The Effect of Acute Mild Dehydration on Blood Pressure Control

NCT03560869

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HUMAN SUBJECTS PROTOCOL
University of Delaware

Protocol Title: Sympathetic Reactivity to Water Restriction in Young and Older Adults

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Investigator Assurance:
By submitting this protocol, I acknowledge that this project will be conducted in strict accordance with the procedures described. I will not make any modifications to this protocol without prior approval by the IRB. Should any unanticipated problems involving risk to subjects occur during this project, including breaches of guaranteed confidentiality or departures from any procedures specified in approved study documents, I will report such events to the Chair, Institutional Review Board immediately.

1. **Is this project externally funded?** ■ YES □ NO
   If so, please list the funding source:
   NIH Grant 1R01HL128388-01A1

2. Research Site(s)
■ University of Delaware
□ Other (please list external study sites)

Is UD the study lead? ■ YES □ NO (If no, list the institution that is serving as the study lead)

3. **Project Staff**
Please list all personnel, including students, who will be working with human subjects on this protocol (insert additional rows as needed):

NAME	**ROLE**	**HS TRAINING COMPLETE?**
Joseph Watso	Principal Investigator	Yes
William Farquhar	Co-Investigator (Advisor)	Yes
Megan Wenner	Co-Investigator	Yes
Matthew Babcock	Research Assistant	Yes
Kamila Migdal	Research Assistant	Yes
Austin Robinson	Research Assistant	Yes
Wendy Nichols	Research Nurse	Yes
Liza Walker	Lab Coordinator	Yes

4. **Special Populations**
Does this project involve any of the following:

Research on Children?
No

Research with Prisoners?
No

If yes, complete the Prisoners in Research Form and upload to IRBNet as supporting documentation

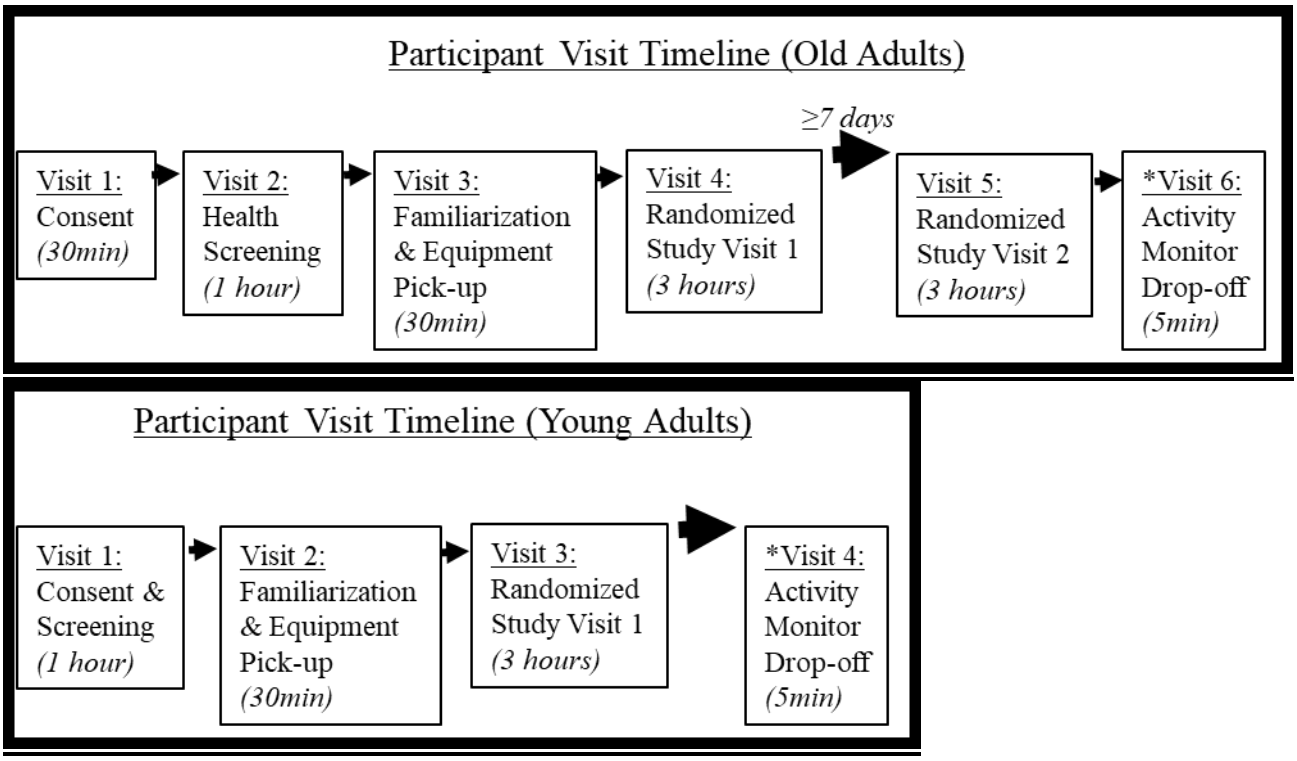
Research with Pregnant Women?
No

Research with any other vulnerable population (e.g. cognitively impaired, economically disadvantaged, etc.)? please describe
No

