

# Reducing Stereotypic Behavior with a Ketogenic Diet

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## Background

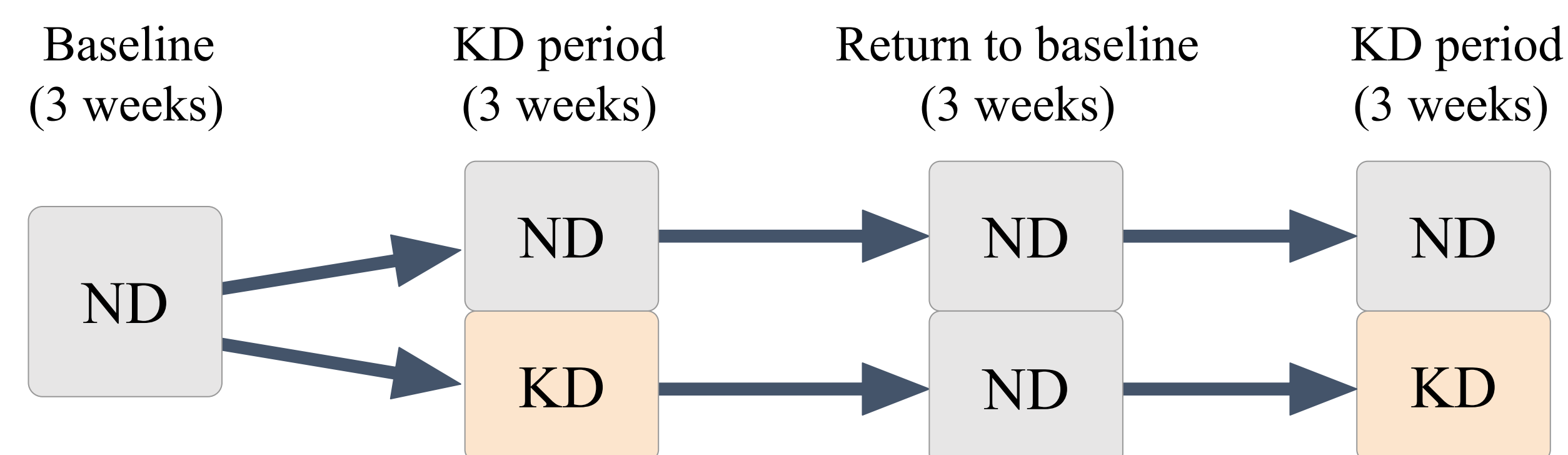
- Now a popular fad diet, the ketogenic diet (KD) is a high-fat, low-carb diet.
- For decades KD has been used for treatment of intractable epilepsy. In animal models, KD has been used in the treatment of dementia, obsessive-compulsive disorder, and autism spectrum disorder.
- These disorders are associated with stereotypic behaviors (repetitive, invariant behaviors that have no apparent function) that are often life-impairing and stigmatizing. However, little is known about their underlying mechanisms and no effective pharmacological treatments are available.
- Here, we present a novel application of KD to reduce stereotypic behavior in an inbred strain of mice (FVBN/J) that displays a prominent repetitive circling behavior. Using an ABAB design (see *Figure 1*), we compared stereotypic behavior between assessment periods on normal food diet (ND) and on KD.
- Following Golgi-Cox histochemistry, the density of dendritic spines (see *Figure 5*) in the dorsal and ventral striatum will be investigated as one potential neurobiological mechanism for these effects.

## Ethogram

Category	Behavior	Label	Description
Stereotypic	Spinning	S (L/R, W/N)	Turns in full circle at least three times without stopping. Specify left/right and wide/narrow.
	Flipping	O (F)	Alone or with bars, flipping at least three times.
	Bar biting	O (B)	Biting or gnawing on the bars of the cage top.
	Twirling	O (T)	Circling while suspended from cage, at least three times
Active	Eating	A (E)	Consuming food from cage top or in cage.
	Drinking	A (D)	Drinking from water bottle tap.
	Grooming	A (G)	Licking paws or fur, rubbing paws on face or head, etc.
	Roaming	A (R)	Normal active behavior, not included in another label.
	Inactive	Inactive	I

*Table 1.* Behavioral ethogram used to score subject behavior via instantaneous focal sampling. Observations were conducted every 10 minutes for 1 hour, 3 times per week.

