The Ketogenic Diet Rescues *Fmr1*^{KO} Phenotypes: Does the Gut Microbiome Play a Role?

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Background: In addition to intractable epilepsy, the ketogenic diet (KD) is showing beneficial effects in cancer, obesity, diabetes and neurodegeneration. We sought to determine if the KD attenuated disease phenotypes in *Fmr1*^{KO} mice, a mouse model for the neurodevelopmental disorders fragile X syndrome and autism.

Methods: *Fmr1*^{KO} mice were weaned onto KD versus control diet at postnatal day 18 and tested for seizure susceptibility in the audiogenic-induced seizure (AGS) assay and for alterations in circadian hyperactivity by actigraphy after chronic treatment.

Results: We typically observe 50-90% reduction in wild running, which precedes seizures in the AGS assay, and 100% rescue of seizure and death outcomes in response to acute treatment with metabotropic glutamate receptor 5 (mGluR$_5$) inhibitors in *Fmr1*^{KO} mice. In response to the KD, we observed 3% wild running, seizures and deaths in response to 110 dB audiogenic stimulation in male mice. In contrast, the KD did not attenuate seizure phenotypes in female *Fmr1*^{KO} mice, indicating a strong sex-specific difference in response to KD. We have tested several thousand mice in the AGS assay over the past decade in response to over two dozen pharmaceutical, dietary or genetic interventions. The attenuation in male mice in response to KD is comparable to or better than the best mGluR$_5$ inhibitors we have tested. This is the first time we have observed a strong sex-specific response to an intervention. In addition to juvenile seizures, the KD affects diurnal rest-activity rhythms in adult *Fmr1*^{KO} mice and wild type (WT) littermates. Actigraphy is a sensitive, noninvasive, reliable biomarker to measure rest-activity cycles. There are contradictory reports regarding hyperactivity in *Fmr1*^{KO} mice. We binned our data into 6-hr quadrants (first and second half of the light cycle and first and second half of the dark cycle) and observe a highly reproducible, statistically significant 40% increase in activity in *Fmr1*^{KO} mice during the 6am-noon quadrant, which is the first half of the light cycle. If we analyze in 12-hr bins, the difference is masked. Mice are nocturnal; thus, this period corresponds to the animals having trouble "*falling asleep*" and correlates with sleep problems in children with fragile X. In response to the KD, we replicated this finding (p=0.04), and activity was significantly decreased in *Fmr1*^{KO} mice in response to the KD in 3 of 4 binned timeframes (12am-6am, 6am-noon and 6pm-midnight).

Conclusion: The mechanism underlying the success of the KD and ketosis is not understood, but most likely involves the restoration of aberrant energy metabolism. A likely effector includes the gut microbiome. In future studies, we plan to determine the effect of the gut microbiome in mediating positive effects of the KD. Questions we propose to ask include: How does a germ-free background affect *Fmr1*^{KO} phenotypes? How does transplantation of human fragile X microbiota into gnotobiotic mice affect molecular, seizure and behavioral outcomes? Is the composition of the gastrointestinal microbiota altered in *Fmr1*^{KO} mice and fragile X patients? Do pre- and probiotics affect fragile X phenotypes? Do antibiotics other than minocycline improve fragile X phenotypes? How does the gut microbiome affect blood-based metabolites in fragile X? Our long-term goal is to understand the effects of diet and the microbiome on the development of neurological outcomes to better inform nutrition guidelines, particularly in the case of developmental disabilities such as fragile X syndrome.

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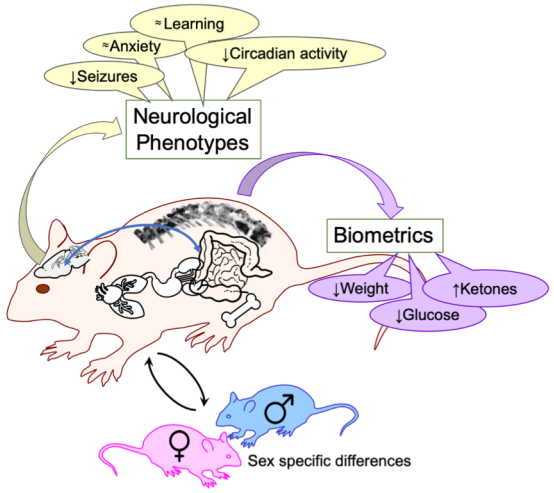

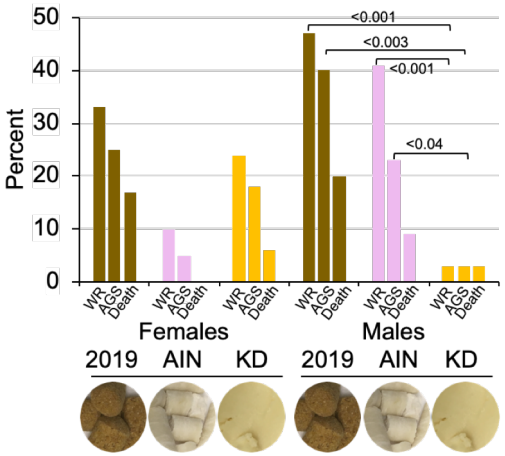

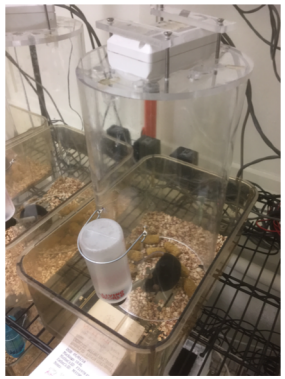

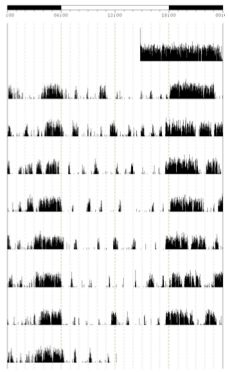

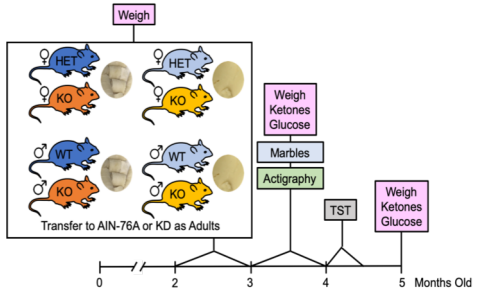

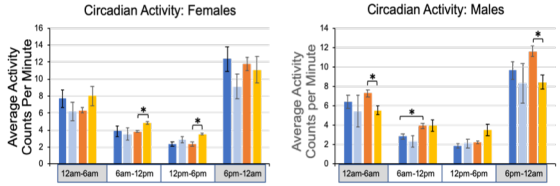

- The KD rescues AGS in male *Fmr1*^{KO} mice as well as the best mGluR5 inhibitors.
- The KD affects diurnal rest activity rhythms in young adult, adult and aged adult *Fmr1*^{KO} mice.
- No significant dietary effects on learning & memory, autism or depression tests (data not shown).
- Large dietary effect on weight gain (data not shown).
- Sex-specific differences.

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