation.

Based on this knowledge, providing additional alternative anaplerotic fuel, which can increase the amounts of C4 intermediates of the TCA cycle in the brain, is a biochemically valid approach to improve energy metabolism in “epileptic” brains (Brunengraber and Roe, 2006). Thus, we tested the metabolic effects and anticonvulsant profile of triheptanoin treatment as an anaplerotic approach, which at that time had already been explored in a few animal models and in patients with various genetic metabolic disorders. Please also note two reviews on the effects of other approaches to supplement TCA cycle substrates (Kovac et al., 2013; Tan et al., 2015).

**TRIHEPTANOIN ALTERS BRAIN ENERGY METABOLISM IN VARIOUS SETTINGS, INCLUDING EPILEPSY MODELS**

Few publications shed light on the metabolic effects of triheptanoin or its metabolite heptanoate on the brain.

An elegant study by the group of Professor Pascual (Marin-Valencia et al., 2013), infused $^{13}$C-labeled 5,6,7-heptanoate into the jugular vein of mice. The $^{13}$C-carbons were largely found in brain glutamine, but not glutamate, indicating that astrocytes primarily metabolize heptanoate and its C5 ketone metabolites.

McDonald et al. (2014) compared the amounts of various hippocampal metabolites within energy metabolism pathways (adenosine nucleotides, NAD+, NADH, and NADPH), glycolysis, the pentose phosphate pathway, and the TCA cycle in healthy mice after feeding triheptanoin and trioctanoin (the triglyceride of capric and oleic acids) for 3 weeks. While the even medium chain triglyceride altered the levels of various metabolites, no significant changes were found with triheptanoin, indicating that it would have few metabolic side effects.

A recent study in patients with Huntington’s disease patients found that 1-month triheptanoin treatment corrected the energetic response to brain activation (Adanyeguh et al., 2015). In untreated Huntington’s disease patients, a visual stimulus fails to alter the ratio of inorganic phosphate to phosphocreatine, while healthy control and treated patients showed an increase in this ratio, indicating that triheptanoin could restore metabolism of high-energy phosphates.

Two studies (Hadera et al., 2014; Willis et al., 2010) aimed to increase the understanding of the metabolic effects of triheptanoin treatment in mouse “epileptic” brain tissue. In the pilocarpine model, mice that experience status
epileptics (SE) show one motor seizure per day on average in the chronic stage of the model, while mice that did not get SE (no SE) do not show any seizure activity or neuropathological changes (Benson et al., 2015; Borges et al., 2003). Willis et al. (2010) compared the levels of various forebrain metabolites related to the TCA cycle in the chronic stage of this model. Forebrain levels of malate and propionyl-CoA were reduced in SE versus no SE mice on control diet, but this was prevented by triheptanoin feeding, indicating that triheptanoin can improve a low capacity of the TCA cycle. Hadera et al. (2014) followed the metabolism of [1,2-13C]glucose in the same mouse model to be able to evaluate the activities of pyruvate dehydrogenase and pyruvate carboxylase, the latter of which provides C4 carbons to the TCA cycle. The analyses of 13C label incorporation into TCA cycle intermediates showed that the percentage of enrichment for two 13C atoms in malate, citrate, succinate, and GABA was reduced in control treated SE mice versus no SE mice. Except for succinate, triheptanoin feeding alleviated these reductions in SE mice, which provides additional evidence that triheptanoin can increase the metabolism of glucose via the TCA cycle. The author believes that an adequate ATP supply is very important in “epileptic” tissue to keep neuronal membrane potentials stable and prevent seizure generation. Taken together, all studies are consistent with the brain being able to metabolize heptanoate or “C5 ketones,” and improvements in cases of impaired energy metabolism could be detected.

ANTICONVULSANT EFFECTS OF TRIHEPTANOIN AND CLINICAL TRIALS

Triheptanoin shows a unique anticonvulsant profile. It was found to be anticonvulsant in various mouse seizure models (McDonald et al., 2014; Thomas et al., 2012; Willis et al., 2010) (Figure 34.3), with efficacy in chronic mouse seizure models. In the chronic corneal kindling model we found a reproducible delay in the kindling process in CF1 mice (Willis et al., 2010), which is similar to effects found with established anticonvulsant drugs in the rat kindling model, namely phenobarbital and low concentrations of valproate (Brandt et al., 2006; Matagne et al., 2008; Silver et al., 1991). Protective effects of various compounds in kindling models correlate well with efficacy in humans against absence seizures. Also, in a mouse model for genetic absence seizures (Tan et al., 2007), namely mice with a missense (R43Q) mutation in the GABA<sub>A</sub> receptor γ2 subunit, triheptanoin decreased the number and duration of spike wave discharges (SWD), resulting in a halved time with seizures (Kim et al., 2013) (Figure 34.3C). In chronically epileptic mice after picrotoxin-induced SE, triheptanoin reproducibly increased the pentylenetetrazole (PTZ) seizure threshold (Figure 34.3B). Efficacy in this model suggests efficacy against drug-resistant seizures, based on the finding that a similar second hit rat model is resistant to valproate, phenytoin, and phenobarbital (Blanco et al., 2009; Borges and Sonnewald, 2011). In the acute maximal electroshock threshold test (MEST), a test for the efficacy against generalized seizures, we observed a reproducible increase of the critical current at which 50% of mice seize (Thomas et al., 2012) (CC50, Fig 34.3A). Effects in other acute seizure models have been variable, for example, in the 6-Hz model the threshold to motor seizures was only elevated in some experiments, but not others (McDonald et al., 2014; Thomas et al., 2012). Similarly, anticonvulsant effects in models using the GABA<sub>A</sub> receptor channel blockers, fluoroethyl or PTZ, have been inconsistent (Thomas et al., 2012). This lack of consistent effects in acute mouse models is not surprising, because energy metabolism in healthy mice is likely to be optimal and unlikely to require additional fuel and/or anaplerosis. In summary, triheptanoin’s anticonvulsant profile suggests efficacy against a variety of seizure types, including focal and general motor seizures, absence seizures, and possibly pharmacoresistant motor seizures (Smith et al., 2007; White, 2003).

Currently, there are three clinical trials of triheptanoin in adult and children with medically refractory epilepsy in Australia (http://www.anzctr.org.au/). The first phase IIa randomized double-blind placebo controlled study to evaluate the safety and tolerability of oral triheptanoin as an add-on treatment to adolescent and adult patients with medically refractory epilepsy took place with Professor O’Brien as the lead doctor at the Royal Melbourne Hospital. Results are currently being analyzed. Patients who took part in this trial have been invited to continue in an open-label extension study of oral triheptanoin as an add-on treatment. This study recruited patients in 2015/2016 and is going well. In addition, a trial in Brisbane is evaluating the effects of triheptanoin in children ages 3–18 with medically refractory epilepsy and has stopped recruiting.
TRIHEPTANOIN AND OTHER DISORDERS

Metabolic Disorders: Glucose Transporter Type 1 Deficiency, Long Chain Fatty Acid Oxidation Disorders, Pyruvate Decarboxylase Deficiency

While this review focused on epilepsies of various or unknown etiologies, triheptanoin is also being developed as a new treatment for D-glucose transporter type 1 deficiency (Pascual et al., 2014). In this genetic disorder glucose cannot be taken up into the brain, which results in epileptic seizures around age 3–4 and later in paroxysmal exercise-induced dyskinesias, which can be very disabling (Klepper and Leimendecker, 2007; Sul et al., 2009). The treatments for this disorder are discussed in an other chapter in this book (Klepper, chapter 5).

Originally, triheptanoin was given to patients with rare inborn metabolic disorders, including long chain fatty acid oxidation disorders due to deficiencies in different enzymes, namely carnitine palmitoyltransferase I or II, very long-chain acyl-CoA dehydrogenase, L-3-hydroxy acyl-CoA dehydrogenase, and mitochondrial trifunctional protein (Roe et al., 2002; Roe et al., 2008). The use of dietary or body fat as energy sources is preempted in these disorders, which can result in severe clinical problems, such as cardiomyopathy, intermittent rhabdomyolysis, hypoglycemia, and sudden death. Treatment with traditional medium chain triglycerides has been unsatisfactory in the past, while triheptanoin as an alternatively medium chain and anaplerotic fuel, which is also gluconeogenic, can decrease hospitalizations due to major clinical manifestations and incidences in hypoglycemia (Roe and Brunengraber, 2015; Roe and Mochel, 2006; Roe et al., 2002; Vockley et al., 2015). Another metabolic disorder that may benefit from triheptanoin is pyruvate carboxylase deficiency (Breen et al., 2014; Mochel et al., 2005).

Other Disorders: Neurological Conditions, Muscle Disorders, Cardiac Hypertrophy

Triheptanoin appears to be a promising treatment for other neurological disorders, including Canavan disease, stroke, ALS, Alzheimer’s and Huntington’s disease, autism, and glycogenoses. In three neurological disorders models, oral triheptanoin feeding was found to prevent neuronal cell death, and it is conceivable that increased energy levels can protect cells from degeneration.

The pediatric leukodystrophy Canavan disease is caused by mutations in aspartoacylase, an enzyme that is largely found in oligodendrocytes and catalyzes the hydrolysis of neuronally derived N-acetylaspartate to provide acetyl groups for lipid synthesis. In nur7 mutant mice containing a nonsense mutation in the aspartoacylase gene, early triheptanoin treatment prevented the loss of oligodendrocytes, dysmyelination, and impairments in motor function (Francis et al., 2014). The early loss of various brain metabolites in this model, such as ATP and acetyl-, malonyl-, and propionyl-CoA, was largely prevented, indicating that triheptanoin can rescue metabolic deficits also in oligodendrocytes.

The brain infarct area after middle cerebral artery occlusion (MCAO), a mouse model of stroke, was smaller when mice were fed triheptanoin before the insult (Schwarzkopf et al., 2015). In addition, mitochondrial functions were preserved in mitochondria isolated from brains 1 hour after onset of MCAO (Schwarzkopf et al., 2015), indicating that improved mitochondrial energetic functions contribute to triheptanoin’s neuroprotective effects.

Similarly, in the ALS mouse model overexpressing the human SOD1 G93A mutation, triheptanoin treatment resulted in 33% less motor neuron loss in the L4-L5 spinal cord, and loss of motor functions was significantly delayed (Tefera et al. PLOS One, in press).

A study in an Alzheimer’s disease model indicated that triheptanoin in the context of a ketogenic diet increased the expression of the mRNA levels of Sirt1, Pparg, Sod1, and Sod2 (Aso et al., 2013). As sirtuin 1 and PPARy are involved in the regulation of lipid and glucose metabolism as well as mitochondrial respiration and oxidative stress, this suggests that the neuroprotective effects of triheptanoin may also include other mechanisms that can improve energy metabolism, such as reduced oxidative stress and preserved mitochondrial functions.

Adult polyglucosan body disease (APBD) is a rare autosomal recessive disorder due to partial deficiency of the glycogen brancher enzyme (GBE) and leads to deposition of polyglucosan bodies in neurons and glia. Similar to other glycogenoses, for example, Pompe’s disease, in which glycogen cannot be metabolized, it is thought that the progressive deposition of polysaccharides causes cells to be disrupted. The APBD patients show gradual progression with difficulty walking, impaired balance, neurogenic bladder, weakness, and, in about half of the cases, dementia. Five APBD patients...
treated with triheptanoin experienced stabilization of disease progression or some functional improvement (Roe et al., 2010).

There is increasing hope that Huntington’s disease patients might benefit from long-term triheptanoin therapy, as there is evidence that triheptanoin improves energy metabolism in patients in the CNS and also skeletal muscle (Adanyeguh et al., 2015; Mochel et al., 2010). Moreover, improvements in muscle function were found in skeletal muscle in a Rett syndrome model (Park et al., 2014) and rat heart (Nguyen et al., 2015). In Sydney, a pilot clinical study is currently taking place with patients with inclusion body myositis and Pompe’s disease (http://www.anzctr.org.au/; Corbett et al., 2015).

Among the autism spectrum disorders, Rett syndrome is a genetic disorder largely caused by mutations in the X-linked gene for the transcription factor methyl-CpG binding protein 2 (MeCP2), resulting in severe disability regarding cognitive and motor function. In male MeCP2-deficient mice triheptanoin increased life span and improved social interaction and motor function as well as mitochondrial morphology in skeletal muscle (Park et al., 2014). The mutant mice also showed increased adiposity and lower glucose tolerance and insulin sensitivity, which all improved with triheptanoin treatment, indicating that this medium chain triglyceride can counteract obesity and normalize glucose metabolism.

It had been known for some time that propionate is an anaplerotic substrate for the heart, for example (Martini et al., 2003). Recently, the effects of triheptanoin in a rat model of cardiac hypertrophy were assessed. A 30% triheptanoin diet reduced left ventricular hypertrophy and improved diastolic function and myocardial glucose oxidation (Nguyen et al., 2015), indicating that anaplerotic actions of heptanoate or C5 ketones can benefit the ailing heart.

Studies on the effect of triheptanoin on alterations in fatty acid composition have just begun. Traditionally, it was thought that medium chain fats do not get elongated and subsequently stored. However, in mice, feeding of even and uneven MCT for 1 year altered the fatty acid profile of liver and heart significantly, with unusual fatty acids becoming detectable, for example, C15, C17:1, and C22:1 with triheptanoin and C22:5n3 with triocotanoin (Tucci et al., 2015). The potential impacts of these changes remain to be explored.

CONCLUSIONS

Triheptanoin was first used as a novel alternative approach to satisfy energy needs in patients with rare metabolic enzyme deficiencies, and many patients with fatty acid oxidation disorders depend on its availability. Within the last decade new research has shown promising effects of triheptanoin in a variety of human disorders and their animal models, including epilepsy. Larger-scale controlled clinical trials are now needed to prove triheptanoin’s safety and tolerability, and there is hope that a variety of beneficial effects reported so far can be confirmed.

DISCLOSURE/CONFLICT OF INTEREST

KB applied for two full US patents on the use of triheptanoin for epilepsy and ALS.

ACKNOWLEDGMENTS

I am grateful for funding by the American Epilepsy Foundation, Parents against Childhood Epilepsy, UniQuest, the Thrasher Research Fund, and Ultragenyx Pharmaceuticals, Inc., for funding ongoing clinical trials of triheptanoin in adults and children with treatment resistant epilepsy. I also thank the Australian National Health and Medical Research Council (grant 1044407) for funding my laboratory research.

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Amino Acids in the Treatment of Neurological Disorders

ADAM L. HARTMAN, MD

INTRODUCTION

Studies of the therapeutic mechanisms of nutrient-based therapies mainly focus on fats and carbohydrates (Masino and Rho, 2012). The role of protein, the third major diet component, has not been studied as thoroughly as fats and carbohydrate, but its relevance was demonstrated by the finding that protein must be restricted for systemic ketosis to occur in the ketogenic diet (Laeger et al., 2014; Yudkoff et al., 2007). The building blocks of proteins, amino acids, have been studied in a variety of neurological disorders. This chapter focuses on the therapeutic use of naturally occurring proteinogenic amino acids (and some of their D-enantiomers) for two model neurological disorders, epilepsy and traumatic brain injury. Organic acids (e.g., taurine) and modified/synthetic amino acids are not discussed.

SEIZURES AND EPILEPSY

Seizures and epilepsy (the propensity for recurrent unprovoked seizures) represent one of the most common neurological disorders in all age groups. Current treatments still leave 20-30% of patients without adequate seizure control. This has fueled interest in drug discovery for epilepsy, including amino acids.

L-Amino Acids

L-leucine and L-isoleucine (300 mg/kg, a high dose) protect against seizure onset by prolonging the latency to onset of spike-wave discharges and clinical tonic-clonic seizures induced by the GABA-A receptor antagonist pentylenetetrazol (PTZ); L-leucine also decreases the duration of spike-wave discharges, but neither amino acid affects seizure duration (Dufour et al., 1999). Interestingly, α-ketoisocaproic acid, a metabolite of L-leucine that results from a transamination reaction that also produces L-glutamate, is inactive against PTZ. Also, each of the branched-chain amino acids (L-leucine, L-isoleucine, and L-valine) increase the latency to onset of seizures induced by another GABA-A receptor antagonist, picrotoxin (Skeie et al., 1994).

Using a different seizure test and dosing paradigm, we showed that pretreatment with L-leucine terminates seizures induced by the excitotoxin kainic acid; L-valine was ineffective, suggesting that in this test, there is not a class effect of branched-chain amino acids (Hartman et al., 2015). Interestingly, L-leucine was ineffective in terminating kainic acid-induced seizures when it was injected after seizure onset.

Glycine is somewhat unique among amino acids in that it can have either excitatory or inhibitory effects, depending on which receptor is involved: generally speaking, glycine binding to NMDA receptors in the brain is excitatory but binding to glycine receptors in the spinal cord is inhibitory (Johnson and Ascher, 1987; Werman et al., 1968), although there are exceptions to this general rule. Inhibition of the glycine transporter (GlyT1) protects against acute and chronic seizures in rodents (Kalinichev et al., 2010b; Shen et al., 2015). However, binding of glycine to the N-methyl-D-aspartate (NMDA) receptor likely does not mediate this effect (Zellinger et al., 2014), and in fact, inhibitors of this site have acute antiseizure effects (Bristow et al., 1996; Nichols and Yielding, 1998). The exact role of glycine in seizure initiation versus the progression or propagation of seizure activity remains unclear.

These data suggest a number of potential mechanisms for an antiseizure effect. First, the amino acids may be working through either cell surface transporters or receptors, as in the case of glycine and the GlyT1 transporter. Alternatively, they may activate intracellular amino acid “sensors,” with downstream effects on signaling pathways.
L-leucine activates the master integrator of cellular metabolism, mammalian target of rapamycin complex 1 (mTORC1) via Sestrin2 (Bar-Peled and Sabatini, 2014; Cota et al., 2006; Sancak et al., 2008; Wolfson et al., 2016). However, data suggest that mTORC1 activation is not the mechanism for seizure control of L-leucine, because pathologically increased mTORC1 activity has been measured in both humans and rodent models of tuberous sclerosis complex (Orlova and Crino, 2010; Zeng et al., 2008) as well as some chemoconvulsant rodent models of temporal lobe epilepsy; similarly, pharmacological inhibition of mTORC1 decreases seizures in some models (Buckmaster and Lew, 2011; Huang et al., 2010; Sosanya et al., 2015; Zeng et al., 2009). Thus, it is unlikely that L-leucine, an mTORC1 activator, protects against seizures by increasing activity in this particular pathway. Nonetheless, the role of L-leucine in mTORC1-related disorders is unknown. This mechanism also does not explain the antiseizure effect of L-isoleucine and L-valine.

Another potential mechanism for the action of L-leucine (and other amino acids) on seizure activity is activation via amino acid transporters. Examples include LAT1/SLC7A5, which has implications for transport of other small molecules into the brain (Geier et al., 2013), and B0AT2/SLC6A15 (Broer et al., 2006; Haggblund et al., 2013). Interestingly, LAT1/SLC7A5 is expressed in pathological balloon cells in tissue from patients with tuberous sclerosis (which supports the role of this transporter in cell growth, one of the cardinal abnormal features in this disease) (Lim et al., 2011). An antiseizure mechanism may involve countertransport of other amino acids, which would in turn lead to decreased synaptic concentration of excitatory amino acids and a resulting decrease in seizure activity (Yudkoff et al., 2007; Yudkoff et al., 2005). A number of other amino acid transporters also may transport leucine (Box 35.1). Further testing of these transporters (using either pharmacological inhibitors or genetically modified organisms) is needed to determine their importance in chronic epilepsy.

L-serine is less well known for a role in neurotransmission than its D-enantiomer. Mouse lacking the ASC1 transporter (alanine-serine-cysteine) have seizures (Xie et al., 2005). Indicating stereospecific requirements, L-serine does not potentiate NMDA-induced seizures, although D-serine, an endogenous ligand of this receptor, does (Singh et al., 1990a). D-serine is discussed below.

L-arginine gained attention because it is the metabolic precursor of nitric oxide, which itself has shown mixed results in seizure and epilepsy studies (Banach et al., 2011). Not surprisingly, similarly mixed results have been shown with L-arginine. In an electrical kindling paradigm of epilepsy, L-arginine did not affect acquisition of kindling or seizure severity in rats (Herberg et al., 1995). In a developmental model of seizures, L-arginine prolonged PTZ-induced EEG-recorded seizure duration in postnatal day #10 (P10) rat pups, although somewhat paradoxically, P21 rats had increased survival (with no change in seizure duration) (de Vasconcelos et al., 2000). The type of seizure (i.e., tonic vs. tonic-clonic) also was different in L-arginine-treated P21 pups compared with controls, suggesting a form of neuromodulation that needs further clarification (and as the authors note, this may have an impact on survival).

D-Amino Acids
Our studies of L-leucine led us to consider transporter-mediated effects or contaminants in commercial preparations of L-leucine. The most abundant other amino acid “species” in this preparation was the enantiomer, D-leucine, which represented up to 0.5% of the L-leucine in our commercial source (Sigma-Aldrich technical information). Surprisingly, D-leucine terminates the behavioral manifestations of kainic acid–induced seizures, even when administered after seizure onset (Hartman et al., 2015). As mentioned previously, L-leucine was ineffective when given after seizure onset in this test. D-leucine (administered in drinking water for 14 days) also protects against seizures in the 6-Hz electroshock test, which models focal-onset seizures, demonstrating seizure protection in a different assay. Although D-leucine

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**BOX 35.1**

**NEUROLOGICALLY IMPORTANT L-LEUCINE TRANSPORTERS**

(NUMERICAL ORDER, MODIFIED FROM GALLUS.REACTOME.ORG)

<table>
<thead>
<tr>
<th>SLC3A2 (ATA2)</th>
<th>SLC6A14</th>
</tr>
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<tbody>
<tr>
<td>SLC6A15</td>
<td>SLC7A5</td>
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<tr>
<td>SLC7A6 (γLAT2)</td>
<td>SLC7A7 (γLAT1)</td>
</tr>
<tr>
<td>SLC7A9</td>
<td></td>
</tr>
</tbody>
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Chapter 35: Amino Acids in the Treatment of Neurological Disorders 347
is more ketogenic than L-leucine (i.e., its degradation results in the production of ketone bodies but not glucose) (Embden, 1908), D-leucine treatment does not induce systemic ketosis (Hartman et al., 2015). D-leucine does not bind to the kainic acid receptor (thus eliminating competition on the receptor as a mechanism of seizure protection), nor does it bind to a panel of other CNS receptors or transporters. D-leucine is a known ligand of the taste receptors Tas1R2/R3 (which are expressed in the hippocampus, a major seizure-generating region of the brain), but it is unclear whether this represents the mechanism of D-leucine antiseizure action (Bassoli et al., 2014; Shin et al., 2010). Other ligands of this receptor have been shown to have limited antiseizure activity in the maximal electroshock test (Talevi et al., 2012), supporting a potential role for this receptor in epilepsy.

