Alcohol-mediated Gut Dysbiosis Leads to Reduced Nitric Oxide Bioavailability and Vascular Dysfunction

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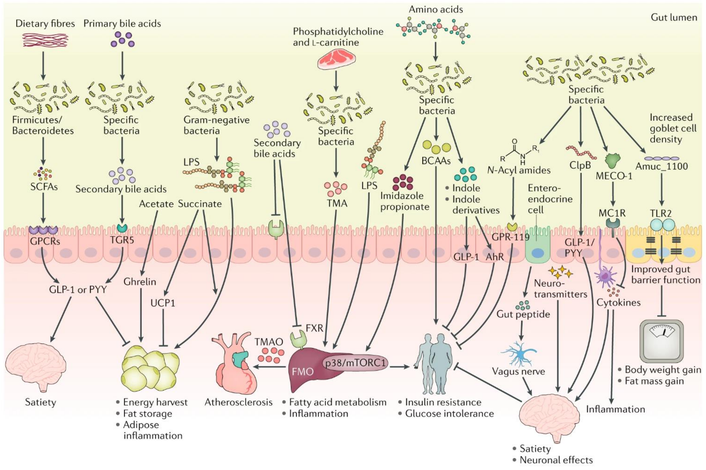
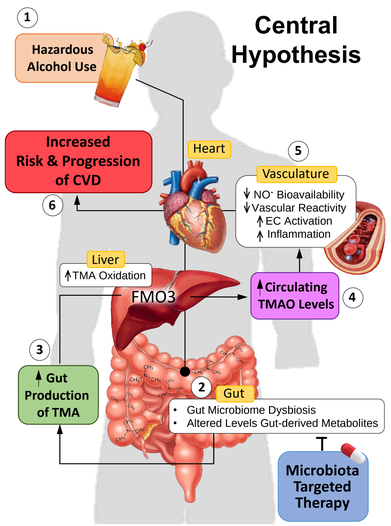
Introduction

Chronic and hazardous alcohol use has adverse effects on cardiovascular function and homeostasis leading to increased risk of cardiovascular disease (CVD). Hazardous alcohol use has also been linked to gut dysbiosis and alteration in gut derived metabolites. However, it is unknown if alcohol mediated gut dysbiosis has effects on vascular endothelial function and subsequent development of CVD.

Gut-derived Metabolites and Systemic Physiological Effects



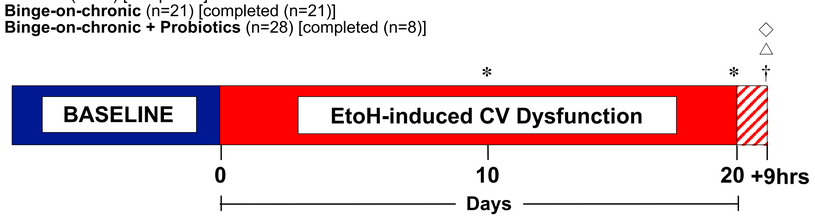
Central Hypothesis



Central Hypothesis. (1) HAU leads to gut dysbiosis **(2)** and altered gut-derived metabolites, like TMA **(3)**. TMA is rapidly oxidized in the liver by FMO3 and result in an increase in circulating TMAO levels **(4)**. High TMAO is linked to **(5)** vascular dysfunction which increases the risk and progression of CVD **(6)**. MBTT (blue box) which inhibits gut dysbiosis and TMA production may result in reduced TMAO levels, improved vascular function and overall cardiovascular health.

Study Experimental Protocol

Pair-fed (n=20) [completed]
Binge-on-chronic (n=21) [completed (n=21)]
Binge-on-chronic + Probiotics (n=28) [completed (n=8)]

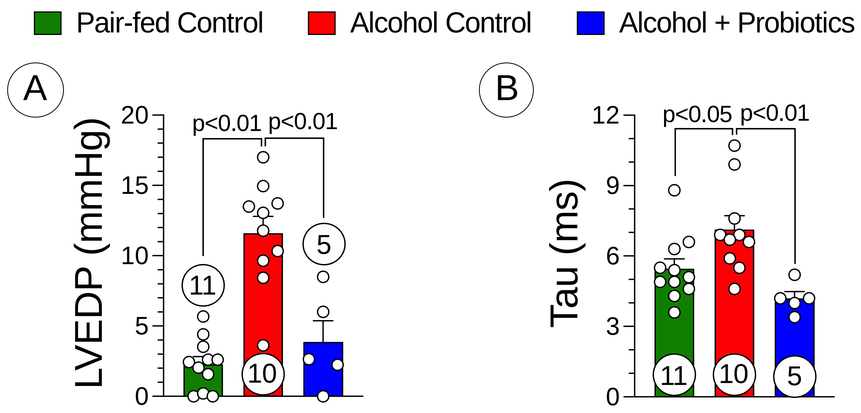


* **Binge EtoH (4g/kg)**
◇ **Cardiac Echocardiography**
△ **Invasive Hemodynamics**

