Study Protocol, Statistical Analysis Plan, and

Informed Consent

Cover Page

NCT03567434

Randomized, Double-Blind, Placebo-Based Study to Determine Effect of Evening Alcohol on Sympathetic Neural Activity and Baroreflex Function in Binge Drinkers

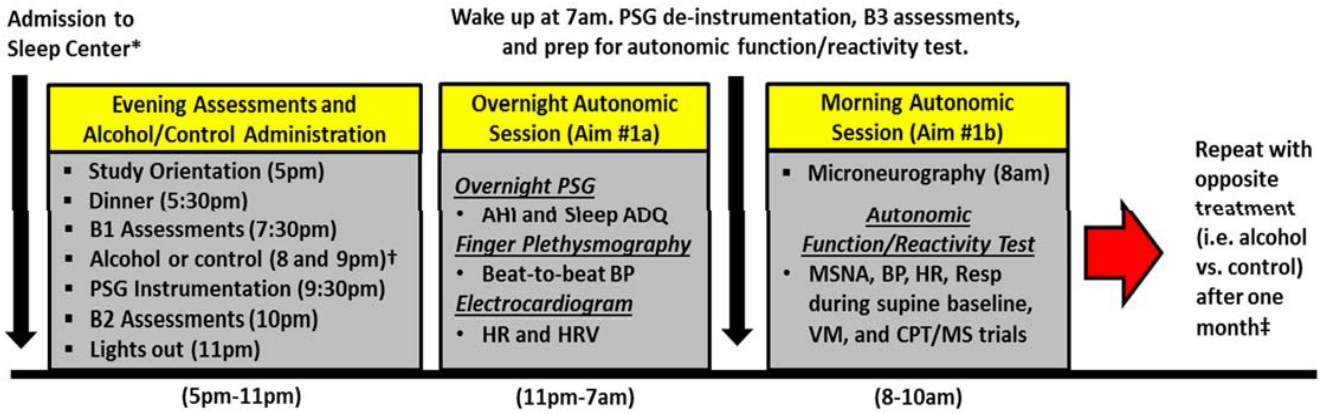
February 21, 2022

Study Protocol

Following enrollment in the study, participants were asked to complete one at-home, overnight sleep apnea test (ApneaLink, Resmed, San Diego, CA, USA) to screen for sleep-disordered breathing. If eligible following the at-home apnea testing (apnea-hypopnea index < 30 events/hour), participants were asked to report to the sleep laboratory at 9PM on a separate occasion to perform a familiarization overnight polysomnography (PSG) sleep study. This night was used to confirm the absence of sleep disordered breathing or other sleep disorders (i.e., restless leg syndrome, etc.) and to familiarize the participants with equipment and procedures.

After completing the familiarization night, participants were scheduled to return to the sleep laboratory on two occasions separated by one month. On each day of testing, meals throughout the day (i.e., breakfast, lunch, snack, and dinner) were provided based on participant caloric needs following consultation with a registered dietitian. Participants were assigned to a fluid control or alcohol condition separated by 1-month using a randomized, fluid-controlled, crossover design. Random assignment was performed by random selection of slips of paper with either experimental condition out of a cup by a blinded experimenter. In either condition, participants were asked to arrive to the laboratory at 4PM to assess breath alcohol concentration (Intoximeters, Inc., St. Louis, MO, USA) and hydration status via urine specific gravity (Schmidt Haensch, Berlin, Germany). During the alcohol condition, alcohol administration began at 8PM. Alcohol doses were diluted in a 1:3 ratio of 95% ethanol to fruit juice (orange or cranberry juice based on participant preference). A total dose of 1g/kg for males and 0.85g/kg for females was used. These doses were separated into a total of 6 aliquots, 3 of which were administered every 5 minutes starting at 8PM and another 3 starting at 9PM. In the fluid control condition, the same volume of fluid (i.e., juice) was ingested at each time point, but beverages did not contain alcohol but were masked with an alcohol mist. Participants were provided ad libitum water. The volume of fluid intake and urine output was monitored throughout the duration of the experimental visit. Following final dose administration, participants were instrumented with overnight PSG equipment. Following a venous blood draw and breathalyzer reading at 10PM for blood/breath alcohol concentration, participants entered bed at 10:45PM followed by lights out at 11PM. All participants were provided an 8-hour sleep opportunity.

