Working Memory, Locomotor Activity, and Neuronal Density in Old Mice Fed a Ketogenic Diet

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Background

- The ketogenic diet (KD), is a high-fat, low-carb diet which has recently become popular as a way to improve memory and cognitive function. There is little research done on the benefits of KD and to what extent it benefits memory function.
- We modeled the effects that KD has on performance of old mice during a working memory task and locomotor activity. As a control, half of the mice were fed normal chow (SD), while the other half were fed KD.
- In experiment 1, the Barnes Maze was used to assess working memory (Figure 1). Using spatial cues, the mouse escapes to a target hole located in the Maze, and each day the target location is changed. KD mice showed a lower latency to escape (Figure 2) and a tendency to make fewer mistakes (Figure 3) .
- To detect if there were diet-induced differences in activity, experiment 2 utilized a 1hr locomotor test at the start of the diet period (baseline) (Figure 4) and again at the end of the diet period (KD) (Figure 5) but found no differences between mice on KD and mice fed a normal diet.
- In experiment 3, we used a cresyl violet stain to assess neuronal density in regions of the brain involved in memory, that included the prefrontal cortex and hippocampus, where higher density was hypothesized to be found in KD mice.
- Overall, we investigated one potential mechanism for our findings that show KD improves working memory performance without affecting locomotor activity
- Results support the potential for using KD as a treatment for disorders with memory impairments.

Figure 1. The Barnes Maze assessed working memory across the span of 4 days, consisting of 5 trials per mouse, per day. The first day, cue day, has a visual cue which indicates the target hole. The target hole is then moved each day for the next three days, and working memory is assessed by looking at both latency, and total amount of errors.

Experiment 1: Working Memory

- 11 old mice, 7 KD and 4 SD, were assessed in the working memory task
- The Barnes Maze was used to trace latency time to the target hole (Figure 2) and total number of errors (Figure 3) across four days of testing, including one cue day.
- Average latency time and total number of errors for all KD and SD mice were calculated for each day and graphically analyzed.

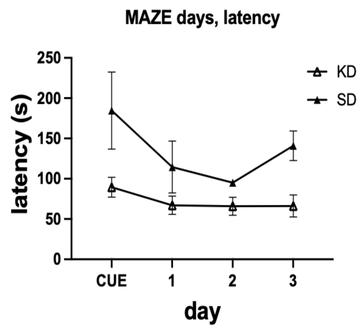

Figure 2. Latency (s) to find the target hole on a Barnes Maze for mice fed KD and SD. Mice fed KD had overall lower latencies compared to mice fed SD (Diet: ($F(1, 8) = 32.3$, $p = 0.0005$). KD mice performed better in the working memory task.

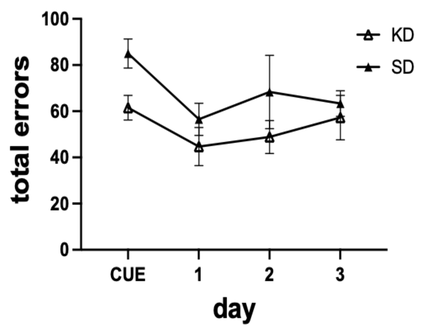

Figure 3. Total errors during Barnes Maze of mice on KD and SD. Mice fed KD had a tendency to make less total errors across the four days of working memory test (Diet: ($F(1, 8) = 3.8$, $p = 0.085$).

Experiment 2: Locomotor

- Locomotor activity was observed and assessed through one hour interval sessions.
- Automated photo-beam breaks were indicative of activity levels (see figure 4 and figure 5).
- We were specifically looking for the effects of a ketogenic diet on levels of horizontal activity.

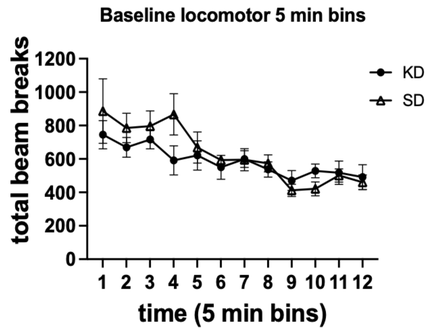

Figure 4. Baseline locomotor test. All mice decreased locomotor activity across the 1h test (Time: $F(2.5, 22.6) = 9.9$, $p = 0.0004$). There were no differences between groups at baseline.

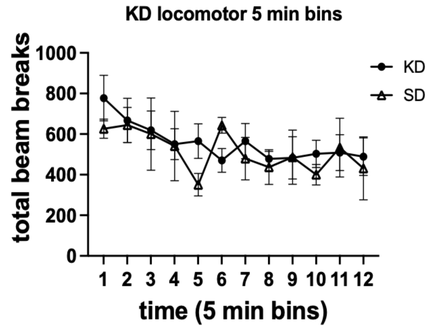

Figure 5. Locomotor test after 3 weeks of KD. All mice decreased locomotor activity across the 1h test (Time: $F(4, 28) = 3.3$, $p = 0.023$). There was no effect of diet.

Experiment 3: Neuronal Density

- Neuronal density of the prefrontal cortex and hippocampus were analyzed using cresyl violet staining. The densities were calculated utilizing cell count and areas of tissue samples selected within ImageJ.
- An increased neuronal density signifies better memory. As such, we expected an increase in density amongst those mice fed a KD diet.

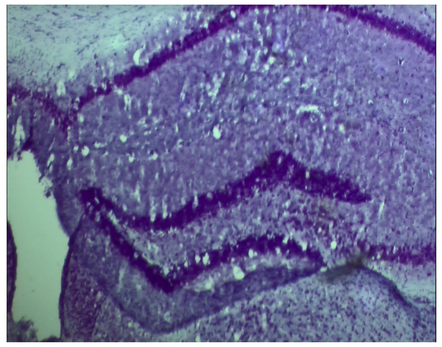

Figure 6. Hippocampus stained in cresyl violet. 5x magnification.

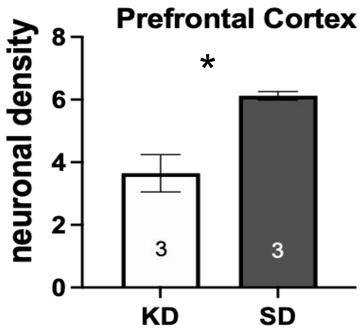

Figure 7. Neuronal density of the prefrontal cortex. Analysis showed a decrease in neuronal density in mice fed the KD diet (Diet: ($t(1,4) = 4.04$, $p = 0.015$).

Discussion

- Experiment 1, the Barnes Maze working memory experiment, exhibited significant findings of KD mice performing better in the working memory task than SD mice, as indicated by the lower latency times and fewer total errors across all four days of testing.
- Experiment 2, the locomotor experiment, assesses differences in activity levels. The findings were insignificant, as decreased beam breaks over time were recorded throughout the experiment for both KD and SD diets.
- Experiment 3, the neuronal density analysis, quantified the prefrontal cortex neuronal density. The findings showed a decrease in density amongst the mice that were fed a KD diet.

Future Directions/ limitations

- The first limitation is that only old mice ran the experiments and that younger mice were not included.
- The second limitation is that only 7 KD diet mice and 4 SD mice were in the experiment making it impossible to assess sex effects.
- A third limitation is that the sample size for the experiment was only 11 mice in total, which is relatively small.
- Neuronal density findings are based on a small sample of mice (N=6) and should be replicated. The hippocampus analysis is still in progress.
- Future research should replicate these experiments using equal sample sizes of different ages, genders, and breeds of mice.
- Further research is needed to decipher KD's exact mechanism.