

**Western Diet and Chronic Alcohol Consumption Have a Systemic
Impact on the Stool and Plasma Metabolome in a Non-human
Primate Model.**

Sydney Smith

Research Advisor: Dr. Shawn Campagna, Dr. Courtney Christopher

Spring 2025

©University of Tennessee, Knoxville

College of Arts and Sciences

Department of Chemistry

Abstract

The gut microbiome holds a significant amount of power in influencing metabolic pathways, immune function, and chronic disease development in the human body. Its behavior and composition are largely impacted by what nutrients the organism consumes on a regular basis. This metabolomics study investigates how the standard western diet (WD) and chronic binge alcohol (CBA) consumption alter the stool and plasma metabolome using a non-human primate (NHP) model. The method of analysis was high resolution liquid chromatography-mass spectrometry (LC-MS) on NHP extracted stool and plasma metabolites that were analyzed on a 7-month WD with or without CBA and compared to baseline samples. The results showed that both WD and CBA significantly altered metabolite abundances, impacting critical reaction pathways involved in purine metabolism, neurotransmitter synthesis, and antioxidant production. The addition of CBA habits and increased WD duration intensified these effects, leading to a larger imbalance in gut microbial composition and contributing to metabolic dysregulation. Furthermore, alcohol consumption alone affected metabolites in the stool metabolome involved in immune response and neurochemical regulation. These findings emphasized the complex relationship between diet, alcohol, and gut microbiota, revealing potential ways that these factors contribute to the onset of chronic diseases like obesity, diabetes, gastrointestinal and neurological disorders, and weakening of the body's immune defense. Despite these newer insights, the complexity of the gut microbiome is still much unexplored and ongoing research is still under way to fully understand the long-term health implications of dietary and alcohol-related shifts in gut metabolism.

Table of Contents

I.	Introduction.....	4
II.	Experimental.....	6
III.	Results and Discussion.....	9
IV.	Conclusion.....	24
V.	Acknowledgments.....	25
VI.	References.....	27

Introduction

Within the last 10 years, the importance of the gut microbiome and its role in human health began to be recognized and truly investigated. Home to millions of diverse microorganisms, the gut microbiome plays a significant role in regulating human health and host metabolism, directly linking it with the development of chronic diseases.¹ For example, any imbalance or shift in the gut microbiome can lead into gut dysbiosis, a disruption that causes negative health effects. Some of its responsibilities include regulating the immune system, synthesizing vitamins, producing hormones and signaling molecules, controlling neurotransmitters, digesting nutrients, and maintaining metabolic homeostasis. These responsibilities explain how the gut microbiome plays such a large role in the potential development of diseases like gastrointestinal inflammatory conditions and neurological, cardiovascular, and respiratory illnesses.¹

The overall composition and function of the gut microbiome is highly sensitive and depends on multiple factors like diet, exercise, ageing, genetics, stress, etc. In a healthy gut, the microbes interact and work together to create a balance, but if gut dysbiosis occurs, the ratio of harmful microbes to symbiotic microbes increases, leading to negative imbalance. Every individual has a unique gut microbiome, based off habitual activities and genetic factors. Dietary habits play one of the largest roles in shaping the gut microbiome composition and activity, which in turn directly affects the metabolites circulating throughout the human body's systems. The nutrients consumed through dietary sources are digested and metabolized by microbes in the gut and can either serve as beneficial or detrimental to the overall gut and host health.² For example, chronic binge alcohol consumption alters gut microbial composition and affects the immune system's response abilities, leading to negative health consequences.³ Alcohol-induced changes to the intestinal microbiota has previously been linked to liver disease, alcohol-induced oxidative

stress, and intestinal hyperpermeability to certain bacteria.³ Diet is such a large determinant of the human microbiome and is connected to so many hidden aspects of human health; however, because of its vastness and large diversity, much is still unknown about how specific diets can target metabolite quantities in the body.

Another common example of how diet can completely alter the gut microbiome is seen in much of the United States population, where obesity, diabetes, and cardiovascular health conditions have been on the rise due to the popularity of the “western diet” (WD).² A western diet is defined by its high-sugar, high-fat, and low fiber characteristics; when combined with low activity levels and sedentary habits, the health effects are even greater. It is the leading cause for increased rates of obesity and diabetes, as well as high cholesterol levels and heart disease. Increased alcohol consumption is also associated with a WD. The goal of this study was to identify how WD combined with chronic alcohol consumption alter the gut and plasma metabolome of non-human primates (NHPs), as well as identify signatures unique to WD or unique to chronic alcohol consumption.

In the past decade, much has been discovered about the gut microbiome and its importance in regulating mammalian physiology; however, there is still a vast amount unexplored due to the extreme complexity of the gut microbiome. Studying the metabolome tends to become complex due to its greatly diverse and unknown elements; therefore, there can be some complications when it comes to identifying and quantifying compounds.⁵ Mass-spectrometry based metabolomics is a growing analytical technique used to quantify and detect metabolite or lipid concentrations in a sample.⁵ Using liquid chromatography mass spectrometry to analyze extracted stool and plasma samples, metabolites were detected and compared between experimental groups for 40 male non-human primate rhesus macaque samples. Once again, this study specifically compared WD and

chronic alcohol consumption with control groups to research the specific effects of these habits on the stool and plasma metabolome.

Experimental

All experiments were reviewed and approved by the Institutional Animal Care and Use Committee at both the Tulane National Primate Research Center (TNPRC) in Covington, LA, and Louisiana State University Health Sciences Center in New Orleans, LA and adhered to the NIH guidelines for the use of experimental animals. This study collaborated with Tulane University's National Primate Research Center and LSU Health, who hold the responsibility for the non-humate primate study and sample delivery. A total of 40 male rhesus macaques (*Macaca mulatta*) were studied during the 5-year funding period. Study objectives for the RCs were achieved using a 2x2 repeated measures design with chronic binge alcohol (CBA) and Western Diet (WD) being the manipulated variables. Macaques will be purchased and housed in the Division of Animal Care (DAC) for the duration of the study. Animals were selected based on three criteria:

- 1) Adult male macaques, 5-9 years old, from Specific Pathogen Free breeding colonies
- 2) Macaques will be deemed to be in excellent health based on a complete physical examination performed at the primate centers.
- 3) Macaques will be negative for SRV and STL V.

Study design: Macaques were quarantined for 3 months before participating in the study. During this period, macaques received a high fat and high sucrose diet (WD, Teklad TD.07802). The standard laboratory diet given (TD.07802) consists of 4.3 Calories/g, which contained 42.3% fat, 15.8% protein, and 41.9% carbohydrate.⁶ Macaques were fed ad libitum, and the amount consumed was monitored and recorded twice daily. The proposal to purchase 20 animals in years one and two (ten each year) and a second cohort in years three and four was to ensure that animal

studies were staggered to ensure optimal care and attention to detail to the controlled experimental procedures.

Alcohol Administration: Alcohol or vehicle was administered via surgically implanted gastric catheters as described: Briefly, alcohol (30%; w/v in water) was infused over a 30 min period, 5 days per week to produce blood alcohol concentrations of 45-55 mM (~200 mg%). Control monkeys received an isovolumic water infusion instead of alcohol.