Produced by bacteria, D-leucine is found in food products, particularly those that are plant-based (Ekborg-Ott and Armstrong, 1996; Mutaguchi et al., 2013). D-leucine has been isolated from rat and mouse hippocampus, mouse neocortex, and other areas of the brain at lower concentrations (Hamase et al., 1997; Hamase et al., 2001). Newly-synthesized mammalian proteins do not include D-leucine (Fox et al., 1998). The only other data on therapeutic use of D-leucine were in analgesia studies, some of which also included use of D-phenylalanine in combination with D-leucine (Cheng and Pomeranz, 1980; McKibbin and Cheng, 1982; Ninomiya et al., 1990). Importantly, the doses required of each amino acid in the analgesia studies were ~80x greater than the lowest effective dose in our seizure studies, suggesting a different mechanism for D-leucine in terminating seizures.

Other D-amino acids may play a role in seizure activity, although the literature is mixed in terms of efficacy. D-serine binds to the glycine site on the NMDA receptor, and decreased endogenous concentrations have been shown to be partly responsible for cognitive dysfunction in rats with epilepsy induced by pilocarpine status epilepticus (Klatte et al., 2013; Schell et al., 1995). Results of studies on the anticonvulsant effects of D-serine have been mixed. D-serine protects weakly in the maximal electroshock test, where it also potentiates the effects of some antiseizure drugs (Kaliničev et al., 2010a; Peterson, 1991). D-serine increases afterdischarge thresholds in amygdala-kindled rats (Loscher et al., 1994). Conversely, serine racemase knockout mice, which have decreased extracellular levels of D-serine in the dentate gyrus, are relatively protected against seizures induced by PTZ (Harai et al., 2012; Singh et al., 1990a). Similarly, exogenously administered D-serine potentiates seizures induced by PTZ (Singh et al., 1990a). D-serine does not have an effect on spike wave discharges in GAERS (Genetic Absence Epilepsy Rats from Strasbourg) Wistar rats (Koerner et al., 1996). D-serine potentiates seizures induced by NMDA, as might be expected (Singh et al., 1990a). In studies designed to test the efficacy of antiseizure medicines at the D-serine/glycine binding site on the NMDA receptor, D-serine decreases the antiseizure effect of the clinical medicine felbamate, the experimental compound L-687,414, and the opioid kappa-receptor agonist CI-977, among others (De Sarro et al., 1994; Singh et al., 1990b; Tricklebank et al., 1994; White et al., 1995). Notably, the latter experiments were not designed to directly test the antiseizure efficacy of D-serine but rather used it as a coactivator of NMDA receptors.

D-alanine binds to the D-serine/glycine site on the NMDA receptor and may play a role in circadian endocrine function (Kleckner and Dingleidine, 1988; Morikawa et al., 2008). Treatment with D-alanine does not change seizure-related parameters in fully amygdala-kindled rats (Groucher and Bradford, 1991). A high dose (1,400 mg/kg) of exogenously administered D-arginine has CNS depressant effects and increases the latency to onset of PTZ-induced convulsions (but 700 mg/kg was ineffective) (Navarro et al., 2005).

D-amino acids are considered by some to be “unnatural,” but the extant literature shows that other endogenous D-amino acids have biological functions. These are discussed briefly here for the sake of interest and completeness, although they do not have a reported direct role in epilepsy. D-aspartate has neurotransmitter properties and may play a role in neurodevelopment, learning, and neuroendocrine function (D'Aniello et al., 2000; D'Aniello et al., 2011); it also decreases chemically induced schizophrenia-like symptoms in rodents (Errico et al., 2011; Errico et al., 2008). The brain concentration of D-proline is highest in pineal and pituitary tissue, but its endogenous function has not been reported (Hamase et al., 2005). D-glutamate has been detected in subsets of neuronal cell bodies in the mesencephalon and thalamus by immunohistochemical techniques, but its role is unknown (Mangas et al., 2007). D-methionine (1 mM) protects cultured auditory neurons against cisplatin-induced neurotoxicity (Gopal et al., 2012). A role for these D-amino acids in seizures has not been reported. Interestingly, plasma concentrations of D-serine, D-aspartate, D-alanine, D-leucine, and D-proline are reduced
in the rat beta-amyloid hippocampus injection model of Alzheimer’s disease (Xing et al., 2016). Further work should identify whether D-amino acid detection will be useful as a biomarker for either identifying the disease or monitoring disease progression (Box 35.2).

**TRAUMATIC BRAIN INJURY (TBI)**

Traumatic brain injury represents one of the major causes of injury and disability in all age groups (although the causes vary between age groups). Branched chain amino acids (particularly leucine) represent a major source of glutamate in the central nervous system (Sakai et al., 2004). Glutamate serves as an excitatory neurotransmitter, an excitotoxin, and an energy store (used indirectly for generation of ATP). In the mouse lateral fluid percussion model of traumatic brain injury, hippocampal concentrations of each of the branched chain amino acids (total levels, i.e., L- and D-amino acids) are decreased; supplementation of these amino acids for 5 or 10 days (but not shorter durations) reverses injury-induced deficits in both anterograde and retrograde memory (Cole et al., 2010; Elkind et al., 2015). In human trials, supplementation of all three branched chain amino acids led to a substantial improvement in Disability Rating Scale scores in patients with traumatic brain injury (Arch Phys Med Rehab 86, 1729–1735).

L-serine is neuroprotective in a mouse weight-drop model in the acute phase of TBI (measured morphologically), and the mechanism may involve activation of glycine receptors (Zhai et al., 2015). L-serine also decreases astrocitosis, microglial activation, and concentrations of inflammatory cytokines in the later phases of this model of TBI (Zhai et al., 2015).

### CONCLUSIONS

Select amino acids have been shown to protect against seizure activity and the sequelae of TBI. Interestingly, these compounds have a variety of different mechanisms in these disorders, including receptor binding and transporter effects, and as metabolic intermediates for other signaling molecules. They also may affect intracellular signaling pathways, although evidence for the latter is scant at this point. Further research will elucidate additional roles for these amino acids in the treatment of neurological disorders.

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**BOX 35.2**

**D-AMINO ACIDS WITH ANTISEIZURE EFFECTS**

- D-leucine
- D-serine
- D-arginine


Chapter 35: Amino Acids in the Treatment of Neurological Disorders


INTRODUCTION
Conventional anticonvulsant medications reduce neuronal excitability through effects on ion channels or synaptic function. In recent years, it has become clear that metabolic factors also play a role in the modulation of neuronal excitability (Reid et al., 2014). This volume contains many examples of potentially beneficial metabolic treatments for epilepsy and other neurological disorders. In particular, the high-fat, low-carbohydrate ketogenic diet (KD) is effective in controlling seizures in many children whose seizures are refractory to anticonvulsant medications (Neal et al., 2008). However, the mechanisms of action of the KD and its variants (e.g., medium chain triglyceride diet, modified Atkins diet, low glycemic index treatment) are very complex and are not fully characterized (Rho and Stafstrom, 2012; Lutas and Yellen, 2013; Gano et al., 2014). Possible mechanisms include reduction of excitability by ketone bodies or fatty acids, altered neurotransmitter synthesis or action, improved mitochondrial function, or a combination of these or other factors. The key observation that ingestion of small amounts of carbohydrate by children on the KD results in loss of seizure control (Huttenlocher, 1976) led to the idea that carbohydrate restriction could exert a protective effect against seizures (Greene et al., 2003). In addition to limiting carbohydrate intake, restricting calorie intake also suppresses seizures and affords neuroprotection (Greene et al., 2003; Ingram and Roth, 2011; Pani, 2015; Yuen and Sander, 2014), and in fact, the KD was initially formulated to mimic the physiological effects of fasting. Although some data supports intermittent fasting for seizure control (Hartman et al., 2013), fasting is not a feasible long-term treatment option.

As an alternative to fasting, the KD restricts dietary carbohydrates and generates ketone bodies as the proximate energy source, thereby reducing glycolysis (Figure 36.1). This effect and the observation that minimal carbohydrate intake can abolish seizure control achieved by the KD suggests that inhibitors of glycolysis may mimic some of the favorable therapeutic effects of the diet. Among compounds that inhibit glycolysis, the glucose analog 2-deoxy-D-glucose (2DG) is a promising novel agent for seizure protection. 2DG differs from glucose by removal of a single oxygen atom from the 2-position (Figure 36.2A). 2DG is taken up by cells and undergoes phosphorylation at the 6-position to 2DG-6P, but glycolytic flux is reduced because 2DG-6P cannot undergo isomerization by phosphoglucone isomerase (Figure 36.2B). Uptake of glucose and 2DG is enhanced in energetically active cells. 2DG has been used for decades as a fluorinated positron emitted tracer (F18-2DG) for measurement and imaging of regional glucose utilization by positron emission tomography (PET) (Wree, 1990). 2DG has also been investigated as an adjuvant chemotherapeutic agent for several types of cancer, since rapidly dividing, metabolically active neoplastic cells with enhanced glucose uptake are vulnerable to glycolytic inhibition by 2DG (Pelicano et al., 2006; Cheong et al., 2011).

ANTICONVULSANT ACTIONS OF 2DG
We tested the effects of 2DG in several models of acute seizures in vivo and in vitro (Stafstrom et al., 2009). When exposed to elevated extracellular potassium (K+*, 7.5 mM), hippocampal CA3 neurons in slices from adult rats developed high-frequency interictal (epileptiform) bursts at a frequency of about 30 per minute. Addition of 2DG (10 mM) to the bathing medium reversibly reduced the burst frequency by about 50% (Stafstrom et al., 2009). Similarly, 2DG reduced epileptiform bursts induced by bath application of the chemical convulsants bicuculline, a γ-aminobutyric acid
section IV: Ketone-Based Metabolism

(GABA) receptor antagonist, or 4-aminopyrididine, a potassium channel blocker. In slices from juvenile animals (P10-13), 7.5 mM K⁺ induced prolonged ictal discharges in area CA3 for 10–30 seconds; 2DG decreased the occurrence of these ictal bursts. Therefore, 2DG has an anticonvulsant effect on both interictal and ictal epileptiform activity in the CA3 region of hippocampus.

![Diagram of glucose metabolism and points at which interventions could affect neuronal excitability and seizure control.](image)

2DG inhibits glycolysis by blocking the phosphoglucose isomerase step. The ketogenic diet (KD), via ketone bodies, bypasses glycolysis by providing acetyl-CoA (ACoA) to the TCA (tricarboxylic acid cycle) after glycolysis. Anaplerosis refers to the "refilling" of intermediate compounds depleted from the TCA cycle. Other abbreviations: β-OHB, beta-hydroxybutyrate; AcAc, acetoacetate; PDH, pyruvate dehydrogenase. Reprinted with permission from Rho and Stafstrom, 2012.

![Diagram of glucose, 2DG, and the glycolytic pathway.](image)

Phosphorylation of 2DG yields 2DG-6P, which cannot undergo isomerization by glucose-6-phosphate (glucose-6-P) isomerase (GPI) to fructose-6-phosphate (fructose-6-P), thereby preventing subsequent steps of glycolysis. (b) Schematic diagram of key steps of glycolysis illustrating the rate-limiting step involving phosphofructokinase, which is inhibited by pyruvate, the end product of the pathway. Oxidation of phosphoenol-pyruvate (structure not shown) to pyruvate generates nicotinamide adenine dinucleotide (NADH) before entry into the tricarboxylic acid (TCA) cycle. Reprinted with permission from Stafstrom et al., 2009.
An anticonvulsant effect was also seen in in vivo seizure models. Kindling refers to the progressive decrease in seizure threshold in response to repeated subconvulsive electrical stimuli. Kindled seizures can be elicited by stimulation of many brain pathways. We assessed the effect of 2DG on kindling development by stimulation of the perforant path or the olfactory bulb (Garriga-Canut et al., 2006; Stafstrom et al., 2009). 2DG was administered at a dose of 250 mg/kg intraperitoneally (i.p.) 30 minutes prior to daily kindling stimulation of either pathway. In response to kindling of the perforant path, but not the olfactory bulb, 2DG-pretreated rats displayed an increase in mean afterdischarge (AD) threshold over time, defining a region-specific anticonvulsant effect.

In addition, an anticonvulsant effect of 2DG was seen in two other acute seizure models in animals. First, in the 6 Hz stimulation model (Barton et al., 2001), psychomotor seizures were induced by corneal stimulation using electrical pulses at a frequency of 6 Hz. Pretreatment with 2DG resulted in seizure protection in 75% of rats tested (Stafstrom et al., 2009). Second, in Fring's mice with audiogenic seizures, 50% of mice were protected from sound-induced seizures after pretreatment with 2DG (Stafstrom et al., 2009). However, 2DG did not protect against pentylenetetrazole- or maximal electroshock-induced seizures in rats. Therefore, anticonvulsant effects of 2DG were demonstrated in several but not all in vivo seizure models, with a pattern of effectiveness unlike that afforded by any currently available antiseizure medicine. For example, no conventional antiseizure medication exhibits region-specific activity, as indicated by 2DG's differential action on perforant path versus olfactory bulb. Thus, 2DG's mechanisms are likely to be broad, affecting seizure-induced plasticity across many seizure types and syndromes, but an exact correlation with human epilepsies is not yet possible (Holmes and Zhao, 2008).

Not all studies have confirmed that 2DG exerts an anticonvulsant effect. In the presence of 2DG, latency to seizure onset was slightly decreased in mice when chemoconvulsants (pentylenetetrazole, kainic acid) were coadministered intravenously with 2DG (Gasier et al., 2010). While this outcome has been interpreted as a possible proconvulsant effect, an alternative explanation is that the well-documented acute increase in cerebral blood flow induced by 2DG enhances brain delivery of parenteral convulsants such as pentylenetetrazole and kainic acid, thereby shortening the latency to initial seizure manifestations as a threshold outcome measure.

It is worth mentioning that the KD and 2DG both suppress seizure activity, but our studies with 2DG were not intended to investigate KD mechanisms. For instance, 2DG does not cause ketosis. Both 2DG and the KD suppress seizures in Fring's mice and in the 6-Hz model (Hartman et al., 2008), but the KD is effective in the MES model while 2DG is not. The former two models are used to model focal onset seizures, whereas the MES is considered to mimic generalized tonic-clonic seizures. Kindling is abrogated by both the KD and 2DG, though exact kindling protocols differ widely among studies (Hori et al., 1997; Garriga-Canut et al., 2006; Stafstrom et al., 2009). Therefore, 2DG and the KD are not comparable directly, though both modify seizure susceptibility by altering metabolic pathways involved in energy regulation. Therefore, potentially useful clinical information can be gained from studies of both compounds.

**ANTIEPILEPTIC ACTIONS OF 2DG**

An antiepileptic (or antiepileptogenic) effect is defined as the slowing or prevention of epilepsy development. Antiepileptogenesis is “disease-modifying,” meaning that the process of epilepsy development is hindered. Using the kindling model, with stimulation of either the olfactory bulb or perforant path, we investigated whether 2DG mediates an antiepileptogenic effect. Kindling progression was quantified by the number of ADs required to elicit specific stages of seizure severity, using the Racine scoring scale (Racine, 1972). Pretreatment with 2DG at doses ranging from 37.5 mg/kg intraperitoneally (i.p.) (we found this to be the minimally effective dose) up to 250 mg/kg i.p., given 30 minutes before each kindling stimulation, resulted in an ~2-fold increase in the number of ADs required to achieve class III, IV, or V kindled seizures in both regions (Garriga-Canut et al., 2006; Sutula and Franzoso, 2008; Stafstrom et al., 2009). These results indicate a disease-modifying antiepileptic action. Note that the olfactory bulb kindling experiments described in the preceding paragraph, testing the anticonvulsant effects of 2DG, showed no significant effect of this compound on kindled seizure threshold. Therefore, 2DG has different anticonvulsant effects in different brain regions, whereas its antiepileptic effects were similar in hippocampus and olfactory bulb. Furthermore, 2DG retarded kindled seizure progression when administered immediately after or 10 minutes after a kindled seizure, raising the possibility that 2DG can be used as a “postseizure”
treatment (e.g., for clusters of seizures or even status epilepticus) (Sutula and Franzoso, 2008; Stafstrom et al., in preparation).

2DG exerts neuroprotective actions in several other models as well. In hippocampal cell cultures, 2DG increased neuronal resistance to oxidative and metabolic insults by inducing stress proteins, and in rats, 2DG alleviated kainic acid seizure-induced memory deficits and hippocampal neuron loss (Lee et al., 1999). In another model, 2DG pretreatment of mice undergoing bilateral carotid artery occlusion protected against seizures that typically occur in this protocol (Redjak et al., 2001). Numerous mechanisms of 2DG-mediated neuroprotection have been hypothesized, including activation of adenosine monophosphate (AMP)-activated protein kinase (Park et al., 2009), reduction in oxidative stress (Yao et al., 2011), and disruption of glycosylation causing protein unfolding (Zhang et al., 2014), to name a few.

**POSSIBLE MECHANISMS OF 2DG EFFECTS**

The acute and chronic actions of 2DG probably involve different cellular and molecular mechanisms. The chronic antiepileptic effects of 2DG have been associated with decreased expression of brain derived neurotrophic factor (BDNF) and its receptor, tyrosine kinase B (trkB), which are required for kindling progression (He et al., 2004). 2DG suppression of seizure-induced increases in BDNF and trkB is mediated by the transcriptional repressor neuron restrictive silencing factor (NRSF) and its nicotinamide adenine dinucleotide hydride (NADH)–sensitive corepressor carboxy-terminal binding protein (CtBP) acting at the promoter regions of BDNF and trkB genes. In pathological conditions such as seizures, glycolysis is enhanced to meet the energy demands of activated neurons. The increase in glycolysis elevates NADH, which in turn causes dissociation of CtBP from NRSF, thus decreasing transcriptional repression and resulting in increased expression of BDNF and trkB. In the presence of 2DG, which reduces NADH levels as a consequence of glycolytic inhibition, the NRSF-CtBP complex maintains repression of BDNF and trkB, and kindling progression is slowed (Garriga-Canut et al., 2006; Huang and McNamara, 2006).

Compared to its pivotal role in mediating chronic 2DG effects, NRSF is not required for the antiepileptic effect of the KD (Hu et al., 2011). The antiepileptic effect of 2DG was abolished in mice with conditional knockout of NRSF, but the KD continued to afford protection against kindling progression in these transgenic animals.

Others have reported evidence that the effect of 2DG is mediated through up-regulation of K$_{ATP}$ channel subunits Kir6.1 and Kir6.2 (Yang et al., 2013). K$_{ATP}$ channels are closed in the presence of intracellular ATP and open when intracellular ATP is depleted. Open K$_{ATP}$ channels efflux K$^+$, hyperpolarizing the cell and decreasing its excitability. It has been hypothesized that ketones, by decreasing glycolysis and thus ATP production, may lower cellular excitability (Ma et al., 2007). Decreased ATP levels, perhaps restricted to submembrane compartments adjacent to K$_{ATP}$ channels, might lead to enhanced K$^+$ efflux and hyperpolarization. Such effects could be limited to certain neuron types (e.g., substantia nigra, dentate gyrus) that function as a gate for pathological discharges (Ma et al., 2007; Tanner et al., 2011; Lutas and Yellen, 2013).

The rapid onset of anticonvulsant effects of 2DG in vitro and in vivo suggests that 2DG may be exerting direct actions at the synaptic or membrane levels. The effects of 2DG on excitatory synaptic transmission were investigated in pilot experiments using hippocampal slices (Pan et al., 2008; Pan et al., 2014; Rutecki et al., in preparation). In hippocampal area CA3, there was no effect of 10 mM 2DG on the frequency or amplitude of spontaneous excitatory postsynaptic currents (sEPSCs). However, after the induction of epileptiform bursting in this region by application of elevated (7.5 mM) K$^+$, 2DG reduced sEPSC frequency and amplitude, suggesting that the effects of 2DG are activity-dependent—2DG is taken up by actively firing cells and works preferentially in conditions of intense neuronal activity such as seizures. The same dose of 2DG had no effect on miniature EPSCs isolated by exposure to tetrodotoxin, which blocks sodium channels and thus reduces neuronal activity-mediated synaptic release of neurotransmitters, but miniature EPSCs were significantly reduced by 10 mM 2DG in the elevated K$^+$ condition. These effects are not a general consequence of glycolysis inhibition, since other glycolysis blockers depress sEPSCs in both normal and epileptic slices (Pan et al., 2009; Devinney et al., 2009). 2DG also reduces spontaneous inhibitory postsynaptic current (sIPSC) frequency in conditions of elevated K$^+$ (7.5 mM), but to a lesser extent than sEPSCs, resulting in a net reduction in excitatory transmission (Pan et al., 2015). 2DG has no significant effects on intrinsic membrane properties, and its acute effects on synaptic transmission appear to be
presynaptic based on analysis of miniature EPSCs (Pan et al, 2015).

Therefore, one of the unique features about 2DG is its use-dependence. That is, 2DG is taken up only by neurons that are metabolically active, as occurs in areas of circuitry involved in seizure activity. This represents a distinct advantage when considering the goal of a medication in targeting only brain areas displaying pathological seizure activity.

The acute anticonvulsant effects of 2DG may be influenced by other, as yet undetermined metabolic or electrophysiological consequences of glycolytic inhibition. For example, 2DG’s effects might be spatially limited to certain submembrane compartments, it might alter systemic lipid metabolism, or it might modify mitochondrial metabolism in a manner that subsequently influences neuronal excitability.

Another compound in the glycolytic pathway, fructose-1,6-diphosphate (FDP), has been shown to exert acute anticonvulsant activity in several seizure models in adult rats including kainic acid, pilocarpine, and pentylentetrazole (Lian et al., 2007). In that study, the effectiveness of FDP as an anticonvulsant surpassed that of 2DG, KD, and valproate. The mechanism of FDP’s anticonvulsant effect is unclear. Fructose-1,6-diphosphate increases glucose flux from glycolysis into the pentose phosphate pathway (PPP) (Figure 36.1), and NADPH generated in the PPP reduces glutathione, resulting in an anticonvulsant action. Therefore, FDP may exert an endogenous anticonvulsant action (Stringer and Xu, 2008). Subsequent studies have established that FDP retards kindling progression by attenuating BDNF and trkB expression (Ding et al., 2010), like 2DG. Furthermore, during the kindling process, FDP inhibits the kindling-induced down-regulation of the expression of potassium-chloride cotransporter 2 (KCC2) and decreases the expression of sodium-potassium-chloride cotransporter 1 (NKCC1), suggesting that FDP might alter the switch between GABAergic excitation and inhibition (Ding et al., 2013). These findings remain to be verified, but together, results from several laboratories have identified modification of glycolysis as a possible novel mechanism for treatment of seizures.

