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Effects of a Ketogenic Diet on Stereotypic Behavior in Mice

Molly Brady

sponsored by Allison Bechard, PhD & Terry Bazzett, PhD

ABSTRACT

Stereotypic behaviors are repetitive, invariant, and purposeless actions resulting from central nervous system dysfunction. As one of the diagnostic criteria for autism, stereotypic mice have been used as a model for investigating mechanisms underlying autism. The ketogenic diet (keto diet) is a high fat, low carbohydrate diet that changes the body's main source of energy from glucose to ketones. It has numerous beneficial effects, including reducing self-directed repetitive behavior and increasing sociability. In this study, aged mice were fed a keto diet for seven weeks to assess its effects on stereotypic behavior and sociability. Home cage observations for stereotypic behaviors and a three-chamber social assay were used to evaluate behavior before and after administration of the keto diet. Brains were processed for immunohistochemistry of Delta-FosB, a transcription factor produced from chronic activation of striatal neurons. The keto diet decreased stereotypy across the test period, however, social behavior did not change significantly. Immunohistochemistry of Δ FosB in the nucleus accumbens was inconclusive and warrants further investigation.

INTRODUCTION

Stereotypic behavior is highly repetitive, purposeless actions and is seen in human disorders such as autism spectrum disorder (ASD), as well as in caged animals. Stereotypy can result from an underlying central nervous system disorder or pharmacological treatment with stimulants, and is often associated with neurological changes in the basal ganglia (Phillips et al., 2016). The rise in prevalence of autism has increased the need for mouse models to investigate mechanisms and treatments. As autism has a complicated symptomatology, using rodent models to analyze core behavioral symptoms such as excessive motor movement, reduced sociability, and abnormal development can lead to insights regarding underlying pathology and potential treatments (Bey & Jiang, 2014).

The keto diet has gained attention in recent years due to a wide variety of associated health benefits. A keto diet is characterized by increased fat and protein consumption and decreased carbohydrate intake. Practical use includes enhanced weight loss, decreased

epileptic seizures, decreased risk of cardiovascular disease, and increased sociability in ASD individuals (Gogou & Kolios, 2018; Paoli, Rubini, Volek, & Grimaldi, 2013; Williams & Cervenka, 2017). Recent studies administered a keto diet to both ASD children and adults with significant improvements in core autistic features (Lee et al., 2019).

Ketosis is the process of burning fats for energy rather than glucose, and results from cellular alterations that lead to metabolic and behavioral changes. While the central nervous system typically relies on glucose as an energy source, lack of dietary carbohydrates necessitates an alternative energy source. Fat is broken down by the liver and astrocytes into ketone bodies (acetoacetate, β -hydroxybutyric acid, and acetone), which are then used as a source of energy for the central nervous system (Boison, 2017). As a result, brain-derived neurotrophic factor (BDNF) and neurogenesis increase, while oxidative stress and neuroinflammation decrease. Cognitive processes benefit, especially working and spatial memory (Masino & Rho, 2012). Further, the keto diet leads to improved mitochondrial functioning and altered NADH dehydrogenase complex mRNA gene expression, which have implications for decreased cellular stress (Cooper et al., 2018). These findings exhibit the metabolic changes that can ameliorate neuronal loss and allow more efficient central nervous system (CNS) functioning (Wang et al., 2018).

The beneficial use of a keto diet for autism is still an emerging area of interest. While the disorder is prevalent and well-studied, the causes are still yet unknown beyond both environmental and genetic influences. Beyond behavioral deficits, autism has been associated with metabolic dysfunction and can be comorbid with epilepsy or other seizure disorders (Napoli, Duenas, & Giulivi, 2014). Thus, the ketogenic diet benefits multiple aspects of autism through similar mechanisms, leading to overall improvements in both behavioral symptoms such as social anxiety, stereotypy, and cognitive functioning.

