Alcohol Related Alterations in TNF - α In Binge and Moderate Drinkers

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Alcohol is one of the most popular recreational drugs with roughly 70% of Americans reporting alcohol use within the last year (NIH, 2020). It is known that alcohol indirectly influences the immune system via its effects on the hypothalamic–pituitary–adrenal (HPA) axis, resulting in a dampening of the immune response (Eskandi & Sterberg 2002). Additionally, alcohol has direct effects on inflammatory and immune processes (Molina, et. Al., 2010), i.e., alcohol directly decreases white blood cell function (Gacouin, et. al, 2012) and quantity (Szabo & Mandrekar, 1999).

For example, alcohol also decreases expression of TNF-α through activation of its molecular inhibitor. TNF-α is a pro-inflammatory cytokine prone to decreased expression following the consumption of alcohol due to the interaction of alcohol with the NF-B inhibitor. TNF-α is a pro-inflammatory cytokine produced by macrophages/monocytes during infection which participates in a wide array of signaling pathways within cells. TNF-α is also an important protein in resistance to cancer and infection (Idriss & Naismith, 2000). Alcohol interacts with the NF-B inhibitor, decreasing phosphorylation and allowing it to bind to NF-B- thereby inhibiting the production and expression of TNF-α. This binding inhibits proper production of TNF-α in chronic drinkers. Importantly, these immunosuppressant qualities of alcohol consumption have been linked to greater susceptibility to respiratory infections (Happel and Nelson, 2005, Szabo and Mandrekar 2009).

Because of the tendency of a binge drinker to consume more alcohol on a more regular basis when compared to a moderate drinker, we hypothesized that the expression of TNFα following consumption of alcohol will be significantly less than moderate drinkers after alcohol cue exposure and alcohol consumption. Understanding the potential negative ramifications of alcohol consumption on the immune system is especially relevant as the COVID19 pandemic continues on college campuses.

Table 1. Demographics. This table breaks down the demographics of selected individuals. (Total N=64 and the * symbol notates a significant difference between BH/MD groups at p=0.05.)

<table>
<thead>
<tr>
<th>SUBJECT VARIABLE</th>
<th>MODERATE SOCIAL DRINKERS (N=32)</th>
<th>BINGE/HEAVY SOCIAL DRINKERS (N=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GENDER</td>
<td>FEMALE: 14 (44%)</td>
<td>16 (48%)</td>
</tr>
<tr>
<td></td>
<td>MALE: 17 (55%)</td>
<td>17 (52%)</td>
</tr>
<tr>
<td>RACE</td>
<td>AFRICAN AMERICAN*: 5 (16%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td></td>
<td>CAUCASIAN: 23 (72%)</td>
<td>32 (97%)</td>
</tr>
<tr>
<td></td>
<td>ASIAN AMERICAN*: 2 (10%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>HISPANIC: 5 (16%)</td>
<td>5 (16%)</td>
</tr>
<tr>
<td>AGE*</td>
<td>28 (7)</td>
<td>23.73 (4.5)</td>
</tr>
<tr>
<td>YEARS OF EDUCATION*</td>
<td>16.84 (2.5)</td>
<td>15.76 (2.6)</td>
</tr>
<tr>
<td>DRINKING VARIABLES</td>
<td>NUMBER OF ALCOHOL FIRST DEGREE RELATIVES</td>
<td>0.26 (0.58)</td>
</tr>
<tr>
<td></td>
<td>YEARS OF REGULAR DRINKING</td>
<td>7.56 (8.89)</td>
</tr>
<tr>
<td></td>
<td>DRINKING DAYS IN PAST MONTH*</td>
<td>9.89 (13.35)</td>
</tr>
<tr>
<td></td>
<td>TOTAL AMOUNT CONSUMED IN PAST MONTH*</td>
<td>23.46 (13.35)</td>
</tr>
<tr>
<td></td>
<td>CANADIAN QF'S USUAL NUMBER OF DRINKS*</td>
<td>2.45 (0.93)</td>
</tr>
<tr>
<td></td>
<td>CANADIAN QF'S MAX NUMBER OF DRINKS*</td>
<td>4.07 (1.83)</td>
</tr>
</tbody>
</table>

Participants were recruited based on their responses to a survey screening for drug use and abuse, alcohol use and abuse, history of mental health disorders, as well as age, race, and weight. Using parameters set by the NIH, the participants were classified into either the binge or moderate group. The classification was not mentioned to participants, and both groups experienced equal treatment. The group classified as “binge” was composed of participants who consumed 15+ alcoholic beverages a week for men, and 12+ alcoholic beverages a week for women. In these use patterns, we also looked to identify “binge” occurrences where the participant consumed 5-7+ drinks in one instance roughly once a month. The group classified as “moderate” consumed less than 15 drinks a week for men, and less than 12 drinks a week for women.