2DG AS A POTENTIAL CLINICAL AGENT

Preclinical studies to evaluate safety and toxicity of 2DG have demonstrated that 2DG is well tolerated in rats and dogs at doses associated with anticonvulsant and antiepileptic effects. We used both acute and chronic protocols to test the effects of 2DG on spatial learning and memory in the Morris water maze (Ockuly et al., 2012). For acute testing, 2DG was injected 15 minutes prior to the water maze trial each testing day. For chronic testing, 2DG (250 mg/kg or 500 mg/kg i.p., twice daily) was injected daily for 14 days before water maze testing began. Neither protocol caused a difference in the latency to platform acquisition (spatial learning) or retention of platform location (probe test) by either dose of 2DG, suggesting that 2DG has no obvious deleterious effect on spatial memory or learning.

Rats were also tested on the open field test, which assesses exploratory activity and has also been regarded as a measure of anxiety (Ockuly et al., 2012). Rats were pretreated (30 min prior to open field testing) i.p. with saline, 50 mg/kg 2DG, or 250 mg/kg 2DG in a crossover design. The exploratory activity (number of lines crossed in the open field arena) did not differ between the saline- and 50 mg/kg 2DG-treated groups, but the rats receiving 250 mg/kg 2DG had decreased motor activity (fewer lines crossed). When the saline group was crossed over to receive 2DG 250 mg/kg and the prior 250 mg/kg group was crossed over to saline, the results reversed—rats receiving 250 mg/kg 2DG had diminished open field activity and the group now receiving saline had increased motor activity. Therefore, the 250 mg/kg dose of 2DG caused a transient, reversible decrease in exploratory activity. Taken together, these findings suggest that 2DG has no permanent adverse behavioral effects on spatial learning and memory, exploratory activity, or anxiety at doses that suppress seizures and retard epilepsy progression, supporting its potential for clinical use.

Some animal studies have shown that 2DG at high doses is associated with adverse cardiac effects. Oral or intravenous doses of 2DG 250–2,000 mg/kg given to rats or mice over 7 days caused a dose-dependent fall in mean arterial pressure, decreased respiratory rate, and increased mortality (Vijayaraghavan et al., 2006). Detailed pathological evaluation of cardiac tissue after chronic oral 2DG ingestion in two rat strains revealed cardiotoxic effects with vacuolization of cardiac myocytes, increased incidence of pheochromocytomas, and reduced life spans (Minor et al., 2010). Cardiac effects of 2DG in rats had features consistent with autophagy, a process by which a cell degrades unnecessary cellular components in response to nutrient stress. Comprehensive preclinical safety and toxicology studies have subsequently demonstrated reversible species-specific cardiac toxicity at high doses in F344 rats but not in Beagle dogs; this toxicity was detectable by monitoring
plasma N-terminal probrain natriuretic peptide (NT-proBNP) (Terse et al., 2016).

Experience regarding human safety of 2DG has been obtained in cancer trials (Pelicano et al., 2006). Tumor cells are dependent on glycolysis to support their metabolic requirements. By reducing glycolysis, 2DG deprives rapidly growing cells of their required cellular fuel (Cheong et al., 2011). For example, 2DG inhibits breast cancer cell growth in a dose-dependent manner and causes cell death through apoptosis (Aft et al., 2002). In combination with adriamycin, 2DG slows the growth of solid tumors such as osteosarcoma (Maschek et al., 2004). In patients with advanced prostate cancer, a 2-week dose-escalating study revealed dose-limiting toxicity of grade 3 asymptomatic QTc prolongation at a dose of 60 mg/kg, but doses of 45 mg/kg were well tolerated (Stein et al., 2010).

**CONCLUSIONS**

The inhibition of glycolysis with compounds such as 2DG represents a novel therapeutic approach to epilepsy. The effects of 2DG in animal models are summarized in Table 36.1. 2DG exerts acute anticonvulsant effects in vitro that are independent of...
the method of seizure induction. 2DG has acute anticonvulsant effects in vivo in several models of seizure induction and also possesses novel chronic antiepileptic effects against progression of seizures and the adverse consequences of seizure-induced plasticity that are associated with alterations in neuronal gene expression. Finally, 2DG has a favorable preliminary toxicity profile. Together, these observations support 2DG or similar agents as feasible for treating epilepsy in patients. The detailed anticonvulsant and antiepileptic mechanisms of 2DG actions remain to be clarified.

ACKNOWLEDGMENTS
This work was supported by The Charlie Foundation (CES), NIH RO1 25020 (TPS), the Epilepsy Research Foundation New Therapy Development Project, and the Wisconsin Alumni Research Foundation.

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Ketogenic Diets as Highly Effective Treatments for Diabetes Mellitus and Obesity

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INTRODUCTION
The prevalence of overweight and obesity has dramatically increased in the past 40 years, with levels now reaching epidemic proportions in both developed and developing countries (Lobstein and Baur, 2005; Mastorakos et al., 2010). Obesity brings with it an increased risk for premature mortality and development of comorbidities including hypertension, dyslipidemia, and type 2 diabetes mellitus (T2DM) (Stein and Colditz, 2004). Obesity has been shown to be one of the main etiological factors in the development of T2DM, and the current epidemic of the disease is believed to be largely attributable to the increased incidence of obesity (The Look AHEAD Research Group, 2003). Epidemiological studies have shown that the risk of developing T2DM increases exponentially with a body mass index (BMI) >28kg/m² (Clark, 2004). And T2DM is fast becoming a serious public health problem all over the world—the disease is expected to affect 380 million people globally by 2025 (Tahran et al., 2010).

Once developed, T2DM brings with it many health, social, and economic burdens (Huang et al., 2010). In particular, individuals with the disease can experience a life expectancy shortened by up to 10 years when compared with individuals without the disease, due to an increased risk of both macrovascular and microvascular complications (Bottomley and Raymond, 2007). As the worldwide prevalence of the disease increases, so too does the demand for and cost of medical care in order to treat this progressive disease (Brandt et al., 2003; Bottomley and Raymond, 2007). Reports show that effective glycemic control is one of the strongest predictors in decreasing annual healthcare costs among individuals with T2DM (Hansen et al., 2010). As well as having direct costs to society and to an individual's health, T2DM brings with it many indirect costs. These include loss of productivity for employers and loss of income for both employer and employee due to chronic sickness and absence from work, early retirement, or premature mortality (Bottomley and Raymond 2007).

Type 2 diabetes mellitus has long been regarded as a chronic progressive condition that is heterogeneous in nature (Lim et al., 2011; Nyenwe, 2011). It is a complex disorder in which the interaction of both the environment and an individual's genetics results in the development of insulin resistance (IR) and, eventually, β-cell dysfunction (Leahy, 2005). The β-cell function declines over a period of time, and the development of IR to T2DM is progressive, with some evidence showing it can take up to 12 years for the disease to develop (Nyenwe, 2011). Obesity worsens IR, as adipose tissue releases increased amounts of hormones (e.g., adiponectin), pro-inflammatory cytokines (e.g., tumor-necrosis-α), and other factors that are involved in the development of IR (Kahn et al. 2000). As obesity becomes a chronic state, there is an increase in the release of nonesterified fatty acids (NEFAs), particularly from intra-abdominal fat (Leahy, 2005). This is believed to be one of the crucial factors linking obesity, specifically central obesity, to IR and eventually T2DM (Stumvoll et al., 2005). Insulin resistance is thought to impair liver and pancreatic β-cell function, consequently reducing the target tissues' sensitivity to insulin (Tahran et al., 2010). In addition to defective insulin action and secretion, nonsuppressible glucagon secretion after a meal is also evident in individuals with T2DM (Nyenwe, 2011).

With the significant health, social, and economic burdens associated with T2DM, it is clear that devising effective interventions for treating
and, most importantly, preventing the disease are imperative. A fundamental approach for the effective prevention and treatment of T2DM is weight management (Miles et al., 2002; Nyenwe, 2011). Currently, it is understood that a moderate weight loss of between 5% and 10% of body weight has been shown to offer improvements in glycemic control, blood pressure, and dyslipidemia in patients with T2DM.

**ORIGINS OF THE USE OF KETOGENIC DIETS TO TREAT OBESITY**

One of the first descriptions of a low-carbohydrate weight-loss program was written in 1863 by William Banting, called *A Letter on Corpulence* (Banting, 1863). As a result of Banting’s letter, a low-carbohydrate, high-fat diet was a widely recommended diet for obesity until the mid-20th century. John Yudkin, a professor of nutrition in the United Kingdom, brought attention to carbohydrate restriction as a method of losing weight, and suspected that sugar was a culprit in modern illnesses (Yudkin, 1959).

**RANDOMIZED, CONTROLLED TRIALS OF CARBOHYDRATE RESTRICTION FOR OBESITY**

Marginalized as a “fad diet,” the low-carbohydrate diet was forgotten as a mainstream treatment for obesity in the last half of the 20th century. Over the last 15 years, several independent groups have reexamined low-carbohydrate diets and given them the new name of “carbohydrate-restricted diets,” or “ketogenic diets” if the dietary carbohydrate is sufficiently low to cause an increase in blood or urine ketone bodies (typically < 20 grams of dietary carbohydrate/day). These recent studies for the treatment of obesity have been summarized in several meta-analyses (Nordmann et al., 2006, Hession et al., 2008, Santos 2012, Krieger 2006, Hu 2012, Johnston 2014). These studies are notable in that, despite the higher fat content of the low-carbohydrate diet, the adverse changes in serum cholesterol that were predicted from other diet studies did not occur. The low-carbohydrate diet improves the cardiometabolic risk factors by lowering serum triglyceride and raising HDL-cholesterol, both components of the metabolic syndrome (Table 37.1; Volek and Feinman, 2005). There are very few dietary disease endpoint outcome studies, but a low-carbohydrate diet was an intervention arm in a study assessing carotid intimal thickness after 2 years (Shai et al, 2010). In this study, all diet interventions, including the low-carbohydrate diet led to significant regression of measurable carotid intimal thickness. The effect appeared to be mediated by weight loss–induced decline in blood pressure.

**ORIGINS OF THE USE OF KETOGENIC DIETS TO TREAT DIABETES MELLITUS**

The discovery of insulin and dissemination of its use in the early 1920s was truly miraculous for individuals with type 1 diabetes mellitus (T1DM), who had no endogenous insulin production. Insulin treatment was quickly incorporated into clinical practice (Bliss, 1982). The widespread use of insulin and subsequently developed antiglycemic medications for T2DM,

**TABLE 37.1 OUTPATIENT RANDOMIZED, CONTROLLED TRIALS OF CARBOHYDRATE VERSUS FAT RESTRICTION FOR OBESITY: RESULTS**

<table>
<thead>
<tr>
<th>Reference</th>
<th>n</th>
<th>Weight</th>
<th>LDL</th>
<th>Trig</th>
<th>HDL</th>
<th>Weight</th>
<th>LDL</th>
<th>Trig</th>
<th>HDL</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sondike</td>
<td>30</td>
<td>-4.1 kg</td>
<td>-17%*</td>
<td>-6%</td>
<td>+2%</td>
<td>-9.9 kg</td>
<td>+4%</td>
<td>-48%*</td>
<td>+4%</td>
<td>3 months</td>
</tr>
<tr>
<td>Brehm</td>
<td>42</td>
<td>-3.9 kg</td>
<td>-5%</td>
<td>+2%</td>
<td>+8%</td>
<td>-8.5 kg</td>
<td>0%</td>
<td>-23%*</td>
<td>+13%</td>
<td>6 months</td>
</tr>
<tr>
<td>Samaha</td>
<td>132</td>
<td>-3.1 kg</td>
<td>-3%</td>
<td>+2%</td>
<td>-12%</td>
<td>-5.1 kg</td>
<td>+6%</td>
<td>-29%*</td>
<td>-2%</td>
<td>12 months</td>
</tr>
<tr>
<td>Foster</td>
<td>63</td>
<td>-4.5 kg</td>
<td>-6%</td>
<td>+1%</td>
<td>+3%</td>
<td>-7.3 kg</td>
<td>+1%</td>
<td>-28%*</td>
<td>+18%*</td>
<td>12 months</td>
</tr>
<tr>
<td>Yancy</td>
<td>119</td>
<td>-6.5 kg</td>
<td>-3%</td>
<td>-15%</td>
<td>-1%</td>
<td>-12.0 kg</td>
<td>+2%</td>
<td>-42%*</td>
<td>+13%*</td>
<td>6 months</td>
</tr>
<tr>
<td>Brinkworth</td>
<td>40</td>
<td>-11.5 kg</td>
<td>+3%*</td>
<td>-12%*</td>
<td>0%*</td>
<td>-14.5 kg</td>
<td>+3%*</td>
<td>-35%*</td>
<td>+21%*</td>
<td>12 months</td>
</tr>
<tr>
<td>Shai**</td>
<td>211</td>
<td>-2.9 kg*</td>
<td>-0.1%</td>
<td>-2%*</td>
<td>+17%*</td>
<td>-4.7 kg</td>
<td>-3%</td>
<td>-13%*</td>
<td>+22%*</td>
<td>24 months</td>
</tr>
</tbody>
</table>

* p < .05 for between group comparison
** Study also included a third Mediterranean diet intervention (not shown here)
however, have not allowed individuals with diabetes to live entirely normal lives. Like T1DM, T2DM is known as a chronic disease with high rates of retinopathy, nephropathy, and vasculopathy (Feudtner, 2003). Importantly, the pathophysiology of the majority (95%) of individuals with diabetes today is related to insulin resistance (T2DM), not to insulin deficiency (T1DM). Today, individuals with either T1DM or T2DM are instructed to self-monitor their blood glucose and self-administer subcutaneous insulin to achieve normoglycemia. Despite intensive management with insulin and oral medications, normoglycemia remains an elusive goal (DCCT 1993, UKPDS 1998).

In hindsight, the rapid adoption of insulin therapy and the hope that insulin would “cure” T2DM may have led to a premature departure from successful treatments for T2DM prior to the discovery of insulin. In the pre-insulin era, the pharmacological treatment of diabetes mellitus included a combination of modalities including alcohol, opiates, arsenic, and potassium bromide. More potent than medication, though, was the high-fat, low-carbohydrate diet. From a textbook written in 1877: “There are few diseases which present to the practitioner so clear an indication of what is to be done . . . a Diabetic should exclude all saccharine [sugary] and farinaceous [starchy] materials from his diet” (Morgan, 1877).

The leading authorities in the early 1900s, Frederick Madison Allen and Elliott P. Joslin, published several studies supporting the use of a low-carbohydrate, high-fat diet for diabetes. Allen used a pancreatectomized dog model of diabetes and observed that feeding a high-carbohydrate diet led to glycosuria. If, however, the dogs were fed a high-fat, low-carbohydrate diet, the glycosuria was no longer detectable. In the treatment of diabetes in humans, Allen employed fasting, then a stepwise reintroduction of macronutrients to find the threshold at which glycosuria developed (Allen, 1914, 1915a, 1915b, 1920). Using this method, the average diet recommendation for diabetes was a diet containing 70% fat, 18% protein, 4% alcohol, and only 8% of calories from carbohydrate (Allen et al., 1919; Westman et al., 2006).

Like Allen, Joslin recommended a 70% fat, 10% carbohydrate diet for the treatment of diabetes (Joslin, 1928). Joslin categorized carbohydrate-containing foods by their carbohydrate content, and advised his patients to eat vegetables with less than 5% carbohydrate content (Joslin, 1919). According to his classification, vegetables with carbohydrate content from 1% to 3% included lettuce, cucumbers, spinach, asparagus, rhubarb, endive, dandelion greens, swiss chard, celery, and mushrooms. Vegetables with carbohydrate content from 3% to 5% were tomatoes, brussels sprouts, watercress, sea kale, okra, cauliflower, eggplant, cabbage, canned string beans, broccoli, and canned artichokes. Non-carbohydrate-containing foods were not limited (meat, eggs, fish).

So, before the modern medical therapies of medication and insulin, the latter being discovered in 1921, the leaders in diabetes used a low-carbohydrate, high-fat diet for the treatment of diabetes.

**DIETARY RECOMMENDATIONS FOR DIABETES IN THE POST-INSULIN ERA**

The discovery and therapeutic use of insulin changed the management of T1DM and T2DM, including its dietary management (Franz et al., 2002). Sansum achieved the absence of glycosuria with increased levels of dietary carbohydrate by increasing the dose of insulin (Sansum, 1928). From the calculated means of the cases reported, an increase in dietary carbohydrate from 41.9 g to 196 g could be made with an increase of insulin from 20.4 units to 85.6 units/day on average. It was also observed that in patients already on insulin, glycosuria remained absent and carbohydrate could be increased without increasing the insulin dosage if the caloric intake was reduced (Richardson, 1929; Rabinowitch, 1930). From the calculated means of the cases reported, an increase from 73g to 144g of carbohydrate could be made by reducing the caloric intake from 1,788 kcal to 1,427 kcal on average. However, it is interesting to note that Richardson studied individuals with T1DM. In regard to T2DM, Richardson wrote, “it has been evident that with patients who are fat or markedly overweight it is not possible to make this change in the fat and carbohydrate of the diet. The tolerance has been exceeded and sugar in the urine has resulted in all of those cases which we have tried” (Richardson, 1929). Higher carbohydrate intakes could be consumed, but glycosuria would appear unless the insulin dosage was increased, or the total caloric intake was decreased.

Note that these observations were made using glycosuria as the measure of glycemic control. While individual variability exists, glucose does not typically appear in the urine until the serum level is greater than 180 mg/dL (Buse et al., 2003).
Chapter 37: Ketogenic Diets as Highly Effective Treatments


After the use of insulin became routine, the amount of carbohydrate in the “diabetic diet” was gradually increased. Although carbohydrate counting was still an important part of the diabetic diet recommended by the American Dietetic Association as late as 1950, by 1971 the guidelines read “Important dietary concepts have developed in the last decade which require some alteration in long-held precepts. There no longer appears to be any need to restrict disproportionately the intake of carbohydrate” (Bierman et al., 1971). (It is interesting in retrospect that there are no references included in this 1971 position paper.)

It appears that the change in guidelines to soften the limitation of dietary carbohydrate was based on a few small studies. In 1963, a study of insulin-dependent diabetic patients compared two 2,200-kcal eucaloric diets containing two relatively high levels of carbohydrate (41% vs. 64%), and found that both diets led to similar glucose control (Stone and Connor, 1963). Blood glucose measurement was not used, however, and good glucose control was defined as no glycosuria and few hypoglycemic episodes. According to the authors, “In practical terms, this meant the avoidance of more than minimal glycosuria, the avoidance of more than 10 gm. of glucose per twenty-four-hour specimen of urine and of more than one mild hypoglycemic episode every two weeks.”

In 1971, a paper was published involving 13 patients with mild diabetes who were given liquid formula diets containing 85%CHO/15%PRO/0%FAT or 45%CHO/15%PRO/40%FAT diet for a period of 8–10 days (Brunzell et al., 1971). The carbohydrate was either dextrose or a mixture of dextrins and maltose; calories were adjusted to maintain a constant body weight. On the 85% carbohydrate diet, the fasting blood glucose was 91 mg/dL and the fasting insulin was 16.2, compared with 100.2 mg/dL and 20.8, respectively, on the lower carbohydrate diet. The conclusion was that the glucose control was similar for a moderate or high carbohydrate diet.

It appears that the recommendation of a 55% carbohydrate diet was based on extremely small studies that used either glycosuria or hypoglycemia as the measure of diabetes control (not precise enough by today’s standards) and compared relatively high levels of carbohydrate intake, which does not take into account the clinical experience of low-carbohydrate diets prior to the discovery of insulin. Clearly, the discovery and clinical application of insulin therapy revolutionized the treatment of diabetes mellitus due to insulin deficiency, and no one would question the use of insulin for T1DM. For diabetes mellitus related to insulin resistance, however, it is not clear that medication therapy (including insulin) is superior to a high-fat, low-carbohydrate diet for glycemic control and avoidance of long-term complications. This study was never done. It can be argued that because the pathophysiology of T2DM involves insulin resistance and hyperinsulinemia, using insulin therapy is counterintuitive.

Several limitations temper our ability to directly apply this historical information today. Due to the possibility of spectrum bias, it is difficult to ascertain the severity of diabetes that was treated by Allen and Joslin. Their patients may have had less severe or progressed T2DM and therefore might not be representative of the diabetic population of that time, since individuals with more severe disease are likely to have died. There was also no formal distinction between type 1 and type 2 diabetes mellitus at the time, though it was noted that children and young adults presented with weight loss (probably type 1), while older patients were often obese (probably type 2). In the sample of Allen’s patients, with a mean age of 40, most of these patients probably had type 2 diabetes.

In summary, one of the widely recommended treatments of diabetes mellitus in the early 1900s before the introduction of medication therapy was a high-fat, low-carbohydrate diet.


The amount of glucose in the human bloodstream is regulated very closely, and chronic, small elevations of serum glucose lead to glucose intolerance, T2DM, and the associated complications. The amount of serum glucose in an adult with a serum glucose of 100 mg/dL and 5–7 liters of blood is about 5–7 grams (about the amount contained in a heaping teaspoon of table sugar or a few medium-sized strawberries). Normal serum glucose ranges from 80 to 99 mg/dL, and when a fasting serum glucose is elevated to 100–124 mg/dL, the diagnosis of impaired fasting glucose is made. When the fasting glucose is above 124 mg/dL or a random glucose is elevated above 200 mg/dL with common symptoms such as polydipsia or polyuria, the diagnosis of T2DM is made (American Diabetes...
Association, 2010). These small changes in concentration in serum glucose represent changes in the amount of glucose in the blood of only a few grams.

The “glycemic index” is a concept that categorizes single foods containing carbohydrate on the basis of the rise in blood glucose after the ingestion of a standard amount of carbohydrate from the particular food (Jenkins et al., 1981; Holt et al., 1997; Ludwig, 2002). This glucose response is then compared with the glucose response to a standard weight of white bread or glucose. The “glycemic load” is a variation of the “glycemic index” in that the usual serving size of the food is the unit of comparison, in the attempt to make the construct more clinically useful. One problem with these constructs is that the glycemic effect of a meal is a function of not only the glycemic index of the carbohydrate, but also of the other macronutrients and foods ingested at the same time. Despite these criticisms, low glycemic index diets can lead to improvement in diabetic control (Brand-Miller et al., 2003). However, the magnitude of this effect on glycemic control in patients with T2DM is modest, with a typical response of 0.43% reduction compared with higher glycemic diets. Because the “low glycemic” diets in these studies have contained from 40% to 60% of calories from carbohydrate, it is possible that a more potent effect on lowering blood glucose may be observed with a reduction in the percentage of carbohydrate and not just the glycemic index.