Metabolite Extractions: All samples were extracted and analyzed at the Biological and Small Molecule Mass Spectrometry Core at the University of Tennessee Knoxville (RRID: SCR_021368). During the metabolomic extraction, samples were kept at 4 °C. Roughly 50 mg of stool and 100 µL of plasma were aliquoted for extraction. The exact mass or volume of each sample was recorded and used for sample normalization. Water-soluble metabolites were extracted using an acidic acetonitrile extraction procedure adapted from Rabinowitz and Kimball.⁷ Briefly, metabolites from the aliquoted samples were extracted using 1.3 mL of 4:4:2 acetonitrile: methanol: water with 0.1 M of formic acid. All solvents were HPLC grade. Samples mixed with extraction solvent were then kept at –20 °C for 20 min. Following this, the mixture was centrifuged for 5 min, and the supernatant was collected. The remaining sample was re-extracted with 300 µL of extraction solvent. After an additional 20 min at –20 °C and 5 min of centrifugation, the supernatant was collected and combined with the supernatant previously collected. The combined supernatants for each sample were dried under pure nitrogen. Once dried, 300 µL of LC-MS-grade water was added to each sample in preparation for mass spectral analysis.

UHPLC-HRMS: A ultra-high-performance liquid chromatography—high resolution mass spectrometry (UHPLC-HRMS) method was used for global metabolomic analysis.⁸ Prior to

analysis, metabolites were kept at 4 °C in an UltiMate 3000 RS autosampler (Dionex, Sunnyvale, CA, USA). Chromatographic separation was accomplished using a previously described 25 min gradient elution, reverse phase ion-pairing method with a water:methanol solvent system, a tributylamine ion pairing reagent, a Synergi 2.6 µm Hydro RP column (100 mm × 2.1 mm, 100 Å; Phenomenex, Torrance, CA, USA), and an UltiMate 3000 pump (Dionex).⁹ The metabolites in the chromatographic eluent were then ionized via negative mode electrospray ionization (ESI) prior to full scan mass spectral analysis with an Exactive Plus Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) as previously described.⁸

Metabolomics Data Processing: A package from ProteoWizard, msConverter, was used to convert raw mass spectral files to mzML files. All mzML files were imported into an open-source software, metabolomics analysis and visualization engine (El-MAVEN) where metabolites were manually identified using an in-house library based on exact mass (± 5 ppm) and retention time (± 2 min).⁸ Metabolite peaks were integrated, and raw peak intensities for identified metabolites were exported from El-MAVEN to a csv file. Prior to statistical analysis, raw spectral data were normalized by either the sample mass or the volume used for extraction.

Statistical Analysis: Either a One-Way Analysis of Variance (ANOVA) followed by Tukey multicomparisons test, Fisher's test or an Unpaired t-test were used on demographics, substance use and HIV data, using the Prism v.9 software. The normalized data were imported into MetaboAnalyst 5.0 and were filtered via the interquartile range (IQR), log transformed, and Pareto scaled prior to statistical analysis.⁹ Partial least squares discriminant analyses (PLS-DAs) were performed in MetaboAnalyst 5.0. For each biological matrix, PLS-DA was used to compare the global metabolomes of control participants, participants on western diet, and participants

consuming alcohol and western diet. Within each biological matrix, PLS-DA pairwise comparisons were used to elucidate the impact of a long term vs. short term western diet, chronic alcohol consumption, or both simultaneously on the metabolome by comparing these to the control samples. Variable importance in projection (VIP) scores were assigned to each metabolite to indicate the importance of each metabolite in contributing to the separation between experimental groups. VIP scores > 1 indicate that a metabolite significantly contributes to the separation of groups in the PLS-DA model based on identified metabolite profiles. Volcano plots were also used to visualize metabolites that were statistically ($p < 0.1$) significantly (fold change $> |2|$) different between the pairwise comparisons.

Results and Discussion

Alcohol consumption (EtOH) combined with a western diet (WD) significantly affected metabolite abundances and altered metabolic pathways detected in non-human primate stool and plasma samples. A total of 164 metabolites were identified in the stool and plasma samples. Compared to the baseline group, samples from experimental groups had many metabolites differ significantly in abundance (VIP score > 1 and $p < 0.05$), with a smaller population also being altered in some way by the addition of CBA or WD habits. As the duration on a WD and alcohol consumption increased, the changes on the global gut microbiome also increased. The composition of the gut metabolome on a 3-month WD, 7-month WD, and 7-month WD with chronic binge alcohol consumption beginning at 3 months were all significantly shifted from baseline samples. In this study, the stool metabolome and plasma metabolome were analyzed separately.

Stool Metabolome:

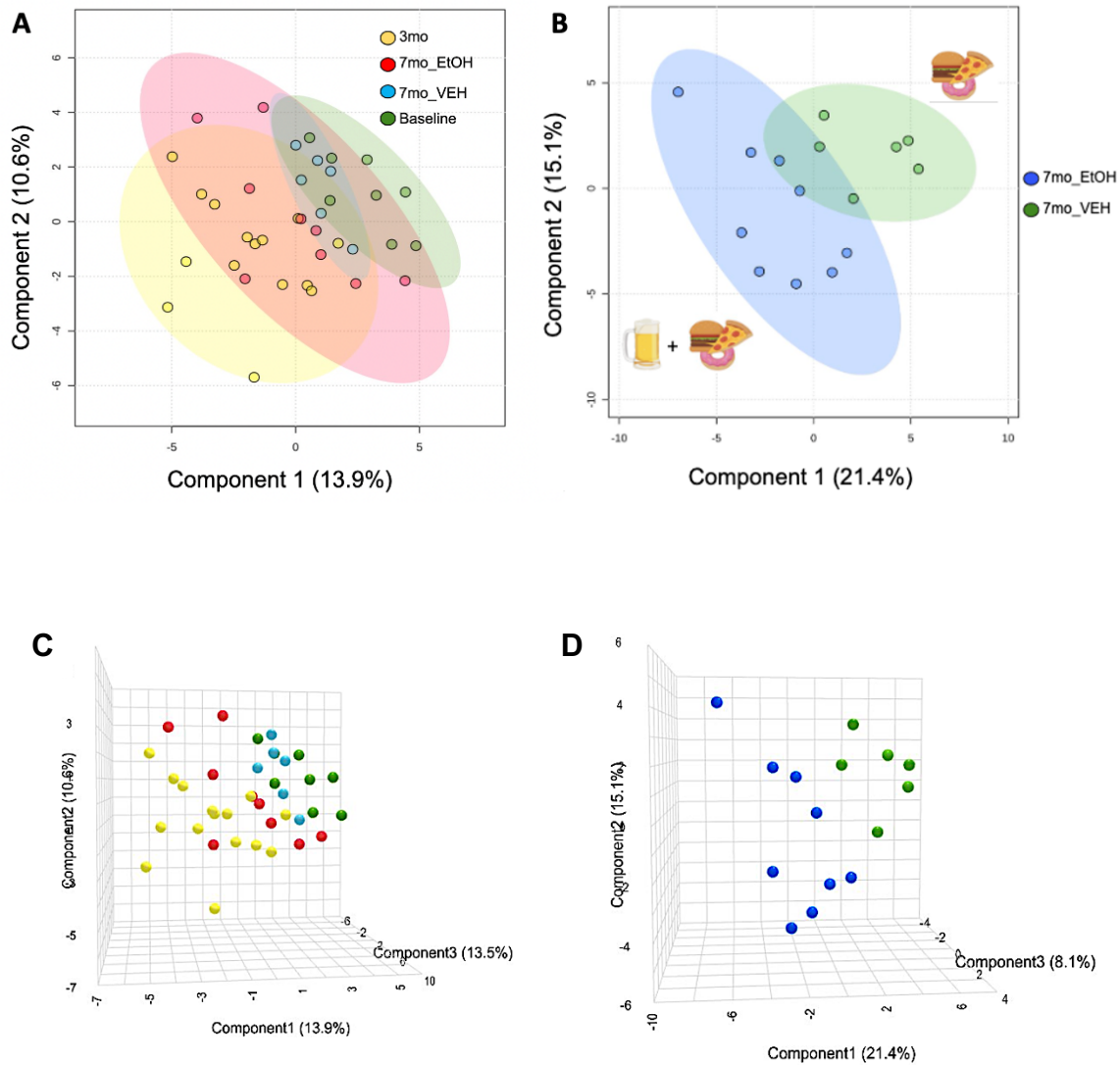


Figure 1: The PLS-Da plots above prove that western dietary habits and chronic alcohol consumption significantly changed the composition of the gut microbiome within the stool samples. **(A)** Baseline is separate/defined from experimental groups in the stool metabolome. **(B)** The addition of alcohol after 3 months on a WD significantly altered the metabolite composition of the samples and shifted the stool metabolome. A 3D PLS-DA visual can give more information

and more detail on the group separations, shown in **C** and **D**. “Component 1” and “Component 2” referenced in each PLS-Da plot are arbitrary units, unrelated to specific variables, to show overall differences between sample profiles through separation.

