Ketogenic Diets for Rodents: Diverse Applications for Neurological and Metabolic Diseases

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The ketogenic diet (KD) was originally developed in the early 1920s as a treatment for pediatric epilepsy (1). It is characterized by very high levels of fat, minimal carbohydrate, and adequate protein. In the absence of glucose oxidation, ketone bodies (β-hydroxy-butyrate, acetoacetate, and acetone) are formed as a byproduct of fatty acid oxidation in order to serve as an alternative source of fuel for the brain and peripheral tissues. KDs restrict carbohydrate consumption in order to limit circulating levels of glucose and, therefore, promote production and utilization of ketone bodies as a primary fuel source (2). This state of ketosis mimics starvation as ketone bodies are naturally produced in response to prolonged fasting or intense exercise (3).

KDs for rodents are quite different compositionally compared to human ketogenic diets (4). Human KDs can vary in terms of the macronutrient distribution (carbohydrate content ranging from less than 30 g/day up to 30% of energy intake) and types of fatty acids consumed (medium-chain triglycerides or long-chain triglycerides) (5). In contrast, ketogenic rodent diets contain very little carbohydrate (ranging from <1% up to 5% of total energy) and reduced levels of protein. Fatty acid composition of rodent diets can also vary depending on the sources of fat used (4). Figure 1 depicts the differences in energy composition of low-fat, high-fat, and ketogenic diets.

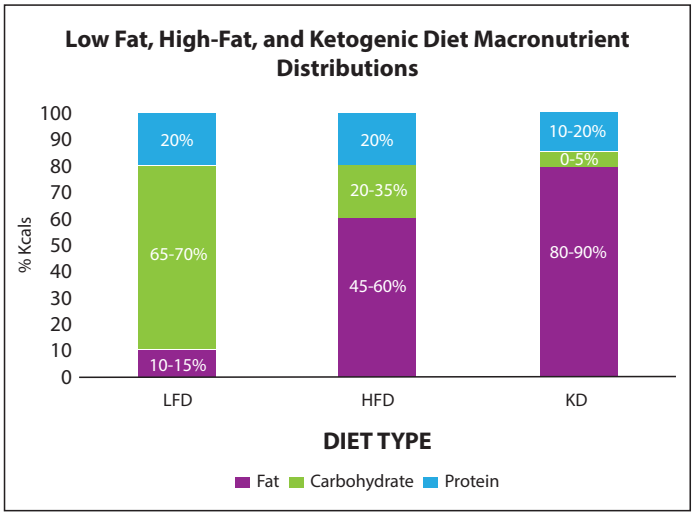

Figure 1

Though used as an epilepsy therapy for nearly a century, only recently have KDs been implicated as a potential treatment method for other neurological and metabolic diseases (2). Indeed, rodent models are currently being used to study the effects of ketosis on other conditions including Alzheimer's disease (AD), diabetes, and cancer (5). In this review, we discuss the use of KDs in a variety of pre-clinical models, as well as technical considerations for their implementation.

Neurological Disorders and Diseases

KDs are best known for their use in the treatment of neurological and neurodegenerative diseases, such as epilepsy, AD, Autism Spectrum Disorder, and Parkinson's disease (6,7). The mechanism by which KDs exert their neuroprotective effects is not fully understood. However, it is hypothesized that the multilateral, systemic effects of ketosis on metabolism, inflammation, and gene regulation collectively function to mitigate neurological damage (7).

Alzheimer's Disease. AD is a neurodegenerative disease characterized by progressive memory loss, brain atrophy, and other cognitive deficits such as disorientation and personality changes (7). Pathologically, it is associated with the deposition of amyloid plaques formed of amyloid β-peptide and neurofibrillary tangles (NFTs) formed from tau protein (7,8). AD, sometimes called "Type 3 Diabetes Mellitus", is accompanied by reduced glucose uptake in the brain as well as neural insulin resistance. It is believed that loss of glucose over time, in addition to NFT and amyloid β production, leads to neuronal damage and eventual cell death (9). KDs, therefore, are often implemented as way to provide nutritional support for the brain by circumventing glucose pathways.

Rodent models of AD largely rely on the use of genetic modifications as rodents do not naturally develop plaques or NFTs. Thus, models vary in nature depending on the specific element of AD the investigator wishes to study (8). KDs fed to animal models have been shown to improve disease markers such as motor function, cognition, brain vascular function, and amyloid β (10). Indeed, female APP/V717I mice fed a KD exhibited increased β-hydroxybutyrate (BHB) and decreased amyloid β deposition compared to chow fed animals in as little as 43 days (11). However, these findings are not consistent across models. Others have shown conflicting results with little improvement in APP/PS1 and Tg4510 mice fed a KD for 3 months (12). Such discrepancies imply that the efficacy of a KD on Alzheimer's related symptoms is highly dependent on strain. It is also likely that KD choice, control diet choice, and sex contribute to variability between reported findings.