In this study, a keto diet was administered to a group of mice exhibiting various behavioral deficits associated with mouse models of autism including stereotypical motor movements, decreased sociability, and susceptibility to seizures (Bey & Jiang, 2014). The strain of mouse used, FVB/NJ, is not commonly used as a mouse model of autism spectrum disorder; however, the mice involved in the study had a history of stereotypy and antisocial behavior within the lab. Further, this strain of mice is also especially seizure-prone (Goelz et al., 1998). Thus, the combined behavioral and neurological symptoms warranted therapeutic investigation with a ketogenic diet to determine whether stereotypy and antisocial behaviors would decrease with consumption of the diet. Prior research has shown positive results in autism spectrum disorder mouse models, including increased sociability, decreased self-directed repetitive behavior, decreased anxiety, and higher nociceptive (pain) thresholds (Ruskin et al., 2013; Castro et al., 2016). However, few studies have examined the effects in older populations of mice, focusing rather on adolescent or young adult mice (Ruskin, Fortin, Bisnauth, & Masino; 2017). Regarding beneficial effects on non-stereotypic

mice, ketogenic diets have been shown to improve longevity of mice, preservation of physiological functions in year-old C57BL/6J mice, and increase locomotor activity and alertness (Roberts et al., 2017). As there is support for benefits of a keto diet in both younger autism spectrum disorder-model mice and aged healthy models, the current study focused on the potential effects of administering a keto diet to aged mice exhibiting stereotypic behavior.

MATERIALS AND METHODS

Subjects

Fifteen-month-old FVB/NJ mice (JAX stock #001800) were used in the experiment. All subjects were inbred from mice obtained from Jackson Labs (Bar Harbor, Maine). The animals were housed individually and kept under a 12-hour light/dark cycle. All animals received ketogenic paste (F3666; Bio-Serv, Flemington, NJ) in a temperature-controlled vivarium with ad libitum access to food and water. All procedures were performed in accordance with the Institutional Animal Care and Use Committee of the State University of New York at Geneseo, and were consistent with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Materials

The ketogenic diet consisted of 75.1% fat, 8.6% protein, and 3.2% carbohydrate. HealthyWiser urinalysis strips were used to ensure all animals reached ketosis. Food consumption and body weight were monitored throughout the study, and all measures were taken to minimize animal suffering.

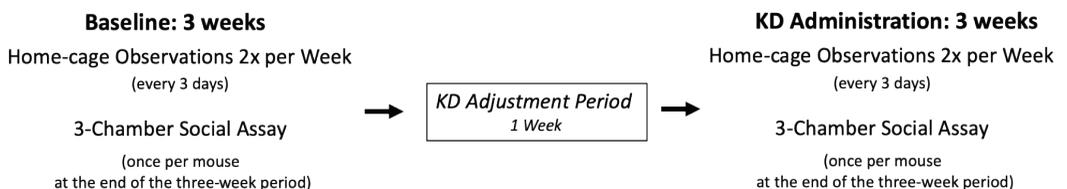


Figure 1. Methods used to investigate effects of a ketogenic diet before and after administration.

Behavioral Analysis

Mice were analyzed for overall sociability and stereotypy levels using a three-chamber apparatus and home-cage observations. All behavioral observations occurred for three weeks before ketogenic diet administration, and three weeks while consuming the ketogenic diet, as seen in Figure 1. To test sociability, each mouse underwent three 10-minute phases in which it could pass freely between chambers. Empty inverted wire pencil holders (10 cm tall and wide) were placed in the left and right chambers. In the first phase, the test animal explored the apparatus, and the wire cages were empty to account for side bias. In the second phase, a C57/BL6 mouse of the same

sex (JAX stock #000664) was placed in the wire cage within the first chamber, and the test mouse was allowed to roam and interact freely as a test of sociability. In the third phase, a second C57/BL6 mouse of the same sex was placed in the last wire cage in the third chamber to test for social novelty preference. Coders were blind to the condition.

