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The Characterization of Diet Influences on Ethanol Consumption in Mice

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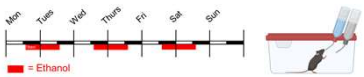
Introduction

The gut-brain axis is a distinct, yet uncharacterized tract of the nervous system that provides direct communication between the myenteric and the central nervous systems. The gut-brain axis is implicated in numerous underlying pathological phenomena, such as depression, Parkinson's disease, and autoimmune disorders. Signaling along the gut-brain axis is primarily mediated by the Vagus nerve, which projects to the Nucleus Tractus Solitarius (NTS). From the NTS, projections link to higher order brain structures, namely reward regions, such as the paraventricular nucleus of the hypothalamus, Locus Coeruleus and the Prefrontal cortex.

Alcohol Use Disorder is a complex and widespread disease with limited pharmacotherapeutic options. Emerging evidence indicate that the gut microbiome influences alcohol intake. Chronic alcohol use leads to gut dysbiosis which is correlated with psychological symptoms such as depression and increased alcohol craving. Diet is a potent regulator of the gut microbiome. In this study, we investigated the role of various rodent diet formulations on alcohol consumption and preference in C57BL/6J mice. This study builds on accidental preliminary findings implicating a strong link between standard rodent diets and alcohol drinking. In this study we sought to confirm these preliminary findings and extend them by determining whether diet influences on alcohol consumption were a) reversible b) resistant to quinine adulteration of alcohol and c) secondary to alterations in taste preference. The overarching hypothesis is that diet formulations differentially influence the composition of the gut microbiome, which in turn alters signaling across the gut-brain axis to influence alcohol intake.

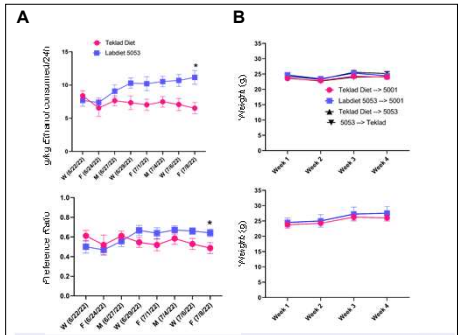
Methods

Two-bottle-choice intermittent access alcohol consumption
The rodent diets analyzed were LabDiet 5001 (LD 5001), LabDiet 5053 (LD 5053), and Teklad (TK). Voluntary alcohol consumption was measured using an intermittent access (IA) two-bottle-choice protocol, providing mice access to 15% alcohol and water every other day for 24h per session as shown below.



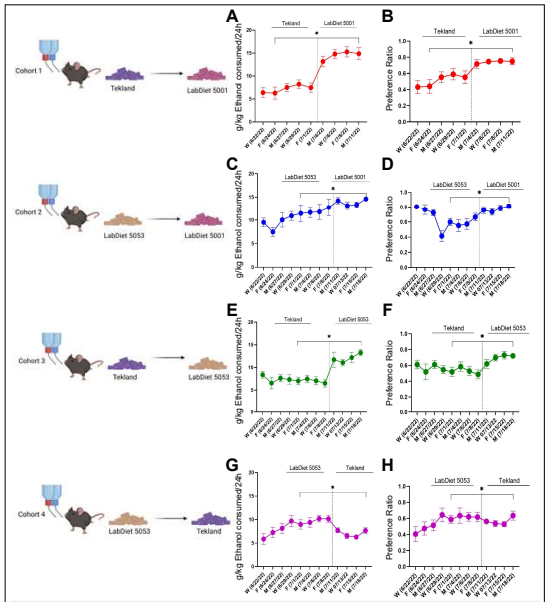
Administration of Tastants
Mice had continuous access to sucrose, saccharin, and quinine. Mice were presented with each concentration of tastant for 1-2 days.
Stool collection
Stool samples were collected to analyze metabolites and bacterial colonies inhabiting the gut.