Participants came into the AUBIE lab on 2 non-consecutive days and administered a urine drug screen and a breathalyzer breath alcohol content test. Next, baseline blood samples were collected by a registered nurse prior to the alcohol taste test and placed in an ice bath for preservation prior to centrifugation. Next, during
The Alcohol Taste Test, participants are presented with three mugs of beer. Participants are instructed to drink enough to determine if they are same beer or different beers. Following the ATT, the participant was monitored for 45 minutes and a final blood sample was collected.

The blood samples were then spun in the centrifuge at 3000 RPMs for 12 minutes at 4°C to separate the blood components. The plasma from the samples was then pipetted into participant specific 5mL test tubes and brought to Dr. Beck’s lab at VCOM Auburn to be analyzed via an ELISA assay to determine and quantify the presence of TNF-α within participant samples. ELISA assays are an efficient method in quantifying blood proteins. A blood sample is tagged using antibodies that select for a specific blood protein, TNF-α, and are run through software that can detect the quantity of TNF-α by identifying the number of fluorescent antibodies.

There were no significant difference of baseline levels of TNF-α between the two drinking groups, as shown in Figure 1. This demonstrates that there two groups were young and healthy, i.e., without a health condition affecting baseline immune activity.

Additionally, the response elicited by the binge drinking group is indicative of a priming effect, whereby initial exposure to reminders of drinking led to more drinking. The moderate drinking group does not elicit a priming response and experiences a release of TNF-α following the alcohol stimulus. The moderate group releases TNF-α following the consumption of alcohol. This response, by the moderate drinking group, is a healthy, unadjusted response to alcohol. The binge group, however, experiences the priming effect by releasing TNF-α initially when exposed to the alcohol cues, but before the consumption of alcohol.

Through the analysis of blood TNF-α via ELISA assays, it was found that TNF-α levels following exposure to alcohol cues differed significantly between groups. The analysis indicated that TNF-α levels in binge drinkers increased when exposed to alcohol visual cues but decreased following consumption. On the other hand, it was found that TNF-α levels in moderate drinkers decreased when exposed to alcohol cues but increased following consumption.

**Statement of Research Advisor**

As part of a larger project funded by NIH, TNF-α response to alcohol cues and consumption were measured in binge drinkers and social drinkers. During the COVID19 pandemic, Josh noted the importance of a healthy immune response in fighting the virus. He therefore proposed to examine the differences in TNF-α response between the groups and found preliminary evidence that binge drinkers may have a comprised immune response to alcohol.

-Sara K. Blaine, PhD, College of Arts and Sciences

**References**


Authors Biography

Joshua Enger is a senior year of Biomedical Science at Auburn University. He recognized the importance of immune function during the COVID-19 pandemic and developed this project for his Undergraduate Research Fellowship. He plans on continuing his research in medical school.

Benjamin Campbell is a post-graduate research assistant. He has compiled statistics for all facets of the research study and contributed his technical knowledge of computer programming and lab management.

Dr. Darren Beck is a member of the faculty of VCOM studying Cell biology and physiology. ELISA assays were run in his lab and under his supervision.

Clayton Ridner, is a graduate student in the Cognitive and Behavioral Neurosciences Program at Auburn in Fall 2020. He performed psychological interviews of participants and supervised experimental procedures.

Lily Crone, is a junior-year student pursuing a bachelor’s of science in Neuroscience from Auburn University. Lily worked as a research assistant, handling participant blood samples and compiling data.

Juliet Wilson is a rising third year medical student at Edward Via College of Osteopathic Medicine. She performed ELISA assays.

Summer West is a rising third year medical student at Edward Via College of Osteopathic Medicine. She performed ELISA assays.

Austin McClanahan is a rising third year medical student at Edward Via College of Osteopathic Medicine. He performed ELISA assays.
Dr. Sara Blaine is an Assistant Professor in the Department of Psychological Sciences as an Assistant Professor in 2019. TNF-α levels in response to alcohol cues and consumption were already being measured via her NIH funded Study, R00 AA25401.