Experimental Neurology 306 (2018) 149–157

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research paper

A ketogenic diet reduces metabolic syndrome-induced allodynia and promotes peripheral nerve growth in mice

Michael A. Cooper[a], Blaise W. Menta[a], Consuelo Perez-Sanchez[c], Megan M. Jack[b], Zair W. Khan[a], Janelle M. Ryals[a], Michelle Winter[d], Douglas E. Wright[a,*]

[a] Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS 66160, United States
[b] Department of Neurosurgery, University of Kansas Medical Center, Kansas City, KS 66160, United States
[c] Department of Integrative and Molecular Physiology, University of Kansas Medical Center, Kansas City, KS 66160, United States
[d] Kansas Intellectual and Developmental Disabilities Research, University of Kansas Medical Center, Kansas City, KS 66160, United States

ARTICLE INFO

Keywords:
Ketones
High-fat
Pain
Exercise
Diabetes
Ketogenic
Mice
DRG

ABSTRACT

Current experiments investigated whether a ketogenic diet impacts neuropathy associated with obesity and prediabetes. Mice challenged with a ketogenic diet were compared to mice fed a high-fat diet or a high-fat diet plus exercise. Additionally, an intervention switching to a ketogenic diet following 8 weeks of high-fat diet was performed to compare how a control diet, exercise, or a ketogenic diet affects metabolic syndrome-induced neural complications. When challenged with a ketogenic diet, mice had reduced bodyweight and fat mass compared to high-fat-fed mice, and were similar to exercised, high-fat-fed mice. High-fat-fed, exercised and ketogenic-fed mice had mildly elevated blood glucose; conversely, ketogenic diet-fed mice were unique in having reduced serum insulin levels. Ketogenic diet-fed mice never developed mechanical allodynia contrary to mice fed a high-fat diet. Ketogenic diet fed mice also had increased epidermal axon density compared all other groups. When a ketogenic diet was used as an intervention, a ketogenic diet was unable to reverse high-fat fed-induced metabolic changes but was able to significantly reverse a high-fat diet-induced mechanical allodynia. As an intervention, a ketogenic diet also increased epidermal axon density. In vitro studies revealed increased neurite outgrowth in sensory neurons from mice fed a ketogenic diet and in neurons from normal diet-fed mice given ketone bodies in the culture medium. These results suggest a ketogenic diet can prevent certain complications of prediabetes and provides significant benefits to peripheral axons and sensory dysfunction.

1. Introduction

The growing epidemic of obesity and diabetes has led to a dramatic increase in various pain syndromes and a personal as well as economic burden. A common complication associated with diabetes and metabolic syndrome is a loss of small fibers in the skin and increased pain (Callaghan and Feldman, 2013; Smith and Singleton, 2013). With the development of metabolic syndrome, some have proposed that there is a loss of axonal regenerative capacity leading to a loss of small fibers that can occur with diabetes (Singleton et al., 2015). Prediabetes can be modeled in rodents using a high-fat diets and consumption of high-fats and carbohydrates lead to metabolic alterations and changes in sensory function similar to changes human patients, including obesity, elevated blood glucose, insulin resistance, and mechanical allodynia (Groover et al., 2013; Guilford et al., 2011; Hoke, 2012; Obrosova et al., 2007). Physical activity can improve many of these symptomatic changes; including reversing mechanical allodynia induced by a high-fat diet (Groover et al., 2013). However, the mechanism(s) by which exercise leads to metabolic and sensory nerve benefits is poorly understood.

Previous research has demonstrated that exercise and a high-fat diet create distinctive metabolic phenotypes both systemically and in the peripheral nervous system (Cooper et al., 2016). Both exercise and a ketogenic diet intervention are attractive approaches as both can increase fat oxidation (Horowitz and Klein, 2000; Paoli, 2014). Additionally, both exercise and ketogenic diet can stimulate anti-inflammatory signaling cascades and reduce chronic inflammation that occurs in response to a high-fat diet (Ruskin et al., 2009).