5. **RESEARCH ABSTRACT** Please provide a brief description in LAY language (understandable to an 8th grade student) of the aims of this project.

The purpose of this study is to investigate the effect of short-term water restriction on blood pressure responses to various maneuvers in old adults. A secondary purpose is to investigate if daily physical activity levels affect blood pressure control in young adults. Under various hydration states, the body compensates by managing blood electrolyte and body water levels to maintain blood volume and blood pressure balance. This compensation is thought to be diminished with aging. Previous studies have shown that dehydration status impairs how our body controls the sympathetic nervous system and blood pressure under resting conditions. The purpose of this study is to assess how hydration (mild dehydration vs. normal hydration) status affects the blood pressure responses during different tests that stimulate the sympathetic nervous system in old adults. Handgrip exercise with arm cuff occlusion, the hand in cold water test, and a breath hold will be used to stimulate sympathetic responses. These tests will provide specific insight into how hydration status affects how our body controls blood pressure.

6. **PROCEDURES**
Describe all procedures involving human subjects for this protocol. Include copies of all surveys and research measures.



*If necessary. Seven-day physical activity monitoring may be done between earlier visits when possible based on scheduling.

Participants enrolled prior to February 2018, who have indicated they can be contacted for future research studies, will have the opportunity to undergo activity monitoring for seven days. They

will come in for two visits (one for pick-up, one for drop off) to obtain the activity monitor, wear it for seven consecutive days, then return it.

Total Visit Time Summary (up to 6 total visits: ~8 hours total time commitment for Old adults; and up to 4 total visits: ~5 hours total time commitment for Young Adults)

Old Adults: Following the participant's initial consenting visit (up to 30 minutes), the participant will return for a screening visit with the Nurse Managed Primary Care Center at STAR Campus, Newark, DE (approximately 1 hour). The next visit will be a familiarization visit (30 minutes), which will allow for the participant to practice the exercise trial that will be used during the study visits. Following the participant's familiarization visit, we will schedule two study visits separated by one week. Each study visit will require approximately 3 hours.

Young Adults: Participants will come in for a consent and screening visit (approximately 1 hour). Young participants will be screened by a member of the Cardiovascular Physiology Research Team at STAR Campus, Newark, DE. The next visit will be a familiarization visit (30 minutes), which will allow for the participant to practice the exercise trial that will be used during the study visits. Following the participant's familiarization visit, we will schedule one study visit that will require approximately 3 hours.

Hydration Protocols

Three days prior to each study visit old participants should follow instructions for consuming recommended sodium diet (2,300 milligrams). They will be assigned to a normal hydration and water restriction protocol for each of the two study visits, in a random order. Young adults will complete only a normal hydration protocol. The table below indicates the fluid intake participants will be asked to follow for each protocol by day. Participants will drink 23mL water per kg of body weight for the normal hydration condition, and 10mL water per kg bodyweight for the water restricted condition. Below is the intake plan for both conditions.

	Normal Hydration	Water Restriction
Day 1	54oz	54oz
Day 2	54oz	40oz
Day 3	54oz	24oz
Morning of Visit	8oz	Avoid fluids

*based on a 70kg (154lb) participant. This will be customized for each participant based on their body weight.

Water and food will not be provided to the participants, they will be given a water bottle with liquid measure marks to maintain the recommended water intake. They will be given information on how to correctly complete a food log while using instructions to avoid high dietary sodium. Participants will also be given a list of foods that are high in water content to avoid 24 hours prior to their study visit. They will be given a protective case and be instructed on how operate the blood pressure cuff that they will wear for the 24 hours preceding each study visit. This blood pressure cuff will measure blood pressure every 20 minutes throughout the day and every 30 minutes during night time. A 3-day diet record will also be kept during the 3 days prior to data collection visits. Participants will also be instructed to avoid caffeine, alcohol, and moderate-to-vigorous (running, lifting weights, etc.) exercise 72 hours prior to their data collection visits.

Consent visit for old adults (30 min)
A laboratory member will explain the informed consent document to the participant. We will also answer any questions the participant may have. We will bring participants to the Nurse Managed Primary Care Center to schedule a screening visit.

