INFORMED CONSENT TO PARTICIPATE IN RESEARCH

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

Principal Investigator(s): Joseph Watso, BS (PI); William Farquhar, PhD (Advisor)

You are being invited to participate in a research study. This consent form tells you about the study including its purpose, what you will be asked to do if you decide to take part, and the risks and benefits of being in the study. Please read the information below and ask us any questions you may have before you decide whether or not you want to participate.

WHAT IS THE PURPOSE OF THIS STUDY?

The purpose of this study is to look at the effects of reduced water intake on blood pressure responses to various tests. The body manages salt and water levels in the blood to maintain blood volume and blood pressure balance. Not drinking enough water impairs how our body controls blood pressure. The purpose of this study is to assess how water intake affects the blood pressure during different tests in young and older adults. Hand grip exercise with arm cuff occlusion, the hand in cold water test, and a breath hold will be used. These tests will provide insight into how water intake status affects how your body controls your blood pressure.

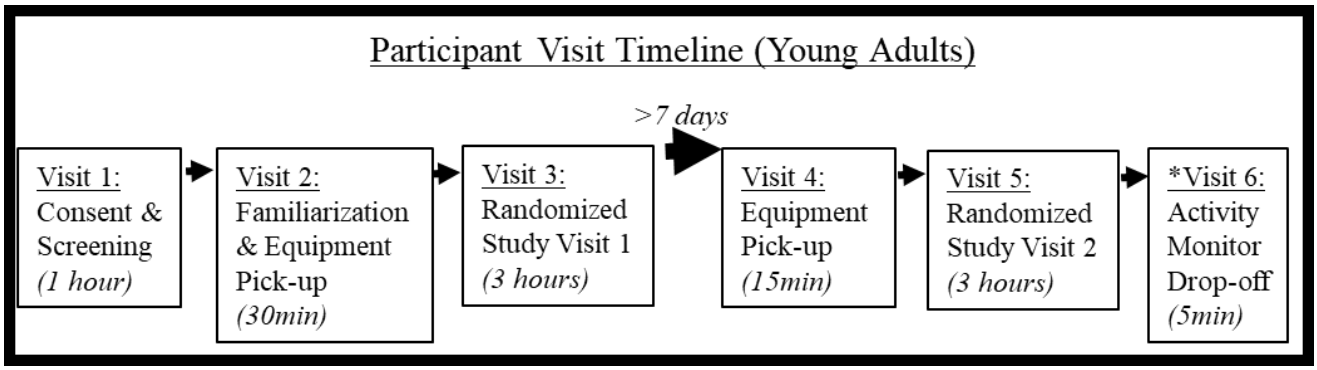
You will be one of about 80 people in this study (one of 40 young adults).

WHY ARE YOU BEING ASKED TO PARTICIPATE?

You are being asked to participate because you are between the ages of 20-35 years old with a body mass index (ratio of height and weight) score between 18.5 and 29.9. In order to qualify for this study, you must not be a smoker and must have blood pressure within specific limits (systolic 90-139 mmHg and diastolic blood pressure 50-89 mmHg). You must also have no known heart diseases, metabolic diseases, cancer, kidney disease, and/or other chronic diseases. In addition, individuals that are pregnant, plan on becoming pregnant, or currently receiving hormone replacement therapy do not qualify.

WHAT WILL YOU BE ASKED TO DO?

As part of this study you will be asked to return to the STAR Campus, Newark, DE for up to 6 total visits, with the first being this consent visit. The total time commitment for this study is up to ~8 hours, including this consent visit.



*If necessary. Seven-day physical activity monitoring may be done during washout period between visits three and four.

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Water intake Protocols

Three days prior to each study visit you will be asked to eat a recommended sodium diet (2,300 mg). You will be assigned to follow a normal water intake and reduced water intake protocol for each of the two study visits, in a random order. The table below indicates the fluid intake you will be asked to follow by day. You will be asked to drink 23mL water per kg of body weight per day for the normal water intake state, and 10mL water per kg body weight per day for the reduced water intake protocol. Below is the intake plan for both.