Upon awakening at 7AM, participants' urine specific gravity was once again assessed, and another venous blood draw was performed to ensure that blood alcohol concentration had returned to baseline. Next, participants were asked to lie supine on a cushioned tilt-table for autonomic and cardiovascular testing. Recordings of beat-to-beat blood pressure via finger plethysmography (NOVA; Finapres Medical Systems, Amsterdam, The Netherlands), heart rate (HR; electrocardiography), respiration (pneumobelt), and muscle sympathetic nerve activity (MSNA) were established. After all autonomic and cardiovascular variables were obtained, participants rested for 10 minutes of non-recorded rest. Following this non-recorded period, three supine brachial blood pressures were taken using an automated sphygmomanometer (Omron HEM-907XL, Kyoto, Japan) after which 10-minutes of quiet, resting baseline data were obtained.



Statistical Analysis Plan

All data were analyzed using SPSS statistical software (Version 29.0, IBM Corp.). Shapiro-Wilk and skewness assessment were used to assess normality. Paired T-tests were utilized to assess differences in cardiovascular and sympathetic measures at rest. An α-value of 0.05 was used throughout.

Published Results

We have 21 peer-reviewed publications published so far related to this project:

Bigalke, J. A., Durocher, J. J., Greenlund, I. M., Keller-Ross, M., & Carter, J. R. (2023). Blood pressure and muscle sympathetic nerve activity are associated with trait anxiety in humans. American journal of physiology. Heart and circulatory physiology, 324(4), H494–H503. https://doi.org/10.1152/ajpheart.00026.2023

Bigalke, J. A., Cleveland, E. L., Barkstrom, E., Gonzalez, J. E., & Carter, J. R. (2023). Core body temperature changes before sleep are associated with nocturnal heart rate variability. Journal of applied physiology (Bethesda, Md. : 1985), 135(1), 136–145. https://doi.org/10.1152/japplphysiol.00020.2023

Bigalke, J. A., Greenlund, I. M., Nicevski, J. R., Tikkanen, A. L., & Carter, J. R. (2022). Sympathetic neural reactivity to the Trier social stress test. The Journal of physiology, 600(16), 3705–3724. https://doi.org/10.1113/JP283358

Bigalke, J. A., Greenlund, I. M., Nicevski, J. R., Smoot, C. A., Oosterhoff, B., John-Henderson, N. A., & Carter, J. R. (2021). Blunted heart rate recovery to spontaneous nocturnal arousals in short-sleeping adults. American journal of physiology. Heart and circulatory physiology, 321(3), H558–H566. https://doi.org/10.1152/ajpheart.00329.2021

Bigalke, J. A., Greenlund, I. M., & Carter, J. R. (2020). Sex differences in self-report anxiety and sleep quality during COVID-19 stay-at-home orders. Biology of sex differences, 11(1), 56. https://doi.org/10.1186/s13293-020-00333-4

Carter, J. R., Knutson, K. L., & Mokhlesi, B. (2022). Taking to "heart" the proposed legislation for permanent daylight saving time. American journal of physiology. Heart and circulatory physiology, 323(1), H100–H102. https://doi.org/10.1152/ajpheart.00218.2022

Carter, J. R., Mokhlesi, B., & Thomas, R. J. (2021). Obstructive sleep apnea phenotypes and cardiovascular risk: Is there a role for heart rate variability in risk stratification?. Sleep, 44(5), zsab037. https://doi.org/10.1093/sleep/zsab037

Greenlund, I. M., & Carter, J. R. (2022). Sympathetic neural responses to sleep disorders and insufficiencies. American journal of physiology. Heart and circulatory physiology, 322(3), H337–H349. https://doi.org/10.1152/ajpheart.00590.2021

Greenlund, I. M., Smoot, C. A., & Carter, J. R. (2021). Sex differences in blood pressure responsiveness to spontaneous K-complexes during stage II sleep. Journal of applied physiology (Bethesda, Md. : 1985), 130(2), 491–497. https://doi.org/10.1152/japplphysiol.00825.2020

Greenlund, I. M., Bigalke, J. A., Tikkanen, A. L., Durocher, J. J., Smoot, C. A., & Carter, J. R. (2021). Evening binge alcohol disrupts cardiovagal tone and baroreflex function during polysomnographic sleep. Sleep, 44(11), zsab130. https://doi.org/10.1093/sleep/zsab130

Greenlund, I. M., Cunningham, H. A., Tikkanen, A. L., Bigalke, J. A., Smoot, C. A., Durocher, J. J., & Carter, J. R. (2021). Morning sympathetic activity after evening binge alcohol consumption. American journal of physiology. Heart and circulatory physiology, 320(1), H305–H315. https://doi.org/10.1152/ajpheart.00743.2020