Self-directed grooming and stereotypy levels were quantified through hour-long observational periods. Each mouse was observed for a one-minute period in succession. Each mouse was given one behavioral rating (as specified in the ethogram in Table 1), per focal period, for a total of six ratings throughout one hour. Sessions took place during active hours every three or four days over a three-week period.

Behavior	Abbreviation	Definition
Spinning	S	Mouse turns in a complete circle at least three times in a row without stopping.
Other	O	Any other abnormal behavior repeated three times in a row without stopping; most often includes hanging from the cage top and biting the bars or backflipping.
Active	A	Any normal, active behavior, including roaming cage, eating, grooming, etc.
Inactive	I	Laying down, not moving.

Histology

After a total of seven weeks of behavioral observations, all animals were sacrificed and underwent perfusion. Four animals were transcardially perfused, while the other six were extracted and drop-fixed in 4% paraformaldehyde (PFA) for four weeks. For all brains, PFA was refreshed twice within the first 24 hours to aid fixation and transferred to 30% sucrose in phosphate buffered saline (PBS) for 48 hours before snap-freezing in isopentane. Brains were stored at -20 degrees C, then sliced at 30 µm using a Leica cryostat into wells of PBS stored at 4 degrees C.

The ΔFosB immunohistochemical procedure (using FosB Monoclonal Rabbit IgG Antibody (ThermoFisher Scientific) was obtained from relevant studies (Phillips et al, 2016; Werme, et al., 2002). All procedures were conducted at room temperature. Slices were exposed to H₂O₂ (0.3% in PBS) for 15 minutes, then blocked (PBS, 1.5% Triton X and 3% normal donkey serum) for 2 hours, and incubated in primary antibody (diluted 1:1000 in PBS with 0.3% Triton X and normal donkey serum) overnight. Samples were then incubated in biotinylated goat anti-rabbit secondary antibody (1:200, Vector Laboratories, Burlingame, CA) for 2 hours and treated with an avidin/biotin peroxidase complex (Vectastain ABC Kit, Vector Laboratories) for 90 minutes. Slices were then stained for 8 minutes in 0.06% diaminobenzidine and

0.1% H₂O₂ in PBS. Finally, the slides were rinsed in increasing concentrations of ethanol (70%, 95%, 100% each for one minute), dipped in xylene for 10 minutes, then cover-slipped using DPX Mountant (Fisher Scientific).

RESULTS

Behavioral Analysis

Overall levels of stereotypy were reduced across all stereotypic mice ($p = 0.045$, $n = 10$) as seen in Figure 1. However, when spinning or other stereotypic behavior were examined alone, there was not a significant decline.

The three-chamber social assay did not yield significant changes in sociability with ketogenic diet consumption. Test mice preferred to spend more time with a stimulus mouse than an empty chamber regardless of diet condition ($p = 0.012$, $p = 0.005$). There was no significant preference for the familiar or new stimulus mouse in either diet condition (Figure 2). Self-directed repetitive grooming behavior also did not decrease significantly.