Screening visit for old adults (1 hour)
Participants will be asked to avoid caffeine, alcohol, and exercise in the 24 hours prior to this visit. The following information will be obtained to confirm inclusion criteria are met: a complete medical history using a questionnaire, a PAR-Q (physical activity readiness questionnaire), height, weight, waist circumference, hip circumference, a resting electrocardiogram (10 self-adhesive electrodes will be placed on their chest), resting blood pressure, a body fat assessment, and a blood sample (obtained with a needle from a vein in the arm). If a participant answers "yes" to one or more questions on the PAR-Q they will be enrolled into the study on a case-by-case basis. The blood sample will be used to obtain an assessment of liver function, a lipid profile, a complete blood count, glucose, sodium, potassium, chloride, and kidney function. About 2 teaspoons (1.0 fluid oz.) will be sampled. All information will be reviewed by the Nurse Managed Primary Care Center and by the study investigators, and only those with no signs or symptoms of disease (example: chest pain, dizziness, or shortness of breath, etc.), blood pressure within specific limits, blood work within normal limits, and a resting electrocardiogram within normal limits, will be accepted into the study. Also, the medical history form and PAR-Q will be reviewed by the study investigators and Nurse Managed Primary Care Center to determine if the participant qualifies for the study. In addition, participants will be excluded if they are obese (body mass index greater than or equal to 30 kg/m^2), if they use any tobacco products. For women, menopause will be confirmed prior to testing. All medications will be reviewed on a case-by-case basis by the Nurse Managed Primary Care Center and study investigators to determine eligibility for study participation. The investigator(s) will discuss the results of these tests with the participants, and upon their request, we will make copies of these results for them.

Combined consent and screening visit for young adults (1 hour)
A laboratory member will explain the informed consent document to the participant. We will also answer any questions the participant may have. Participants will complete a PAR-Q. Participants will be screened at the Cardiovascular Physiology Research Laboratory at the University of Delaware. This involves completing a medical history questionnaire, measuring resting blood pressure, height and weight. Investigators review all information and only those with no signs or symptoms of disease will be asked to participate (example: history of chest pain, dizziness, or unusual shortness of breath, etc.). Female participants will have a urine pregnancy test administered to ensure that they are not pregnant. Individuals taking hormonal contraceptives will be permitted to participate in the current study. Additional exclusion criteria include: if resting blood pressure is outside specific limits (systolic blood pressure 90-139mmHg and/or diastolic blood pressure 50-89mmHg), if hypotension is present (systolic blood pressure <90mmHg and/or diastolic blood pressure <50mmHg), a history of cancer, diabetes, or any other chronic disease; a history of any heart disease; hormone therapy; use of tobacco or nicotine products; or a body mass index greater or equal to $30kg/m^2$. In the event that one of the test results is abnormal or a positive pregnancy test, they will be referred to their personal physician.

Familiarization & equipment pick-up visit (30 min)
All participants will come to the Cardiovascular Physiology Lab (STAR Campus Lab 128) for protocol familiarization. During the familiarizations, participants will practice a handgrip exercise and post exercise arm cuff occlusion trial to simulate a data collection visit. Participants will be provided with a special backpack designed to carry the plastic urine container and blood pressure monitor.

Experimental visit (3-hour visit)
Participants will arrive to the Cardiovascular Physiology Laboratory (STAR Campus Lab 128) fasted 4 hours. Participants will hand in the equipment backpack. The urine sample will be analyzed for salt content, as well as for the concentration of several hormones that control salt and water balance. Some of these analyses will be done immediately in the lab, and some of the urine sample will be stored in a freezer for future analysis. The participants name will not appear on the stored urine sample; rather, the sample will be coded with a number.

Urine Analysis
Upon arrival to the Cardiovascular Physiology Laboratory, participants will collect a spot urine sample during the same time they void prior to the start of the testing visit. This will be used to analyze hydration status and check pregnancy for pre-menopausal female adults. In the event of a positive test, they will be notified and be excused from further participation. We will also refer them to their personal physician.

Catheter Insertion & Blood Sample
A research nurse or Dr. Farquhar will place a catheter into the participants' dominant arm. We will insert one catheter into the dominant arm in retrograde fashion, leaving a small flexible tube in their vein. About 7.5 tablespoons (3.8 fluid oz.) will be sampled from the intravenous catheter (IV), and some of the blood will be stored in a freezer for future analysis of hormones that control salt and water balance in the body. Blood samples from the catheter placed in their dominant arm will be collected prior to handgrip exercise, immediately after handgrip exercise during post exercise arm cuff occlusion, and after cuff deflation. The catheter will be removed at the end of each study visit.