Holwerda, S. W., Carter, J. R., Yang, H., Wang, J., Pierce, G. L., & Fadel, P. J. (2021). CORP: Standardizing methodology for assessing spontaneous baroreflex control of muscle sympathetic nerve activity in humans. American journal of physiology. Heart and circulatory physiology, 320(2), H762–H771. https://doi.org/10.1152/ajpheart.00704.2020

Kerkering, E. M., Greenlund, I. M., Bigalke, J. A., Migliaccio, G. C. L., Smoot, C. A., & Carter, J. R. (2022). Reliability of heart rate variability during stable and disrupted polysomnographic sleep. American journal of physiology. Heart and circulatory physiology, 323(1), H16–H23. https://doi.org/10.1152/ajpheart.00143.2022

Lee, E., Anselmo, M., Tahsin, C. T., Vanden Noven, M., Stokes, W., Carter, J. R., & Keller-Ross, M. L. (2022). Vasomotor symptoms of menopause, autonomic dysfunction, and cardiovascular disease. American journal of physiology. Heart and circulatory physiology, 323(6), H1270–H1280. https://doi.org/10.1152/ajpheart.00477.2022

Lindsey, M. L., Kleinbongard, P., Kassiri, Z., Carter, J. R., Hansell Keehan, K., Ripplinger, C. M., LeBlanc, A. J., Brunt, K. R., & Kirk, J. A. (2023). We asked and you answered. American journal of physiology. Heart and circulatory physiology, 324(5), H657–H658. https://doi.org/10.1152/ajpheart.00084.2023

Lindsey, M. L., Kassiri, Z., Hansell Keehan, K., Brunt, K. R., Carter, J. R., Kirk, J. A., Kleinbongard, P., LeBlanc, A. J., & Ripplinger, C. M. (2021). We are the change we seek. American journal of physiology. Heart and circulatory physiology, 320(4), H1411–H1414. https://doi.org/10.1152/ajpheart.00090.2021

Lindsey, M. L., Carter, J. R., Ripplinger, C. M., Kassiri, Z., Hansell Keehan, K., Brunt, K. R., Kirk, J. A., Kleinbongard, P., & LeBlanc, A. J. (2023). Sex still matters in cardiovascular research. American journal of physiology. Heart and circulatory physiology, 324(1), H79–H81. https://doi.org/10.1152/ajpheart.00643.2022

Lindsey, M. L., Kassiri, Z., LeBlanc, A. J., Ripplinger, C. M., Kirk, J. A., Carter, J. R., Kleinbongard, P., & Brunt, K. R. (2023). Spring cleaning: freshening up the portfolio.

American journal of physiology. Heart and circulatory physiology, 324(6), H840–H842. https://doi.org/10.1152/ajpheart.00219.2023

Lindsey, M. L., LeBlanc, A. J., Ripplinger, C. M., Carter, J. R., Kirk, J. A., Hansell Keehan, K., Brunt, K. R., Kleinbongard, P., & Kassiri, Z. (2021). Reinforcing rigor and reproducibility expectations for use of sex and gender in cardiovascular research. American journal of physiology. Heart and circulatory physiology, 321(5), H819–H824. https://doi.org/10.1152/ajpheart.00418.2021

Pabon, E., Greenlund, I. M., Carter, J. R., & de Wit, H. (2022). Effects of alcohol on sleep and nocturnal heart rate: Relationships to intoxication and morning-after effects. Alcoholism, clinical and experimental research, 46(10), 1875–1887. https://doi.org/10.1111/acer.14921

Informed Consent

SUBJECT CONSENT FORM FOR PARTICIPATION IN HUMAN RESEARCH MONTANA STATE UNIVERSITY - BOZEMAN

PROJECT TITLE: **Alcohol and Neurovascular Control in Humans**

PROJECT DIRECTOR: Jason R. Carter, PhD, Dept of Health & Human Development
Sleep Research Laboratory, MSU-Bozeman, 59717
Phone: 406-994-2891
E-mail: jcarter@montana.edu

PURPOSE OF STUDY:

Binge alcohol consumption can lead to negative health consequences. Our laboratory is interested in the impact of an evening binge alcohol consumption on overnight sleep and the nervous and cardiovascular systems the next morning.

