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Different ketogenesis strategies lead to disparate seizure outcomes

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ABSTRACT

Background: Despite the introduction of new medicines to treat epilepsy over the last 50 years, the number of patients with poorly-controlled seizures remains unchanged. Metabolism-based therapies are an underutilized treatment option for this population. We hypothesized that two different means of systemic ketosis, the ketogenic diet and intermittent fasting, would differ in their acute seizure test profiles and mitochondrial respiration.
Methods: Male NIH Swiss mice (aged 3–4 weeks) were fed for 12–13 days using one of four diet regimens: ketogenic diet (KD), control diet matched to KD for protein content and micronutrients (CD), or CD with intermittent fasting (24 h feed/24 h fast) (CD-IF), tested post-feed or post-fast. Mice were subject to the 6 Hz threshold test or, in separate cohorts, after injection of kainic acid in doses based on their weight (Cohort I) or a uniform dose regardless of weight (Cohort II). Mitochondrial respiration was tested in brain tissue isolated from similarly-fed seizure-naïve mice.
Results: KD mice were protected against 6 Hz-induced seizures but had more severe seizure scores in the kainic acid test (Cohorts I & II), the opposite of CD-IF mice. No differences were noted in mitochondrial respiration between diet regimens.
Interpretation: KD and CD-IF do not share identical antiseizure mechanisms. These differences were not explained by differences in mitochondrial respiration. Nevertheless, both KD and CD-IF regimens protected against different types of seizures, suggesting that mechanisms underlying CD-IF seizure protection should be explored further.

1. Introduction

In 2015, 1.2% of the US population had the diagnosis of epilepsy, defined as the recurrence of unprovoked seizures, the occurrence of a single seizure in the setting of high risk for further seizures, or diagnosis or an epilepsy syndrome (Fisher et al., 2014; Zack and Kobau, 2017). Approximately 25% of people with epilepsy never achieve seizure freedom with currently prescribed medications and a substantial number have fluctuating seizure control (Berg and Rychlik, 2015; Brodie et al., 2012; Choi et al., 2016). This number has not changed significantly over the last 50–60 years despite a dramatic increase in the number of new medications (Chen et al., 2018). An intensive search for new therapies is underway. One modality, metabolism-based therapy, is underutilized and remains a leading option for patients with medically intractable epilepsy who are not candidates for resection surgery (Freeman et al., 2007). The most commonly used form of metabolism-based therapy is the high-fat, low-carbohydrate ketogenic diet, which is nearing the 100th anniversary of its current clinical formulation (Bailey et al., 2005; Koppel and Swerdlow, 2017).

Despite the longevity of the ketogenic diet in clinical use, a number of questions remain to be answered. The anti-seizure mechanism of the ketogenic diet has been attributed to a number of factors but unifying explanations remain elusive, partly because of interspecies differences in metabolic physiology (Masino and Rho, 2012; Rogawski et al., 2016). It is still unclear whether the ketogenic diet exerts disease-modifying effects in epilepsy and other non-paroxysmal diseases. The critical components of the diet that lead to seizure control remain unknown, despite elegant studies of its fat and carbohydrate components (Masino and Rho, 2012). Reasons for lack of efficacy and loss of seizure control over time in some animal models and in patients also have not

Abbreviations: KD, ketogenic diet fed *ad lib*; CD, control diet fed *ad lib*; CD-IF-feed, intermittent fasting, tested post-feed; CD-IF-fast, intermittent fasting, tested post-fast

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been identified (Hartman et al., 2008; Marsh et al., 2006; Todorova et al., 2000). Interestingly, many of these questions also apply to a number of medicines that are currently used to treat seizures, particularly those shown to have multiple antiseizure mechanisms and so-called "honeymoon" periods of seizure control (Berg and Rychlik, 2015; Brodie et al., 2012; Choi et al., 2016; Rogawski et al., 2016).

One common misconception is that the ketogenic diet is synonymous with fasting. This likely results from the fact that the ketogenic diet was designed to mimic the induction of systemic ketosis, similar to what is seen in fasting (Bailey et al., 2005; Koppel and Swerdlow, 2017). Our group has shown that the ketogenic diet and intermittent fasting do not share identical acute antiseizure mechanisms (Hartman et al., 2010). However, our comparisons between the ketogenic diet and standard rodent chow did not control for differences in protein content. We also did not examine seizure outcomes on the day after a fast (i.e., while mice were in systemic ketosis), which might be a more parallel comparison with the ketogenic diet.

Because of their central role in both metabolism and cell death, mitochondria have been proposed as a major organelle involved in the antiseizure and disease-modifying aspects of the ketogenic diet (Bough et al., 2006). Mitochondria play a number of different physiological roles but mitochondrial respiratory control is thought to be one of the more reliable methods to examine functional outcomes of integrated carbohydrate and fat metabolism (Brand and Nicholls, 2011). Mitochondrial gene expression has been shown to be altered by chronic ketogenic diet feeding (Bough et al., 2006). Whether this leads to a change in protein expression is unclear but physiological outcomes such as inhibition of mitochondrial pore transition (one step in cell death) have been demonstrated (Kim et al., 2015).