In the event a research nurse or Dr. Farquhar are unavailable, a lab member trained in phlebotomy will perform a baseline blood draw and we will miss the post exercise blood samples.

Measurements for neural circulatory control- Blood Pressure, Heart Rate, Respiration and Blood Flow Measurements
Blood pressure will be measured at the middle finger of the non-dominant hand, on a beat-by-beat basis. The finger pressure will be calibrated to brachial artery pressure according to the manufacturer's recommended calibration instructions before the protocol begins. Additionally, brachial blood pressure will be measured at rest and after exercise. A three-lead ECG will be used to measure heart rate, we will place 4 sticky pad electrodes on their shoulders (2) and stomach (2). Breathing frequency and depth will be measured by placing a respiratory band around their lower chest. An ultrasound probe will be used to measure blood flowing in their

upper leg (femoral artery). We will be measuring beat-by-beat blood pressure, heart rate, respiration, and blood flow continuously throughout the visit.

Microneurography

Figure 1: Example of fine wire electrode.

To assess acute sympathetic responses during exercise, we will directly record their nervous system activity at the peroneal nerve using the technique of microneurography. The peroneal nerve runs on the outside of the lower limb, near the fibular bone. The microelectrode will be inserted through the skin just below the knee. A reference microelectrode will also be inserted into the skin above the placement of the microneurography electrode. The insertion point will be determined by external electrical stimulation to elicit involuntary muscle twitches of the lower leg. When the nerve has been located, the electrode will be used to record nerve activity. The fine wire electrode will be removed following each study visit. Dr. William Farquhar, Dr. Megan Wenner, or Dr. Austin Robinson will be performing the microneurography technique.

Tests of Sympathetic Reactivity

Isometric Handgrip Exercise and Post Exercise Ischemia

Participants will perform a handgrip exercise bout lasting 2 minutes at approximately 30%. Maximal handgrip strength of the dominant hand will be measured by having the subject squeeze the handgrip device as hard as possible three-five times. The highest value will be used as the maximum, and this will be used to calculate relative work rates of approximately 30%. In our experience, most participants perceived work rate during the handgrip exercise tends to be "moderate" to "moderately hard." During the last 3 seconds of exercise, a cuff on their upper dominant arm will be inflated and will remain inflated for 3 minutes and 15s. The cuff will be deflated and data collection will continue during recovery for 2 minutes. During handgrip exercise, they will be encouraged to stay relaxed and to breathe normally, so as to not inadvertently dislodge the fine wire electrode from the nerve recording site. Dislodging the fine wire electrode does not pose any risk to the subject, but it does prevent us from obtaining a usable nerve recording. We will also use the Borg rating of perceived exertion (RPE) scale to determine how hard participants perceive they are working. Using the Borg scale, subjects will report an RPE between 6 and 20 during each minute during exercise.

Hand in Cold Water Test

A small bucket will be filled with a slurry ice and water mixture. The participant's dominant hand will be submerged in the cold water for 2 minutes and data collection will continue during recovery for an additional 2 minutes.

Voluntary End-Expiratory Apnea

Participants will be asked to exhale all of the air from their lungs, then be asked to hold their breath for as long as they can (typically lasting 30s). After participants can no longer hold their breath, they will resume normal breathing.

Participants will be offered water to consume at the end of their data collection visit. Participants will leave with an accelerometer after the first (young adults) or second (old adults) experimental visit and return for a brief (≤5 minutes) visit to return the accelerometer.

7. **STUDY POPULATION AND RECRUITMENT**

Describe who and how many subjects will be invited to participate. Include age, gender and other pertinent information.

An estimated 80 participants (40 young adults & 40 older adults) will be recruited to participate from the community surrounding the University of Delaware, Newark, DE. Participants will be recruited by flyers posted in different community locations. Also, flyers will be distributed to students in the department of Kinesiology & Applied Physiology via e-mail. We will IRB approved language from the informed consent, recruitment scripts, and flyers to recruit via UD classifieds, social media, postings in the Newark community, and at community events. The population being asked to participate are men and women between the ages of 20-35 and 55-75 years old with a body mass index (ratio of height and weight) score between 18.5 and 29.9. Also, participants must be non-smokers with blood pressures within specific limits (systolic 90-139 mmHg and diastolic blood pressure 50-89 mmHg) and have no known cardiovascular diseases, metabolic diseases, cancers, kidney diseases, and/or other chronic diseases. In addition, individuals that consume nicotine, are pregnant, plan on becoming pregnant, or currently receiving hormone replacement therapy do not qualify.