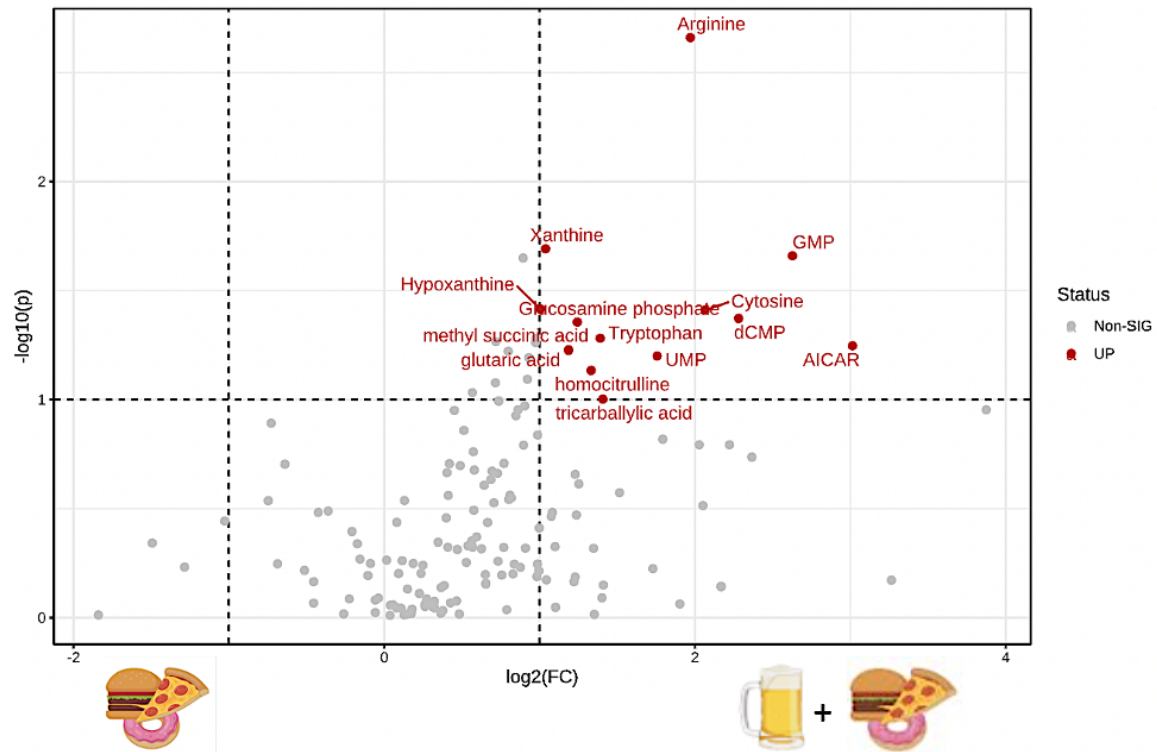


Figure 2: Volcano plot of data from graph B (Fig. 1) shows relative VIP significance of metabolites altered in the stool metabolome by the addition of alcohol after 3 months of WD.

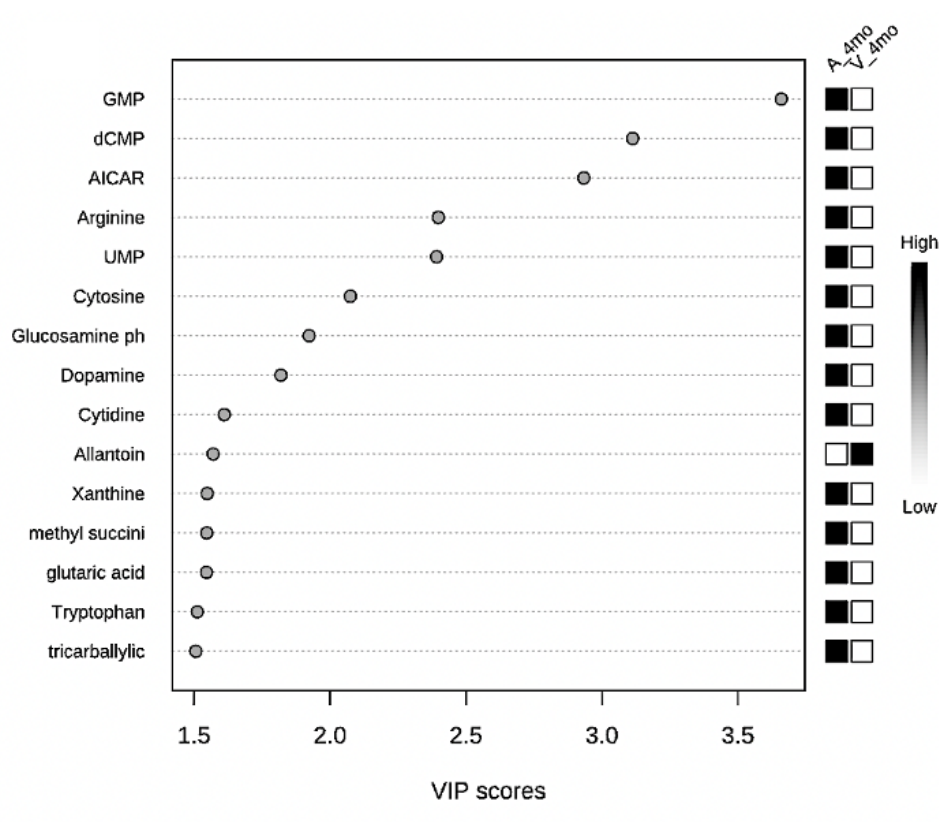


Figure 3: Extension of Fig. 2, shows metabolites with VIP scores greater than 1.5, indicating significant differences in abundance after 4 months of chronic alcohol consumption, compared to without.

The addition of alcohol to a western diet (EtOH+VEH) significantly altered the global stool metabolome. A 7-month duration on WD, with the addition of alcohol at 3 months, significantly altered UMP, GMP, arginine, AICAR, xanthine, tryptophan, and dopamine in stool when compared to 7-month WD samples excluding EtOH (Figure 1C and 1D). These changes would most significantly affect processes like purine degradation, antioxidant production, and tryptophan metabolism.