	Normal water intake	Reduced water intake
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 changed for you based on your body weight.

Water and food will not be provided to you. You will be given a water bottle with liquid measure marks to drink the correct amount of water. You will be shown how to correctly maintain a food log. You will be shown how to avoid high dietary sodium. You will also be given a list of foods that are high in water content to avoid 24 hours before your study visit. You will be given a case and be told how to operate the blood pressure cuff that you will wear for the 24 hour period before each study visit. This blood pressure cuff will measure blood pressure every 20 minutes during the day and every 30 minutes during night time. A 3-day diet record will also be kept during the 3 days prior to each of the data collection visits. You will also be asked to avoid caffeine, alcohol, and moderate-to-vigorous (running, lifting weights, etc.) exercise 72 hours prior to the data collection visits.

Visit 1: Consenting & screening (1 hour)

We will explain the informed consent and answer any questions you may have.

You will be screened at the Cardiovascular Core Laboratory at the University of Delaware. This involves completing a medical history questionnaire and physical activity readiness questionnaire (PAR-Q), measuring resting blood pressure, height and weight. We will review all of your information and if you are free from signs or symptoms of disease will be asked to participate (example: history of chest pain, dizziness, or unusual shortness of breath, etc.). If you answer "yes" to one or more questions on the PAR-Q we will review enrollment into this study on a case-by-case basis. For women, a urine pregnancy test will be administered to ensure you are not pregnant. Those currently taking hormonal contraceptives are able to participate in the study. Additional exclusion criteria include: 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 than or equal to 30kg/m2. In the event that one of the test results is abnormal or a positive pregnancy test, you will be referred to your personal physician.

Visit 2: Hand grip practice & Equipment pick-up (30 min)

You will practice a hand grip exercise and post exercise arm cuff occlusion trial to simulate a data collection visit.

You will be provided with a special backpack designed to carry the plastic urine container and blood pressure monitor. The urine sample will be analyzed for salt content and hormones that control salt and water balance. Some of these analyses will be done immediately in the lab, and some samples will be stored in a freezer for future analysis. Your name will not appear on the stored urine sample; rather, the sample will be coded with a number.

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Visit 3: Study visit (randomized water intake) (3 hours)

You will arrive to the Cardiovascular Physiology Laboratory (STAR Campus Lab 128) fasted 4 hours. You will hand in the equipment backpack.

Urine Analysis

Upon arrival to the laboratory, you will be asked to provide a spot urine sample. This will be used to analyze hydration status and check pregnancy for women.

Catheter & Blood Sample

A research nurse or Dr. Farquhar will place a catheter into your dominant arm. We will insert one catheter into the dominant arm, leaving a small flexible tube in their vein. About 7.5 tablespoons (3.8 fluid oz.) will be sampled from the catheter. A blood sample will be collected prior to hand grip exercise, right after hand grip 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 our research nurse or Dr. Farquhar are unavailable, a lab member trained in phlebotomy will use a butterfly needle to collect a baseline blood sample. About 3.5 tablespoons (1.8 fluid oz.) will be sampled from the butterfly needle. The needle will be removed immediately after blood collection. If this is the case, we will not sample blood during post exercise arm cuff occlusion and after cuff deflation.

Measurements - Blood Pressure, Heart Rate, Respiration and Blood Flow Measurements

Blood pressure will be measured at the middle finger of your non-dominant hand, on a beat by beat basis. Additionally, brachial blood pressure will be measured during the visit. We will place 4 sticky pad electrodes on your shoulders (2) and stomach (2) to measure heart rate. Breathing frequency will be measured by placing a band around your lower chest. An ultrasound probe will be used to measure blood flowing in your upper leg (femoral artery). We will be measuring beat by beat blood pressure, heart rate, respiration, and blood flow continuously throughout the visit.

Microneurography

To assess sympathetic responses during exercise, we will record nervous system activity at the peroneal nerve using the technique of microneurography. The peroneal nerve runs on the outside of your 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 main 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 this technique.

Figure 1: Example of fine wire electrode.