Epilepsy. For the last century, KDs have been implemented as a treatment for drug-resistant epilepsy. Epilepsy is a complex disease with multiple clinical presentations that have poorly understood underlying mechanisms. Given this, there is no one animal model that encompasses the entirety of the disease and an array of models exist, including but not limited to the use of chemoconvulsants, electrical stimulation, and genetic manipulations (13). The anti-convulsant properties of KDs have been investigated in a number of models. Indeed, CD-1 mice and Wistar rats with chemically-induced convulsive seizures fed a 94 kcal% fat diet demonstrated both a decrease in spontaneous seizure activity and an increase in seizure threshold (14). Further, the same KD improved longevity and delayed the onset of both more frequent and more severe seizures in Kcna1-null mice, which are prone to sudden unexpected death in epilepsy (15). However, the beneficial effects of KDs on seizure activity seem to be dependent on the model chosen. EL mice fed a KD show a delayed onset of severe seizures, but this response does not appear to be as robust since an fed KD did not reduce seizure susceptibility (16,17).

Recent data suggest a relationship between the gut microbiome and drug-resistant epilepsy as patients with drug-resistant epilepsy have significantly different microbial profiles from drug-sensitive patients (18). Moreover, mice fed a KD have increased levels of Akkermansia muciniphila and Parabacteroides merdae compared to control fed ones in the feces as measured by 16S rDNA sequencing (19). Olson et al. demonstrated that mice with a knock down of the microbiota via antibiotics were not protected from seizures by a KD but that recolonization with these species restored protection. These data indicate that the gut microbiota may be a critical element of KD seizure reduction (19).

Metabolic Disorders

Refined carbohydrate sources, such as sucrose and fructose, have been linked to adverse metabolic outcomes. As such, diets low in carbohydrate, such as the KD, have been explored as dietary intervention strategies (4).

Obesity. KDs have proven themselves to be effective tools for weight loss in rodent models in some instances. Mice fed a KD have demonstrated significantly reduced weight gain compared to chow diets and western-type diets rich in refined carbohydrates and elevated fat levels. These effects can be seen in as little as 5 weeks. Increased energy expenditure is one potential mechanism for the observed weight loss effect associated with KDs. In fact, KD-fed animals reportedly have increased expression of genes related to beta-oxidation and reduced expression of genes related to lipid synthesis (4).

Despite promising findings of reduced weight gain in animals fed a KD, other studies focused on body composition and fat distribution have reported that mice and rats fed a KD diet have greater fat mass compared to chow-fed counterparts despite reduced weight gain overall. Moreover, long term studies of rodents on KDs suggest that KDs cause immediate and rapid weight loss, but after 20+ weeks on a KD, weights return to, or even exceed, baseline values (4). Other reports have suggested that obesity-related outcomes in mice do not translate to the human population, which is a common shortcoming of rodent models (20).

Diabetes. Although KDs may exert positive short-term effects on weight management in rodents, adverse metabolic side effects may also be observed. Mixed results have been observed in terms of glucose homeostasis in rodents fed KDs. Ob/ob mice have shown improved glucose tolerance and inulin sensitivity in conjunction with weight loss (20,21) and db/db mice have demonstrated a reversal of diabetic neuropathy on KDs (22). Other studies have shown the opposite effects. In fact, multiple studies have reported glucose intolerance and insulin resistance with or without concurrent weight loss (20). A possible reason for this adverse observation is that glucose produces post-prandial thermogenesis to a greater extent than lipids. As such, post-prandial metabolic rates are increased when a diet containing higher levels of carbohydrate are included (22). Another possible explanation for the discrepancies between results may be related to a difference in the expressed phenotypes of hepatic and skeletal muscle tissues. In a study of KD-fed wild type mice utilizing a hyperinsulemic-euglycemic clamp model, hepatic insulin resistance was observed, but systemic insulin resistance was much less severe. It is quite possible that sensitivity to glucose and insulin in animals fed KDs is tissue-specific. More research is needed to validate these findings (21).

Non-Alcoholic Fatty Liver Disease. Given that weight loss is a recommended method for management of NAFLD and KDs may be a beneficial weight loss tool, efforts have been made to determine whether KD-induced weight loss may also mitigate symptoms of NAFLD. Interestingly, a study of C57Bl/6 mice fed a KD for 12 weeks indicated that while the KD had a positive impact on weight management, they were also glucose intolerant and demonstrated symptoms of NAFLD. Hepatic lipid accumulation was observed after just 3 weeks on the KD. At the end of the study, the mouse livers exhibited ER-stress, inflammation, and macrophage accumulation, while counterparts on a western-type diet only demonstrated steatosis. Since KDs are low in total protein, it is speculated that a potential reason for NAFLD onset was due to low levels of dietary methionine along with an insufficient amount of dietary choline (21). Methionine and choline deficient diets are traditionally used to induce NAFLD in rodent models; KDs may inadvertently also cause these symptoms depending on the precise KD formulation. This could potentially confound study results in rodent models of KD and metabolic disorders. However, the translatability of rodent NAFLD models to humans is also not well understood, and methionine and choline deficient diets for induction of NAFLD in rodent models are heavily criticized in terms of human relevance (23).