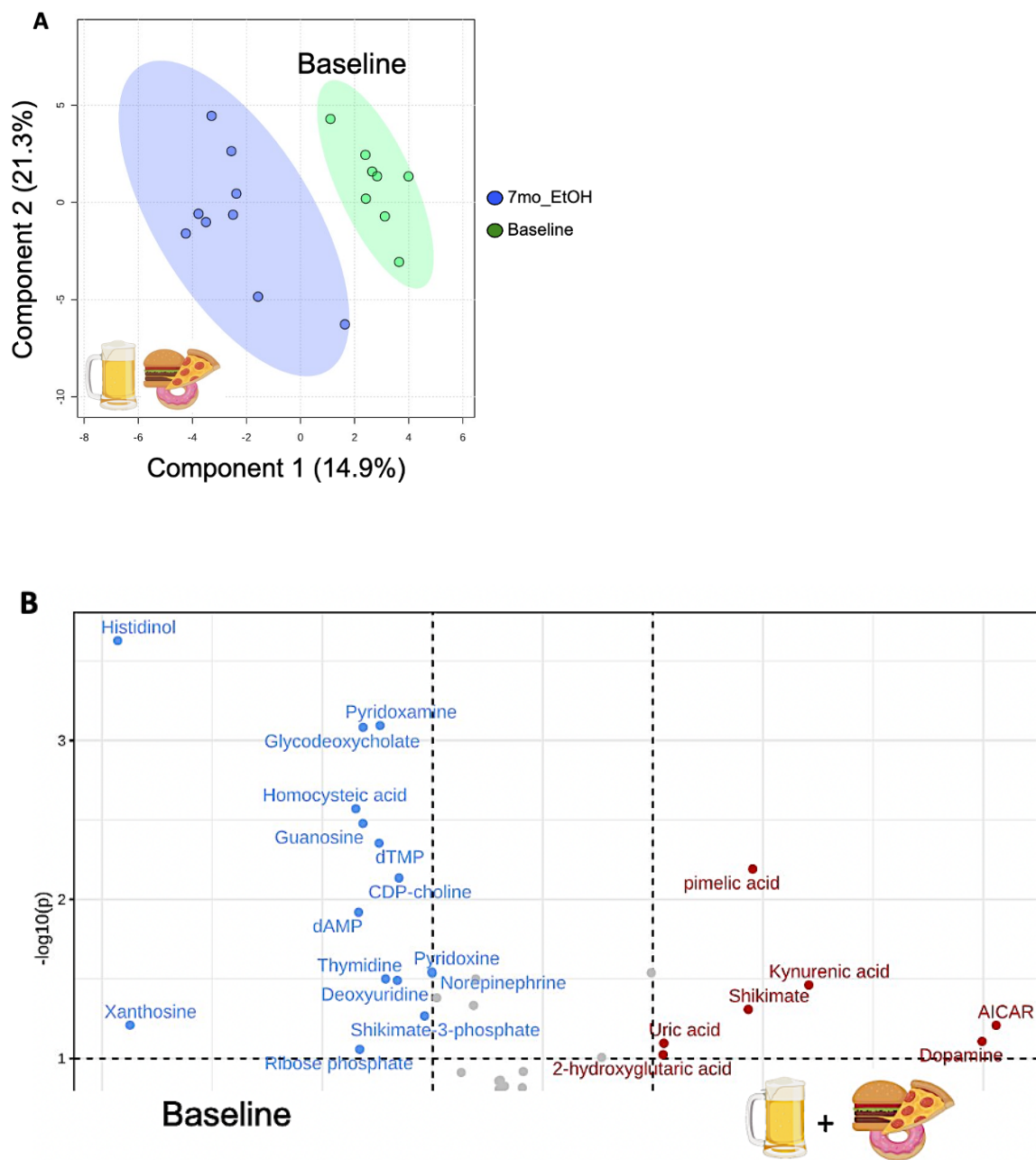


Figure 4: (A) Baseline samples do not overlap with WD+ETOH samples, proving a significant shift in stool metabolome composition. (B) Volcano plot of data from graph A shows relative VIP significance of metabolites altered by comparing baseline stool samples with WD+ETOH sample.

Figure 4, graph A shows the complete separation of baseline samples with EtOH+VEH samples in the metabolome, which was after the addition of alcohol to a WD. The 7-month WD+EtOH vs. baseline comparisons show the main metabolites that were significantly altered included pimelic acid, AICAR, kynurenic acid, shikimate, and dopamine. These metabolites are involved in pathways like purine degradation, kynurenine metabolism, oxidative dysfunction, energy metabolism, and purine biosynthesis. AICAR, 5-Aminoimidazole-4-carboxamide-1-beta-4-ribofuranoside, is a metabolite directly involved in the pentose phosphate pathway (PPP) that regulates glucose metabolism and stimulates AMP activated protein kinase (AMPK).

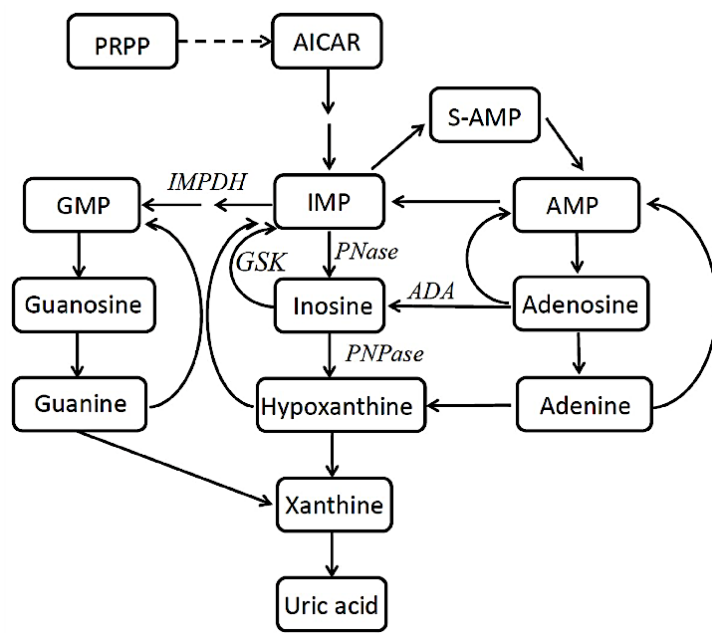


Figure 5: AICAR's metabolic reaction pathway, part of the pentose phosphate pathway.

AMPK is a key regulator of energy homeostasis and metabolism within the body. Natural optimization of AMPK is advised to avoid obesity by aerobic exercise and mediated caloric restriction. It is currently being researched as a potential pharmacological treatment to type 2 diabetes.¹¹ The WD+EtOH samples showed a shift in AICAR abundance, which would then lead to shifts in AMPK and alter the PPP; therefore, a western diet and chronic alcohol consumption

could be causative factors in developing obesity and type 2 diabetes by disrupting energy metabolism and decreasing AICAR and AMPK abundances within the body.

Stool metabolites altered exclusively by high alcohol consumption include dopamine, tryptophan, kynurenic acid, indole, xanthurenic acid, methionine, and cysteine. Shifts in these metabolite abundances could affect pathways like tryptophan and tyrosine biosynthesis (Indole-tryptophan breaks down into indole), methionine, cysteine, and pyrimidine metabolism. Indole is identified as a signaling molecule to initiate immune cell recruitment and response; more recent studies also show that it has a role in maintaining intestinal barriers to help with internal inflammation.¹² If tryptophan's pathway was altered to result in less overall indole formation, the body's immune response could be compromised. Interference of indole production could also result in intestinal barrier degradation, leading to internal inflammation which has many health consequences alone. Kynurenine has a unique reaction pathway within the body related to tryptophan metabolism. Changes in tryptophan abundances would directly lead to less production of kynurenic acid.