Mice fed LabDiet 5053 consume significantly more alcohol



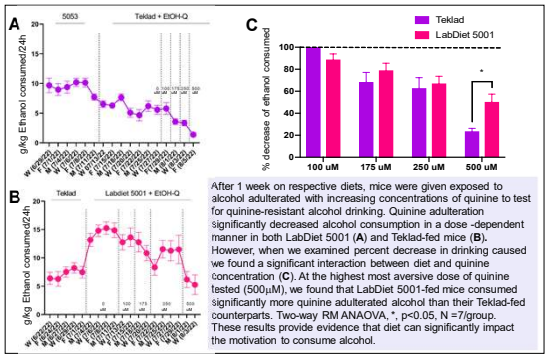
A) Ethanol consumption and preference was significantly increased in LD 5053-fed mice compared to Teklad (Two-Way RM ANOVA, *, p<0.05, N =14/group). **Figure B** depicts body weights (grams) of all animals. Top: ethanol mice. Bottom: taste perception mice. No significant differences in body weights were observed between the two diets. These results suggest that different commercial rodent diet formulations can significantly impact alcohol consumption and reward.

The effects of diet on alcohol consumption are reversible



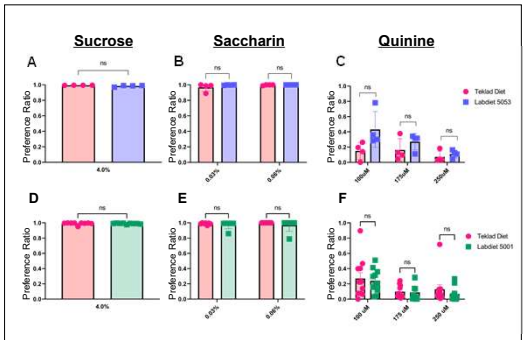
Switching diets 48h before alcohol exposure from Teklad to LabDiet 5001 (**A and B**) or LabDiet 5053 (**E and F**) significantly increased alcohol consumption and preference. Switching from LabDiet 5053 to 5001 (**C and D**) resulted in a small but significant increase in alcohol consumption and preference. However, switching from LabDiet 5053 to Teklad significantly decreased alcohol consumption and preference (**G and H**) (*, p<0.05, One way ANOVA of last 4 session before and first 4 sessions after diet switch, N =7/group). These results suggest that effects of diet on alcohol consumption are reversible and not due to novelty of the diet.

Diet-induced increases in alcohol consumption is resistant to quinine



After 1 week on respective diets, mice were given exposed to alcohol adulterated with increasing concentrations of quinine to test for quinine-resistant alcohol drinking. Quinine adulteration significantly decreased alcohol consumption in a dose -dependent manner in both LabDiet 5001 (**A**) and Teklad-fed mice (**B**). However, when we examined percent decrease in drinking caused we found a significant interaction between diet and quinine concentration (**C**). At the highest most aversive dose of quinine tested (500μM), we found that LabDiet 5001-fed mice consumed significantly more quinine adulterated alcohol than their Teklad-fed counterparts. Two-way RM ANAOVA, *, p<0.05, N =7/group. These results provide evidence that diet can significantly impact the motivation to consume alcohol.

Diets do not alter sweet and bitter taste perception



Taste perception experiments were performed to rule out the possibility that diet-induced changes in taste perception underlie changes in alcohol consumption. To do this, mice on LabDiet 5053 and Teklad (n=4/group), or LabDiet 5001 and Teklad (N =11/group) were given continuous access to **(A, D)** 4% sucrose **(B, E)** saccharin (concentrations at 0.03% and 0.06%), and **(C, F)** quinine (concentrations at 100uM, 175uM, and 250uM). There were no differences in sucrose or saccharin preference between Teklad-, LabDiet 5053-, and LabDiet 5001-fed mice **(A, B, D, E)**. Quinine preference decreased in Teklad-, LabDiet 5053-, and LabDiet 5001- fed mice in a dose-dependent manner (**C,F**). These results provide evidence that diet-induced changes in alcohol consumption are not a consequence of altered taste perception

Summary

Conclusions

- Alcohol consumption and preference was increased in mice that were fed LabDiet 5001 or 5053 when compared to Teklad.
- The effects of diet on alcohol intake were reversible suggesting that the increase in alcohol intake was not due to novelty preference.
- There were no significant differences in body weights between mice fed the different diets.
- There were also no significant differences in sucrose, saccharin, and quinine consumption or preference in mice fed with Teklad or LabDiet 5053, and LabDiet 5001-fed mice.
- Alcohol consumption in mice fed LabDiet 5001 was significantly more quinine-resistant at the highest concentration of quinine tested.
- Our results rule out confounding factors like taste and novelty preference and provide preliminary evidence for diet influencing the motivation to consume alcohol.

Future directions

- Gut microbiome and metabolite differences as a result of diet changes

Acknowledgements

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