Attach all recruitment fliers, letters, or other recruitment materials to be used. If verbal recruitment will be used, please attach a script.

Describe what exclusionary criteria, if any will be applied.
- Outside the age ranges of 20-35 or 55-75
- Those with a body mass index score under 18.5 or over 29.9
- Smokers and nicotine users
- Resting blood pressure outside specific limits (those with systolic outside the range of 90-139 mmHg or diastolic outside the range of 50-89mmHg)
- Those who are pregnant, plan on becoming pregnant, or currently receiving hormone replacement therapy

Describe what (if any) conditions will result in PI termination of subject participation.

A person will be excluded for adverse side effects to the water restriction protocol, a positive pregnancy test, recent significant change in body weight or change in health status.

8. **RISKS AND BENEFITS**

List all potential physical, psychological, social, financial or legal risks to subjects (risks listed here should be included on the consent form).

There are no known risks associated with obtaining consent, height, weight, a resting ECG, resting blood pressure, urine analysis or using ultrasound to measure blood flow.

Hydration Protocol
There are some risks when old participants are asked to limit water intake during the water restriction protocol. During the water restriction protocol participants may develop a dry mouth, develop thirst, become tired, develop a headache, develop constipation, or become dizzy or light headed. This protocol is designed to induce mild dehydration and we have identified strategies and remedies to prevent moderate to severe dehydration. First, participants will not be exercising prior to the water restriction therefore activity will not contribute to the mild dehydration. Second, participants will be told to swish water in their mouth and spit it out as needed to reduce thirst. If participants do begin to feel the more severe forms of dehydration (severe headache, extreme thirst, very dry mouth) they will be instructed to consume 8oz of water and if symptoms are not resolved within 30 minutes, participants will be instructed to consume another 8oz of water. We anticipate quick resolution of any dehydration related symptoms after the consumption of 8oz of water, however if symptoms are present after 30 minutes they will be instructed to contact the investigators and if necessary seek medical attention.

The technique of mild dehydration through short-term water restriction is an accepted and safe technique. Included is a list of five published papers where this technique has been used (1-5):
1. Armstrong, L. E., et al. "Thermal and Circulatory Responses during Exercise: Effects of Hypohydration, Dehydration, and Water Intake." Journal of applied physiology (Bethesda, Md.: 1985) 82.6 (1997): 2028-35. Print.
2. Charkoudian, N., et al. "Interactions of Plasma Osmolality with Arterial and Central Venous Pressures in Control of Sympathetic Activity and Heart Rate in Humans." American journal of physiology.Heart and circulatory physiology 289.6 (2005): H2456-60. Print.
3. Judelson, Daniel A., et al. "Effect of Hydration State on Strength, Power, and Resistance Exercise Performance." Medicine and science in sports and exercise 39.10 (2007): 1817. Print.
4. Perry, Blake G., et al. "Mild Dehydration Modifies the Cerebrovascular Response to the Cold Pressor Test." Experimental physiology 101.1 (2016): 135-42. Print.
5. Rabbitts, J. A., et al. "Influence of Endogenous Angiotensin II on Control of Sympathetic Nerve Activity in Human Dehydration." The Journal of physiology 587.Pt 22 (2009): 5441-9. Print.

Blood Pressure Monitoring & Urine Collection
Participants may have minor discomfort when the ambulatory blood pressure cuff is inflated (they may feel numbness and tingling in their arm and hand). Due to potential difficulty faced by female adults, a urine funnel collection tool will be given to assist with urine collection. The ambulatory blood pressure cuff may cause participants to wake up during the night but this is limited by readings only occurring every 30 minutes as opposed to the daytime readings occurring every 20 minutes.

Catheter Insertion & Blood Sample
Participants may have pain and/or bruising at the spot where blood is taken or where the IV is placed in the arm (during dehydration there is a risk for increased discomfort), and there is a small risk of infection (this will be minimized by using sterile, single-use IV's). Signs of infection include pain, swelling, and redness. If these signs occur, they will be told that they should contact the study investigators. Fainting sometimes occurs during or shortly after blood

is drawn.

Tests of Sympathetic Reactivity

Assessment of blood pressure, heart rate and respiration
Participants may have minor discomfort removing the self-adhesive electrodes. They may have minor discomfort when the blood pressure cuff is inflated (they may feel numbness and tingling in their arm and hand) and when the upper arm cuff is inflated after handgrip exercise. In addition, following cuff deflation, they may feel a "flushed" sensation in the lower arm, which may also cause minor discomfort.