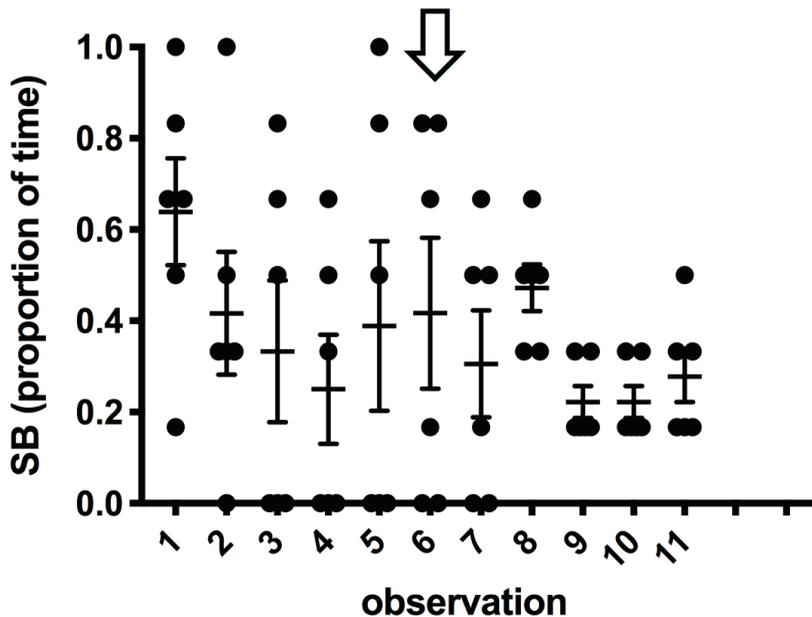


Figure 2. Overall levels of stereotypy significantly decreased with consistent consumption of KD. Arrow represents introduction of KD.

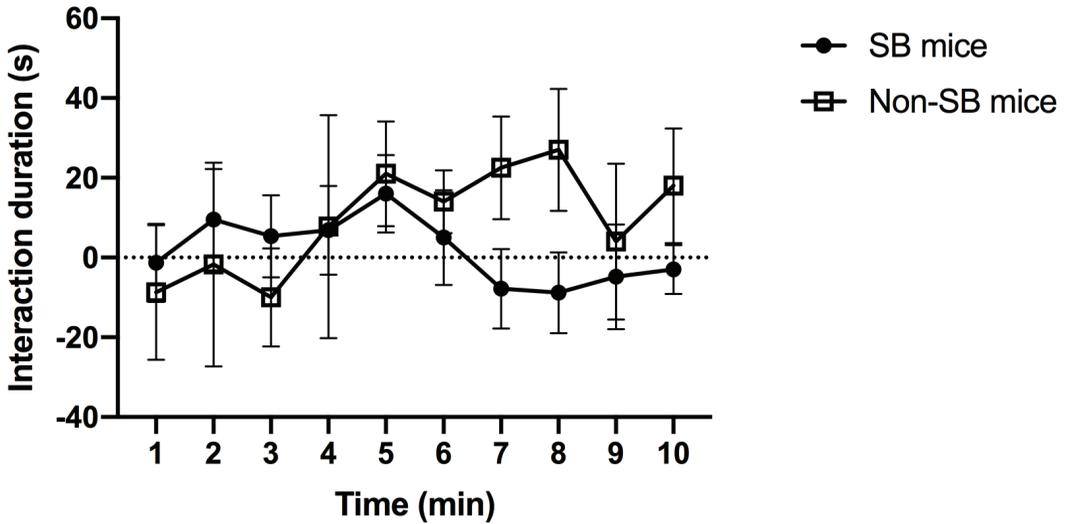


Figure 3. A delta comparison of non-significant interaction duration for new and familiar stimulus mice before and after administration of KD.