Cancer. Unlike normal cells, cancer cells chiefly generate ATP through glycolysis rather than oxidative phosphorylation and not all cancers are able to metabolize ketone bodies (24). Thus, it is believed that KDs can reduce cancer/tumor proliferation by starving the cancer of glucose. There is evidence to demonstrate the anti-cancer effects a KD in models of glioblastoma, neuroblastoma, prostate, colon, pancreatic, lung, breast, stomach, and liver cancers, indicating potentially widespread efficacy of this approach (24). In addition to slowing tumor growth, KDs have also been shown to improve cachexia and increase the efficacy of chemotherapy and radiotherapy in animal models (25). While promising, these findings are not ubiquitous across all cancer types and models. Evidence in rat and mouse models of kidney cancer demonstrated significant adverse effects. Male and female Long Evans Tcs2+/- rats, which can develop renal cell carcinoma, fed a ketogenic diet for 4-8 months showed significantly enhanced tumor growth (26). Likewise, KDs had a pro-cancer effect on mice with human melanoma xenografts, increasing both the size and growth rate of tumors on two separate KD formulas (27). Taken together, investigators should carefully consider the type of cancer and the underlying genetic mechanism of that cancer when selecting a diet for their studies.

Control Diets

Choosing a proper control is a critical element in the experimental design process for any study. One benefit of using a purified ingredient laboratory animal diet is that it gives the researcher the ability to tightly control many elements of the formula, leaving only the variable(s) in question different between control and experimental groups. Selection of an improper control diet can introduce extraneous and unwanted variables that can make data interpretation difficult for investigators (28). Indeed, this may be one factor contributing to inconsistent findings across research groups as there are numerous instances in the literature indicating that a grain-based (GB) diet was used as the control for a KD (4).

One common selection for a control diet is a GB diet, colloquially referred to as “chow”, “standard diet/chow”, or “normal diet/chow”. These diets are made with a combination of animal by-products and whole plant based ingredients (such as corn, wheat, and alfalfa). The ingredients they use make GB diets an affordable option and animal vivariums typically stock GB diets as the main food source for the animals they house. However, GB diets introduce a significant amount of variation as the nutritional quality of the ingredients can vary depending on where and when they are harvested and how they are processed (28).

Purified Diets Paste or Pellet vs Chow Based Diets

Despite their ubiquitous use, GB diets are compositionally different from most KDs, which are frequently made with purified ingredients. In selecting a control diet for a KD, one should consider the ingredient compositions of both diets, their macronutrient profiles, and the micronutrient (vitamins and minerals) mixes. Other factors, such as sucrose, fiber, and non-nutrients (e.g. phytoestrogens), can also introduce additional variables. If you need help selecting a properly matched control for your ketogenic diet study, the nutritional scientists at Research Diets, Inc. are glad to discuss options based on the specific KD you select for your study.

Technical Considerations

Typically, rodent ketogenic diets contain 10-20 kcal% protein and 80-90 kcal% fat, with very few calories from carbohydrate. The fat in KDs is normally derived from a source that can be solid or semi-solid at room temperature (eg. shortening, lard, and cocoa butter) to avoid leakage of the oil.

The amount of protein in the diet is an important consideration when designing or choosing a KD. Rodents can produce glucose from protein through gluconeogenesis during fasting or when dietary carbohydrate is scarce. It has been found that plasma BHB induction is higher and faster when the protein content in the diet is lower (e.g. 5 kcal% protein and 95 kcal% fat vs. 13 kcal% protein and 87 kcal% fat) (29). Importantly, however, ketogenic diets with 5 kcal% protein can cause weight loss. The weight loss seems to occur only when the protein level in the ketogenic diet is lower than 10 kcal% since mice fed a ketogenic diet with 10 kcal% protein and 90 kcal% fat do not exhibit weight loss (30). When protein level is maintained at 10 kcal%, plasma BHB is negatively correlated with carbohydrate content, as zero carbohydrate diet generated the highest levels and increasing carbohydrate content gradually reduced plasma BHB levels. There is no difference in plasma BHB levels after there is more than 15 kcal% carbohydrates (30). Further, since rodents are nocturnal, it has been shown that plasma BHB induction by ketogenic diet is highest during the nighttime feeding period (30).

Due to the very high levels of fat found in ketogenic diets, several important considerations should be made regarding the physical properties of the diet when planning your study. First, the great majority of ketogenic diets are not available in the traditional pelleted form. Rather, they will be produced as a thick paste, which must be administered in cups on the cage floor or in a J-shaped hanging feeder, rather than in the food hoppers. To prevent diet form as a confounding variable in your study, you may wish to order your control diet in powder form as well, despite the fact that it may be available in pelleted form.

If obtaining a pelleted ketogenic diet is important for your research goals, diets formulated with cocoa butter as the primary fat source allow us to overcome this obstacle. The higher melting point of cocoa butter allows us to pellet the diet even at concentrations upwards of 90 kcal%. However, it may also be important to consider the fatty acid composition of the fat sources you. One of the nutrition scientists at Research Diets, Inc. would be more than happy to discuss custom options for your specific research needs.

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