**FIGURE 37.1** Fasting and Postprandial Glucose Response of a Ketogenic Diet 24-h glucose response. The open circle–solid line represents the mean glucose concentration at several time points during the first 24 h of both days during which the standard diet was ingested (i.e. day 1 of each arm of the study). The triangle–dotted line represents the mean glucose concentration during the last 24 h on a carbohydrate-free diet. The closed circle–solid line represents the mean glucose concentration during the last 24 h of the fast (energy-free) diet. B, L, D, indicate the times at which breakfast, lunch, and dinner were ingested. The net area response (Left Insert) indicates the area under the curve using the fasting concentration as baseline. Different letters on bars indicate statistically significant differences (Friedman: \( P < 0.0012 \)). The total area response (Right Insert) indicates the area under the curve, using zero as baseline. Different letters on bars indicate statistically significant differences (Friedman: \( P < 0.0001 \)) (Nuttall, Almokayyad, Gannon, 2015).
GLYCEMIC EFFECT OF DIETARY CARBOHYDRATE

In controlled studies, consuming foods of lower glycemc index versus foods of higher glycemic index leads to a reduction in postprandial glucose and insulin. When the glycemic concept is taken further and applied to the reduction of the amount of carbohydrate to less than 20–40 g/day—all of the carbohydrate-containing foods are “very low” to “no” glycemic index foods. (Fat has a glycemic index of 0 because the consumption of fat does not raise the blood glucose.) When the carbohydrate intake is this low, the glucose and insulin responses to the meals are even lower. In fact, the fasting and postprandial glucose and insulin levels after carbohydrate-deficient meals are almost as low as total fasting (not eating anything at all) (Figure 37.1; Nuttall et al., 2015). The lack of postprandial rise in glucose and insulin for an extremely low carbohydrate diet (<20 g/day) has been replicated in two other studies in subjects without diabetes (Bisschop et al., 2000; Noakes et al., 2006).

In summary, lowering the glycemic index and the absolute amount of carbohydrate in the diet can have a profound effect on lowering the blood glucose; dietary protein has a small effect on blood glucose; dietary fat has none. This is the rationale for use of a low-carbohydrate diet for the treatment and prevention of diabetes.

CONTROLLED OUTPATIENT STUDIES OF LOWER CARBOHYDRATE DIETS FOR TYPE 2 DIABETES MELLITUS

From 1998 to 2004, several controlled studies showed that lowering the dietary carbohydrate can lead to a reduction in fasting blood glucose and HbA1c and that this effect can be independent of weight loss (Table 37.2). However, none of these studies lowered the carbohydrate intake to the levels of the ketogenic diet, which had been employed clinically in the early 1900s, as discussed earlier. A review of existing studies in 2008 concluded that a low-carbohydrate diet would be useful for the treatment of T2DM (Dyson, 2008).

METABOLIC WARD STUDY OF THE KETOCNIC DIET FOR TYPE 2 DIABETES MELLITUS

A major limitation of out-patient clinical trials is that the dietary intake is estimated by self-reported intake questionnaires and may not reflect actual dietary intake. This limitation was overcome in a carefully controlled metabolic ward study among individuals with T2DM. In this study, an ad libitum low-carbohydrate, ketogenic diet (<20 g/day) led to a spontaneous reduction in caloric intake and improvement in insulin sensitivity measured by insulin clamp technique (Boden et al., 2005). Additionally, serum glucose levels improved substantially and consistently enough in the 10 subjects that hemoglobin A1c improved significantly from baseline after only 14 days on the low-carbohydrate diet.

RANDOMIZED, CONTROLLED TRIALS OF CARBOHYDRATE RESTRICTION FOR TYPE 2 DIABETES MELLITUS

There has been a recent interest in studying the use of low-carbohydrate diets for the treatment of T2DM. Several of the recent studies have limited carbohydrate intake to the degree that was used by Allen and Joslin (Table 37.3, Daly 2006, Westman 2008, Gulbrand 2012, Saslow 2014, Tay 2014, Yamada 2014, Mayer 2014). The consistent finding is that the lower carbohydrate, even ketogenic, diets lead to a greater improvement in T2DM than the diets containing higher levels of carbohydrate. Lowering dietary carbohydrate intake demonstrated benefits on glycemic control beyond its weight loss effects in some of the studies, while at the same time lowering antiglycemic medication requirements. Two meta-analyses have also summarized these studies (Kirk 2008, Dyson 2008).

PILOT STUDY OF A KETOGENIC DIET FOR WEIGHT LOSS IN OBESITY VERSUS TYPE 2 DIABETES MELLITUS

While clinical experience suggests that weight loss is slower for individuals with T2DM than obesity alone, there are few studies directly comparing this clinical observation for ketogenic diets. One of this chapter’s authors (EM) conducted a retrospective cohort study to assess the rate of weight change that occurred between a group of overweight or obese type 2 diabetics compared with age-, weight-, and gender-matched control groups of overweight or obese individuals (unpublished data).

The intervention was the Go Lower Low Carbohydrate Diet, which provided around 10% of total energy from carbohydrates, 30% of total energy from protein, and 60% of total energy from fat. All meals were delivered to individuals, providing them with breakfast, lunch, dinner, and up to 2 snacks per day. Weekly support was provided to participants with additional nutrition support.
# Table 37.2 Controlled Studies of Lower Carbohydrate Diets for Type 2 Diabetes Mellitus

<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>BMI</th>
<th>KCAL</th>
<th>CHO</th>
<th>PRO</th>
<th>FAT</th>
<th>Duration</th>
<th>Post Glucose</th>
<th>Pre HgbA1c</th>
<th>Post HgbA1c</th>
<th>Weight Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gutierrez</td>
<td>19</td>
<td>27.9</td>
<td>Varied</td>
<td>55</td>
<td>20</td>
<td>25</td>
<td>12 weeks</td>
<td>183</td>
<td>8.1</td>
<td>8.9</td>
<td>-0.8</td>
</tr>
<tr>
<td>1998</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>192</td>
<td>9.9</td>
<td>8.1</td>
<td>-1.4</td>
</tr>
<tr>
<td>Heilbronn</td>
<td>35</td>
<td>33</td>
<td>1541</td>
<td>73</td>
<td>17</td>
<td>10</td>
<td>12 weeks</td>
<td>131</td>
<td>8.5</td>
<td>7.0</td>
<td>-6.6</td>
</tr>
<tr>
<td>1999</td>
<td></td>
<td></td>
<td>1596</td>
<td>50</td>
<td>18</td>
<td>32 MUF</td>
<td></td>
<td>119</td>
<td>7.8</td>
<td>6.8</td>
<td>-6.6</td>
</tr>
<tr>
<td>2002</td>
<td>45</td>
<td>22</td>
<td>1440</td>
<td>60 HGI</td>
<td>22</td>
<td>18</td>
<td>12 weeks</td>
<td>110</td>
<td>6.7</td>
<td>6.1</td>
<td>-4.8</td>
</tr>
<tr>
<td>Heilbronn</td>
<td></td>
<td></td>
<td>1440</td>
<td>60 LGI</td>
<td>22</td>
<td>18</td>
<td></td>
<td>116</td>
<td>6.7</td>
<td>6.0</td>
<td>-4.4</td>
</tr>
<tr>
<td>Gannon</td>
<td>12</td>
<td>31</td>
<td>2250</td>
<td>55</td>
<td>15</td>
<td>34</td>
<td>5 weeks</td>
<td>114</td>
<td>8.0</td>
<td>7.7</td>
<td>0</td>
</tr>
<tr>
<td>2003</td>
<td></td>
<td></td>
<td>2235</td>
<td>40</td>
<td>30</td>
<td>30</td>
<td></td>
<td>114</td>
<td>8.1</td>
<td>7.3</td>
<td>0</td>
</tr>
<tr>
<td>Rizkalla</td>
<td>12</td>
<td>31</td>
<td>2291</td>
<td>38 HGI</td>
<td>20</td>
<td>37</td>
<td>4 weeks</td>
<td>176</td>
<td>7.5</td>
<td>7.6</td>
<td>-0.6</td>
</tr>
<tr>
<td>2004</td>
<td></td>
<td></td>
<td>2222</td>
<td>36 LGI</td>
<td>21</td>
<td>37</td>
<td></td>
<td>165</td>
<td>7.6</td>
<td>7.2</td>
<td>-0.6</td>
</tr>
<tr>
<td>Gannon</td>
<td>8</td>
<td>31</td>
<td>2825</td>
<td>55</td>
<td>15</td>
<td>30</td>
<td>5 weeks</td>
<td>198</td>
<td>9.8</td>
<td>9.8</td>
<td>-1.8</td>
</tr>
<tr>
<td>2004</td>
<td></td>
<td></td>
<td>2835</td>
<td>20</td>
<td>30</td>
<td>50</td>
<td></td>
<td>126</td>
<td>9.8</td>
<td>7.6</td>
<td>-1.8</td>
</tr>
</tbody>
</table>

**BMI** = body mass index, **HGI** = high glycemic index, **LGI** = low glycemic index, **MUF** = high monounsaturated fat, **SF** = high saturated fat
# TABLE 37.3 OUTPATIENT RANDOMIZED, CONTROLLED TRIALS OF CARBOHYDRATE RESTRICTION FOR TYPE 2 DIABETES MELLITUS

<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>BMI</th>
<th>Carbohydrates</th>
<th>Duration</th>
<th>Start HgbA1c</th>
<th>End HgbA1c</th>
<th>Difference HgbA1c</th>
<th>Difference Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daly</td>
<td>102</td>
<td>36</td>
<td>170 grams/day</td>
<td>3 months</td>
<td>9.0</td>
<td>8.8</td>
<td>-0.2</td>
<td>-0.9</td>
</tr>
<tr>
<td>2006</td>
<td>110</td>
<td>36</td>
<td>170 grams/day</td>
<td>3 months</td>
<td>9.0</td>
<td>8.5</td>
<td>-0.5</td>
<td>-3.6</td>
</tr>
<tr>
<td>Westman</td>
<td>49</td>
<td>38</td>
<td>100 grams/day</td>
<td>6 months</td>
<td>8.3</td>
<td>7.8</td>
<td>-0.5*</td>
<td>-6.9</td>
</tr>
<tr>
<td>2008</td>
<td>20</td>
<td>38</td>
<td>100 grams/day</td>
<td>6 months</td>
<td>8.8</td>
<td>7.3</td>
<td>-1.5*</td>
<td>-11.0</td>
</tr>
<tr>
<td>Guldbrand</td>
<td>61</td>
<td>32</td>
<td>225 grams/day</td>
<td>24 months</td>
<td>7.2</td>
<td>7.4</td>
<td>+0.2</td>
<td>-4.0</td>
</tr>
<tr>
<td>2012</td>
<td>90</td>
<td>32</td>
<td>225 grams/day</td>
<td>24 months</td>
<td>7.5</td>
<td>7.5</td>
<td>-0.0</td>
<td>-4.0</td>
</tr>
<tr>
<td>Saslow</td>
<td>34</td>
<td>36</td>
<td>138 grams/day</td>
<td>3 months</td>
<td>6.9</td>
<td>6.9</td>
<td>0*</td>
<td>-2.6</td>
</tr>
<tr>
<td>2014</td>
<td>58</td>
<td>36</td>
<td>138 grams/day</td>
<td>3 months</td>
<td>6.6</td>
<td>6.0</td>
<td>-0.6*</td>
<td>-5.5</td>
</tr>
<tr>
<td>Tay</td>
<td>115</td>
<td>34</td>
<td>205 grams/day</td>
<td>6 months</td>
<td>7.3</td>
<td>6.6</td>
<td>-0.7*</td>
<td>-12</td>
</tr>
<tr>
<td>2014</td>
<td>57</td>
<td>34</td>
<td>205 grams/day</td>
<td>6 months</td>
<td>7.4</td>
<td>6.2</td>
<td>-0.6*</td>
<td>-12</td>
</tr>
<tr>
<td>Yamada</td>
<td>24</td>
<td>25</td>
<td>203 grams/day</td>
<td>6 months</td>
<td>7.7</td>
<td>7.5</td>
<td>-0.2*</td>
<td>-1.4</td>
</tr>
<tr>
<td>2014</td>
<td>126</td>
<td>25</td>
<td>203 grams/day</td>
<td>6 months</td>
<td>7.6</td>
<td>7.0</td>
<td>-0.6*</td>
<td>-2.6*</td>
</tr>
<tr>
<td>Mayer</td>
<td>46</td>
<td>40</td>
<td>156 + O grams/day</td>
<td>12 months</td>
<td>7.6</td>
<td>7.7</td>
<td>+0.1*</td>
<td>-8.1</td>
</tr>
<tr>
<td>2014</td>
<td>76</td>
<td>40</td>
<td>156 + O grams/day</td>
<td>12 months</td>
<td>7.6</td>
<td>6.9</td>
<td>-0.7*</td>
<td>-7.5</td>
</tr>
</tbody>
</table>

*p < .05 for between group comparison.

n = sample size, O = Orlistat
provided by qualified nutritionists. Between weeks 9 and 12, only breakfast and snacks were provided, which required individuals to prepare two meals per day.

A sample of 20 participants (10 obese with diabetes and 10 obese without diabetes) was identified for inclusion in this study. At baseline, the mean age was 55.5 years, mean BMI was 36.0 kg/m², and 70% were female, and the mean HgbA1c was 12.3%. After 3 months of following the dietary protocol, obese nondiabetic participants had lost significantly more weight than obese type 2 diabetic participants (-20.56 ± 8.8 kg versus -12.84 ± 8.1 kg; \( p = .05 \)). However, there was no significant difference between these groups in mean weight change at 12 months. These results are in line with other previous studies that have demonstrated that patients with type 2 diabetes do not lose as much weight as their nondiabetic counterparts (Khan et al., 2000; Miles et al., 2002).

**NONRANDOMIZED STUDIES USING THE KETOGENIC DIET IN TYPE 2 DIABETES MELLITUS**

In order to assess the effect of the ketogenic diet on T2DM in clinical practice, one of the authors (ECW) performed chart reviews of physicians who had used carbohydrate restriction for many years in their clinical practices (Hickey et al., 2003; O’Neill et al., 2003; Vernon et al., 2003; Vernon et al., 2004) (Table 37.4). Nielsen also reported the results of long-term follow-up in a clinical setting (Nielsen and Joensson, 2008). These cases show that reversal of T2DM is possible in the context of selection of highly motivated individuals, or as a result of programs that have excellent adherence.

One of these research projects was a prospective study which enrolled 28 overweight subjects with T2DM into a 16-week single-arm diet intervention trial at the Durham Veterans Affairs Medical Center (Yancy et al., 2003). At diet initiation, diabetic medication was reduced to avoid hypoglycemia. At baseline, the mean age was 56.3 years and the mean BMI was 42.2 kg/m². After 16 weeks, the mean hemoglobin A₁c decreased by 15% from baseline (7.4% to 6.3%, \( p < .001 \)). Medications for diabetes were discontinued in 7 subjects, reduced in 10 subjects, and unchanged in 5 subjects. The mean body weight decreased by 6.5% from 130.9 kg to 122.4 kg (\( p < .001 \)). Fasting serum triglyceride decreased 40% (2.61 mmol/L to 1.54 mmol/L, \( p < .001 \)), while other serum lipid measurements did not change significantly.

**CASE REPORT OF REMISSION OF SEVERE TYPE 2 DIABETES MELLITUS WITH KETOGENIC DIET**

One of the authors (ECW) recently treated an otherwise healthy 60-year-old Caucasian male with a BMI of 26.9 kg/m² who had a new onset of T2DM. The initial hemoglobin A₁c was 10.5% on routine annual testing, and serum glucose levels were also reflective of hyperglycemia. He was given instruction on how to follow a low-carbohydrate, ketogenic diet (<20 grams of carbohydrate/day), and the repeat hemoglobin A₁c at 1, 5, 12, and 24 months was 6.4%, 5.5%, 5.6%, and 5.5% respectively (Figure 37.2). The current recommended approach to manage severe diabetes mellitus is to start chronic insulin therapy. This case report suggests that potent lifestyle approaches like the ketogenic diet may be useful instead of medication therapy.

**CONCLUSION**

In summary, lifestyle modification that reduces carbohydrate intake is effective in the treatment of obesity, T2DM, and metabolic syndrome.
A low-carbohydrate, ketogenic diet combines two approaches that, on their own, improve blood glucose control: weight loss and reduction of glycemic index/load diet response to dietary intake.

As for most ongoing lifestyle change programs, research efforts to improve dietary adherence are needed. We have found that introducing the concept that T2DM is possibly reversible, and that individuals may be able to have normal blood glucose off medications is highly motivating, and the reduction or elimination of medications may improve long-term adherence. Most individuals with T2DM have been told repeatedly that they will always have diabetes and always require medication—but this is clearly not the case in some individuals with amelioration of the underlying insulin resistance.

The underlying principle of carbohydrate restriction and the history of using the low-carbohydrate diet for T2DM suggest that the low-carbohydrate approach may play an important nonpharmacologic role in reversing the current epidemic of “diabesity” (Accurso et al., 2008; Feinman et al., 2015; Khazrai et al., 2014). Furthermore, it suggests that the common approach of antiglycemic medication + higher carbohydrate diets should be more rigorously tested for the treatment of T2DM.

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Keto-Adaptation in Health and Fitness

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INTRODUCTION
Not completely dissimilar from the gravitational potential energy of a ball sitting atop a tower, our bodies have an abundance of metabolic potential energy (MPE). This MPE can be found in two primary storage forms, carbohydrate and fats. Carbohydrates stored predominantly as glycogen in skeletal muscle and liver represent approximately 2,000 kcals of energy, whereas fat stores in adipose tissue are at least an order of magnitude greater. Thus, from an evolutionary standpoint, dietary carbohydrate more aptly represents a single matchbook of MPE, better suited for transient fight-or-flight responses and not the primary fuel source. Since storage is limited, when carbohydrate is consumed it must be oxidized or converted to something else. The glucose-insulin axis supports this hierarchy of fuel use, as acute elevations in insulin following consumption of carbohydrate promptly inhibit both adipose tissue lipolysis and systemic fat oxidation (Bonadonna et al., 1990; Jensen et al., 1989). Thus, when energy expenditure is high there is a constant need to supply a steady source of carbohydrate-based fuel. As exemplified by the metaphor “hitting the wall,” when carbohydrate stores are near depletion during exercise, the body cannot simply transition to using fat efficiently, unless, the person has undergone a very low-carbohydrate diet for several weeks, which results in a complex set of coordinated adaptations that dramatically accelerate the body’s ability to access and use lipid-based fuels. The rest of this chapter discusses the concept that humans function more efficiently when they are utilizing predominantly fat for fuel.

Modern diets emphasize carbohydrate-dense foods, and therefore bias metabolism toward carbohydrate oxidation while impairing fat oxidation. Whereas some individuals can maintain weight and metabolic health consuming low-fat/high-carbohydrate diets, the majority cannot, as evidenced by the fact that half the adults in the United States are prediabetic or diabetic (Menke et al., 2015).

The parallel increase in dietary carbohydrate and the sharp rise in obesity and diabetes in the United States is prima facie evidence that carbohydrate intake has an important effect on health status. The mechanistic links between dietary carbohydrate, oversecretion of insulin, and exacerbation of insulin resistance make the overconsumption of sugars and starches the most obvious driver of the obesity and diabetes epidemics. Intuitively, decreasing carbohydrate is an obvious solution. To what extent should carbohydrate be decreased? The answer is not simple and varies from person to person and even within an individual over time. For many with insulin resistance (i.e., carbohydrate intolerance), a modest reduction in dietary carbohydrate may be all that is necessary to maintain metabolic health. For others with a higher degree of insulin resistance, effective metabolic correction requires keeping carbohydrates under 40–50 g/day for an extended period of time, resulting in keto-adaptation.

Keto-adaptation is a process that sustains optimum fuel flow to all organs through use of metabolic pathways we have acquired over 2 million years as hunters/gatherers/herders. The most important aspect of keto-adaptation is the ability of the brain to displace glucose for ketones as the primary fuel source. Pioneering work in the 1960s and 1970s examining starvation ketosis showed that the brain will switch to preferential use of ketone oxidation (~60%) for derivation of energy (Cahill et al., 1970; Cahill and Owen, 1968; Owen et al., 1967). This preferential use of ketone bodies as a primary fuel source provides both an energy- and neuroprotective effect (Cahill et al., 1970). Similar effects are observed in non-starvation-induced ketosis, when carbohydrate is restricted and dietary protein and fat are present in the diet.
The metabolic pathways responsible for synthesis, transport, uptake, and oxidation of ketones are all up-regulated. Within days, blood levels of ketones increase from less than 0.1 mmol/L to 0.5 up to 3–5 mmol/L. This range is referred to as nutritional ketosis, and is associated with a positive effect on a broad range of cardiometabolic risk factors. It is noteworthy that ketoacidosis does not occur until ketones are above 10 mmol/L, a level only reached in individuals with insulin insufficiency (i.e., poorly controlled type-1 diabetics). Even in a starvation-induced ketogenic state with extreme exercise, an individual with a functioning pancreas will not experience the increased acidity associated with ketoacidosis.

The near-exclusive reliance on lipid-based fuel is a fundamental characteristic of keto-adaptation, but a new perspective of nutritional ketosis has emerged following the identification of β-hydroxybutyrate (βOHβ) as a potent epigenetic and signaling molecule (Newman and Verdin, 2014a). We now appreciate that keto-adaptation involves cellular adaptations that can phenotypically manifest in different ways between people, probably because of genetic and epigenetic effects. The duration of this chapter focuses on mechanisms associated with keto-adaptation as it pertains to health outcomes, and explores the emerging potential of well-formulated ketogenic diets to extend the physical and cognitive performance of athletes and military personnel. Although many studies have documented acute and short-term responses of ketogenic diets, the precise temporal pattern of responses to nutritional ketosis ultimately leading to the keto-adapted phenotype requires additional work (Figure 38.1).

**KETO-ADAPTATION AND HEALTH IMPLICATIONS**

Ketogenic diets have been shown to improve numerous health outcomes, especially those associated with insulin resistance, the underlying common feature in metabolic syndrome (MetS), type 2 diabetes, cardiovascular disease (CVD), obesity, polycystic ovarian syndrome (PCOS), and many other chronic health conditions.