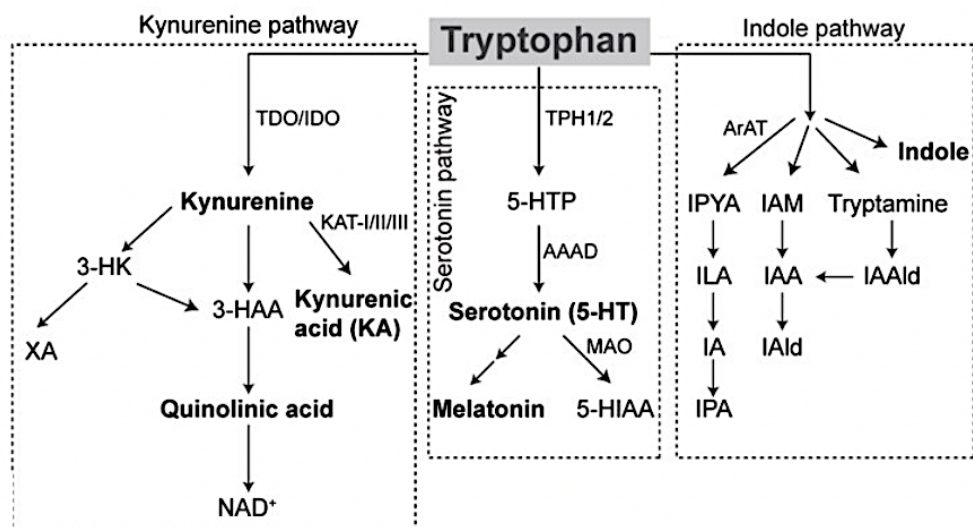


Figure 6: Tryptophan metabolism is directly connected to the indole and kynurenine reaction pathways.

Kynurenic acid and the kynurenine pathway (KP) has been linked to the buildup of neurotoxic substances in the body, as well as the development of neurological disorders like depression and Alzheimer's disease.¹⁴ Dopamine abundances were also altered by high EtOH consumption; low levels of dopamine have been linked to depression and Parkinson's disease. Tryptophan, indole, and kynurenine are all metabolites connected through the tryptophan metabolism pathway. If dysregulated, they also all share a common connection to neurodegenerative and neurological disorders. Therefore, chronic alcohol consumption exclusively altered the abundances of metabolites that could potentially contribute to the development of neurological diseases.

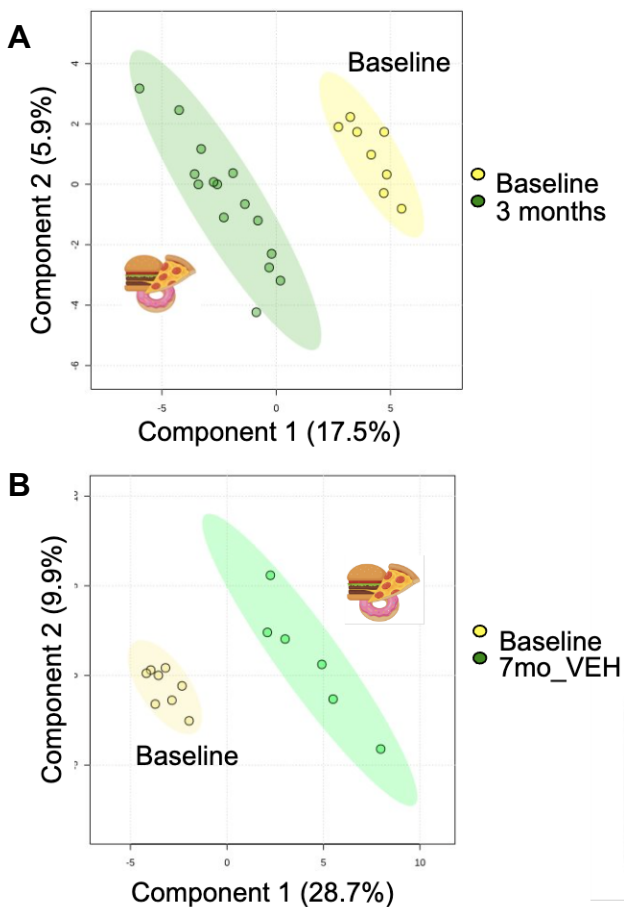


Figure 7: Western diet significantly altered the global stool metabolome with a duration dependent relationship. (A) The baseline vs. 3 months WD, shows the stool metabolome is significantly

different than the baseline metabolome. **(B)** Baseline vs. 7 months WD shows an increased percent difference of stool metabolites.

The data shows metabolites and pathways altered by western diet demonstrated duration dependent behavior. As the duration on a western diet increased from three to seven months, the percent difference in stool metabolome composition also increased, proving a direct relationship of WD duration and shifts within the gut metabolome. Western dietary habits altered the gut metabolome activity by targeting Shikimate metabolism, purine metabolism, vitamin B6 levels, fatty acid biosynthesis, biosynthesis of neurotransmitters, and defense against reactive oxygen species (ROS). Vitamin B6 plays a very important role in many bodily functions, such as co-catalyzing amino acid and fatty acid biosynthesis and defending against ROS. If ROS defenses are impaired, the resulting increase in oxidative stress would start to damage surrounding cells.¹⁵ A short term WD (3-months) significantly altered the abundances of creatinine, creatine, and guanidoacetic acid. A negative shift in creatine and guanidoacetic acid would largely affect reaction pathways in control of replenishing cytoplasmic ATP, regenerating the protective mucosal barrier, and maintaining a steady ATP pool. A long term WD (7-months) showed significant changes affecting the following metabolites: xanthosine, arginine, guanosine, xanthine, biotin, indoleacrylate, hypoxanthine, uracil, anthranilate, xylose, and cysteine. Shifts in these specific metabolites from a long term WD would significantly affect the pentose phosphate pathway (PPP) and purine biosynthesis. The PPP's main product is NADPH, which plays an important role in building DNA/RNA synthesis precursor molecules and regulating the control of reactive oxygen species in cells.

Plasma Metabolome:

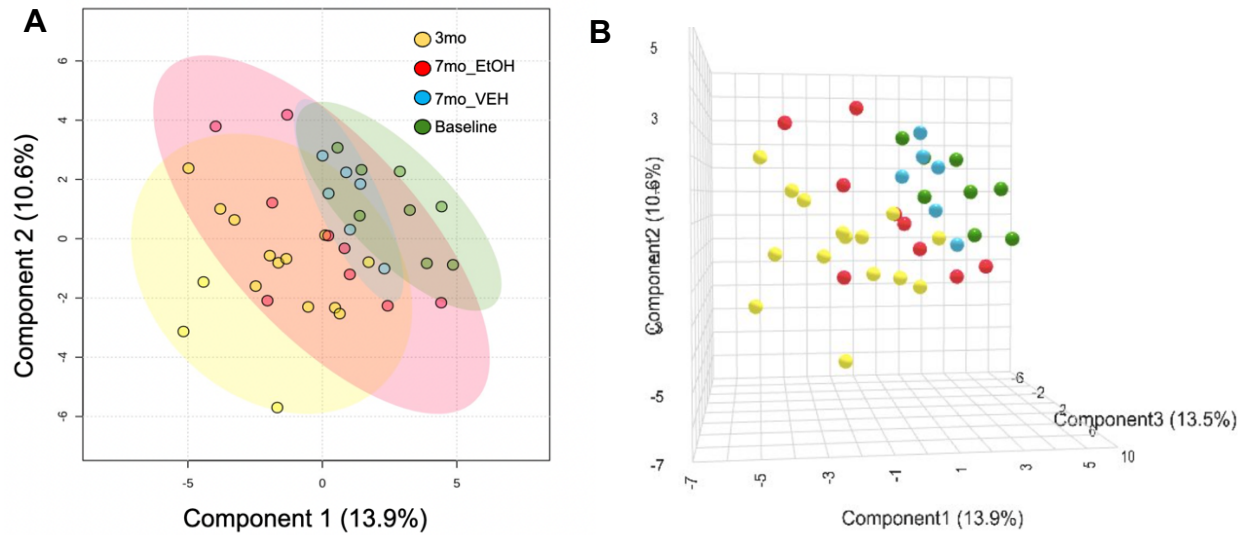
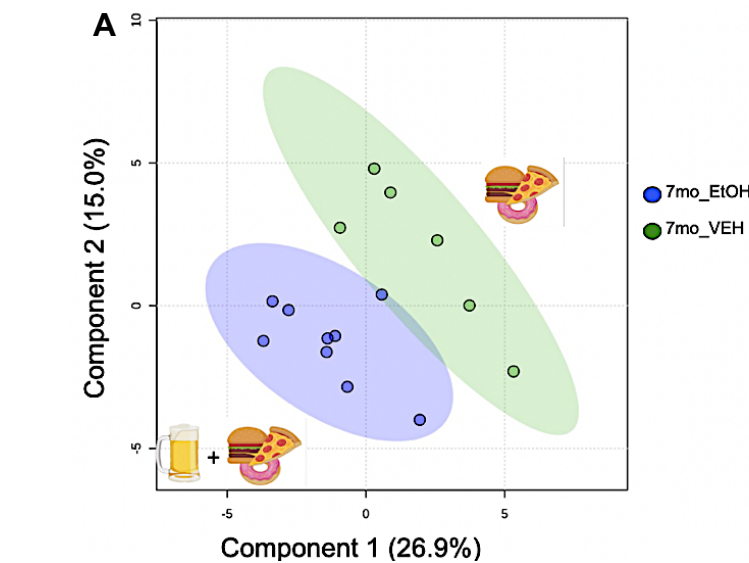
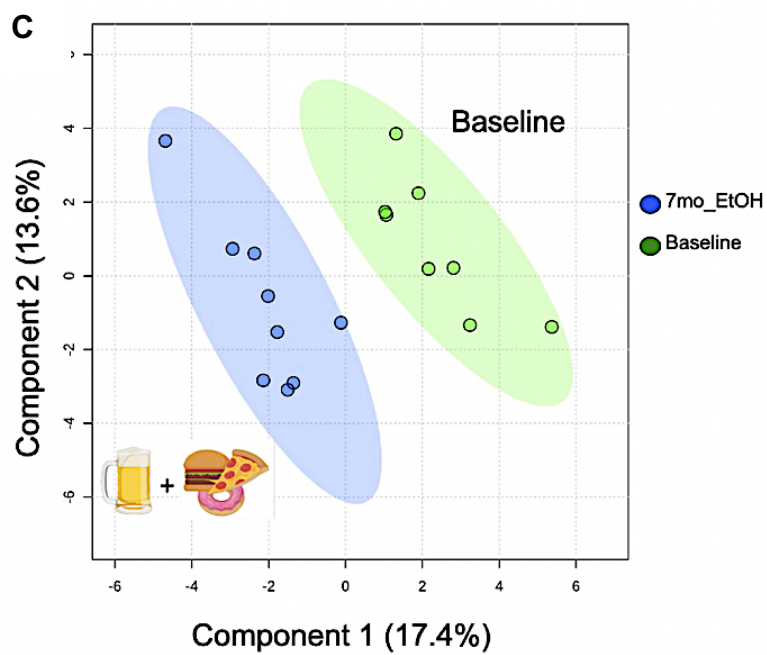
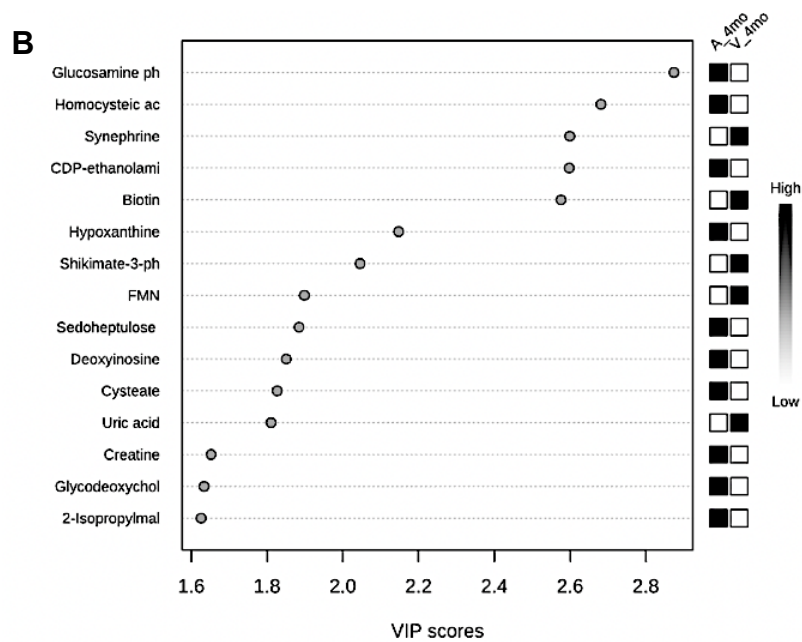


Figure 8: The plasma metabolome was significantly altered by a western diet and chronic alcohol consumption. The 2D (A) and 3D (B) PLS-da figures show baseline samples are compared to experimental groups; there is some overlap between groups, but a significant shift is present.





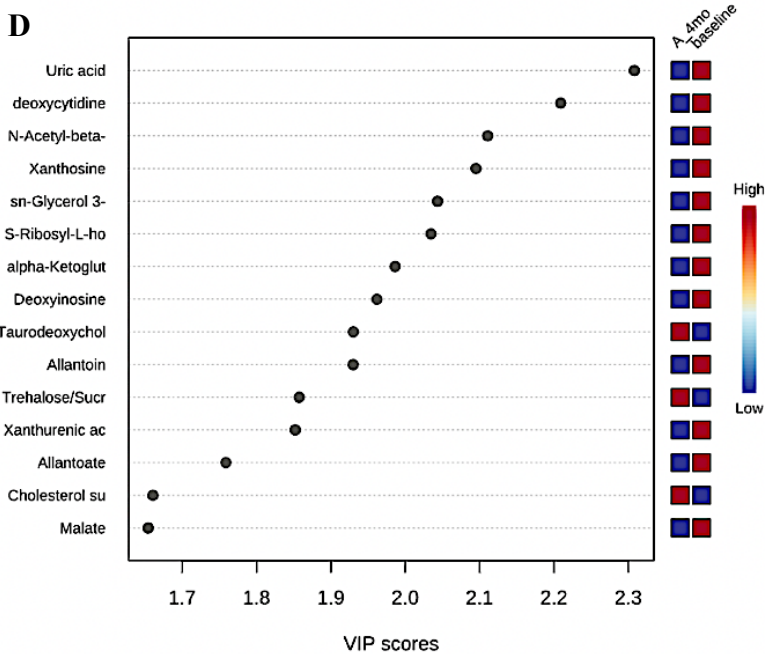


Figure 9: (A) The addition of alcohol after 3-months significantly altered the composition of the plasma metabolome. (B) Using sample groups from graph B, this plot shows the metabolites significantly altered with a VIP score greater than 1.6. (C) Compared to the baseline samples, the WD+ETOH samples were significantly different. (D) Graph E shows metabolites with a VIP score higher than 1.7 from comparison of baseline and 7-month WD+EtOH samples.