High-fat, low carbohydrate “ketogenic” diets are a rapidly emerging intervention for a wide array of clinical diseases (Klein et al., 2014; Masino and Ruskin, 2013; Wheless, 2008). Historically, a ketogenic diet was popularized in the treatment of epilepsy after it was noted that patients who fasted observed reduced seizure occurrence (Wheless, 2008). Relevant to peripheral nerve function, only a limited number of investigations have examined how a ketogenic diet impacts mechanical

* Corresponding author.
E-mail address: dwright@kumc.edu (D.E. Wright).

https://doi.org/10.1016/j.expneurol.2018.05.011
Received 11 April 2018; Received in revised form 8 May 2018; Accepted 9 May 2018
0014-4886/ Published by Elsevier Inc.

and thermal sensation (Galdino et al., 2014; Ruskin et al., 2009, 2013; Ziegler et al., 2005). Caloric restriction increases ketone metabolism and can be a pro-growth signaling agent in the hippocampus and dentate gyrus (Lee et al., 2002a,b). However, the mechanism and function of increased neuronal growth is still under investigation.

Here, we examined several parameters of peripheral nervous system function from mice fed a ketogenic diet. We have previously reported that mice fed a high-fat and carbohydrate-rich diet develop negative symptoms related to peripheral nerve function similar to early peripheral neuropathy in prediabetes (Cooper et al., 2016; Groover et al., 2013; Guilford et al., 2011). In the current study, we provide evidence that mice fed a ketogenic diet fail to develop mechanical allodynia similar to mice fed a high-fat diet. Additionally, after 8 weeks of a high-fat diet, a ketogenic diet can reverse established mechanical allodynia. Lastly, we investigated the potential of a ketogenic diet to promote peripheral nerve outgrowth and noted that a ketogenic diet or ketone body supplementation increases axon growth of primary sensory neurons. Together, these results suggest that a ketogenic diet may be an attractive intervention for metabolic and diabetic peripheral neuropathy.

2. Materials & methods

2.1. Diet and mice

Seven-week-old male C57/BL6 #027 mice were purchased from Charles River (Wilmington, Mass) and maintained on a 12:12 h light/dark cycle in the research support facility at the University of Kansas Medical Center. All mice were given ad libitum access to food and water and were fed either a standard chow diet (8604; Envigo, Madison Wisconsin; 14% kcals from fat, 32% protein, and 54% carbohydrate), a high-fat diet (07011; Envigo; 54% kcals from vegetable shortening (hydrogenated) and corn oil fat, 21% protein and 24% carbohydrate), or a ketogenic diet (96,355; Envigo; 90.5% kcals from vegetable shortening (hydrogenated) and corn oil fat, 9.2% protein, and 0.3% carbohydrate). Standard diet energy was calculated as previously described (Groover et al., 2013).

Two separate experiments to examine the effects of dietary and exercise interventions on peripheral nerves were conducted. The first experiment included mice, referred to as the “challenge cohort”, included control-fed sedentary (CF-Sed); high-fat sedentary (HF-Sed); high-fat exercise (HF-Ex); ketogenic (Keto) groups of mice. After baseline behavioral testing was complete, mice were separated and the groups were then challenged with different diets and/or access to running wheels.

The second experiment included groups of mice referred to as “intervention cohort” in which all mice were fed a high-fat diet for eight weeks (notated as HF-CF-Sed, HF-HF-Sed, HF-HF-Ex, HF-Keto). After eight weeks and baseline behavioral testing, mice were then separated and the groups were given different diets or interventions. Control-fed, high-fat-fed and ketogenic-fed mice were all pair housed for the entirety of the study. Exercise mice were singly housed to allow for analysis of running amounts for each mouse and given access to a voluntary running wheel and were allowed to run for the remainder of the study. All mice were 8 weeks of age at the start of each experiment. A timeline of diet and exercise challenge and intervention cohorts are displayed in Fig. 1. All studies were in accordance with NIH guidelines and conformed to protocols approved by the institutional Animal Care Committee.

