# Drinking in the Dark (DID): A Mouse Model of Keto Influenced Binge-Like Alcohol Intake in the C57BL/6J Mice

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## Background

Alcohol misuse is a public health issue particularly since excessive drinking increased by 21 percent during the COVID-19 pandemic (Julien, 2021). In efforts to find non-pharmacological approaches to solving this ethanol epidemic, animal models such as the C57BL/6J are selectively bred strains of mice commonly used in the literature because they help to study the effects of alcohol on human-like behavior. They are used in this experiment and tested with red LED lighting to maintain their nocturnal habitats which helps promote drinking (Thiele, 2014). Using the DID model will help to simulate binge-drinking in humans. The ketogenic diet has also become a popular trend in western culture. The ketogenic diet is high in fat content and low in carbohydrate and protein content. The literature has also suggested that foods rich in omega-3 fatty acids can help reduce alcohol cravings. Our project is seeking to determine if a high fat KD diet intervention in "alcoholic" aka C57BL/6J mice will help to lower present binge drinking rates that translate to lower alcohol consumption in human behavior. Our hypothesis is that without any intervention, it is expected that binge drinking rates will rise over time; however, with intervention, binge drinking rates will fall; female mice will be more susceptible to binge drinking than male mice; and a KD diet will aid in producing lasting effects of drinking aversion.

## Methods

- Seven C57BL/6J mice (male = 5, female = 2) were randomly assigned to one of two diet groups: KD (ketogenic diet) and SD (standard diet).
- 20-30 minute habituation followed by two hour alcohol consumption assessments were tracked for approximately 8 weeks, 3x/week using sippers filled with 10% EtOH.
- Mice that were selected for standard diet remained on a healthy controlled diet throughout the entire experiment. Whereas, mice that were selected for a keto diet received daily nutrition high in fat halfway through the experiment for approximately 3 weeks starting at week eight. Data for pre/post weight and pre/post alcohol consumption was subsequently recorded into a lab notebook.

## Results

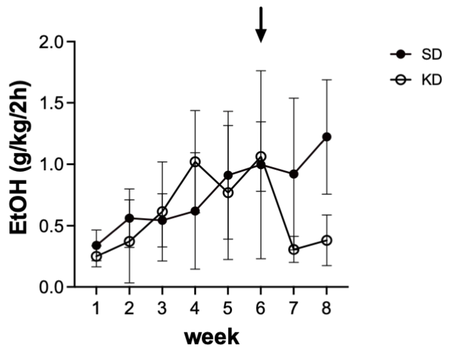


**Fig 1.** Averaged EtOH consumption in g/kg for 2-hour weekly drinking trials. The arrow indicates the point of KD intervention. There is no effect of time (F (2,11) =1.5, P=0.267). There is no significance of diet (F (1,5) =0.09, P=.766). There is no time x diet interaction (F (7,33) =1.2, P=.310).

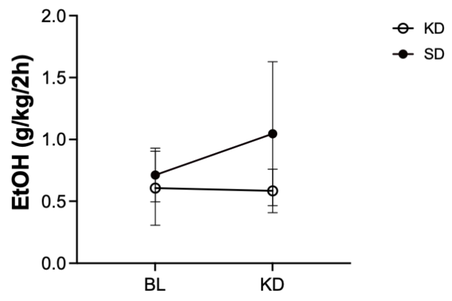


**Fig 2.** Averaged EtOH consumption in g/kg for standard diet and keto diet subjects before and after diet intervention. There is no effect of time (F (1,5) =.56, P=.488. There is no significance of diet (F (1,5) =0.46, P=.527). There is no time x diet interaction F (1,5) =0.73, P=.432).

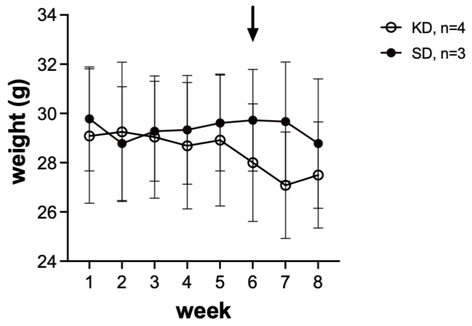


**Fig 3.** Averaged weekly weight of mice in grams. The arrow indicates the point of KD intervention. There is a non-significant trend in time (F (2, 11) = 3.806, P=.052). There is no significance of diet F (1, 5) = 0.070, P=.802). There is a significant time x diet interaction since mice in KD lost more weight in the later sessions than mice in SD; F (7, 35) = 4.28, P=.002.

## Discussion/Conclusion

- There appears to be significance in relation to the effect of time x diet on mice weight increase. However, no significance in diet, time, nor time x diet interactions for the averaged alcohol consumption and a non-significant trend in the averaged weekly weight between mice intervened with a ketogenic diet and mice being controlled for a standard diet.
- The analysis of the averaged weekly weight of mice does suggest considerable data. Since mice eating a ketogenic diet lost more weight in the latter sessions than mice eating a standard diet, there appears to be a substantial time x diet relationship.
- With the ongoing study and the analysis of the data shown, this could apply to humans, suggesting potential effects of ketogenic usage during weight loss attempts.

## Limitations/Future Directions

The study had a small sample size of seven thus there is a chance for a type II error. In the future a larger sample size would be suggested. Additionally, C57BL/6J mice may help to limit a prior confounding factor of voluntary consumption of ethanol inducing pharmacologically insignificant amounts of BECs. However, the literature for binge-alcohol drinking may concludingly reflect human-like alcohol consumption among those who are already predisposed to alcoholism as opposed to those are engaging in excess alcohol consumption because of non-genetic factors such as environmental stress. Future research may consider using a non-alcoholic dependent strain of mice so they may test for the impact of stress on binge drinking.

## References

Julien, J., Ayer, T., Tapper, E., Barbosa, C., Dowd, W., & Chhatwal, J. (2021). Effect of increased alcohol consumption during COVID-19 pandemic on alcohol-associated liver disease: A modeling study. *Hepatology*, *75*(6), 1480–1490. https://doi.org/10.1002/hep.32272

Thiele, T. E., Crabbe, J. C., & Boehm, S. L., 2nd (2014). "Drinking in the Dark" (DID): a simple mouse model of binge-like alcohol intake. *Current protocols in neuroscience*, *68*, 9.49.1–9.49.12. https://doi.org/10.1002/0471142301.ns0949s68