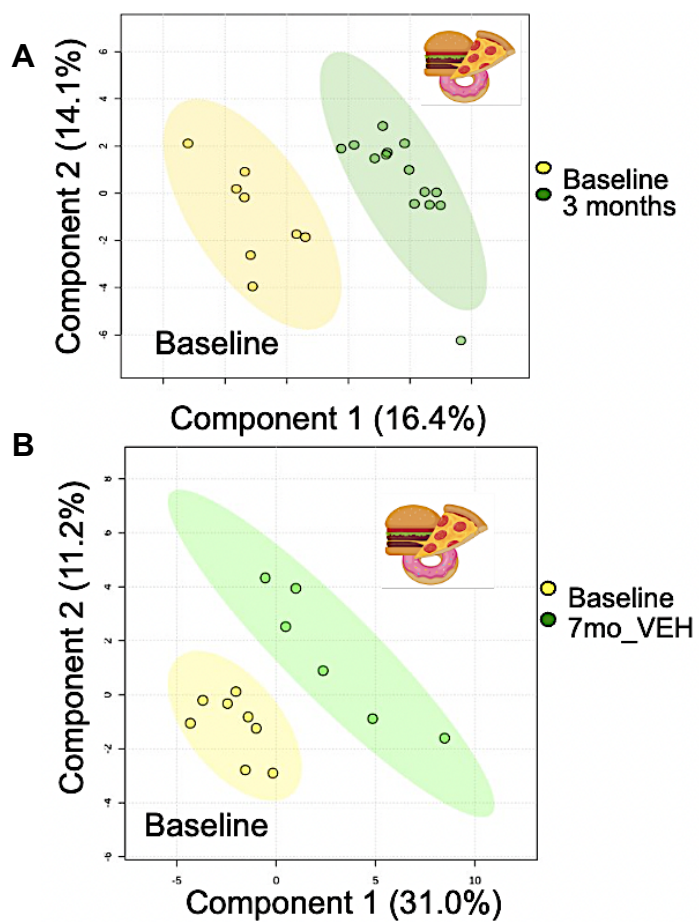


Figure 10: (A) Metabolites in the plasma metabolome were altered differently depending on the duration spent consuming a western diet. (B) The compositional changes of the plasma metabolome significantly increased, as duration on WD increased (3-months WD vs 7-months WD).

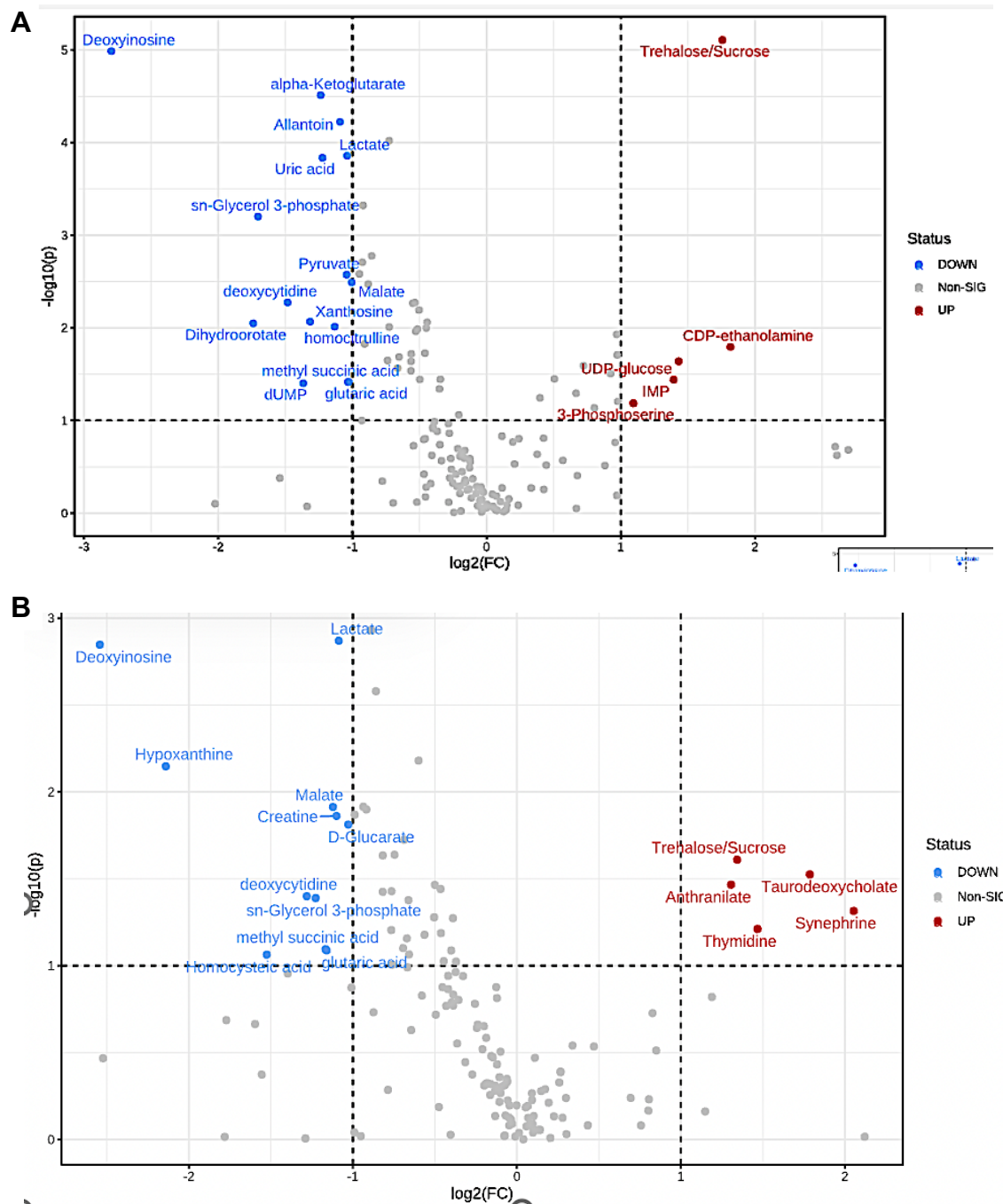


Figure 11: Volcano plots of the data from Fig. 8 shows metabolites significantly shifted from baseline to 3-month (A) or 7-month (B) western diet.

Metabolites that were altered exclusively by alcohol in the plasma metabolome included Vitamin B6, CDP-ethanolamine, biotin, and xanthurenic acid. Vitamin B6 has a large role in the

neurotransmitter synthesis pathway, which xanthurenic acid is an intermediate product of from the kynurenine pathway. The neurotransmitter synthesis pathway begins with the breakdown of tryptophan into kynurenine; vitamin B6 is used as a regulator to break down kynurenine into metabolites like xanthurenic acid and anthranilic acid. If vitamin B6 abundance was significantly altered by chronic alcohol consumption, tryptophan metabolism and neurotransmitter synthesis would be directly affected. Alterations in the tryptophan metabolism pathway have been linked to neurological disorders, like depression, due to the decrease in serotonin levels.¹² Vitamin B6 is also a key regulator in one carbon metabolism (OCM) and the ethanolamine OCM pathway. Biotin is a B-vitamin complex that assists the body with the breakdown of nutrients to functional energy. A shift in biotin abundance in the plasma metabolome would interfere with metabolism pathways and their ability to function normally.

In the plasma metabolome, western diet had duration dependent effects. On a short term WD (3-months), metabolites like methionine, UDP-glucose, IMP, Xanthine, Uracil, and Glutathione disulfide were significantly altered. Changes in these metabolite abundances would all cause the purine synthesis pathway, related to OCM and antioxidants, to be thrown off balance. This imbalance in regulation of the purine-OCM pathway would decrease the bodies antioxidant defenses. A long term (7-month) western diet caused metabolites such as Shikimate-3-phosphate, creatine, anthralanate, and tyrosine to be significantly changed; these metabolites are involved in the Shikimate pathway.