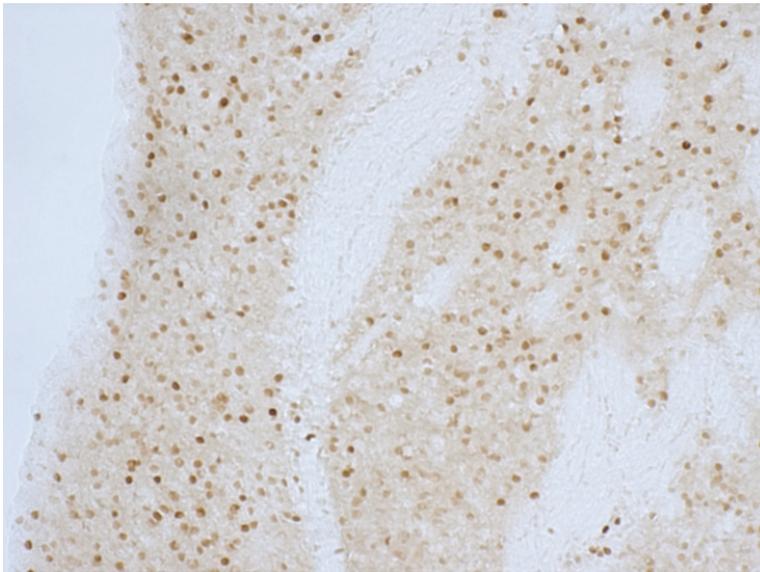


Figure 4. Δ FosB expression in the nucleus accumbens in a positive control.

Histology

Efforts to visualize Δ FosB expression using immunohistochemistry were unsuccessful due to issues with brain preservation. Positive control C57BL/6J mice of similar age were injected with ethanol (1.12 g/kg) 30 minutes prior to euthanasia, then processed with the same protocol described above. Results are displayed in Figure 4, and show successful staining of nucleus accumbens tissue.

DISCUSSION

These findings demonstrate that overall stereotypy decreased with continued consumption of a ketogenic diet. Although the sample size was small, leading to non-significant differences for spinning and all other stereotypy, respectively, a significant decrease occurred when all stereotypic animals' behavior was analyzed together. The cause of the change in behavior may have several underlying mechanisms influenced by the ketogenic diet at multiple target sites. The increase in circulating ketone bodies alters production of inhibitory neural mediators and ion channel modulators in various brain regions, which lead to decreased abnormal signaling and excess activity (Rogawski et al., 2018). Other mechanisms of interest include reduced reactive oxygen species and neuroinflammation, which may lead to increased neuronal inhibition via decreased glutamate release (Ruskin et al., 2013).

Previous studies have shown positive sociability changes in autism spectrum disorder mouse models including social transmission of food preference and the three-chamber sociability assay (Ruskin et al., 2013; Ruskin et al., 2017). Although Ruskin et al. (2017) found sex differences in sociability improvements of in the EL mouse, the current study did not find any significant sex effects (although the small sample size created excessive variance). The current study did not find any significant improvements in sociability after consumption of KD, and there may be multiple contributing factors. Social disposition is prone to impacts and influence during earlier stages in life when the brain is still developing (Verpeut et al., 2016). Thus, sociability is less likely to change as the mouse ages, and the mice in the current study were considerably old, there was likely to be less impact at such a late stage in life. Further, as sociability is a complex behavior, the sample size may have been too low to detect and significant alterations in interactions within the three-chambered test. Finally, as the FVB/NJ mouse is one of the most social species of mouse, overall sociability may have been less subject to influence by the ketogenic diet than other mouse models of autism (Bey & Jiang, 2015).

We would have expected to see heightened expression of Δ FosB in the nucleus accumbens and striatum in mice exhibiting increased levels of stereotypy compared to those who showed little or no stereotypy. Δ FosB is implicated in both addictive behaviors and voluntary movement, and thus would be expected to express in higher concentration in areas involved in movement and reward (Phillips et al., 2016; Werme et al., 2002). Thus, the basal ganglia, specifically the nucleus accumbens, are likely implicated in the observed stereotypy. The inconclusive nature of the current study thus requires replication in order to confirm behavioral findings with increased n-values and improved processing of the brains to support improved histology results.

The current research does, however, provide substantial foundation for further investigation into the neural mechanisms of a novel genetic mouse model for autism spectrum disorder. Human autism spectrum disorder can present in a variety of behavioral and developmental deficits, and the therapeutic impact of a ketogenic diet on the various aspects is still yet to be fully understood. The current findings help to bridge

the gap between rodent research and human research, which will eventually lead to a greater understanding of why the ketogenic diet yields such success in decreasing abnormal behavior. A goal of the research is to provoke further investigation into the functional methods of the diet, which may provide insight regarding the neural basis of autism spectrum disorder itself.