## Methods

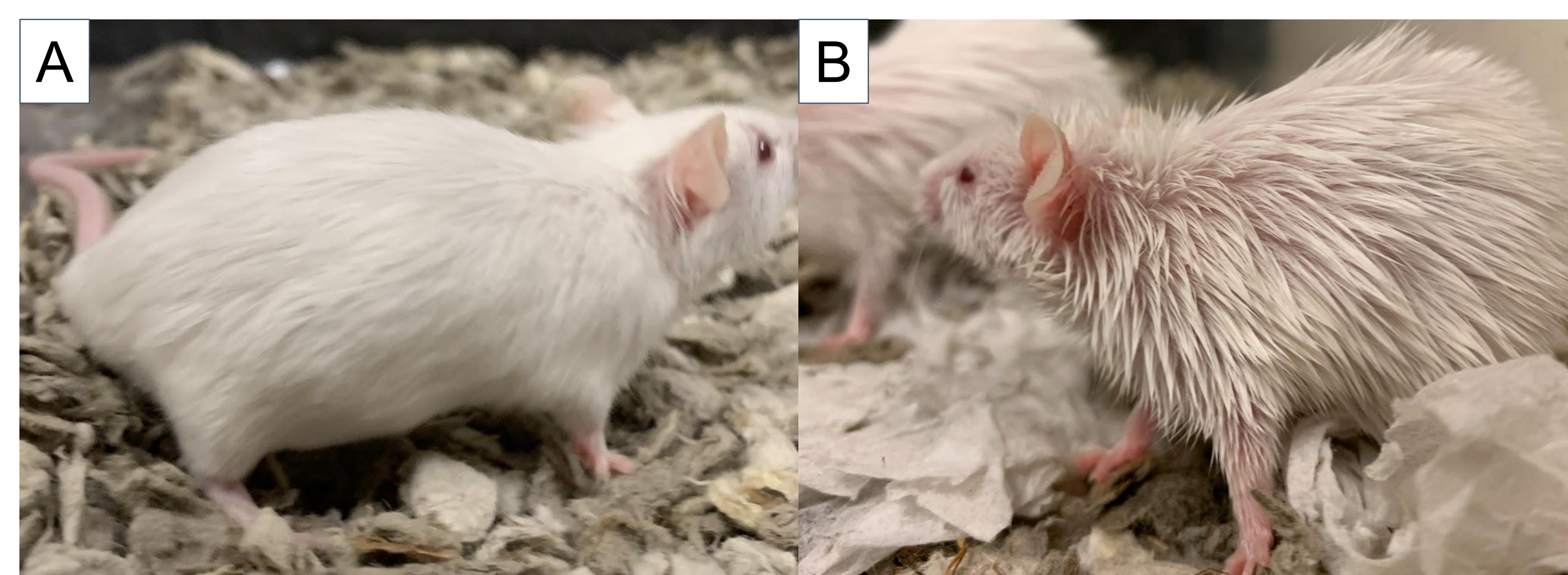


*Figure 1.* Timeline for the experiment. KD is ketogenic diet and ND is normal diet (rodent lab chow). Note that the last two periods were spaced farther apart due to timing of semester break.



*Figure 2.* Shown above are sequenced snapshot images depicting a “spinner” mouse circling in a rightward, narrow turn. Most subjects show a preference for directions, and an occasional preference for size of the circle.