**Ketogenic Diets Improve Insulin Resistance, Metabolic Syndrome, and Type 2 Diabetes**

A primary application of ketogenic diets in adults is the management of insulin-resistant conditions. Insulin resistance is the primary feature underlying type 2 diabetes, but it also exists across a continuum in the general population. It is defined by an impaired functioning of the insulin response at the cellular level, which given the pleiotropic effects of insulin can manifest in many different signs and symptoms. Most commonly, insulin resistance is viewed from the perspective of impaired skeletal muscle glucose uptake and increased hepatic glucose output, manifesting in hyperglycemia.
Thus, individuals with insulin resistance have a fundamental problem metabolizing dietary carbohydrate and maintaining a normal glucose level. Fasting blood glucose values greater than 100 mg/dL are indicative of impaired glucose tolerance, and values greater than 7.0 mmol/L (126 mg/dL) on two occasions indicate type 2 diabetes. Since type 2 diabetes is discussed in a separate chapter, we focus our discussion on the impact of ketogenic diets on milder forms of insulin resistance that manifest in the cluster of disturbances that encompass metabolic syndrome.

Metabolic syndrome is best described as prediabetes and provides an early sign that the body is mismanaging dietary carbohydrate. One aspect of this mismanagement is that a greater proportion of incoming carbohydrate is converted to fat in the liver (Petersen et al., 2007). This greater conversion of dietary carbohydrate into fat (i.e., de novo lipogenesis), much of it entering the circulation as saturated fat, is a metabolic abnormality that causes a significant amount of collateral damage in the body. For all these reasons, insulin resistance is a problem that effectively manifests itself as carbohydrate intolerance. Like other food intolerances, the most logical and effective approach to managing carbohydrate intolerance is to restrict the offending nutrient. When dietary carbohydrate is restricted to a level below which it is not significantly converted to fat (a threshold that varies from person to person), signs and symptoms of insulin resistance improve or often disappear completely. Based on our research, a ketogenic diet can resolve all the signs and symptoms of metabolic syndrome (Volek and Feinman, 2005; Volek et al., 2008).

The main features of metabolic syndrome are abdominal obesity; high blood levels of glucose, insulin, and triglycerides; low HDL-cholesterol; and high blood pressure. Other features not typically included in the diagnosis of metabolic syndrome include elevated constitutive inflammation, vascular dysfunction, and disturbances in fatty acid composition. It is commonly stated that obesity is the cause of metabolic syndrome, but this perspective fails to acknowledge the cause of obesity, which for many involves the overconsumption of dietary carbohydrate relative to a person’s tolerance. Thus, the primary driver of metabolic syndrome is overconsumption of sugars and starches relative to the level a person can metabolize without resorting to increased de novo lipogenesis and other manifestations of carbohydrate intolerance (e.g., increased oxidative stress). Although the molecular details of insulin resistance have not been fully elucidated, a well-formulated ketogenic diet results in broad spectrum improvements across a range of risk factors relative to a traditional low-fat diet in patients with metabolic syndrome, in effect putting the disorder into remission (Volek et al., 2009b). A large number of studies have shown that the features of metabolic syndrome improve, often in dramatic fashion, with adequate carbohydrate restriction (Volek and Feinman, 2005; Volek et al., 2008).

Insulin resistance is one of the primary predictors of nonalcoholic fatty liver disease (NAFLD), which is characterized by increased liver fat, inflammation, and resultant hypertriglyceridemia, all of which increase CVD risk. Although the pathophysiology and mechanisms of NAFLD are yet to be fully elucidated, there is a clear link to increased carbohydrate consumption, de novo lipogenesis, and increased adiposity. Insulin resistance in the peripheral tissues inhibits the cellular uptake of glucose, thus a shunting of dietary carbohydrate toward the liver occurs. Glucose flux into hepatic tissue is not insulin dependent, thus an increased rate of glycogen synthesis or de novo lipogenesis, during the postprandial state, is experienced. Hepatic insulin resistance is associated with increased concentration of malonyl-CoA; inhibition of carnitine palmitoyl transferase 1 (CPT-1), a transporter for fatty acids into the mitochondria; inhibition of beta-oxidation; and increased accumulation of triglycerides, cholesterol esters, and very low density lipoprotein (Browning et al., 2011; Zivkovic et al., 2007). Since keto-adaptation results in significantly decreased de novo lipogenesis and accelerated beta-oxidation, as well as decreased inflammation and serum triglycerides, it would seem obvious to also have beneficial effects on NAFLD. However, only a few studies have examined effects on liver fat. Early during keto-adaptation, approximately 2 weeks, there is significantly decreased hepatic triglyceride content, as compared to a higher carbohydrate diet (Browning et al., 2011). Six months of a ketogenic diet has been demonstrated to improve steatosis, necroinflammatory grade, and centrilobular fibrosis, all of which are histological markers of NAFLD (Tendler et al., 2007).

Weight Loss and Body Composition Responses

Dozens of studies have examined weight-loss responses to low-carbohydrate and low-fat diets over the last 15 years. Although there is great variability in the comparison of diets of any duration, it is noteworthy that low-carbohydrate diets do at least as well and usually better than low-fat diets according to recent meta-analyses (Ajala...
et al., 2013; Bueno et al., 2013). In fact, a recent meta-analysis, which used Bayesian hierarchical modeling to provide an estimate of the likelihood of achieving a desired degree of weight loss, reported that the probability of greater weight loss associated with a low-carbohydrate diet was >99% (Sackner-Bernstein et al., 2015). It is noteworthy that the degree of insulin resistance has an effect on expected weight loss. In a post-hoc analysis of the A to Z study, it was reported that individuals who were insulin sensitive had similar degrees of weight loss on a low- and high-carbohydrate diet after 1 year, but the those with higher levels of insulin resistance lost significantly more weight on a low-carbohydrate diet (McClain et al., 2013).

Several weight-loss studies have also measured changes in fat and lean body mass. Similar to changes in total body mass, low-carbohydrate diets have been shown to result in a greater loss of fat mass (Krieger et al., 2006). Outcomes for lean mass, however, are less clear due to variations in diet and limitations in diet formulation and assessment methods. Over 30 years ago Phinney et al. (1983) reported that nitrogen balance was positive after the first week of a eucaloric ketogenic diet. An important factor not always appreciated in studies of ketogenic diets is the need to adjust electrolyte intake to offset the natriuretic effect of carbohydrate restriction. Unfortunately, the majority of studies conducted since then have not considered this natriuretic effect. However, our research has shown it is possible not only to preserve lean mass during weight loss with a ketogenic diet but also to increase fat mass (McClain et al., 2013). An important consideration is the water retention associated with high-carbohydrate diets and glycogen storage (Olsson and Saltin, 1970), and hence the water loss that accompanies glycogen loss during carbohydrate restriction. Readings from dual-energy X-ray absorptiometry DXA (Going et al., 1993) and biological impedance (Koullmann et al., 2000), which are two commonly used methods of assessing body composition, fluctuate in accordance with body water content. Furthermore, these methods generally include total body water as a component of lean mass. Therefore, the water loss that typically occurs during the initiation of carbohydrate restriction can give a false indication of functional muscle loss.

As previously described, nutritional ketosis is characterized by an elevation in blood ketones and, depending on the extent of carbohydrate restriction, is not always synonymous with a low-carbohydrate diet. Infusion of βOHB, the primary ketone elevated during nutrition ketosis, reduces nitrogen excretion (Sherwin et al., 1975) and leucine oxidation (Nair et al., 1988) and increases muscle protein synthesis (Nair et al., 1988). As such, nutritional ketosis may be an important determinant of lean mass preservation during prolonged periods of energy deficit induced by caloric restriction and/or high energy expenditure.

Preservation of lean mass can further be supported through resistance training. In fact, our research has shown 12 weeks of resistance training to result in increases in lean body mass, along with greater fat loss, during a ketogenic diet versus a low-fat diet (Quann et al., 2007; Volek et al., 2010). Similar results have been observed in response to a ketogenic diet versus a typical Western diet (Rauch et al., 2014).

While the metabolic adaptations that occur during carbohydrate restriction clearly have an important role in weight loss, satiety is an important factor as well. In weight-loss trials comparing low-carbohydrate to low-fat diets, energy deficit for the low-carbohydrate diet is often achieved without intentional restriction, indicating greater satiety. Furthermore, very low calorie diets, which are often ketogenic due to the inherently low level of carbohydrates and protein, are known to reduce appetite. This is supported by a recent meta-analysis in which both ketogenic and very-low calorie diets were considered (Gibson et al., 2015). The similarities in satiety between these two diets, despite the considerable differences in protein, fat, and energy content, suggests that ketosis may suppress appetite. This is supported by studies showing a eucaloric or ad libitum ketogenic diet to be more satiating than nonketogenic diets with higher protein content (Johnstone et al., 2008; Veldhorst et al., 2010).

**Improved Lipoprotein Profile**

Although higher probability of weight loss is a major benefit of a ketogenic diet, there are also profound changes in lipoprotein metabolism (Volek et al., 2008). The reduced conversion of carbohydrate to fat in the liver (lipogenesis) plus the low insulin state enabling accelerated fat oxidation are major factors contributing to the improvements in processing of lipoproteins commonly observed. The most consistent response is a sharp decrease in plasma triglycerides, most dramatically in those with preexisting hypertriglyceridemia. A ketogenic diet also demonstrates a striking reduction in the postprandial lipemic response to a high-fat meal (Volek et al., 2000; Volek et al., 2009b) and has positive effects on postabsorptive and postprandial vascular function (Volek et al., 2009a). Almost
as consistent as their triglyceride-lowering effects, ketogenic diets increase HDL-C. The increase in HDL-C may not occur as quickly as the decrease in triglycerides, but there is some evidence that it develops more slowly over time (Shai et al., 2008). The response in LDL-C concentration to a ketogenic diet is highly variable, with many people showing a decrease and many an increase, with a subset (~15%) showing a marked rise. While there is variability in the LDL-C concentration response, there are more uniform patterns in qualitative features of the LDL particle. Dietary carbohydrate restriction consistently decreases small, dense LDL particles, which are more atherogenic due to their longer residence time in the circulation and greater propensity for oxidation, whereas low-fat/high-carbohydrate diets have the opposite effect. This inverse relationship between dietary carbohydrate content and LDL particle size is evident over a wide range of carbohydrate intakes (Krauss, 2005), and it can have quite dramatic (i.e., positive) effects in response to a ketogenic diet (Volek et al., 2009b).

**KETO-ADAPTATION IN SPORT AND FITNESS**

Beyond the clinical applications of keto-adaptation for promoting weight loss and managing insulin resistant conditions, there is a growing interest in ketogenic diets by athletes and active individuals interested in better health, performance, and recovery.

**Brief History**

More than 70 years ago (Christensen and Hansen, 1939) described that consumption of a high-carbohydrate diet, compared to a high-fat diet, for 1 week prior to a submaximal event was associated with enhanced performance. Nearly 3 decades later, Bergstrom and Hultman (1966, 1967) discovered that muscle glycogen depletion was associated with fatigue, and that a high-carbohydrate diet maintained muscle glycogen and performance. This set the stage for the next 40+ years, during which time the supremacy of carbohydrate has become deeply embedded in the minds of most scientists, coaches, and athletes (Hargreaves et al. 2004). There has been a strong confirmation bias to support high-carbohydrate diets, reinforced by a burgeoning sports beverage industry profiting from this paradigm.

It needs to be acknowledged that a lot of great work has been done by many researchers detailing how carbohydrate affects exercise metabolism and performance, but there has been a surprising lack of recognition, or willful neglect, of the alternative paradigm that deemphasizes carbohydrate. For example, there are few placebo-controlled randomized control trials, few studies of very low carbohydrate intake, and even fewer studies that allowed adequate time to adapt to a low-carbohydrate diet. Carbohydrate loading has not shown improved performance in all studies, despite significantly increased muscle glycogen levels (Burke et al., 2000). The most obvious fact that has been downplayed in prior research has been the profound effect of carbohydrate intake on inhibition of fat utilization, and the perspective that fat is a preferred fuel.

**Fat as a Premium Fuel**

Conventional wisdom has been that carbohydrate is the preferred source of fuel for exercise, but this perspective is rapidly changing considering the many benefits associated with emphasizing fatty acids and ketones as primary fuels, and relegating blood sugar and glycogen to secondary status. Ten hypothetical reasons a high capacity to utilize lipid-based fuels might be beneficial for athletes are listed here:

1. Fat is stored in adipose tissue in greater quantities than carbohydrates by at least an order of magnitude (>20,000 kcal in even the thinnest athlete vs. 2,000 kcal as glycogen). A key element contributing to deteriorating cognitive and physical performance during physically demanding exercise is reduced carbohydrate availability coupled with an inability to effectively utilize the thousands of calories stored as fat. Paradoxically, deteriorating performance associated with glycogen depletion occurs in the presence of an abundance of fuel stored as body fat that the athlete cannot access efficiently, unless they have previously keto-adapted for several weeks.

2. Fat is a more efficient source of fuel than carbohydrates. A molecule of palmitic acid has over twice as many carbons as a molecule of glucose, and over twice the energy per unit weight (i.e., 9 vs. 4 kcal/g). Fat is also more efficient, since it is not stored along with a lot of extra water weight like glycogen. Finally, ketones have been shown to have greater efficiency in providing cellular energy and work output (i.e., more work per unit oxygen consumed) (Sato et al., 1995).

3. Relying on body fat for energy significantly decreases the need to fuel during exercise.
This has physiologic benefits as it avoids the need to digest and absorb nutrients at a time when the majority of blood flow is diverted to active skeletal muscles. It also alleviates the gastrointestinal problems associated with fueling during exercise that are common among endurance athletes.

4. When oxidized inside the mitochondria, ketones generate less reactive oxygen species relative to glucose or other fuels, in essence making them a cleaner burning fuel (Maalouf et al., 2007; Veech, 2004).

5. Deriving greater amounts of energy from fat at higher exercise intensities translates into less reliance on limited glycogen stores, glycolysis, and generation of hydrogen ions.

6. Making fat the primary fuel often results in greater ease of fat loss and improved body composition, which translates into improved efficiency and power-to-weight ratio. Hypothetically this would be advantageous for endurance as well as many strength/power athletes.

7. When ketone concentrations increase to the 1–5 mmol/L range, the brain will begin to preferentially oxidize ketones (Cahill et al., 1970; Owen et al., 1967). In a non-keto-adapted athlete, continuous exercise often results in profound mental confusion (i.e., “hitting the wall” or “bonking”). A brain adapted to extracting the majority of its energy requirements from ketones is resistant to this energy crisis, since ketones are derived from fatty acids and actually increase in concentration as exercise duration increases. A keto-adapted brain is able to tolerate extraordinarily low levels of blood sugar without any signs or symptoms of hypoglycemia (Cahill and Aoki, 1980; Drenick et al., 1972).

8. Independent of its role as a vital fuel source for the brain and other organs/tissues when carbohydrates are limited, βOHb has recently been shown to directly signal cellular processes, acting more like a hormone (Newman and Verdin, 2014a, 2014b). Specifically, it was demonstrated that at blood levels characteristic of nutritional ketosis, βOHb acts as a class I histone deacetylase inhibitor, switching on specific genes that protect cells from free radical damage (Shimazu et al., 2013). Oxidative stress is a root cause of aging and inflammation, and the reduction by βOHb of these processes was remarkable.

9. One of the most common perceived benefits following keto-adaptation is enhanced recovery from exercise. This is frequently demonstrated by an expedited return to training and competition following physically demanding events and long training sessions. This area of research has not been investigated thoroughly, but we speculate that less oxidative stress and the anti-inflammatory effects of a ketogenic diet may contribute to the perceived improvement in recovery by many athletes. Carbohydrate ingestion and acute hyperglycemia activate a host of inflammatory and free radical-generating pathways (Aljada et al., 2006; Dhindsa et al., 2004). Some of these include: stimulation of NADPH oxidase, superoxide generation by leukocytes, TNF-α production, and activation of NF-κB which regulates the transcriptional activity of over 100 pro-inflammatory genes. Consistent with the notion that carbohydrate can aggravate inflammatory balance, many studies have reported that low carbohydrate diets decrease markers of constitutive inflammation in athletes (Phinney et al., 1983) and non-athletes (Youm et al., 2015).

10. A low carbohydrate intake enhances the effects of exercise on several positive cellular adaptations associated with health (Hawley and Burke, 2010). For example, in the post-exercise period consumption of even small amounts of carbohydrate rapidly decreases the release of fatty acids from fat stores and oxidation of fat in the muscle (Long et al., 2008). In some athletes, a surge in insulin may be followed by a low blood sugar eliciting a stress response characterized by a counter-regulatory hormonal response that can manifest as carbohydrate cravings, lethargy, poor physical/mental performance and suboptimal recovery. Carbohydrate consumption during recovery has also been shown to diminish the beneficial effects of exercise on insulin sensitivity and other cardio-metabolic risk markers (Holtz et al., 2008; Stephens and Braun, 2008).
THE KETO-ADAPTED ATHLETE

Although a critical mass of experimental data on ketogenic diets and ketone physiology has been generated in the last decade, demonstrating safety and therapeutic efficacy in managing a range of clinical conditions, there has been little effort focused on studying how ketogenic diets affect performance and recovery. The two notable studies that shed light on the metabolic phenotype of the keto-adapted athlete come from Phinney in 1983 (Phinney et al., 1983) and Volek in 2016 (Volek et al., 2016).

Phinney first demonstrated in a small (n=5) but highly controlled metabolic ward study that 4 weeks of adaptation to a ketogenic diet in high-level cyclists resulted in profound metabolic adaptations and restoration of endurance performance following an initial decline. These cyclists showed a remarkable near exclusive reliance on fat as the sole fuel at 64% maximal oxygen consumption, while significantly decreasing glycogen breakdown and glucose oxidation. For more than 3 decades these findings have been largely ignored but never refuted.

We designed the FASTER (Fat Adapted Substrate Use in Trained Elite Runners) study to validate and extend Phinney’s work by documenting the keto-adapted phenotype in elite athletes after long-term keto-adaptation (Volek et al. 2016). The primary goal was to characterize the metabolic differences between high-caliber ultra-endurance runners habitually consuming a very low-carbohydrate ketogenic diet versus a more traditional high-carbohydrate diet. Ten low-carb and 10 high-carb high-caliber ultra-runners were recruited and studied. They were carefully matched on age, body mass, maximum aerobic capacity, and competition times; the main difference was their habitual diet. The high-carbohydrate group consumed over half their intake as carbohydrate, whereas the low-carbohydrate athletes consumed ~10% of their energy intake from carbohydrate for an average of 20 months. Athletes performed a maximal oxygen consumption test during which peak fat oxidation was determined, and on a separate day they ran on a treadmill at 64% of maximal oxygen consumption for 3 hours.

The most remarkable finding was an extraordinary ability to oxidize fat in low-carbohydrate athletes, which was on average two-fold higher (1.5 vs. 0.7 g/min, respectively). Even the lowest “fat burner” in the low-carbohydrate group exceeded the highest “fat burner” in the high-carbohydrate group. These rates of fat oxidation are even more remarkable considering that the highest values reported in the literature (with the notable exception of the cyclists in Phinney et al., 1983) are 50% lower (i.e., ~1.0 g/min). Fuel use during the 3-hour run was also dramatically different. The high-carbohydrate group used an even mix of fuel, whereas nearly 90% of energy expenditure was derived from fat in the low-carb athletes. This translated into a two-fold greater use of fat (~1.2 vs 0.6 g/min) during exercise. Figure 38.2 shows how this enhanced reliance on fat oxidation could enable endurance athletes to compete without need for exogenous fuel. Serum glycerol concentrations, a marker of adipose tissue lipolysis (fat breakdown), were two-fold higher during exercise and recovery in low-carbohydrate runners, further supporting the dramatically accelerated rates of fat metabolism in the keto-adapted runners (Figure 38.2).

Despite the extraordinary transformation to extract the majority of energy from fat during submaximal exercise in the low-carbohydrate athletes, they showed a surprisingly high level of muscle glycogen at rest, glycogen breakdown during exercise, and synthesis of glycogen during recovery. This result was different from that observed in keto-adapted cyclist after 4 weeks, suggesting that complete adaptations in glycogen metabolism may occur over a longer period than 1 month. Although speculative, we hypothesize there is a need to provide a source of oxaloacetate for optimal Krebs cycle functioning in the keto-adapted state, and breakdown of glycogen, without terminal oxidation, provides a source of carbons for that purpose.

SUMMARY

The long-standing dogma in sports nutrition has been that athletes need to consume a high-carbohydrate diet in order to excel physically and cognitively. This approach may work for some athletes, but for many it is associated with less than desirable health and performance outcomes. Ketogenic diets represent an alternative approach with a rapidly growing level of scientific and observational support. Although it is clear that ketogenic diets are an effective weight-loss intervention often leading to greater fat loss than low-fat diets, it remains to be determined whether the benefit is exclusively a result of carbohydrate restriction or whether nutritional ketosis provides added benefit. Given the new perspective of βOHB as more than a metabolite, there are now more reasons to speculate why a well-formulated ketogenic diet would be associated with more robust health and performance effects. In regard to metabolic syndrome,
Ketogenic diets are more likely than low-fat diets to effect global improvement in the diverse signs and symptoms of this complex disease highly associated with type 2 diabetes. Moreover, treating any of the individual metabolic syndrome markers with carbohydrate restriction holds the promise to benefit the others. Therefore, a ketogenic diet is the preferred primary intervention.

In regard to physical activity, ketogenic diets dramatically transform energy metabolism during exercise and may also facilitate recovery through modulation of inflammation, free radical generation, and antioxidant defense mechanisms. However, as with weight loss, it is not clear whether nutritional ketosis provides added benefit beyond carbohydrate restriction without ketosis. There are many health benefits associated with nutritional ketosis that are relevant for athletes. In regard to exercise performance, field testing indicates a high level of success among many endurance athletes, but there has been very little laboratory testing focused on performance outcomes. Hopefully that will change as we dawn on a new era of sports nutrition that looks beyond the high-carbohydrate paradigm to alternative approaches that emphasize a fat-based metabolism.