Microneurography
Our lab has been utilizing this technique for over 15 years without any major complications. Mild external electrical stimuli will be applied to their lower leg; this may cause some discomfort. There may also be mild discomfort when the small needle is inserted through the skin; however, this needle is very small. Brief sensations of pins and needles and/or cramping are likely to be felt during the nerve search portion of this technique. It is also possible that feelings of muscle weakness and/or pins and needles sensations can be felt after completion of the procedure. There is no specific treatment for these sensations, and in the small number of volunteers that have experienced them (<5% of participants in our laboratory), they have disappeared spontaneously. It is generally thought that the risks of side effects are minimized when no more than 1 hour is used to locate the peroneal nerve with the small needle. There is also a small risk of infection at the site where the fine wire needle is inserted. The fine wire needle will be removed at the end of each study visit.

The technique of microneurography is an accepted and safe technique. Included is a list of ten published papers where this technique has been used (1-10):
1. Charkoudian N, Joyner MJ, Barnes SA, Johnson CP, Eisenach JH, Dietz NM, and Wallin BG. Relationship between muscle sympathetic nerve activity and systemic hemodynamics during nitric oxide synthase inhibition in humans. Am J Physiol Heart Circ Physiol 291: H1378-1383, 2006.
2. Delaney EP, Greaney JL, Edwards DG, Rose WC, Fadel PJ, and Farquhar WB. Exaggerated sympathetic and pressor responses to handgrip exercise in older hypertensive humans: role of the muscle metaboreflex. Am J Physiol Heart Circ Physiol 299: H1318-1327, 2010.
3. Fadel PJ. Dynamic arterial baroreflex function during high intensity exercise in humans: insights into sympathetic control. J Physiol 586: 2667-2668, 2008.
4. Farquhar WB, Wenner MM, Delaney EP, Prettyman AV, and Stillabower ME. Sympathetic neural responses to increased osmolality in humans. Am J Physiol Heart Circ Physiol 291: H2181-2186, 2006.
5. Greaney JL, Ray CA, Prettyman AV, Edwards DG, and Farquhar WB. Influence of increased plasma osmolality on sympathetic outflow during apnea. Am J Physiol Regul Integr Comp Physiol 299: R1091-1096, 2010.
6. Halliwill JR, Morgan BJ, and Charkoudian N. Peripheral chemoreflex and baroreflex interactions
in cardiovascular regulation in humans. J Physiol 552: 295-302, 2003.
7. Joyner MJ, and Wieling W. Increased muscle perfusion reduces muscle sympathetic nerve

activity during handgripping. J Appl Physiol 75: 2450-2455, 1993.
8. Minson CT, Halliwill JR, Young TM, and Joyner MJ. Influence of the menstrual cycle on sympathetic activity, baroreflex sensitivity, and vascular transduction in young women. Circulation 101: 862-868, 2000.
9. Sundlof G, and Wallin BG. The variability of muscle nerve sympathetic activity in resting recumbent man. J Physiol 272: 383-397, 1977.
10. Wallin BG, and Sundlof G. A quantitative study of muscle nerve sympathetic activity in resting normotensive and hypertensive participants. Hypertension 1: 67-77, 1979.

Physical Activity Monitor
There are no known risks for undergoing daily physical activity monitoring.

Handgrip Exercise and Post Exercise Ischemia Trial
There is a risk of an excessive increase in blood pressure and heart rate during the two minutes of handgrip exercise. If the systolic blood pressure reaches > 220 mmHg or diastolic blood pressure reaches 110 mmHg, we will stop testing and allow blood pressure return to normal. Participants may have tightness in the forearm that will occur with the inflation of the cuff during post exercise cuff occlusion. The release of the cuff and influx of blood back into the arm may produce a "pins and needles" or "flushed" sensation. Additional risks associated with this procedure may include: soreness and stiffness of the forearm after the procedure, damage to the veins, thrombosis, and lightheadedness.

Hand in Cold Water Test
The hand in cold water test can cause discomfort. If the hand in cold water test becomes too uncomfortable, we can remove their hand from the cold water at their request at any time. There is a risk of excessive blood pressure increase and heart arrhythmias. We will monitor blood pressure and heart rate throughout.