REFERENCES

- Bey, A. L., & Jiang, Y. (2014). Overview of mouse models of autism spectrum disorders. *Current Protocols in Pharmacology*, 66(5), 1–26. doi:10.1002/0471141755.ph0566s66
- Boison, D. (2017). New insights into the mechanisms of the ketogenic diet. *Current Opinion in Neurology*, 30(2), 187–192.
- Castro, K., Baronio, D., Perry, I. S., Riesgo, R. D. S., & Gottfried, C. (2017). The effect of ketogenic diet in an animal model of autism induced by prenatal exposure to valproic acid. *Nutritional Neuroscience*, 20(6), 343–350. doi:10.1080/1028415X.2015.1133029
- Lee, R. W. Y., Corley, M. J., Pang, A., Arakaki, G., Abbott, L., Nishimoto, M., ... Wong, M. (2018). A modified ketogenic gluten-free diet with MCT improves behavior in children with autism spectrum disorder. *Physiology & Behavior*, 188, 205–211. doi:10.1016/j.physbeh.2018.02.006
- Gogou, M., & Koliou, G. (2018). Are therapeutic diets an emerging additional choice in autism spectrum disorder management? *World Journal of Pediatrics*, 14(3), 215–223. doi:10.1007/s12519-018-0164-4
- Gross, E. C., Klement, R. J., Schoenen, J., D'Agostino, D. P., & Fischer, D. (2019). Potential protective mechanisms of ketone bodies in migraine prevention. *Nutrients*, 11(4). doi:10.3390/nu11040811
- Masino, S. A., & Rho, J. M. (2012). Mechanisms of Ketogenic Diet Action. In J. L. Noebels, M. Avoli, M. A. Rogawski, R. W. Olsen, & A. V. Delgado-Escueta (Eds.), *Jasper's Basic Mechanisms of the Epilepsies* (4th ed.). Bethesda (MD): National Center for Biotechnology Information (US).
- Napoli, E., Dueñas, N., & Giulivi, C. (2014). Potential therapeutic use of the ketogenic diet in autism spectrum disorders. *Frontiers in Pediatrics*, 2, 69. doi:10.3389/fped.2014.00069
- Paoli, A., Rubini, A., Volek, J. S., & Grimaldi, K. A. (2013). Beyond weight loss: a review of the therapeutic uses of very-low-carbohydrate (ketogenic) diets. *European Journal of Clinical Nutrition*, 67(8), 789–796. doi:10.1038/ejcn.2013.116

- Roberts, M. N., Wallace, M. A., Tomilov, A. A., Zhou, Z., Marcotte, G. R., Tran, D., ... Lopez-Dominguez, J. A. (2017). A ketogenic diet extends longevity and healthspan in adult mice. *Cell Metabolism*, 26(3), 539–546.e5. doi:10.1016/j.cmet.2017.08.005
- Rogawski, M. A., Löscher, W., & Rho, J. M. (2016). Mechanisms of action of antiseizure drugs and the ketogenic diet. *Cold Spring Harbor Perspectives in Medicine*, 6(5). doi:10.1101/cshperspect.a022780
- Ruskin, D. N., Svedova, J., Cote, J. L., Sandau, U., Rho, J. M., Kawamura, M., ... Masino, S. A. (2013). Ketogenic diet improves core symptoms of autism in BTBR mice. *Plos One*, 8(6), e65021. doi:10.1371/journal.pone.0065021
- Werme, M., Messer, C., Olsen, L., Gilden, L., Peter, T., Nestler, E., & Brene, S. (2003). Δ FosB regulates wheel running. *The Journal of Neuroscience*, 22(18), 8133-8.
- Williams, T. J., & Cervenka, M. C. (2017). The role for ketogenic diets in epilepsy and status epilepticus in adults. *Clinical Neurophysiology Practice*, 2, 154–160. doi:10.1016/j.cnp.2017.06.001