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Chapter 38: Keto-Adaptation in Health and Fitness

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Advancing the Awareness and Application of Ketogenic Therapies Globally

The Charlie Foundation and Matthew’s Friends

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OVERVIEW
The Charlie Foundation was born out of the desire to spare children from the unnecessary suffering that Charlie Abrahams endured before he achieved seizure freedom with the ketogenic diet. Jim and Nancy Abrahams shared their story in 1994 on Dateline and through a 1999 movie called First Do No Harm. Across the Atlantic in the United Kingdom, Matthew Williams suffered for 6 years with a devastating seizure disorder before becoming seizure-free within 2 weeks of starting the diet. Based on this experience, Matthew’s Friends was founded in 2004 by Emma Williams, Matthew’s mother, with a similar mission as the Charlie Foundation. Despite these dramatic testimonials and dedicated foundations, the diet remained underutilized. Several key breakthroughs came in 2008: The Charlie Foundation had commissioned medical professionals with ketogenic experience to collaborate on guidelines for prescribing the diet. This culminated in a publication in Epilepsia, a prestigious international medical journal. During the same year, a Class I study was published in Lancet Neurology confirming the diet’s efficacy as a treatment for epilepsy; more positive Class I studies followed. Use of ketogenic diet therapy spread rapidly worldwide, and with increased use came a broader understanding of its benefits for other disorders. Less restrictive versions of the diet were developed to meet the needs of older children and adults. In 2012 the Charlie Foundation also began educating all people with epilepsy to eliminate sugar, reduce refined carbohydrates, and choose a predominantly whole foods diet. In addition, both foundations have expanded efforts to address other conditions that can benefit from ketogenic therapies including neurological and neurodegenerative disorders and certain cancers.

GENESIS OF THE CHARLIE FOUNDATION
Charlie Abrahams, the youngest in a family of three children, had no apparent health problems until his first birthday in March 1993, when he experienced his first seizure. This was followed by a series of tumultuous events that changed his life as well as the lives of his siblings, Joseph and Jamie; his parents, Jim and Nancy; and ultimately thousands (possibly millions) of others. His father Jim described those months as follows:

After thousands of epileptic seizures, an incredible array of drugs, dozens of blood draws, eight hospitalizations, a mountain of EEGs, MRIs, CST scans and PET scans, one fruitless brain surgery, five pediatric neurologists in three cities, two homeopaths, one faith healer, and countless prayers, Charlie’s seizures were unchecked, his development “delayed” and he had a prognosis of continued seizures and “progressive retardation.”

Charlie lived that period in his bed, on a blanket, or in an infant seat, where he was safely padded during the violent seizures that caused him to thrash uncontrollably. The adverse effects of the antiseizure medications were nearly as destructive as the seizures themselves. Between seizures, he was groggy, chronically constipated, and in the words of his father, seemingly “drunk.” Charlie also underwent a high-risk brain surgery to attempt to remove the area of the brain suspected to be the source of his seizures. He
Chapter 39: Awareness and Application of Ketogenic Therapies Globally

Experienced zero improvement, and the seizures continued.

Exhausted by the failure of the medical community to provide an effective therapy and reeling from the severe disruption inflicted on the rest of the family, Jim began looking for another option. He spent hours in the medical library of UCLA reading through literature on epilepsy, and it was there that he came across an epilepsy book containing a chapter on ketogenic diet as a treatment for epilepsy. He subsequently discovered a 1992 study from Johns Hopkins University that had been published in Epilepsia documenting 29% seizure freedom with an additional 30% significant improvement in 58 consecutive cases of children who were placed on a ketogenic diet (Kinsman et al., 1992). Notably, these children were as compromised by their disease as Charlie. Jim brought this book and the study to Charlie’s pediatric neurologist. Although he was aware of the diet, he immediately dismissed it as a treatment that “he had never seen work.” Despite this discouragement, Jim and Nancy made the decision to take Charlie to Johns Hopkins Hospital in Baltimore, Maryland. (At the time, Johns Hopkins was one of the few hospitals in the United States that had provided the ketogenic diet continuously since its discovery in 1921.) Dr. John Freeman and the dietitian Millicent Kelly formed the hospital team that implemented Charlie’s ketogenic diet.

Within 3 days of starting this treatment, Charlie was seizure-free. Over the course of the following month he was weaned off of the four failed antiseizure medications. Free from the sedating effects of antiseizure medication, Charlie could finally hold his head up and once again interact with his family. Although the diet required careful measurement and specific foods, Jim described this as a “walk on the beach” when compared with their previous circumstances. This abrupt and almost incomprehensible reversal left the Abrahams with a diverse range of emotions; exhilaration and gratitude over Charlie’s sudden recovery was mixed with anger over the 9 months of unnecessary suffering that he had endured.

Jim and Nancy appeared on NBC-TV’s Dateline in 1994 to recap those months. They soon found their mailbox overflowing with letters from other families, thanking them for bringing the diet out into the light of day. Each letter described a similar journey to the one the Abrahams experienced with Charlie. The names and ages of the kids were different, but the stories were the same. Uncontrolled seizures, multiple medications, trips to the emergency room, mounting medical bills, and a drugged, poorly functioning child in a rapid decline. Adoption of the ketogenic diet was the common denominator, and, for some, their lifesaver.

One letter came from a family who had to mortgage their farm to pay their son’s medical bills. The child was admitted to a hospital for treatment of uncontrollable seizures and placed under heavy sedation. The family requested a trial of the ketogenic diet, but the treating physicians refused to consider this. Instead, they wanted to add more medications and had recommended surgery. With the help of a compassionate nurse, the family made the difficult decision to leave without medical authorization. They flew directly to Johns Hopkins Hospital. Several days later, this child, like Charlie, became seizure-free.

Jim was compelled to use his professional skills as a movie producer and his connections in the industry to find ways to help more families learn about the diet. He wrote, directed, and produced a dramatization of the devastating impact epilepsy can have, and the potential for the diet to stop seizures when nothing else has helped. In 1997 this story aired in a made-for-TV film called First Do No Harm. The film starred Meryl Streep (who received a Humanitas Award for her performance), and the movie became a conduit for spreading the word about the ketogenic diet worldwide. Twenty-two years later, the Charlie Foundation continues to hear from people who learned about the diet through this movie. Despite the diet’s over-90-year history, many physicians first learn about the diet from the families of their patients.

The Abrahams founded the Charlie Foundation to Cure Pediatric Epilepsy in 1994. Funding was provided for public awareness and research in animal models. Videos were developed for families and medical professionals, and the Johns Hopkins team received financial support for a book, The Ketogenic Diet: A Treatment for Epilepsy (now in its 6th edition). Over the last two decades, the Charlie Foundation has organized educational conferences, maintained a website, and trained medical teams at hospitals worldwide.

### GENESIS OF MATTHEW’S FRIENDS

Matthew Williams was born in 1995. He suffers with a catastrophic form of epilepsy called Dravet syndrome, and his seizures started when he was 9 months old. Like Charlie, his seizures were severe, frequent, and uncontrolled by medication. Emma, his mother, asked if Matthew could try the ketogenic diet when he was 2 years old; she was
told the diet “didn’t work.” As none of the medications helped, she continued to ask periodically about the ketogenic diet and was given a variety of responses; she was told the diet was “too hard” and caused “terrible side effects.” Like the Abrahams, Emma battled on, trying innumerable medications that did not help Matthew’s seizures and themselves caused devastating side effects. Neither family was told that it has long been known that once the first medication fails the chance of seizure control with another medication or an added medication is lower, and even lower with successively added drugs (down to 2.7% seizure control at the third round; Mohanraj and Brodie, 2006). There is even evidence that polypharmacy can aggravate seizures (Perucca et al., 1998; Shorvon and Reynolds, 1979). Matthew continued to suffer from thousands of seizures, and his situation did not improve on what Emma termed the “merry-go-round” of medications. Emma was told that if Matthew lived to age 12 he would likely be confined to a residential home.

When Matthew was 7, at a routine appointment Emma insisted on trying the diet—the only other option was new combinations of drugs that had already been proven ineffective and shown to cause terrible side effects in Matthew:

“The side effects of the medications were awful, he was on a considerable amount of medication and his quality of life was so poor already that it really could not have got any worse for any of us.”

Fortunately there was a new option. Six years after Emma first asked about the ketogenic diet, Matthew and 144 other children with severe epilepsy were enrolled in a clinical research trial of the ketogenic diet spearheaded by Dr. Helen Cross at Great Ormond Street Hospital (GOSH), London, England (Neal et al., 2008). Within 2 weeks of starting the diet Matthew’s seizures had reduced by 90% and within 8 months he was off all medication. His behavior improved dramatically. Sadly for Matthew, however, the damage had been done. Years of thousands of seizures had caused terrible brain damage, his family had broken apart, and Emma was now a single mum to Matthew and his younger sister, Alice.

Inspired by Matthew, and hoping she could spare other families from what they had endured, and particularly prevent others from lifelong disabilities, Emma started Matthew’s Friends in 2004—initially running the charity out of her kitchen. She, like Jim and Nancy Abrahams, was shocked and angry at the lack of information and the underutilization of the diet—even when Emma had asked about it years earlier! She was determined to raise awareness and access to the ketogenic diet.

**TEN YEARS OF ACCELERATING AND COMPLEMENTARY EFFORTS**

The past 10 years have been a period of enormous growth and revitalization for the field of metabolic therapy and for both foundations. Beth Zupec-Kania, a registered dietitian and nutritionist, joined the Charlie Foundation in 2006 to develop resources and trainings for ketogenic therapies. She designed an online ketogenic diet calculator program (KetoDietCalculator.org), which allows nutritionists to efficiently create diets including meal plans, special recipes, and infant and feeding tube formulas. A Help Line within the program supports clinicians by answering daily questions. KetoDietCalculator is provided without cost to licensed nutritionists, who can choose to extend access to their patients or clients. It receives regular updates and additions and has been used by over 50,000 people worldwide. Figure 39.1 displays a screenshot of a typical meal (4:1 ratio of fat to non-fat grams) designed using the KetoDietCalculator.

In 2007, the Charlie Foundation estimated that fewer than 15 of the 200 children’s hospitals in the United States had a ketogenic diet therapy program. Frustrated by this lack of progress, the Charlie Foundation commissioned a group of physicians and dietitians to collaborate on methods of providing ketogenic diets. Their report, published in 2008 in *Epilepsia* was titled “Optimal Clinical Management of Children Receiving the Ketogenic Diet: Recommendations of the International Ketogenic Diet Study Group” (Kossoff et al., 2009). This consensus statement became the cornerstone for developing new programs. The report identified which patients were most likely to benefit, outlined methods of starting the diet, and provided monitoring guidelines. The group summarized its conclusions as follows:

The ketogenic diet (KD) should be strongly considered in a child who has failed two to three anticonvulsant therapies, regardless of age or gender, and particularly in those with symptomatic generalized epilepsies. It can be considered the treatment of choice for two distinct disorders of brain metabolism, Glut-1 deficiency syndrome and Pyruvate Dehydrogenase Deficiency Disorder. In the
particular epilepsy syndromes of Dravet syndrome, infantile spasms, myoclonic-astatic epilepsy, tuberous sclerosis complex, the KD could be offered earlier.

In the same year as this collaborative report, a randomized-controlled study of children receiving the classic and medium chain triglyceride-supplemented ketogenic diets was published in Lancet Neurology (Neal et al., 2008). This is the clinical trial in which Matthew Williams participated. Children between the ages of 2 and 16 with refractory seizures continued their current treatment and were randomized to a ketogenic diet or continued standard of care for 3 months. The results were clear: children treated with the diet were significantly improved compared with baseline and with the control group; some had over a 90% reduction in their seizures or were seizure-free. Seizure frequency in children who continued to receive the standard of care worsened compared with baseline, and none had over a 90% reduction or were seizure-free. This Class I study is considered the highest level of conclusive evidence and was the affirmation that doctors and advocates had been waiting for. It had been nearly 90 years since the ketogenic diet was developed and finally a comparison of the diet as treatment for difficult-to-control epilepsy against multiple antiseizure medications proved that the diet was superior in effectiveness. Recently, another Class I study found similar results (Lambrechts et al., 2016).

The combination of the Epilepsia and Lancet Neurology publications gave further credence to the diet, and in 2010 led to the requirement for inclusion of ketogenic diet therapies in Level 3 and 4 Epilepsy Centers in the United States. This distinction is granted by the NAEC (National Association of Epilepsy Centers) to centers that provide medically approved epilepsy treatments.

A SPECTRUM OF KETOGENIC DIET THERAPIES

Expanding use of the ketogenic diet for epilepsy paved the way to variations in the diet, particularly for adults and for older children who find it difficult to adhere to a restrictive diet. Dr. Eric Kossoff at Johns Hopkins developed a modified version of the ketogenic diet known as the modified Atkins diet (Kossoff et al., 2003). At about the same time, Heidi Pfeifer, a Massachusetts General Hospital dietitian, developed the low glycemic index treatment (LGIT; Pfeifer and Thiele, 2005). These two newer diets are similar to the classic ketogenic diet in their high-fat, low-carbohydrate content, but are different in that they could be initiated outside of the hospital and do not require careful weighing of each food. The modified Atkins diet was shown to be effective in a Class I study (Sharma et al., 2013). Clinical reviews began to report the efficacy of the modified Atkins and LGIT diets as being “nearly as effective” as the classic ketogenic diet (Coppola et al., 2011; Karimzadeh et al., 2014; Thibert et al., 2012). These two diets are often easier for older children and adults to manage and are sometimes used as predicates to the classic ketogenic diet.

For now, the classic ketogenic diet remains the most effective diet therapy based on current scientific evidence. However, less strict versions may be more realistic for certain individuals, especially older children and adults. These metabolic diet treatments are referred to collectively as “ketogenic therapies.” The Charlie Foundation published a chart (Table 39.1) that outlines the differences.
<table>
<thead>
<tr>
<th>QUESTIONS</th>
<th>Ketogenic Therapies</th>
<th>MCT Oil</th>
<th>Low Glycemic Index Treatment</th>
<th>Modified Atkins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is medical supervision required?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Is diet high in fat?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Is diet low in carbohydrate?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>What is the ratio of fat to carbohydrate &amp; protein?</td>
<td>4:1, 3:1, 2:1, 1:1</td>
<td>Approximately 1:1</td>
<td>Approximately 1:1</td>
<td>Approximately 2:1</td>
</tr>
<tr>
<td>How much carbohydrate is allowed on a 1000 Calorie diet?</td>
<td>8gm carb on a 4:1</td>
<td>40-50gm</td>
<td>40-60gm</td>
<td>10gm adolescents or 15gm adults for 1 month 20gm afterwards</td>
</tr>
<tr>
<td>How are foods measured?</td>
<td>Weighed</td>
<td>Weighed or measured</td>
<td>Measured or estimated</td>
<td>Estimated</td>
</tr>
<tr>
<td>Are meal plans used?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Optional</td>
</tr>
<tr>
<td>Where is the diet started?</td>
<td>Hospital</td>
<td>Hospital</td>
<td>Home</td>
<td>Home</td>
</tr>
<tr>
<td>Are calories controlled?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Are vitamin and mineral supplements required?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Are liquids (fluids) restricted?</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Is a pre-diet laboratory evaluation required?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Can there be side-effects?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>What is the overall difference in design of these diets?</td>
<td>This is an individualized and structured diet that provides specific meal plans. Foods are weighed and meals should be consumed in their entirety for best results. The ratio of this diet can be adjusted to effect better seizure-control and also liberalized for better tolerance. This diet is also considered a low glycemic therapy and results in steady glucose levels.</td>
<td>An individualized and structured diet containing Medium Chain Triglycerides (MCT) which are highly ketogenic. This allows more carbohydrate and protein than the classic ketogenic diet. A 2008 study showed that both diets are equal in eliminating seizures. A source of essential fatty acids must be included with this diet.</td>
<td>This is individualized but less structured diet than the ketogenic diet. It uses exchange lists for planning meal and emphasizes complex carbohydrates. The balance of low glycemic carbohydrates in combination with fat result in steady glucose levels. It is not intended to promote ketosis.</td>
<td>This diet focuses on limiting the amount of carbohydrate while encouraging fat. Carbohydrate may be consumed at any time during the day as long as it is within limits and should be consumed with fat. Suggested meal plans are used as a guide. Protein is not limited but too much is discouraged</td>
</tr>
</tbody>
</table>
FIGURE 39.2 Charlie Foundation logo.

FIGURE 39.3 Charlie thanking Meryl Streep for her role in First Do No Harm.
between the therapies to assist families and health professionals in selecting the best option.

Both the Charlie Foundation and Matthew’s Friends offer a wealth of recipes and expertise, including demonstration videos. Their tireless dedication to developing and sharing recipes has helped make the diet enormously more palatable and accessible to patients and their families.

**OTHER CONDITIONS THAT MAY BENEFIT**

A study published in 2008 in *Lipids* laid the groundwork for an entirely new application of ketogenic diet therapy. Metabolic syndrome is a diagnosis that includes three or more of the following abnormalities; abdominal obesity, elevated blood pressure, elevated fasting blood glucose, and elevated lipids or triglycerides. This syndrome is currently an epidemic in many areas of the world, including the United States and the United Kingdom. Improvements in metabolic syndrome were significantly better in a group that followed a modified ketogenic diet versus those who followed a low-fat, high-carbohydrate diet (Volek et al., 2008). Due in part to the appetite-suppressing effect of ketosis, fewer calories are consumed—resulting in weight and adipose tissue loss along with lowered blood glucose, triglycerides, and saturated fatty acids.

People inquiring about ketogenic therapy for brain cancer have become the second-largest group (next to epilepsy sufferers and their families) requesting information from both organizations. These individuals often experience seizures; therefore the role that the ketogenic diet offers as an antiseizure, anti-inflammatory, and antitumor therapy offers multifaceted benefits (Seyfried and Mukherjee, 2005; Veech, 2004). Several studies are currently in progress through the National Institutes of Health (NIH) investigating ketogenic diet effects on cancer. Recently Matthews Friends has partnered with the Astro Brain Tumour Fund to help adults and children with brain cancer.

Additional applications of ketogenic diet therapies for other conditions have grown exponentially in recent years. Benefits have been reported in a multitude of neurological disorders and

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**FIGURE 39.4** Charlie reading to his preschool students in Los Angeles.

**FIGURE 39.5** Matthew’s Friends logo
animal models of disorders including autism, certain mitochondrial diseases, diabetes, migraine, Prader-Willi syndrome, neurodegenerative diseases including Alzheimer’s and Parkinson’s, traumatic brain injury, and stroke. New research may expand the mission of the Charlie Foundation and Matthew’s Friends—or may bring new partners supporting metabolic therapies for diverse applications.

In response to the increasing demands for adult resources, the Charlie Foundation produced a guide in 2014 titled “Modified Ketogenic Therapy for Neurologic and Other Conditions.” Intended for use under medical supervision, it describes portion sizes for protein, fat, and carbohydrate and advice for optimal nutrition. The Charlie Foundation has shared resources with oncology medical professionals and expanded its website information to include this new population of users. A new link on the charliefoundation.org landing page has been added to ketogenic diet studies conducted through the NIH for cancer, epilepsy, and other disorders.

Medical supervision for ketogenic therapies and choosing the appropriate diet therapy for each individual is an evolving focus. The spectrum of therapies facilitates selecting the best diet for individuals taking into account their condition, diagnosis, age, and ability to comply. Matthew’s Friends developed a support system to help families prior to and during the diet to enhance the likelihood of success. Adjusting the therapy during the course of treatment to optimize effectiveness is often necessary. Discontinuing the diet is usually the goal in childhood epilepsy: two to three years is the typical course of treatment. However, in some cases of epilepsy and other neurological disorders it may be necessary to continue with a modified version of the diet indefinitely. These discussions are frequently addressed at the Charlie Foundation and have become the topics of collaborative journal articles and professional guidelines spearheaded by nutritionists.

News of ketogenic diet therapies spreads quickly through social media and the popular press. Thousands of copies of the Charlie Foundation’s Parent’s Guide to the Ketogenic Diet have been distributed in English and Spanish. Consultant chef Dawn Martenz and nutritionist Beth Zupec-Kania have collaborated on a new cookbook, Keto Cookbook II (Demos Publications). Cooking videos of the most popular keto recipes have been added to the charliefoundation.org website, and posts of creative snacks and meals are added to the Facebook page regularly. In addition, an App is under development for the modified ketogenic diet. Similarly, Matthew’s Friends is dedicated to developing and publishing delicious keto-friendly meals.

Both the Charlie Foundation and Matthew’s Friends have been continuously supportive of research and have partnered in funding global symposia on ketogenic diets. They stay abreast of new research related to ketogenic diet therapies. A recent study in the journal Obesity (2015) showed dramatic improvements in the health of obese children in just 10 days after eliminating sugar in their diets (Lustig et al., 2016). The metabolic impact of a sugar-free diet is one element of ketogenic diets that can be undertaken by anyone without the need for medical supervision. Eliminating sugar can be a difficult task for most people and requires strong motivation. A 2007 study published in Neuroscience and Behavioral Reviews showed that rats that were fed sugar intermittently had behavioral and neurochemical changes that resemble the effects of substance abuse (Avena et al., 2008). Eliminating refined (processed) foods is a second step that can be taken to improve one’s diet.

The Charlie Foundation has translated these and other similar research findings into pragmatic
guidelines. A new publication, *Does What I Eat Affect My Epilepsy?* outlines steps that can be taken to eliminate sugar and refined foods and to consume a mostly whole foods diet. This has been distributed widely (in English and Spanish) for all people with epilepsy regardless of their interest in ketogenic diet therapy.

For conditions that may benefit from ketogenic therapies, the Foundation designed a Preketogenic Diet. This document is distributed (free) to health professionals to provide to their patients or clients who are potential candidates for ketogenic diet therapy. Similar to the classic ketogenic diet, it eliminates gluten and sugar; however, it is not intended to induce ketosis. Instead, it can be described as a whole foods Mediterranean-style diet that prepares the user for the transition to ketogenic therapy and also aids in determining which diet in the spectrum is best suited for their needs and preferences. In addition, healthcare professionals can use the Preketogenic Diet as a screening tool to identify which individuals are able to adhere to the lifestyle changes required for ketogenic diets.

### The Charlie Foundation and Matthew’s Friends—Looking to the Future

In 2013 the Charlie Foundation renamed itself the Charlie Foundation for Ketogenic Therapies to better define its mission for the future: advocacy, awareness, and education. An expansion of education efforts includes adult-focused media and training. Development of new resources in both English and Spanish remains a goal. A list of current publications is shown in Box 39.1.

Throughout the 22 years of its existence, the Charlie Foundation has continued to receive daily e-mails and letters from families and health professionals requesting assistance. Jim Abrahams has responded to the majority of these requests, triaging some of them to support staff. Although Charlie has been seizure- and drug-free and off of the diet since 1999, Charlie’s family continues to represent ketogenic therapies in their Los Angeles community and on a national level through partnerships with other nonprofits and with supportive commercial organizations. Over 160 medical centers have been trained in the spectrum of ketogenic therapies in the United States and beyond, including Canada, Portugal, Austria, Jamaica, Slovenia, Kuwait, Saudi Arabia, and the Republic of Georgia. Beginning in 2008, the Charlie Foundation has also sponsored and organized global symposia bringing together leading scientists and medical professionals with the intent of advancing research and clinical use of ketogenic therapies. Plans for future symposia are in-the-making through the year 2022. As new clinical and scientific research continues to emerge, the Foundation will respond with further resources to promote safe and effective use of ketogenic therapies and make it less daunting for the user to manage.