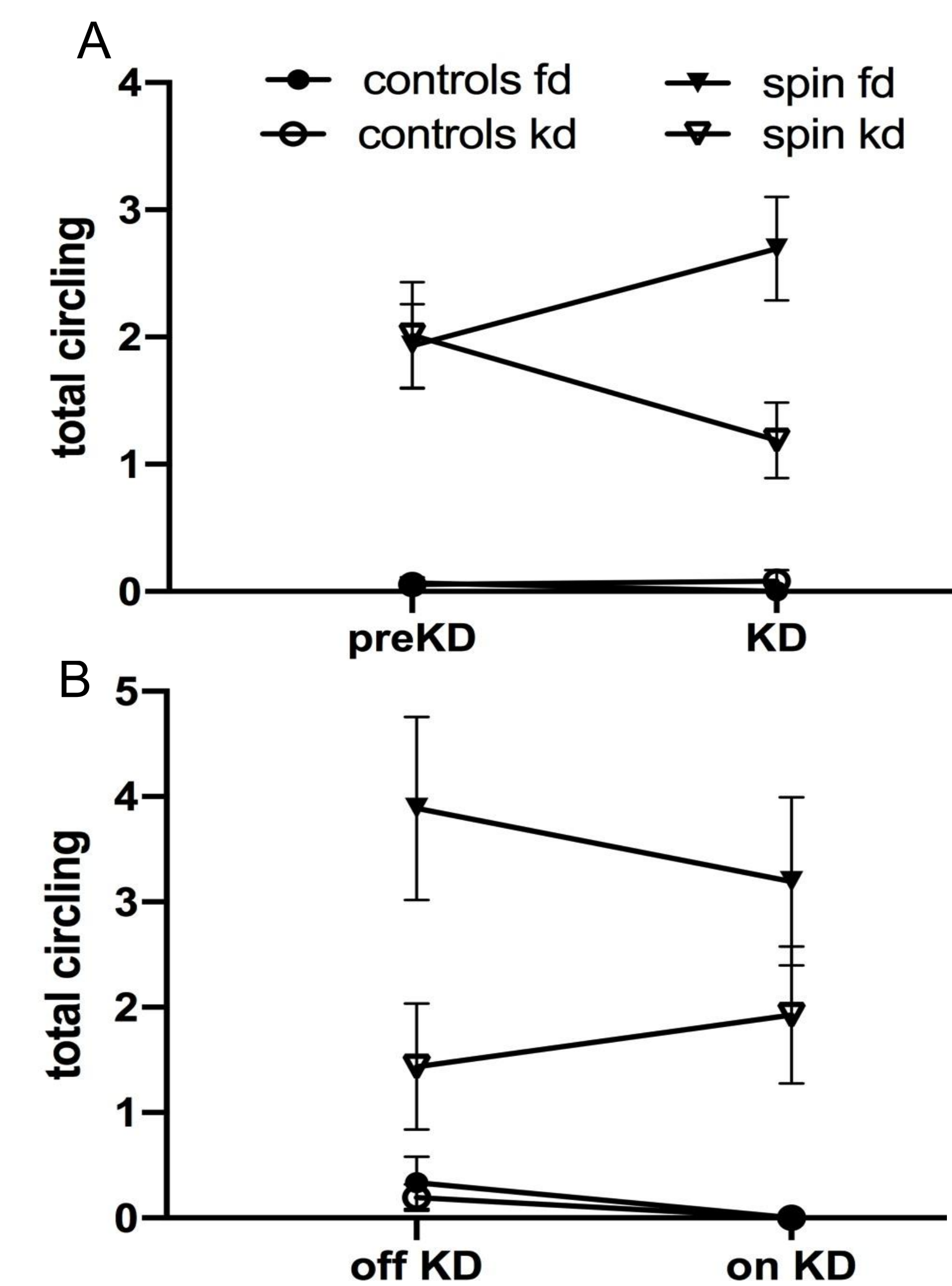
- Adult (6-8 months) females were paired such that a “spinner” mouse (see *Figure 2*) and a non-spinning control mouse from the same litter were housed together (N=14 cages).
- Cages were then split randomly into KD and ND and observed in 3 sessions per week across the 3 week diet period.
- Along with distinct changes in coat (see *Figure 3*), urinalysis was performed to demonstrate ketone bodies were present.
- The proportion of circling for each session was calculated for each mouse and then summed across the entire phase. This Total Circling was the dependent variable then subjected to a Repeated Measures ANOVA with Time and Diet and their interaction as factors in the model.



*Figure 3.* Shown on the left (A) a control mouse on ND, this is how the fur appears in a well-groomed mouse. The right image (B) shows the appearance while on KD. The fur appears greasy due to the animal utilizing its paws to eat then grooming.

## Results

- Phase 1 Data show an interaction between time and diet on stereotypic behavior ( $F(1,12) = 5.95, p = 0.031$ ; see *Figure 4 A*).
- Phase 2 Data show a main effect of Diet (KD vs ND:  $F(1, 19) = 21.1, p < 0.0001$ ) but no Time or Time x Diet significance.



*Figure 4.* Circling behavior at baseline (preKD) and after (KD) of A) Phase 1; return to baseline (off KD) and on KD of B) Phase 2.

## Discussion & Future Directions

- We found KD to be an effective treatment to attenuate the development of a pronounced repetitive circling behavior (Phase 1 conclusion).
- Interestingly, we found mice treated with KD maintained lower levels of circling, even when returned to a normal rodent chow diet (‘off KD’:  $t(11) = 2.3, p = 0.03$ ), and a second KD treatment was not able to further reduce the repetitive circling behavior (Phase 2 conclusion).
- We hypothesize that KD may alter glutamatergic synaptic connections in the basal ganglia via dendritic spine density.
- Future analyses will be performed for dendritic spine density.

*Figure 5.* The image to the right depicts the dendritic spines within the striatum of a mouse. We used a Golgi-Cox staining kit and the image taken on a Zeiss axiocam ICc3 camera with a 40x objective.