Based on our prior work, we hypothesized that there would be a disparity in seizure test outcomes between the ketogenic diet and a control diet (similar in protein content) fed in an intermittent fasting paradigm. Furthermore, there would be a difference between mice fed a ketogenic diet and those intermittently fasted, tested after a night of fasting (i.e., also in systemic ketosis). We also hypothesized that there would be differences in mitochondrial respiratory control between these feeding regimens.

2. Methods

2.1. Mice and diet regimens

Male NIH Swiss mice (NCI, Frederick, MD, U.S.A.) 3–4 weeks of age were acclimatized to the animal care facility for one day and randomly placed into separate cages by Animal Care staff who were not involved in the experiments. Once mice weighed more than 12 g, they were fasted overnight (14–18 h), housed three or four per cage, and the cages were then randomly assigned to one of four different dietary regimens, including: (1) ketogenic diet (KD) fed *ad lib* (i.e., not fasted) as in our prior work (Research Diets #D07091701, New Brunswick, NJ, U.S.A.), (2) control diet fed *ad lib* (CD) (not fasted), with a diet matched to the ketogenic diet for protein, vitamin, mineral and calorie content (Research Diets #D07091702, New Brunswick, NJ, U.S.A.), (3) intermittent fasting of the control diet, tested post-feed (CD-IF-feed, calorie restricted by alternating 24 h fasting and 24 h unrestricted feeding of control diet), and (4) intermittent fasting of the control diet, tested post-fast (CD-IF-fast, calorie restricted by alternating 24 h fasting and 24 h unrestricted feeding of control diet) (Table 1) (Hartman et al., 2010). Cages were matched so that prior to diet initiation, mice had similar weights between diet regimens. Mice were fed their assigned diet for 12–13 days (depending on treatment group) prior to seizure testing. Only male mice were used to facilitate direct comparisons with our prior work. Food consumption was documented by weighing food and/or food hoppers for each cage. Mice were maintained on a 14 h light–10 h dark cycle for the duration of the study. All animal protocols were approved by the Johns Hopkins Animal Care and Use Committee and were in compliance with National Institutes of Health Guide for the Care and Use of Laboratory animals (NIH Publications No. 8023, revised 1978). These experiments complied with ARRIVE guidelines (Kilkenny et al., 2010).

Table 1
Diet comparisons.

Diet	Ketogenic diet (KD)		Control diet (CD)	
	gm%	kcal%	gm%	kcal%
Protein	11	7	6	7
Carbohydrate	0	0	80	83
Fat	72	93	4	10
kcal/gm		6.1		6.1

Both diets contain identical amounts of casein, L-cysteine, cellulose, soybean oil, mineral mix, dicalcium phosphate, calcium carbonate, potassium citrate, vitamin mix, and choline bitartrate. They contain different amounts of lard, corn starch, maltodextrin, and sucrose.

2.2. Seizure tests

2.2.1. 6 Hz threshold test

The 6 Hz threshold test was performed as described previously (Hartman et al., 2010). Researchers were blinded whenever possible but diet regimen assignment was typically apparent based on physical appearance (e.g., greasy fur or weight differences). Experiments were conducted in cohorts of CD vs. CD-IF-fast vs. KD (three separate groups) and one group of CD vs. CD-IF-feed. All mice tested were included in the analysis. All seizure tests were carried out between 10 A.M. and 3 P.M. to minimize potential diurnal variation in seizure susceptibility.

2.2.2. Kainic acid

The kainic acid (KA) test was performed similarly to the technique described previously (Hartman et al., 2010). KA (Tocris Bioscience, Ellisville, MO, U.S.A.) (5.3 mg/ml) dissolved in phosphate buffered saline (PBS; Sigma Aldrich, St. Louis, MO, U.S.A.) was administered via an intraperitoneal injection. Cohort I was injected with KA based on weight (23.5 mg KA/kg mouse). Each mouse in Cohort II was injected with the same amount of KA (0.42 mg), regardless of weight. The amount of kainic acid used was a dose of 23.5 mg KA/kg (mouse weight), with mouse weight represented by the mean weight of mice treated on the four diet regimens in Cohort I and other biochemical experiments. CD, KD, and CD-IF-fast mice were tested on days 12–13 (after 24 h of feeding); CD and CD-IF-feed mice were tested on day 13 (after 24 h of fasting). Each mouse was tested for seizures only once. Seizure behaviors were scored over 2 h after KA injection using a modified Racine scale (the highest score in a given 5-min block was used), as described previously (Hartman et al., 2010). Researchers were blinded whenever possible but diet regimen assignment was typically apparent based on physical appearance (e.g., greasy fur or weight differences). All mice tested were included in the analysis. All seizure tests were carried out between 10 A.M. and 3 P.M. to minimize potential diurnal variation.

2.3. Biochemical studies

Mice in each dietary group were chosen randomly (mouse number selection by someone not involved in the experiments) 1 h before seizure testing for concentration of blood glucose and beta-hydroxybutyrate, as described previously (Hartman et al., 2010).

2.4. Mitochondrial respiration

2.4.1. Reagent and solution preparation

Mitochondria preparation was based on prior work (Frezza et al.,