Matthew’s Friends grew rapidly from Emma’s kitchen table, and in 2011 opened its own clinic. The charity has continued to grow rapidly, and recently expanded into New Zealand and Canada. In 2016 it embarked on a new program called KetoCollege, to offer training for teams around the globe, adding to and complementing the efforts of the Charlie Foundation to train ketogenic diet teams at their home institutions around the world.

Now seizure-free young adults—no longer on a special diet—Charlie Abrahams and Matthew Williams are two clear examples that even catastrophic epilepsy can be cured by a ketogenic diet. A question frequently asked about Charlie, Matthew, and others who have become seizure-free on a ketogenic therapy is “How is it possible that they can come off of the diet and remain seizure-free?” At the present time, there is no clear answer to this question. Emerging genetic research may soon clarify whether early application of ketogenic therapy may either ameliorate the disease or even cure it. The field of nutritional genomics

### BOX 39.1

**Charlie Foundation Education Resources**

- Parent’s Guide to the Ketogenic Diet
- Modified Ketogenic Diet Therapy: 1:1 and 2:1 Prescriptions
- Does What I Eat Affect My Epilepsy?
- Preketogenic Diet; Low-Carb, Gluten-Free, High-Fat
- KetoDietCalculator Guide for the Nutritionist and the User
- Professional’s Guide to the Ketogenic Diet
- Frequently Asked Questions about Ketogenic Diets
- Comparison of Ketogenic Diet Therapies
- Ketogenic Diet Primer for Health Professionals
looks at the effect of nutritional changes on genes and suggests that maladaptive epigenetic changes can be altered by diet. Targeted diet therapies are already being prescribed along with genetic testing for certain metabolic disorders.

Implementation of a restrictive diet is easiest in infants and children (given their reliance on the parents) and becomes more challenging as children gain independence. Early initiation of ketogenic therapies is better tolerated in young children—which may result in improved compliance and outcomes. Nevertheless the diet does work in adults, and has ever-increasing applications.

REFERENCES
Abbott, N. J., 289, 292–294
Abdallah, D. M., 174–175
Abrahams, Charlie, 386
Abrahams, Jim, 3
AC-1202, 319–320
acetone from acetoacetate, see also ketone bodies
acetone from, 113
administration, 124
Alzheimer’s, 122f
brain energy source, 113
brain uptake, 296f
hippocampal slices, 187–190, 189t
inflammation, 150–151
ketogenic diet, 90, 136
Krebs cycle, 243f
mitochondrial metabolism, 315f
NADP system, 263f
neuroprotection, 135
prolyl-hydroxylase inhibition, 220
redox potential, 262t
synthesis and metabolism, 177, 187, 228, 244, 247–248, 248f, 296f
VGLUTs, 281
acetone
from acetoacetate, 113
ketogenesis, 119f
acidosis, 67
actin, 293
Adabi Mohazab, R., 174
adenosine
on inflammation, 151f, 152–153
pain, 201
retaliatory metabolite, 210
adenosine homeostasis
seizures, 209–210
temporal lobe epilepsy, 211–213, 212f
adenosine kinase, epilepsy overexpression, 209–210
adherence, 19
Adk gene, epilepsy, 210–211
adult polyglucosan body disease, triheptanoin, 341–342
adverse effects. see side effects
aerobic fermentation, 80
aerobic glycolysis, 80
aging
cell senescence, 257
neuroprotection, ketosis, 221–222, 221f
reactive oxygen species, 256–257
telomere shortening, 256–257
aging and neurodegeneration, 216–222
cataplerosis–anaplerosis balance, 218
glucose and ketone metabolism, 113–127 (see also Alzheimer’s disease)
glucose metabolism, oxidative stress, 217–218, 218f
HIF1α stabilization, 219–221
ketone bodies, 216–217
ketosis, 217
ketosis neuroprotection, clinical relevance, 218–219, 219f
Ahn, Y., 107
D-alanine, seizure onset protection, 348
Allen, B. G., 92, 367
Allen, Frederick Madison, 364, 365
Alpers disease, status epilepticus, 61
“alternative” ketogenic diets, 5–13. see also specific types
choice, 11–12
effectiveness, vs. classical ketogenic diet, 10–12, 10t
initiation and follow-up, 12–13
low glycemic index treatment, 6f, 9–10, 9b
medium chain triglyceride, 5–7, 6b, 6f
modified Atkins diet, 6f, 7–9, 8b, 8t
Alzheimer, Alois, 242
Alzheimer’s disease, 113–127, 241–249
anaplerosis, 124–125
arteriovenous difference, brain, 114–115, 115t
brain energy status, neuroprotection, and mitochondrial function, 125–126
early brain development and evolution, 126
environmental factors, 241–241–242
genetics, 241
glucose, cerebral use, 243–244
Alzheimer’s disease, (cont.)
glucose, presymptomatic hypometabolism, 116–118, 116f, 117f
glucose, uptake and metabolism, 113–114
hyperketonemia with cognitive deficit, 123–124, 125t
hypometabolism, 216–217
incidence, 241
ketone bodies, available forms, 247–249, 248f, 249f
ketone kinetics and transport, 118–120, 120f, 120t
ketone regional uptake, early disease, 121–122, 121f, 122f
ketones, brain fuel, 113, 118, 119f
ketone supplementation, 318–320, 319f
medium chain triglycerides, dose-response
ketogenesis, 6f, 122–123, 123f
medium chain triglycerides, safety, 126
nutritional and metabolic factors, 241–242
pathophysiology, 242–244, 243f
perspectives, 126–127, 127f
PET-FDG protocol, 114
predisposing factors, 241–242
prevention, 113
risk factors, 113
TCA cycling, 336, 337f, 338f
- antioxidant function, 138
- brain energy source, 115f, 121–122, 121f
- brain uptake, 296f
- cancer, 81–82, 83
- on cellular processes, 381
- hippocampal slices, 187–190, 189f
- inflammation, 150–151, 151f
- ketogenic diet, 90, 281
- liver synthesis, 156
- medium chain triglycerides, 123f
- metabolism and sum reactions, 243, 243f, 246f–248f,
- 247–248
- mineral salts supplementation, with medium chain
- triglycerides, 311–312
- neuroprotection, 124, 124f, 125t, 134

astrocytes
- BAD-altered, metabolic changes, 272
- blood-brain barrier, 290f, 292–293
- athlete, keto-adapted, 382, 383f
- autism spectrum disorder, 101–107
- animal models, 105–107
- definition, 101
- effects, 105
- epidemiology, 101
- etiology, 101
- future research, 107
- ketogenic diet, 103–105
- mitochondrial and metabolic dysfunction, 102–103,
- 104f–105f
- pathophysiology, 101–102

Babayan, Vigen K., 311
BAD, 271
- on KATP channels, 271, 275
BAD channels, metabolic seizure resistance,
- 271–274, 277f
- glucose and ketone body metabolism, 271–272, 282
- KATP channels, 275–277, 276f
- metabolic function alteration, 272, 273f, 277f
- neurons and astrocyte metabolic changes, 272
- vs. other BCL-2 family proteins, 273
Bansal, S., 29
Banting, William, 363
Barborka, C. J., 16–17, 23
basement membranes, blood-brain barrier, 290f, 292
Bastible, C., 16–17
BCL-2-associated agonist of cell death (BAD), 271. see also BAD channels, metabolic seizure resistance
behavioral effects, 231
Bellisario, V., 236
Bergqvist, A. G. C., 69
beta-oxidation metabolic pathway, 248, 249f
β-hydroxybutyrate (BHB), 307. see also ketone bodies
aging brain and Alzheimer’s disease, 113, 115f,
- 121–122, 121f
- aging brain and Alzheimer’s disease, kinetics,
- 120, 120f
- antioxidant function, 138
- brain energy source, 115f, 121–122, 121f
- brain uptake, 296f
- cancer, 81–82, 83
- on cellular processes, 381
- hippocampal slices, 187–190, 189f
- inflammation, 150–151, 151f
- KATP channels, 157
- ketogenesis, 119f
- ketogenic diet, 90, 281
- liver synthesis, 156
- medium chain triglycerides, 123f
- metabolism and sum reactions, 243, 243f, 246f–248f,
- 247–248
- mineral salts supplementation, with medium chain
- triglycerides, 311–312
- neuroprotection, 124, 124f, 125t, 134

amino acids, for neurological disorders, 346–349
seizures and epilepsy
D-amino acids, 347–349, 349b
L-amino acids, 346–347, 347b
traumatic brain injury, 349
AMPA receptor agonists, medium chain fatty acids,
- 331–332, 331f
amyloid precursor protein (APP), 242, 243
amyotrophic lateral sclerosis (ALS), 157–158
triheptanoin, 341
anaplerosis, 124–125, 218
propionyl-CoA carboxylation pathway,
- 336–337, 338f
TCA cycling, 336, 337f, 338f
Angelman syndrome, 52–53
- low glycemic index treatment, 9–10, 9b
anorexigenic food intake, 229, 229f
antiepileptogenesis. see epileptogenesis actions
anti- excitotoxic effects, 137
antioxidants. see also specific types
- mechanisms of action, 257–258, 258f
- neuroprotective mechanisms, 137–138
apolipoprotein E (ApoE), 241
apolipoprotein E-4 (ApoE-4), 241, 242
L-arginine
- seizure onset protection, 347
- traumatic brain injury, 349
ascorbate, 258, 261, 262f
ascorbic acid, 258f, 259
astrocyte-neuron lactate shuttle, 282–285, 283f–285f
- antiepileptic effects, 283–286, 284f, 285f
- electrical regulation, 284, 285f
nutritional ketosis, 377, 379
Parkinson disease, 158
on reactive oxygen species, 83
rodent model, 233f, 235–236
synthesis and metabolism, 187, 228, 296f
D-β-hydroxybutyrate
administration, 124
ionizing radiation protection, 263, 264f
ketogenesis, 119
ketogenic diets, 136
mitochondrial metabolism, 315f
neuroprotection, 135
on redox states, cellular, 261–263, 262f
on transcription, 261, 264f
Blackburn, Elizabeth, 256–257
Blomqvist, G., 121, 121f
blood-brain barrier, 289–299
ketones, 113
metabolic rate, glucose, 115
PPARγ, 170–172, 172f
brain
ketones, 113
metabolic rate, glucose, 115
PPARγ, 170–172, 172f
brain cancer, 392
brain cancer, malignant, 88–94
glioblastoma multiforme, 88
ketogenic diet, humans, 93–94
ketogenic diet, with standard therapies, 90–91
metabolic remodeling, 88
preclinical evidence, 91
standard therapies, 91–93, 92f
mitochondrial dysfunction, 80
nuclear factor-kappa B, 90
PI3K/AKT signaling pathway, 89
radiation therapy, 92
reactive oxygen species, 90
Carballo, R. H., 44–47
ketone supplementation, 320–321
mitochondrial dysfunction, 80
nuclear factor-kappa B, 90
PI3K/AKT signaling pathway, 89
radiation therapy, 92
reactive oxygen species, 90
3-carboxy-terminal binding protein (CtBP), 356
cardiac arrest and resuscitation
  ketosis neuroprotection, 218–219, 219f
  survival after, 217–218, 218f

cardiac hypertrophy, triheptanoin, 342

cardiac side effects, 69–70
cardiomyopathy, 69–70

Castellano, C. A., 121, 121f, 122f

caveolin-based vesicle trafficking, 291f

cell senescence, 257
cerebral metabolic rate, glucose, 115

Champ, C. E., 94

Chang, P., 7, 328–332, 329f, 330t, 331f

Charlie Foundation, 3, 27, 386–392, 391f, 392f
  achievements, 388–389, 389f
  education resources, 393–394, 394b
  future, 394–395
  genesis, 386–387
  history, 386
  ketogenic diet therapy spectrum, 389–392, 390t
  research support, 393–394

childhood absence epilepsy (CAE), 56–57

Chuang, Y. C., 173–174

claudins, 292

clinical trials, 77

cognition, 22

cognitive impairment, mild
  ketogenic treatments, 127, 127t
  medium chain triglycerides, 123f, 124, 125t

cognitive impairment, severe. see Alzheimer's disease

constipation, 67–68

convulsive status epilepticus, 61

Coppola, G., 317

cortical spreading depression, 160

Couch, S. C., 70

counseling, prediet, 27

Crabtree effect, 80

Cross, Helen, 388

D'Agostino, D. P., 91, 310–313, 317

Darlington, C. D., 79

Davidson, T. E., 231

Deanna Protocol, 158

decanoic acid, 331. see also medium chain fatty acids
  AMPA receptors and seizure control, 331
  blood-brain barrier penetration, 328
  mitochondrial proliferation, 332
  seizure control, 330, 332

2-deoxyglucose, 353–359
  anticonvulsant actions, 353–355
  antiepileptic actions, 353–356
  chemical structure, 354f
  glucose metabolism and glycolytic pathway, 353, 354f

depression, 159

diabetes mellitus, Alzheimer's disease and vascular dementia, 242

diabetes mellitus type 2, 362–371
  carbohydrate, glycemic effect, 366f, 367
  carbohydrate restriction, randomized controlled trials, 367, 369t
  dietary recommendations, evolution, 365
  dietary recommendations, post-insulin era, 364
  keto-adaptation, 378
  ketogenic diet
    metabolic ward study, 367
    nonrandomized studies, 370, 370t
    origins and use, 363–364
    pilot study, 367–370
    remission case report, 370, 371f
  ketone supplementation, 321
  low carbohydrate diets, controlled studies, 367, 368t
  nutrition therapy, low glycemic dietary patterns, 365–367
  pathophysiology, 362
  prevalence, 362
  progression and morbidity, 362
  dietary therapy, adults, 16–24. see also specific types
  demand, 17–19
  children transitioning to adult epilepsy
    providers, 17–18
  refractory epilepsy, 18
  super-refractory status epilepticus, 18–19
  effects, adverse
    gastrointestinal, 22–23
    lipids, 23
    menstrual cycle, 23
    other, 23
  effects, beneficial, 22t
    cognition and mood, 22
    weight loss, 22
  history, 16–17
  results
    efficacy, 19–22, 20f, 21f
    feasibility, tolerability, and adherence, 19
  discontinuation, ketogenic diet, 70–71
  prevalence, 66, 67t