The metabolites chosen as points of focus were based on VIP significance scores above 1.5; however, most standard statistical procedures consider anything over a VIP score of 1.0 to be significant. Therefore, the metabolites discussed in this study are not the only ones that shifted significantly between experimental groups, but the ones that changed most significantly.

An example of a metabolite recently detected in the human metabolome that could potentially play a large role in human health is 2,3-dihydroxypropane-1-sulfonate, or DHPS. DHPS enters the human system through diet in the form of sulfoquinovose (SQ): SQ is found in many leafy green vegetables, which when degraded by microbes in the human gut, turns into DHPS.⁴ When DHPS is broken down into hydrogen sulfide, H₂S, and enters systemic circulation, it causes disparate health effects like intestinal inflammation.⁴ The increase of H₂S in the body from individual dietary compounds proves how metabolites, like DHPS, can be significantly affected by human dietary habits. Therefore, this study dedicated extra interest in investigating how western dietary habits and alcohol consumption potentially affected DHPS abundance or the concentration of H₂S circulating the body.

Conclusions

This study evaluated both the combined and separate effects of chronic alcohol consumption and western dietary habits on the gut microbiome to their metabolomic targets and how that could potentially affect human health. Using a non-human primate model, diet and alcohol consumption was monitored, with stool and plasma samples taken intermittently. Metabolites from the samples were extracted and put through ultra-high resolution liquid chromatography mass spectrometry to identify the metabolites and their abundances. This data was statistically analyzed to investigate the significant shifts between experimental group samples.

Alcohol and a western diet each exclusively altered the gut microbiome and metabolites in circulation. When combined, they also had significant affects. In the stool metabolome, EtOH exclusively shifted the abundances of metabolites including dopamine, indole, tryptophan, and xanthine. These metabolites are specifically connected to maintaining the homeostasis of the

body's immune system and neurotransmitters; this is metabolomics-based evidence that alcoholic habits can weaken the body's immune defenses and dysregulate the healthy brain's neurotransmitter levels. Consuming a WD had significant duration dependent and general effects on the microbiome's composition and behavior. Any time spent consuming a regular WD shifted Vitamin B6 abundances in the stool metabolome, which is also a key regulator in neurotransmitter synthesis and defending against reactive oxygen species. A longer term WD targeted metabolites involved in the pentose phosphate pathway and purine synthesis. A short term WD was involved in shifting metabolite abundances involved in the creatine and shikimate pathways. When consuming both alcohol and a WD regularly, a significant change occurred to AICAR abundances in the stool metabolome. Changes to AICAR would also throw AMPK levels off balance and interfere with the body's energy and metabolism; this serves as metabolomics evidence that a western diet and chronic alcohol consumption could contribute to developing diseases like obesity and type 2 diabetes by disrupting energy metabolism and decreasing AICAR and AMPK abundances within the body.

The results showed that the plasma metabolome was significantly shifted by high amounts of EtOH and a western diet. EtOH was proven to exclusively alter the plasma metabolome by targeting metabolites such as vitamin B6 and ethanolamine. Vitamin B6 plays a key factor in regulating the body's production of neurotransmitters; dysregulation of these pathways could lead to malfunctions in the brain like decreased serotonin and cause neurological disorders.

Acknowledgements

First, I would like to thank my research supervisor and thesis mentor, Dr. Shawn Campagna, for allowing me to learn about and assist research with his group. I would also like to

thank Dr. Viktor Nemykin for supervising my thesis writing and being a part of my committee; to Dr. Douglas Stuart, for also taking the time to be part of my thesis committee. Lastly, I would like to thank Dr. Courtney Christopher for letting me assist with her research project and present our findings.

References

1. Vijay, A.; Valdes A.M. Role of the gut microbiome in chronic diseases: a narrative review. *European Journal of Clinical Nutrition*. **2022**, *76*, 489-501.
2. Zmora, N.; Suez, J.; Elinav, E. You are what you eat: diet, health and the gut microbiota. *Nature Reviews Gastroenterology & Hepatology*. **2019**, *16*, 35-56.
3. Engen, P.A. The Gastrointestinal Microbiome: Alcohol Effects on the Composition of Intestinal Microbiota. *Alcohol Res*. **2015**, *37*, 223-36.
4. Hanson, B.T; et al., Sulfoquinovose is a select nutrient of prominent bacteria and a source of hydrogen sulfide in the human gut. *The ISME Journal*. **2021**, *15*, 2779-2791.
5. Alseekh, S.; Aharoni, A.; Brotman, Y.; Contrepois, K.; D'Auria, J.; Ewald, J.; C. Ewald, J.; Fraser, P. D.; Giavalisco, P.; Hall, R. D. Mass spectrometry-based metabolomics: a guide for annotation, quantification and best reporting practices. *Nature Methods* **2021**, *18*, 747-756.
6. Zaparte, A.; Christopher, C.J.; Arnold, C.; Richey, L.; Castille, A.; Mistretta, K.; Taylor, C.M.; Lin, H.; Nelson, S.; Kirwan, J.P. Effects of E-Cigarettes on the Lung and Systemic Metabolome in People with HIV. *Metabolites* **2024**, *14*, 434.
7. Rabinowitz, J.D. and E. Kimball, Acidic Acetonitrile for Cellular Metabolome Extraction from *Escherichia coli*. *Analytical Chemistry*, **2007**, *79*, 6167-6173.
8. Lu, W.; Clasquin, M.F.; Melamud, E.; Amador-Noguez, D.; Caudy, A.A.; Rabinowitz, J.D. Metabolomic analysis via reversed-phase ion-pairing liquid chromatography coupled to a stand alone orbitrap mass spectrometer. *Anal. Chem.* **2010**, *82*, 3212–3221.
9. Pang, Z.; Zhou, G.; Ewald, J.; Chang, L.; Hacariz, O.; Basu, N.; Xia, J. Using MetaboAnalyst 5.0 for LC–HRMS spectra processing, multi-omics integration and covariate adjustment of global metabolomics data. *Nat. Protoc.* **2022**, *17*, 1735–1761.
10. Bazaruto, J.V.; Dearth, S.P.; Tague, E.D.; Campagna, S.R.; Downs, D.M. Untargeted metabolomics confirms and extends the understanding of the impact of aminoimidazole carboxamide ribotide (AICAR) in the metabolic network of *Salmonella enterica*. *Microb. Cell* **2017**, *5*, 74–87.
11. Zhang, B; Zhou, G.; Li, C. AMPK: An Emerging Drug Target for Diabetes and the Metabolic Syndrome, *Cell Metabolism*. **2009**, *9*, 407-416.
12. Ye X; Li H; Anjum K; Zhong X; Miao S; Zheng G; Liu W; Li L. Dual Role of Indoles Derived From Intestinal Microbiota on Human Health. *Front Immunol.* **2022**, *13*.
13. Gao, K.; Mu, C.; Farzi, A.; Zhu, W. Tryptophan Metabolism: A Link Between the Gut Microbiota and Brain, *Advances in Nutrition*, **2020**, *11*, 709-723.
14. Pathak, S.; Nadar, R.; Kim, S.; Liu, K.; Govindarajulu, M.; Cook, P.; Watts Alexander, C.S.; Dhanasekaran, M.; Moore, T. The Influence of Kynurenine Metabolites on Neurodegenerative Pathologies. *Int. J. Mol. Sci.* **2024**, *25*, 853.
<https://doi.org/10.3390/ijms25020853>
15. Snezhkina, A.V.; Kudryavtseva, A.V.; Kardymon, O.L.; Savvateeva, M.V.; Melnikova, N.V.; Krasnov, G.S.; Dmitriev, A.A. ROS Generation and Antioxidant Defense Systems in Normal and Malignant Cells. *Oxid Med Cell Longev.* **2019**.