The hand in cold water test is an accepted and safe test. Included are paper that utilize this technique, including one of the original articles that found two minutes was necessary to observe increased MSNA (1-5):

1. Victor, RG, Leimbach, WN, Seals, DR, Wallin BG, Mark, AL, (1987). Effects of the cold pressor test on muscle sympathetic nerve activity in humans. Journal of Hypertension, 9; 5, 429-436.
2. Young, CN, Stillabower, ME, DiSabatino, A, Farquhar, WB, (2006). Venous smooth muscle tone and responsiveness in older adults. Journal of Applied Physiology, 101 (5): 1362-1369.
3. Pham, I, Nguyen MT, Valensi, P, Rousseau, H, Nitenberg, A, Vicaut, E, Cosson, E, (2015). Noninvasive study of coronary microcirculation response to a cold pressor test. European Journal of Clinical Investigation, 45 (2), 135-143.
4. Dishman, RK, Nakamura, Jackson, EM, Ray, CA, (2003). Blood pressure and muscle sympathetic nerve activity during cold pressor stress: Fitness and gender. Psychophysiology, 40 (3), 370-380.
5. Grewal, S, Sekhon, TS, Walia, L, Gambhir, RS, (2015). Cardiovascular response to acute cold stress in non-obese and obese healthy adults. Ethiopian Journal of Health Science. 25 (1): 47-52.

Voluntary End-Expiratory Apnea
Participants will be asked to exhale maximally and then hold their breath as long as they can, they will resume normal breathing immediately following the breath hold. Participants may feel slight discomfort while holding their breath. They also may feel dizziness immediately following the test.

In your opinion, are risks listed above minimal* or more than minimal? If more than minimal, please justify why risks are reasonable in relation to anticipated direct or future benefits.

The risks associated with the current study are more than minimal due to the mildly invasive nature of short-term water restriction in old adults, intravenous catheter in all adults, and the microneurography technique in all adults. This research study will potentially increase our understanding of the acute effects of dehydration we see and how the sympathetic nervous system is altered by chronic decreased total body water (chronic dehydration) potentially increasing future health risks of a healthy non-hypertensive population. This research will also provide insight on how the body regulates blood pressure throughout the day under various hydration states, and how this is altered during aging.

*(*Minimal risk means the probability and magnitude of harm or discomfort anticipated in the research are not greater than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests)*

What steps will be taken to minimize risks?

In order to minimize risks, the heart rate and blood pressure will be monitored throughout testing. Should we see excessive blood pressure increases or heart rate irregularities, we will terminate the testing visit.

Hydration Protocol
Following the water restriction protocol data collection visit, participants will be told to consume water. If any symptoms of mild dehydration are present, we anticipate quick resolution after the consumption of 8-16oz of water. In the event that someone would require medical treatment, they will be referred to the Nurse Managed Primary Care Center.

As stated, there are potential risks associated with short term water restriction. In order to mitigate these risks and discomfort, they will be instructed to swish water in their mouth, refrain from strenuous exercise, and avoid hot environments for extended periods of time. If participants do begin to feel the more severe forms of dehydration (severe headache, extreme thirst, very dry mouth) they will be asked to consume 8oz of water and if symptoms are not resolved within 30 minutes, participants will be instructed to consume another 8oz of water. If symptoms persist, participants will be instructed to contact the investigators and if necessary seek medical attention.

Old adults may be more susceptible to dehydration related symptoms. We will monitor them closely during the dehydration protocol and instruct them to consume water if they begin to feel more severe symptoms of dehydration (listed above).

Catheter Insertion & Blood Draw
Standard safety precautions will be taken when drawing blood samples and placing IV catheters. To minimize the risk of infection associated with blood collection, only sterile, single use catheters will be used. To minimize the risk of bruising, proper blood collection technique will be utilized.

Tests of Sympathetic Reactivity
Participants will be re-assured that at any time during the protocol, if they feel too uncomfortable to continue than we will stop testing. Blood pressure will be continuously monitored during exercise. If there is an excessive increase in blood pressure, defined as systolic blood pressure above 220 mmHg or diastolic blood pressure above 110 mmHg, exercise will be terminated.

Isometric Handgrip Exercise and Post Exercise Ischemia Trial
Tightness in the forearm will occur with the inflation of the cuff during post exercise ischemia. The release of the cuff and influx of blood back into the arm may produce a "pins and needles" or "flushed" sensation. Additional risks associated with this procedure may include: soreness and stiffness of the forearm after the procedure, damage to the veins, thrombosis, and lightheadedness. If cuff inflation becomes too uncomfortable for the participant, the cuff will be immediately released.

Hand in Cold Water Test
During the test, we will be monitoring the participant's blood pressure and heart rate. Should we see excessive increases in blood pressures or abnormal arrhythmias we will stop the test and begin recovery.

Microneurography
The time limit of one hour for finding a usable recording site will be strictly enforced. In the event a participant experiences adverse symptoms during the procedure, the protocol will be stopped. Participants will be warned about the risks of any discomfort. Participants will also be told that they can discontinue the study at any time. Participants will be directed to monitor for signs of infection (redness, soreness, heat and/or discharge) for 3-5 days post-study at the catheter and microelectrode placement sites. Should signs of infection occur, participants will be asked to contact the study investigators.