DNA methylation levels, epilepsy, 211–213, 212f

Does What I Eat Affect Epilepsy?, 394

Doose syndrome, 35, 46–47

Dravet syndrome, 45–46

Drenick, E. J., 114, 118, 121f

Dressler, A., 43, 45–46

drug resistant epilepsy, 40
  duration, treatment, 31
  dyslipidemia, 229, 230f

education, prediet, 27
  effectiveness, timing, 29–30
  efficacy, 19–22, 20f, 21f
  electrolyte imbalances, 69
  Ellenbroek, J. H., 229, 229f
  El-Rashidy, O. F., 30
  endocrine effects, 227–230, 228t, 229f, 230f, 230t
  endothelial cells, blood-brain barrier, 289–292, 290f, 291f
  energy, brain. see brain energy
energy regulation
dysregulation, 77
long-term, 228–229
short-term, 228
epigenetics, epilepsy, 210–211
epilepsy, see also specific topics
adenosine kinase overexpression, 209–210
Adk gene, 210–211
2-deoxyglucose for, 355–356
DNA methylation levels, 211–213, 212f
energy metabolism and triheptanoin, 337–339, 338f
epigenetics, 210–211
fasting, 209
kainic acid status epilepticus (KASE) model, 210, 211
ketogenic diet mechanisms, 281–282
ketone supplementation, 317
PPARγ, 173–174
epilepsy indications, established, 40–47
Doose syndrome, 35, 46–47
Dravet syndrome, 45–46
infantile spasms, 42–44
Lennox Gastaut syndrome, 44–45
refractory nonsurgical epilepsy, 40–42
epileptogenesis actions, 209–213
adenosine homeostasis and seizures, 209–210
definition, 355
DNA methylation levels, 211–213, 212f
epigenetics, 210–211
ketogenic diet, 137, 165, 198 (see also ketogenic diet; seizure control)
ketogenic diet and adenosine homeostasis, 211–213, 212f
erythropoietin, neuroprotection, 220
Eun, S. H., 43–44
evaluation, pre-treatment, 66–67, 67t, 71t
Evangelou, A., 105
excitotoxic effects, 137
family-centered, team-based approach, 26
fasting
classic ketogenic diet, 28–29
epilepsy, 209
hyperketonemia, 307
neuroprotection, 134
pain, 196–197
spinal cord injury, 134
fats
dietary, as fuel, 380–381 (see also brain energy)
stored, 376
fatty acid metabolism, beta-oxidation metabolic pathway, 248, 249f
Feinman, R. D., 378
fever, on inflammation, 147–148
fever-induced refractory epileptic encephalopathy in school-age children (FIRES), 147
status epilepticus, 60, 61
fibroblast growth factor 21, 147–148
First Do No Harm, 3, 41, 386, 391f
fitness, keto-adaptation, 380–381
food refusal, 70
Freeman, J. M., 3, 40, 387
Freemantle, E., 123, 124f
free radicals. see also reactive nitrogen species (RNS); reactive oxygen species (ROS)
Alzheimer's disease, 243–244
derox state, 261, 262t
removal, in vivo, 263–264
fructose-1,6-diphosphate (FDP), 357
Gahring, L. C., 170–171
gene theory of cancer, 79
Geyelin, H. R., 16
Gibson, C. L., 298
Giménez-Cassina, A., 272–274
glioblastoma multiforme, 88. see also brain cancer, malignant
glioma, malignant, 88. see also brain cancer, malignant
glucagon, 229–230
glucose, 336
blood levels, ketogenic diet, 228, 228t
chemical structure, 354f
decreases, 281–282
pain, 200–201
glucose, brain
cerebral metabolic rate, 115
requirements, 113
glucose, brain, Alzheimer's hypometabolism, presymptomatic, 116–118, 116t, 117f
uptake vs. metabolism, 118
glucose-insulin axis, 376
Glucose/Ketone Index Calculator (GKIC), 82–83
glucose metabolism
BAD on, 271–272
2-deoxyglucose, 353, 354f
oxidative stress, 217–218, 218f
glucose suppression, 313–314
glucose transporters (GLUTs), 294–295, 296f, 297f
GLUT 1 (glucose transporter type 1), 293, 297f
GLUT 1 (glucose transporter type 1) deficiency, 18, 35–37, 308
clinical presentation and phenotype, 35–36, 36f
diagnosis, 35
ketogenic diet efficacy, 20
ketogenic diet therapies, 36–37
ketone supplementation, 317–318
metabolic concept, 35, 36f
open questions, 37
triheptanoin, 341
glutamate, 349
D-glutamate, seizure onset protection, 348
glutathione
mechanisms of action, 259, 261, 262t
on reactive oxygen species, 264
glycemic dietary patterns, diabetes mellitus, 365–367
glycemic index (GI), 9, 365
glycemic load, 366
glycine, seizure onset protection, 346
glycolysis, 336, 354f
glycolytic flux, neuroprotection, 157
glycolytic pathway, 2-deoxyglucose, 353, 354f
Go Lower Low Carbohydrate Diet, 367–370
Gottstein, U., 114
GPR40, 199, 200
GPR41, 201
GPR120, 201
Greenwood, C. E., 231
growth, 70
Gudsnuk, K., 233f
Hadera, M. G., 340
Harmon, Denon, 256
Hayflick, L., 256, 257
HDAC inhibitor, 321
ionizing radiation protection, 264
hepatitis, 69
Herbert, M. R., 105
HIF1α
activation, redox state, 216
ketones, 219
neuroprotection
ketosis, 221–222, 221f
stabilization, 219–221
oxygen homeostasis, hypoxia, 220
regulation, 221
hippocampal slice, 186–193
for ketogenic diet and epilepsy studies, 187
ketogenic diet–fed animal slices, 188f, 189t
ketone body direct application,
187–190, 188f, 189f
temporal lobe epilepsy model, 186–187
whole-cell patch clamp for glucose and ATP control,
188f, 189t, 190–191, 191f
Hippocrates, 16, 28
Hong, A. M., 43
Hong, S., 175
hormonal mechanisms, 227
Hoyer, S., 114–115, 122
Huntington’s disease, triheptanoin, 342
Huttenlocher, P. R., 5, 328
hydrogen peroxide, 264
synthesis, monoamine oxidase, 254–255, 255f
3-hydroxybutyrate ketone monoester (KME),
318–320, 319f
3-hydroxybutyrate methyl ester (HBME), 318
hyperglycemia, 313–314
hyperinsulinemia, 313–314
hyperketonemia
benefits, 307
with cognitive deficit, clinical studies, 123–124, 125f
hyperlipidemia, 69
hypoglycemia, 68
hypometabolism, Alzheimer’s disease, 216–217
hypoxia-inducible factor 1 (HIF-1), 89–90
implementation, 26–31
classic ketogenic diet
caloric restriction, 29
fasting, 28–29
liquid/formula vs. food, 29
education and counseling, predict, 27
effectiveness, timing of determination, 29–30
family-centered, team-based approach, 26
initiation, in-patient vs. out-patient, 27–28
ketogenic diet team, composition, 26–27
ratios, importance, 30–31
response predictor, 31
seizure freedom, 30
treatment duration, 31
infantile spasms, 42–44
infections, 68
inflammasome, 138–139
NOD-like receptor P3, 298
inflammation, 147–153
animal models, neurological disease, 148–150
multiple sclerosis, 148–150
pain, 148
Parkinson’s disease, 150
studies, 148, 149f
clinical evidence, 153
fever, 147–148
fibroblast growth factor 21, 147–148
FIRES, 147
future indications, 153
ketone supplementation, 314–316
mechanisms, 150–153, 151f
adenosine modulation, 151f, 152–153
caloric restriction, 151–152, 151f
ketone bodies, 150–151
mitochondrial membrane potential, 153
polyunsaturated fatty acids, 151f, 152
reactive oxygen species, 151f, 153
uncoupling proteins, 153
neuroinflammation, 297–298
neuroprotection, 138–140
pain, 147, 148, 196 (see also pain)
initiation, in-patient vs. out-patient, 27–28
Inoue, T., 283–286, 284f, 285f
insulin
blood levels, ketogenic diet, 228, 228f
glucose-insulin axis, 376
hyperinsulinemia, 313–314
insulin resistance
brain, Alzheimer’s disease, 243
eketo-adaptation, 377–378
ketone supplementation, 321
nonalcoholic fatty liver disease, 378
weight loss, keto-adaptation, 379
insulin sensitivity
brain ketone use, 118
ketone supplementation, 313–314
International Consensus Statement for Ketogenic Diet (ICSKD), 26
intrauterine growth restriction (IUGR), 234
ionizing radiation
  HDAC inhibitor protection, 264
  ketone ester effects, post-exposure, 266–267, 267f
  ketosis protection, 263
  in vitro studies, 264–266, 265f, 266f
L-isoleucine, seizure onset protection, 346
JAMs, 292–293
Jaworski, D. M., 80–81
Jeong, E. A., 176, 177
Joensson, E. A., 370
Joslin, Elliott P., 364, 365, 367
Juge, N., 188–190
junctional adhesion molecules (JAMs), 292–293
juvenile myoclonic epilepsy (JME), 18, 57–58
kainic acid status epilepticus (KASE) model, 210, 211
Kang, H., 69
Kashiwaya, Y., 314, 316, 318, 321, 322
KATP channels, BAD on, 271
KATP channels, metabolic seizure resistance, 274–277, 277f
BAD disruption on, 275
metabolism and excitability link, 274–275
seizure protection, BAD-elicited, 275–277, 276f
Kelly, Millicent, 387
Kessler, S. K., 30–31
keto-adaptation, 312, 376–383
athlete, 382, 383f
effects, 376–377
fundamentals, 376–377
health implications, 377–380
insulin resistance, metabolic syndrome, diabetes type 2, 377–378
lipoprotein profile, 379–380
weight loss and body composition responses, 378–379
history, 376
nutritional ketosis, 377, 377f
sport and fitness, 380–381
starvation ketosis, 377
Keto Cookbook II, 393
KetoDietCalculator, 388
ketogenesis, 118, 119f
ketogenic diet. see also specific types and topics
history and early research, 3–4, 26
ketosis, 217
mechanisms of action, 90–91 (see also specific types)
pediatrics indications, 4
spectrum, 389–392, 390t
types, 3–4
use, 26
ketogenic diet, advancing awareness, 386–395
brain cancer, 392
Charlie Foundation, 3, 27, 386–392, 391f, 392f (see also Charlie Foundation)
Matthew’s Friends, 386, 388–389, 392f (see also Matthew’s Friends)
metabolic syndrome, 392
other applications, 392–393
research support, 393–394
ketone-based metabolism, 282
ketone bodies and ketones, 271. see also acetoacetate; β-hydroxybutyrate (BHB); specific types
alternative energy substrates, 216–217
Alzheimer’s disease, 244–247, 245f–246f
available forms, 247–249, 248f, 249f
on blood-brain barrier, 298–299
blood levels, ketogenic diet, 228, 228t
brain, 113, 118, 119f
development, ketogenic diet, 228, 228t
uptake, 295–297, 297f
uptake, regional, early Alzheimer’s, 121–122, 121f, 122f
breakdown, 28
exogenous, 307
vs. glucose, 217
as “good medicine,” 81
increased, 281
on inflammation, 138–140, 150–151
kinetics and transport, 118–120, 120f, 120t
metabolism, BAD on, 271–272
neuroinflammation, 297–298
neuroprotection, 126, 156–157, 298
on pain, 200
on radiation effects, post-exposure, 266–267, 267f
on tumors, 91
ketone ester supplementation, 312
ketone salts supplementation, 311–312
ketone supplementation, 310–322
applications, 317–322
Alzheimer’s disease, 318–320, 319f
cancer, 320–321
epilepsy/seizure disorders, 317
GLUT1 deficiency syndrome, 317–318
insulin resistance/type 2 diabetes mellitus, 321
weight loss, 321–322
development and testing, 310–312
1,3-butane diol, 311
formulations, 310
ketone esters, 312
ketone salts, 311–312
medium chain triglycerides, 310–311
βHB mineral salts and medium chain triglycerides, 311–312
therapeutic ketosis from, 310
therapeutic mechanisms, 313–316
glucose suppression and insulin sensitivity enhancement, 313–314
on inflammation, 314–316
metabolic efficiency, enhanced, 314, 315f
mitochondrial health and function, 316
oxidative stress, inhibition, 316
ketosis, nutritional (therapeutic), 217, 307, 310, 379.
see also ketone supplementation
benefits, 377, 377f
β-hydroxybutyrate, 377, 379
ketosis, starvation, 377
kidney stones, 68
Kilaru, S., 47
Kim, D. Y., 190
Kimura, R., 188
kindling, 355
Klein, P., 19
Klement, R. J., 94
Klingenberg, M., 258
Kossoff, E. H., 7, 8b, 20, 30–31, 57, 389
Krebs, H. A., 258–261
Krebs's cycle, 244
Kverneland, M., 58
laboratory, 165–166
lactate dehydrogenase (LDH), 281–286
astrocyte-neuron lactate shuttle, 282–283, 283f
epilepsy
mechanisms, 281–282
metabolic target, 283–286, 284f, 285f
future directions, 286
lactate fermentation, 80
Lambrechts, D. A., 20
Lardy, Henry, 314
Laux, L., 45
Lemmon, M. E., 44
Lennox Gastaut syndrome, 44–45
D-leucine, 347–348
L-leucine, 346–347
leukocyte adhesion molecules, blood-brain barrier, 293
lipids, 23
lipogenesis, 379
lipoprotein profile, keto-adaptation, 379–380
liquid/formula vs. food, classic ketogenic diet, 29
Liu, Christiana, 5
Lomax, M. E., 265–266
long chain fatty acid oxidation disorders, triheptanoin, 341
long-chain triglycerides, 5
low glycemic index treatment (LGIT), 6f, 9–10, 9b
development, 389
initiation and follow-up, 12–13
Luna-Medina, R., 175
Lying-Tunell, U., 114, 115, 115f, 121f, 122
Magee, B. A., 320
Magrath, G., 9
Mantis, J. G., 106
Marie, A., 16
Marin-Valencia, I., 339
Martinez, C. C., 17
Matthew's Friends, 27, 392f, 393f
achievements, 388–389
future, 394–395
 genesis, 387–388
ketogenic diet therapy spectrum, 389–392, 390f
research support, 393–394
Maurois, P., 174
McDonald, T. S., 339
medium chain fatty acids, 329f
AMPA receptor agonists, 331–332, 331f
seizure control, 328–330, 329f, 329t
medium chain triglyceride (MCT) ketogenic diet, 5–7, 6b, 6f
history, 328
initiation and follow-up, 12–13
mechanisms, 328–333
AMPA receptor agonism, 331–332, 331f
implications, 332–333
seizure control, 328–330, 329f, 329t
seizure control, ketones, 332
molecular mechanism, 328–333
medium chain triglycerides (MCTs), 5
coconut oil and palm kernel oil, 123
ketogenesis, dose-response, 6f, 122–123, 123f
products, 123–124, 125f
safety, 126
supplementation, 310–311
supplementation, with βHB mineral salts, 311–312
MELAS, status epilepticus, 61
menstrual cycle, 23
metabolic acidosis, 67
metabolic diseases
autism spectrum disorders, 102–103, 104f–105f
cancer, 79–80
metabolic potential energy, 376
metabolic remodeling, 88
metabolic syndrome
keto-adaptation, 378
ketogenic therapy, 392
plasma markers, ketogenic diet, 229, 230t
metabolism. see also specific types
ketogenic diet mechanisms, 227
ketone supplementation, 314, 315f
metabolism, mitochondrial
acetoacetate, 315f
D-β-hydroxybutyrate, 315f
D-methionine, seizure onset protection, 348
middle cerebral artery occlusion, triheptanoin, 341
migraine, 159–160
mild cognitive impairment
ketogenic treatments, 127, 127f
medium chain triglycerides, 123f, 124, 125f
mitochondrial dysfunction
autism spectrum disorders, 102–103
brain energy status and neuroprotection, 125–126
cancer, 79–80
cytopathies, 27
function, 102
ketone supplementation, 316
membrane potential, inflammation, 153
nervous system, 102
mitochondrial metabolism
acetoacetate, 315f
D-β-hydroxybutyrate, 315f
modified Atkins diet (MAD), 6f, 7–9, 8b, 8t
development, 389
effectiveness, vs. classical ketogenic diet, 10–11, 10t
efficacy, 19–22, 20f, 21t
initiation and follow-up, 12–13
mood, 22
mood disorders, 159
Moorman, M. A., 256, 257
Moreno, S., 170
multiple sclerosis, 148–150
myoclonic astatic epilepsy (MAE), 35, 46–47
Nabbout, R., 45, 60, 61, 63
NADPH, 264
NADP- linked enzymes, liver activity, 259, 259t
NADP system, 261–263, 262f, 263f
D-β-hydroxybutyrate on, 264
Neal, E. G., 3, 4, 10, 10t
Nebeling, L. C., 83, 93
Nei, M., 20
neurodegeneration and aging, 216–222. see also aging
and neurodegeneration
neuroinflammation, 297–298
neurological disorders, 156–160. see also specific types
amino acid treatment, 346–349 (see also amino acids, for neurological disorders)
amyotrophic lateral sclerosis, 157–158
glycolytic flux, 157
ketone bodies, 156–157
migraine, 159–160
mood disorders, 159
neuroprotection, 156–157
Parkinson disease, 158–159
neuron restrictive silencing factor (NRSF), 356
neurons, BAD-altered, metabolic changes, 272
neuropathic pain, 196. see also pain
neuroprotection, 156–157, 298, 376
acetoacetate, 136
aging, 218–219, 219t
aging, ketosis, 221–222, 221f
antioxidants, 137–138
β-hydroxybutyrate, 124, 124f, 125t, 134
α-β-hydroxybutyrate, 134
cardiac arrest and resuscitation, 218–219, 219f
erthropoietin, 220
fasting, 134
glycolytic flux, 157
HIF1α, 219–222, 221f
inflammation, 138–140
ketone bodies, 156–157, 298
ketones, 126
neurological disorders, 156–157
Parkinson’s disease, 158–159
polyunsaturated fatty acids, 156–157
PPARγ, post-SE and during SRS, 171, 172f
spinal cord injury, drug candidates, 133–134
stroke, 218–219, 219f
vascular endothelial growth factor, 220–221
neuroprotection, mechanisms, 216, 221–222, 221f
anaplerotic, 136
anti-excitotoxic, 137
antioxidant, 137–138
inflammation, 138–140
non-anaplerotic, 137
neurovascular unit, 289, 290f
Newport, M. T., 318, 320
NF E2-related factor 2 (Nrf2) transcription factor, 138,
172, 221, 266
Nielsen, J. V., 370
Nizamuddin, J., 69
NLRP3 inflammasome, 138–139, 149t, 150, 151f,
153, 197, 298, 315–316
NOD-like receptor P3 (NLRP3) inflammasome, 138–
139, 149t, 150, 151f, 153, 197, 298, 315–316
nonalcoholic fatty liver disease, insulin resistance, 378
nonconvulsive status epilepticus, 61–62
nonsurgical epilepsy, refractory, 40–42
Nordli, D. R., 43
NORSE, status epilepticus, 60, 61
Nrf2, 138, 172, 221, 266
nuclear factor-kappa B, 90
Numis, A. L., 43–44
nutritional ketosis. see ketosis, nutritional
Nylen, K., 106
obesity, 362–371
carbohydrate restrictions, randomized controlled trials, 363, 363t
diabetes mellitus type 2, 362 (see also diabetes mellitus type 2)
ketogenic diet
origins and use, 363
pilot study, 367–370
prevalence, 362
occludin, 292–293
Ogawa, M., 115, 121f, 122
Oguni, H., 46
Olovnik, A. M., 256
oncogenic paradox, 80
orexigenic food intake, 229, 229f
osteopenia, 70
overweight, prevalence, 362
Owen, O. E., 114, 118, 121f, 376
oxidative phosphorylation, 80
oxidative stress
on glucose metabolism, 217–218, 218f
ketone supplementation, 316
oxygen free radicals. see also reactive oxygen species (ROS)
redox state, 261, 262t
pain, 196–201
adenosine transmission, 201
calorie restriction, 197–198
economic cost, 196
fasting, 196–197
glucose, 200–201
pain, (cont.)
  GPR40, 199, 200
  GPR41, 201
  GPR120, 201
inflammation, 147, 148, 196
inflammatory, 196
ketogenic diet, 198–199, 198f, 199f
ketone bodies, 200
neuropathic, 196
polyunsaturated fatty acids, 200
quality of life, 196
severe, treatment, 196
pancreatitis, 69
Paoli, A., 228–229, 228f
Parent’s Guide to the Ketogenic Diet, 393
Parkinson’s disease
  on inflammation, animal models, 150
neuroprotection, 158–159
Pasteur effect, 80
Patel, S. P., 136
pentose phosphate shunt (PPP), 354f, 357f
pentylenetetrazole (PTZ), 317, 346f
pericytes, blood-brain barrier, 290f, 292f
peroxisome proliferator-activated receptors (PPARs)
  historical overview, 167
  isoforms and domains, 168, 169f
  polyunsaturated fatty acids, 200
  structure and functional diversity, 167–168
peroxisome proliferator-activated receptor γ (PPARγ), 7, 137, 151, 151f, 152, 157, 167–179
  activation, 168
  agonists, 174
  neuroprotection post-SE and during SRS, 175
  pretreatment, acute seizure models, 174
  on seizure thresholds and kindling models, 174–175
  on SRS and spatial learning, 175
brain, 170–172, 172f
epilepsy, 173–174
  functional consequences, 177–178
  gene regulation and activity, 169–170, 169f
  ketogenic diet
    activation, evidence, 175–176
    activation, potential mechanisms, 176–177
    regulation, 173–177
    mechanisms of action, 178–179, 179f
  neuroprotection, homeostatic, 171, 172f
splice variants and activity, 168–169, 169f
  structure and domains, 168, 169f
Pfeifer, Heidi, 389
Phinney, S. D., 379, 382
PI3K/AKT signaling pathway, 89
Pires, M. E., 43
plasmalemna vesicle-associated protein number, 293
polycystic ovary syndrome, 117
polyunsaturated fatty acids (PUFAs)
  on inflammation, 151f, 152
  neuroprotection, 156–157
  pain, 200
peroxisome proliferator-activated receptors, 200
pregnancy
  establishment and maintenance, 233–234
  before and during gestation, 233f, 234–236, 235f, 235t, 236f
Pre-ketogenic diet, 394
presenilin 1/2 genes, 241
Prins, M. L., 298–299
D-proline, seizure onset protection, 348
prolyl-hydroxylase (PHD), 220, 221, 221f
propionyl-CoA carboxylation pathway, anaplerosis, 336–337, 338f
puberty, ketogenic diet long-term effects, 231–234, 232f, 232t, 233f
pyruvate dehydroxylase deficiency, triheptanoin, 341
pyruvate dehydrogenase deficiency, 50
inhibition, Alzheimer’s disease, 242, 243
QT interval prolongation, 69
radiation, ionizing
  HDAC inhibitor protection, 264
  ketone ester effects, post-exposure, 266–267, 267f
  ketosis protection, 263, 264f
  in vitro studies, 264–266, 265f, 266f
  radiation therapy, cancer, 92
Raj, K. N., 30
Rasmussen syndrome, status epilepticus, 61
ratios, importance, 30–31
reactive nitrogen species (RNS), 92, 254–268
damage caused by, 255–256, 255f
origins, reactions, and target molecules, 254–255, 255f, 256f
reactive oxygen species (ROS), 254–268
in aging, 256–257
Alzheimer’s disease, 243–244
D-β-hydroxybutyrate
  on cellular redox states, 261–263, 262f, 263f
  on transcription, 261t, 264
cancer, 90
  cellular redox states vs. standard redox potentials, 258–261, 259f, 259t, 261t, 262t
  damage, 255–256, 255f, 256f
  enzymes destroying, 264
  on inflammation, 151f, 153
  mechanisms of action, 257–258, 258f
  origins, reactions, and target molecules, 254–255, 255f, 256f
  redox state of oxygen free radicals and other reactants, 261, 262t
removal, in vivo, 263–264
removal, NADPH generation, 264
redox potentials, standard, vs. cellular redox states, 258–261, 259f, 259t, 261t, 262t
redox states, cellular
  D-β-hydroxybutyrate on, 261–263, 262f, 263f
  oxygen free radicals and other reactants, 261, 262t
  vs. standard redox potentials, 258–261, 259f, 259t, 261t, 262t
radiation therapy, cancer, 92
radiation, ionizing
Index

refractory epilepsy, 18
refractory nonsurgical epilepsy, 40–42
renal calculi, 68
reproduction, 227–237
behavioral, 231
endocrine, 227–230, 228, 229f
high-fat/ketogenic diets, 227
long-term effects, 233–234
pregnancy
  establishment and maintenance, 233–234
  before and during gestation, 233f, 234–236, 235f, 235t, 236f
puberty, 231–233, 232f, 232t, 233f
reproduction, 233–234
response predictor, 31
resting energy expenditure (REE), 29
Rett syndrome, 50–51, 106
triheptanoin, 342
Richardson, R., 364
Rouach, N., 282–283
Ruskin, D. N., 106
Sada, N., 283–286, 284f, 285f
S-adenosylhomocysteine (SAH) hydrolase, 212, 212f
S-adenosylmethionine (SAM) dependent transmethylation pathway, 211–212, 212f
Samoilova, M., 188
Sansum, W. D., 364
Sarruf, D. A., 170
Scheck, A. C., 83, 91–92, 92f
Schmid, W., 267
Schoeler, N. E., 41
Schrödinger, Erwin, 257
SCN1A gene mutations, 45
screening, pre-treatment, 66–67, 67t, 71t
medical, 66
social, 66–67
seizure. see also epilepsy
  fasting, 209
  freedom from, 30
  ketone supplementation, 317
seizure control. see also epileptogenesis actions
  ketones, 332
  medium chain fatty acids, 328–330, 329f, 329t
  senescence, cell, 257
D-serine, seizure onset protection, 348
L-serine
  seizure onset protection, 347
  traumatic brain injury, 349
severe myoclonic epilepsy of infancy (SMEI), 45–46
Seyfried, T. N., 80
Sharma, S., 3, 10, 10t
Shimazu, T., 81
side effects, 66–71
  acidosis, 67
  cardiac, 69–70
  constipation, 67–68
  electrolyte imbalances, 69
  food refusal, 70
growth, 70
hepatitis, 69
hyperlipidemia, 69
hypoglycemia, 68
infections, 68
kidney stones, 68
osteopenia, 70
pancreatitis, 69
vitamin deficiencies, 68–69
vomiting, 67
Simard-Tremblay, S., 47
Ske2a1, 293
social screening, 66–67
somatic mutation theory, 79
Spilioti, M., 105
spinal cord injury, 133–141
epidemiology, 133
inflammation, 138–140
intermittent fasting, 134
ketogenic diet, mechanisms
  anaplerotic, 136
  anti-excitotoxic, 137
  antioxidant, 137–138
  non-anaplerotic, 137
ketogenic diet, outcome, 134–135
ketogenic diet, translation to clinical setting, 140–141
management, acute, 133
neuroprotective drug candidates, 133–134
rehabilitation, 133
spontaneous recurrent seizures, PPARγ agonists on, 175
sport, keto-adaptation, 380–381
Stafstrom, C. E., 192
status epilepticus (SE), 60–63
  cause, 60
  challenges, 61t, 62–63
  convulsive, 61
  duration, 60
  efficacy, ketogenic diet, 60, 61t
  implementation, 62, 62f
  incidence, 60
  nonconvulsive, 61–62
  super-refractory, 60
  treatment, first-line, 60
types, 60
stoke
  neuroprotection, 218–219, 219f
  triheptanoin, 341
Sturge-Weber syndrome, 53
succinic semialdehyde dehydrogenase (SSADH) deficiency, 105–106
sugar, dietary, children, 393
superoxide dismutase (MnSOD), 138, 264
oxidative stress, 172f
PPARγ deficiency on, 172
reactive oxygen species, 254–255, 255f, 256t, 264
super-refractory status epilepticus (SRSE), 18–19, 60
Sussman, D., 234–235
Index

Tanner, G. R., 190
Taub, K. S., 30
TCA cycle, 336, 337f, 338f
team-based approach
family-centered, 26
team composition, 26–27
telomere shortening, 256–257
temporal lobe epilepsy
hippocampus model, 186–187
ketogenic diet and adenosine homeostasis,
211–213, 212f
Thakur, K. T., 19
therapeutic ketosis, 310. see also
ketone supplementation
Thio, L. L., 188
thioredoxin, 259, 259f, 261
tight junctions, blood-brain barrier, 290f, 292–293
tocopherol, 258, 258f
transcription, α-β-hydroxybutyrate on, 261f, 264
transmethylation pathway, 211, 212f
transporters. see also specific types
blood-brain barrier, 290f, 291f, 293, 296f
traumatic brain injury, 133–141, 298
amino acid treatment, 349
on inflammation, 138–140
ketogenic diet, translation to clinical setting, 140–141
ketogenic diet effects, 135–136
ketogenic diet mechanisms
anaplerotic, 136
anti-excitotoxic, 137
antioxidant, 137–138
non-anaplerotic, 137
triglycerides, 5
triheptanoin, 317–318, 336–342
anaplerosis, 336–337, 337f, 338f
anticonvulsant effects, 338f, 340
brain energy metabolism, 339–340
cardiac hypertrophy, 341
epilepsy, energy metabolism,
337–339, 338f
GLUT1 deficiency, 341
long chain fatty acid oxidation disorders, 341
muscle disorders, 342
neurological conditions, 341–342
pyruvate carboxylase deficiency, 341
tuberous sclerosis complex, 51–52
tumor metabolism, 88–90, 89f
uncoupling proteins, on inflammation, 153
unlimited protein diet. see modified Atkins diet (MAD)
vascular endothelial growth factor (VEGF),
neuroprotection, 220–221
Veech, R. L., 81, 258–260, 259f, 307, 314, 318
Verdin, Eric, 316
vesicular glutamate transporters (VGLUTs), 190, 193, 286
acetoacetate on, 281
functions, 281
ketogenic diet, 282
Vigiano, A., 317
Villeneuve, N., 61
Vining, E., 70
vitamin deficiencies, 68–69
Volek, J. S., 378, 379–380, 382, 383f
vomiting, 67
Warburg, Otto, 88
Warburg effect, 80, 82, 88–90, 89f
Warburg’s theory, 79–80
weight loss, 22
keto-adaptation, 378–379
ketone supplementation, 321–322
Westman, E. C., 367–370, 370f
Wilder, R. M., 16, 26, 165
Williams, Matthew, 386, 387–388, 393f
Williams, S., 70
Williams-Karnesky, R. L., 210, 211–212
Willis, S., 340
Winocur, G., 231
Wirrell, E. C., 28
Worden, L. T., 71
Yudkin, John, 363
zona occludin proteins, 293
Zuccoli, G., 93
Zupec-Kania, Beth, 388, 393