† **Histological, Molecular, Ex vivo Analysis**
- Vascular Reactivity
- Circulating and Tissue Nitrite Levels
- NOS isoform gene expression (Heart, Liv., Kid.)
- Circulating & Liver TMAO Levels Conversion enzymes & by-products
- Gut Microbiome Profiling
- Gut Microbiota Metabolomics
- Tissue Metabolomics
- Plasma Inflammatory Markers
  - TGFβ, IL-6
- EC Activation Marker
  - PECAM, ICAM, ET1

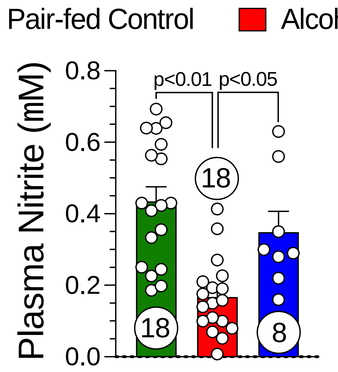
Methods. We studied C57BL/6 mice at 10 weeks of age for these studies. Mice underwent binge-on-chronic alcohol feeding (n=18), pair-fed (n=18) or binge-on-chronic alcohol + probiotics (daily) (n=8) for 20-days. Vascular reactivity, plasma nitrite, measured by HPLC, and oxidative stress were measured. Another cohort of mice (n=11-12 per group) received broad-spectrum antibiotics for 10 days followed by a cecal microbiota transplant from either pair-fed or binge-on-chronic fed mice. Plasma nitrite levels were measured to assess nitric oxide bioavailability and vascular function.

Results



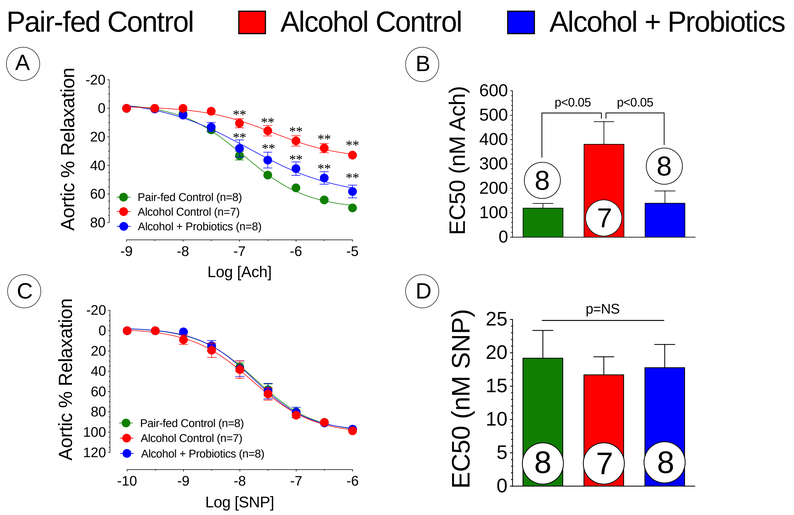
Invasive Hemodynamics. At 20-days into the study invasive hemodynamics were acquired by placement of a solid-state pressure transducer into the left ventricle via cannulation of the right carotid artery. **(A)** Left ventricular end-diastolic pressure (LVEDP) and **(B)** the Tau constant. Pair-fed control (n=11). Alcohol control (n=10). Alcohol + probiotics (n=5). LVEDP was significantly elevated in the Alcohol control group when compared to Pair-fed control. When probiotic was administered in conjunction with alcohol feeding the LVEDP was significantly attenuated. The same observation was observed for the relaxation constant Tau.

Results



Circulating Nitrite Levels. Plasma nitrite levels were measured using high performance liquid chromatography (HPLC). Samples were acquired at the 20-day time point. Alcohol controls and a significant reduction in the circulating nitrite levels compared to pair-fed controls. This reduction in the alcohol-fed controls was significantly attenuated in the presence of probiotic administration. Pair-fed control and Alcohol control (n=18). Alcohol + probiotics (n=8).

Results



Aortic Vascular Reactivity. Thoracic aorta was isolated and dissected into rings for vasorelaxation experiments. **(A)** Aortic % relaxation with increasing doses of acetylcholine (ach). **(B)** Concentration of Ach at 50% the maximal relaxation. **(C)** Aortic % relaxation with increasing doses of sodium nitroprusside (SNP). **(D)** Concentration of SNP at 50% the maximal relaxation. Vasorelaxation was significantly blunted in the alcohol control animals. This loss of endothelial dependent vasorelaxation was significantly restored in the presence of probiotic treatment. There was no change in endothelial independent vasorelaxation with SNP. Pair-fed control (n=8). Alcohol control (n=7). Alcohol + probiotics (n=8).

Conclusions

Binge-on-chronic alcohol induced reduced nitric oxide bioavailability, impaired endothelial-based vascular dysfunction and increase oxidative stress (data not shown). Daily probiotic administration was able to attenuate these findings. The adoptive transfer of microbiota content from binge-on-chronic alcohol mice reduced plasma nitrite levels demonstrating that the alterations to the gut microbiome due to alcohol is sufficient to reduce nitric oxide bioavailability and potentiate vascular dysfunction.