Describe any potential direct benefits to participants.
None

Describe any potential future benefits to this class of participants, others, or society.
The current proposal will give insight into the effect of decreased water intake on sympathetic nerve activity (commonly referred to as the flight or fight response) and blood pressure responses in older adults. We will increase the understanding of how the body maintains cardiovascular homeostasis by managing blood electrolyte and body water levels under various hydration states. These tests will provide specific insight into whether hydration status directly impacts the sympathetic nervous system and reflexes related to the sympathetic nervous system. Additionally, this investigation will provide novel information on cardiovascular regulation during mild dehydration in aging.

If there is a Data Monitoring Committee (DMC) in place for this project, please describe when and how often it meets.
No

9. **COMPENSATION**
Will participants be compensated for participation?
Yes

If so, please include details.
Old adults:
$150 for full completion of the research protocol (6 visits).
$130 if they complete one of the two three-hour experimental visits, and not physical activity monitoring.

Young adults:
$85 for full completion of the research protocol (4 visits).
$65 if they complete one of the one three-hour experimental visits, and not physical activity monitoring.

10. **DATA**
Will subjects be anonymous to the researcher?
No

If subjects are identifiable, will their identities be kept confidential? (If yes, please specify how)

Individual identity will be known only to the investigators. Participants will not be individually identified, except by participant number. All identifying information will be separated into a different locked filing cabinet. A participant number provided to each individual participant will be associated with all data collected. A participant key linking participant ID and name will be kept on a password-protected computer managed by the Principal Investigator.

How will data be stored and kept secure (specify data storage plans for both paper and electronic files. For guidance see http://www.udel.edu/research/preparing/datastorage.html)

The paper files are stored in a locked cabinet. Digital files containing only de-identified data are stored on a password-protected computer or password protected encrypted file. While the results of the research may be published, participants' names and identities will not be revealed.

How long will data be stored?
All data will be stored in a locked cabinet or password protected computer indefinitely.

Will data be destroyed? □ YES **X** NO (if yes, please specify how the data will be destroyed)

We propose keeping the data indefinitely, but destroying the document linking the participant names/identifying information to the data, 5 years after study closeout.

Will the data be shared with anyone outside of the research team? □ YES ■ NO (if yes, please list the person(s), organization(s) and/or institution(s) and specify plans for secure data transfer)

How will data be analyzed and reported?

Coded data will be analyzed using several statistical software packages. The results and interpretation will be published in peer-reviewed journals.

11. **CONFIDENTIALITY**
Will participants be audiotaped, photographed or videotaped during this study?
No

How will subject identity be protected?
Participants will not be individually identified, except by a participant number known only to the investigators.

Is there a Certificate of Confidentiality in place for this project? (If so, please provide a copy).
No

12. **CONFLICT OF INTEREST**
(For information on disclosure reporting see:
http://www.udel.edu/research/preparing/conflict.html)

Do you have a current conflict of interest disclosure form on file through UD Web forms?
No

Does this project involve a potential conflict of interest*?
No

* As defined in the University of Delaware's Policies and Procedures ,a potential conflict of interest (COI) occurs when there is a divergence between an individual's private interests and his or her professional obligations, such that an independent observer might reasonably question whether the individual's professional judgment, commitment, actions, or decisions could be influenced by considerations of personal gain, financial or otherwise.

If yes, please describe the nature of the interest

13. **CONSENT and ASSENT**

__x__ Consent forms will be used and are attached for review (see Consent Template under Forms and Templates in IRBNet)

____ Additionally, child assent forms will be used and are attached.

____ Waiver of Documentation of Consent (attach a consent script/information sheet with the signature block removed).

____ Waiver of Consent (Justify request for waiver)

14. **Other IRB Approval**
Has this protocol been submitted to any other IRBs?

No

If so, please list along with protocol title, number, and expiration date.

15. **Supporting Documentation**
Please list all additional documents uploaded to IRBNet in support of this application.

1. **Protocol (TC and clean)**
2. **Young adult consent (TC and clean)**
3. **Old adult consent (TC and clean)**
4. **Young adult advertisement with tabs (new)**
5. **Young adult advertisement without tabs (new)**
6. **Old adult advertisement with tabs (TC and clean)**
7. **Old adult advertisement without tabs (TC and clean)**
8. **Recruitment scripts (TC and clean)**
9. **Normal hydration protocol packet (clean)**
10. **Water restriction protocol packet (clean)**
11. **Medical history form (clean)**
12. **PAR-Q (clean)**