To participate, you must be between the ages of 21-45 years old, and must not meet any of the following exclusion criteria:

- Smoker
- Body mass index >35 kg/m^2
- Have been told by a doctor you have diabetes or are taking any cardiovascular or nervous system medications
- Unable to refrain from use of alcohol a minimum of 24 hours prior to testing protocol
- Are female and have irregular menstrual cycles or are pregnant
- Are female and taking hormonal contraceptives (within past 6 mo)
- Have a moderate-to-severe Alcohol Use Disorder
- Demonstrate substantial facial “flushing” after 1-2 drinks
- Have been diagnosed with severe sleep apnea
- Have bullous lung disease, bypassed upper airway, or pneumothorax.
- Have chronic low blood pressure or regular difficulties standing
- Have had prior cerebral spinal fluid leaks, abnormalities of the cribiform plate, prior history of head trauma, and/or pneumocephalus

STUDY PROCEDURES & POTENTIAL RISKS:

Orientation:

1. You will undergo an orientation to outline the study design and expectations. To do this, you will report to the Sleep Research Laboratory (CFT-5; Room 205) at Montana State University. You will be provided an oral overview of the research study, and we will answer any questions at this meeting. We will determine if you meet primary inclusion/exclusion criteria.

2. If you meet inclusion/exclusion criteria, and remain interested after this orientation, you will complete and sign this approved IRB consent form.

Study Design and Testing:

1. You will be fitted with a wristwatch that will track your sleep-wake activity for 3-5 consecutive nights for baseline measurements (similar to a "FitBit"). You will also wear a device for 1 night that will help us determine if you have sleep apnea. You will be provided both verbal and written instructions on proper use of this equipment.

2. Following these home sleep tests, you will be scheduled for three overnight visits to the Montana State Sleep Research Laboratory. Each day of testing, you will report to the Sleep Research Laboratory (CFT-5; Room 205) at 7:30AM, and we will give you breakfast and a to-go lunch box. You will then go about your normal daily routines.

3. At ~4PM, you will report back to the Montana State Sleep Research Laboratory. Urine samples will be collected to determine your hydration status, and blood will be drawn to check for alcohol, testosterone, progesterone, and estradiol. We will also perform alcohol breathalyzer tests, which require blowing into a mouthpiece. Measurements will be done to estimate blood pressure near your heart and how well your arteries stretch. A ~5PM, you will be provided dinner.

- ***Potential Risk*: A needle will be placed in your arm for the blood draws. There is a very small risk of infection or bruising of the arm. To combat this, sterile techniques will be used. Some individuals can get lightheaded with blood draws. If this happens, we will lie you down on the bed until you feel better. We will also provide you the option to do the blood draw lying down as opposed to sitting position as this can also help.**

4. At 7:00 PM, you will complete questionnaires and computerized tests to assess your attention and reaction time. Devices to monitor your blood pressure and heart rate will be placed on your finger (i.e., finger cuff) and upper chest (i.e., surface electrodes.)

5. For the alcohol testing night, you will be provided a dose of alcohol known to mimic 'binge' drinking. This dose of alcohol is a "*4-5 drink equivalent*", and will be provided to you in two evenly divided doses at 8 and 9 PM. The alcohol beverage will be diluted in a 1:3 mixture with either cranberry juice or orange juice, depending on your preference. The fluid control session will consist of the cranberry or orange juice plus 1% alcohol added as a taste mask, in the same volume. All beverages will be sprayed with an alcohol mist that has a strong alcohol scent. Beverages will be served in opaque lidded cups. At both times (8 and 9PM), the drinks will be divided into thirds and you will have 5 min to consume each of the smaller (i.e., 1/3 size) drinks. A breathalyzer will be used every 15 min from 8 – 11 PM.

- ***Potential Risk:* Mild intoxication levels will occur during the alcohol session. If at any time you are uncomfortable, or experience any negative side effects (e.g. speech problems, visual problems, dizziness, nausea, profuse sweating), the experiment will be stopped. Because alcohol will be consumed in the evening, and your heart rate will be monitored during the entire night with a laboratory technician, risks are minimal.**

6. At approximately 9:15 PM, various wires and electrodes will be placed on your head, face, thorax, abdomen, and legs measure your sleep from 11:00 – 7:00AM, a procedure called polysomnography. Wires and electrodes on your face will measure your brain waves. Breathing will be monitored from lightweight belts across your thorax and abdomen, and a nasal cannula (similar to what a person uses for oxygen) just under your nose for airflow. Leg movements will be measured from electrodes placed on your shin. You will repeat the questionnaires, arterial blood pressure measurements, and performance tests at 9:45 PM. A blood draw will occur at ~10:00PM to check for blood alcohol content. At ~10:45 PM, you will rest quietly in bed, with lights-out at 11:00 PM. From 11:00 PM – 7:00 AM, you will sleep while we record your blood pressure, heart rate, and sleep measurements. A trained monitoring technician will ensure signal quality throughout the night, and will assist you with requested bathroom visits.

7. You will be woken at 7:00 AM, and blood alcohol content will be checked with a breathalyzer and venous blood sample, and your hydration status with a urine sample. We will disconnect you from the overnight testing equipment, and have you repeat the questionnaires, blood pressure, and performance tests.