2.2. Blood measurements

Mice underwent assessments for weight and blood glucose (glucose diagnostic reagents; Sigma, St. Louis, MO) biweekly after a 3 h fast (Groover et al., 2013). Additionally, at the time of sacrifice following a 3 h fast, blood was drawn from the chest cavity and allowed to clot for 30 min on ice, spun at 3000 g for 30 min at 4 °C and serum drawn off and frozen at −80 °C until insulin was analyzed by ELISA (Alpco; Salem, NH). Blood ketones (β-Ketone blood test strips; Precision Xtra; Abbott Laboratories; Chicago, IL) were measured at baseline, week 1, 4, 8, and at sacrifice following a 3 h fast.

After 4 weeks, an intraperitoneal glucose tolerance test (IPGTT) was performed after a 6 h fast. Animals were given 1 g glucose/kg body weight. Blood glucose levels were measured via tail clip immediately before glucose injection, and 15, 30, 60, and 120 min thereafter.

2.3. Body composition

Body composition to assess fat mass was measured using the EchoMRI-100 (EchoMRI, Houston, TX). The first cohort of mice's body composition was determined immediately before sacrifice. Mice in the obesity intervention groups had body composition determined biweekly from baseline testing until sacrifice.

2.4. Sensory behavior testing

Sensory behavior assessments were carried out at baseline and biweekly time points for all mice. Dietary fed mice were examined for both mechanical and thermal sensitivity, while mice in the obesity intervention groups were only examined for mechanical sensitivity. Mechanical sensitivity was assessed using Von Frey monofilaments as previously described (Groover et al., 2013). Thermal thresholds were assessed by placing mice in individual clear plastic cages on a Hargreaves's apparatus and a 4.0 V radiant heat source was applied three times to the hind paw as previously described (Groover et al., 2013).

2.5. Intraepidermal nerve fiber (IENF) density

Footpads were collected and processed from all mice using protocols previously described for IENF density (Groover et al., 2013).

2.6. Neurite outgrowth

Lumbar DRGs 4–6 neurons were harvested and dissociated to a single cell suspension as previously described (Malin et al., 2007). Mice were fed the control, high-fat, or ketogenic diet described above for four weeks prior to DRG dissection. Upon plating, all mice were given Nutrient Hams F-12 media with 10 mM glucose for 4 days (Gibco). Additional culture experiments utilized chow-fed mice in which DRG neurons were plated and were given F12 media custom supplemented with varying levels of glucose (0 mM, 5 mM, 10 mM) and/or the ketone (R)-(−)-3–hydroxybutyric acid (5 mM or 10 mM) (Sigma). Ketone levels were selected to mirror glucose levels to reduce energy availability differences effecting neurite outgrowth and to mirror physiological levels seen in human patients on a nutritional ketogenic diet (Koppel and Swerdlow, 2017). Following the 4 days in culture, neurons were fixed with 4% paraformaldehyde for 10 min. Immunohistochemistry was performed with SMI-312 (Covance, Emeryville, CA), a pan-axonal marker, to visualize neurites and counterstained with nuclear marker, Hoechst 33,342 (Invitrogen). Coverslips were mounted on slides and imaged. Neurite outgrowth area was quantified using Image J. A stereological grid was superimposed on images of the cultures, and the number of neurites crossing exactly through intersections of the grid was counted, as was the number of neuronal cell bodies producing neurites. Three regions of interest were imaged per coverslip, and three coverslips per group were analyzed for each animal and the neurite area per neuron was calculated according to the following equation (Blacklock et al., 2005):

$$\frac{\left(\frac{\text{neurite intersections}}{\text{total grid intersections}}\right)\times\text{total grid area}}{\text{neurons extending neurites}} = \text{neurite area } (\mu m^2) \text{per neuron}$$