8. For the nervous system test, you will lie down on a comfortable laboratory bed where we will record your heart rate using an electrocardiogram, blood pressure using a finger cuff, your nervous system using two small leg electrodes, and breathing rate using a belt around your stomach. The two leg electrodes will be inserted near the nerve at the back of your knee. You will be asked to perform three safe stress tasks designed to modestly increase your heart rate and blood pressure. These include forcefully blowing into a tube for 15 seconds (i.e., Valsalva maneuver), 2 min of your hand in a cold-water bucket (i.e., cold pressor test), and 5 min of mental arithmetic (i.e., mental stress test). An investigator will verbally explain and demo these three common tasks prior to beginning any of them.

- ***Potential Risk*: There is a very small risk of infection after insertion of the leg electrode. Sterile techniques will be used to help prevent infection. Also, about 7% of subjects experience some aching or "pins and needles" sensations for a few days after the procedure. There is no specific treatment for these sensations, which are believed to be the result of tissue inflammation. In study volunteers who have experienced these sensations, they disappear spontaneously and completely without treatment within a few days. No reports of complications or infections have been reported after sessions. Dr. Carter and his laboratory have safely performed over 400 successful sessions.**

9. If your results show that alcohol increases the number of times you stop breathing at night, you will be invited for a fourth and final testing session in which you will repeat the alcohol treatment, and will be randomly (i.e., draw from a hat) assigned to either continuous positive airway pressure (CPAP) or "fake" CPAP. CPAP is the typical treatment for sleep apnea.

- ***Potential Risk*: Fake CPAP will not prevent the stopping of breathing like CPAP will. Thus, during fake CPAP these naturally occurring stops in**

breathing can potentially increase blood pressure, activate the nervous system, and increase the risk of adverse cardiovascular events. However, you will be continuously monitored throughout the entire night by a sleep technician who is trained to recognize severe and adverse cardiovascular events. In such cases, AASM guidelines will be followed and emergency medicine will be sought.

COST TO PARTICIPATE? There are no costs to participants.

BENEFITS: There are no direct benefits to you as a volunteer for this study. However, you will be able to learn more about how your sleep is impacted by alcohol consumption.

SUBJECT COMPENSATION: You will receive the following compensation for this project: 1) $50 for the familiarization PSG night; 2) $50 for each completed overnight test session, and $50 for each completed morning autonomic function test session. Payments will be prorated if you withdraw from the study. This compensation plan was approved through the National Institutes of Health.

CONFIDENTIALITY: The data and personal information obtained from this study will be regarded as privileged and confidential with only the Project Director and research team having access to this information. Your right to privacy will be maintained in any ensuing analysis and/or presentation of the data by using alphanumeric identification codes of each person's data. The self-report questionnaires that you provide will be retained within an encrypted computer folder in our lab for 7 years (as required by MSU), after which the document will be destroyed by paper shredding.

FREEDOM OF CONSENT: You may stop testing at any time, or withdraw consent for participation in writing, by telephone or in person without prejudice or loss of benefits (as described above). *Participation is completely voluntary*.

In the UNLIKELY event that your participation in the study results in physical injury to you, the Project Director will advise and assist you in receiving medical treatment. No compensation is available from Montana State University for injury, accidents, or expenses that may occur as a result of your participation in this study. Additionally, no compensation is available from Montana State University for injury, accidents, or expenses that may occur as a result of traveling to and from your appointments at the MSU. In the event that participation in this research directly results in injury to you, the standard medical emergency protocol will be initiated (i.e., calling 911) will be available. *Further information regarding medical treatment may be obtained by contacting the Project Director, Jason Carter (jcarter@montana.edu; 406-994-2891).* You are encouraged to express any questions, doubts or concerns regarding this study. The Project Director will attempt to answer all questions to the best of his ability prior to any testing. The Project Director fully intends to conduct the study with your best interest, safety and comfort in mind. *Additional questions about the rights of human subjects can be answered by the Chairman of the Human Subjects Committee, Mark Quinn, at 406-994-4707, or by email (*mquinn@montana.edu*).*

PROJECT TITLE: Alcohol and Neurovascular Control in Humans

STATEMENT OF AUTHORIZATION

I, *the participant*, have read the Informed Consent Document and understand the discomforts, inconvenience, risks, and benefits of this project. I, _____ *(print your name)*, agree to participate in the project described in the preceding pages. **I certify that I am at least 21 years old**. I understand that I may later refuse to participate, and that I may withdraw from the study at any time. I have received a copy of this consent form for my own records.

Signed: _____ Date: _____
(Participant Signature)