Tests of Sympathetic Reactivity

Hand grip Exercise Trials

You will be asked to perform hand grip exercise. This will last 2 minutes at approximately 30% & 40% of your maximal hand grip strength. Maximal hand grip strength of the dominant hand will be measured by having you squeeze the hand grip device as hard as possible three times. The highest value will be used as

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the maximum, and this will be used to calculate relative work rates. In our experience, most participants perceived work rate during the hand grip exercise tends to be "moderate" to "moderately hard." During the last 3 seconds of exercise, a cuff on your 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 hand grip exercise, you will be encouraged to stay relaxed and to breathe normally, so as to not dislodge the fine wire electrode from the nerve recording site. Dislodging the fine wire electrode does not pose any risk to you, but it does prevent us from obtaining a good nerve recording. We will also use the Borg rating of perceived exertion (RPE) scale to determine how hard you perceive you are working. Using the Borg scale, you 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. Your dominant hand will be submerged in the cold water for 2 minutes and data collection will continue during recovery for an additional 2 minutes.

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

You may leave the first experimental visit with an activity monitor (like a Fitbit) to monitor daily physical activity over a seven-day period before the second three-day hydration protocol. Alternatively, you will leave with an activity monitor after the second experimental visit and return for a brief (≤5 minutes) visit to return the activity monitor.

Visit 4: Equipment pick-up (15min)
You will be asked to pick-up the equipment backpack (plastic urine container and blood pressure monitor).

Visit 5: Study visit (randomized water intake) (3 hours)
You will be asked to complete the other hydration protocol, explained above.

Visit 6: Activity Monitor Drop-off:
If necessary, you will return the activity monitor following a seven-day physical activity monitoring period.

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?
There are no known risks associated with obtaining your height, weight, resting ECG, resting blood pressure, urine collection or using ultrasound to measure blood flow.

Water intake Protocol
There are some risks when you are asked to limit water intake during the reduced water intake protocol. During the reduced water intake you 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. We have strategies to prevent more severe dehydration. First, you will not be exercising prior to the water intake reduction, therefore activity will not add to the symptoms. Second, you can swish water in your mouth and spit it out as needed to reduce thirst. If you do begin to feel the more severe symptoms (severe headache, extreme thirst, very dry mouth) you can consume 8oz of water and if symptoms are not resolved within 30 minutes, you can consume another 8oz of water. We anticipate quick relief of any symptoms after

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you drink 8oz of water, however if symptoms are present after 30 minutes you should contact the investigators and if needed seek medical attention.

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

Catheter & Blood Sample
You may have pain and/or bruising at the spot where blood is taken or where the IV is placed in the arm. During reduced water intake there is a risk for increased discomfort. There is a small risk of infection that will be minimized by using sterile, single-use IV's. Signs of infection include pain, swelling, and redness. If these signs occur, you should contact the study investigators. Fainting sometimes occurs during or shortly after blood is drawn however you will be laying down supine already so there is little to no risk of falling and injuring yourself.

Blood pressure, heart rate and respiration
You may have minor discomfort removing the self-adhesive electrodes. You may have minor discomfort when the blood pressure cuff is inflated. You may feel numbness and tingling in your arm and hand).

Microneurography
Our lab has been using this technique for over 15 years without any major complications. Mild external electrical stimuli will be applied to your 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 feeling 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 feeling can be felt after the procedure. There is no specific treatment for these feeling, and in the very small number of volunteers that have felt them (<5% of participants in our laboratory), they have disappeared naturally. The risks of side effects are lessened 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. We sterilize each needle and only use it once. The fine wire needle will be removed at the end of each study visit.

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

Tests of Sympathetic Reactivity

Hand grip Exercise Trials
There is a risk of an extreme increase in blood pressure and heart rate during the two minutes of hand grip exercise. If the systolic blood pressure reaches > 220 mmHg or diastolic blood pressure reaches 110 mmHg, we will stop testing and allow your blood pressure to return to normal. You 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" feeling. Other risks may

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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 your hand from the cold water at your request. There is a risk of excessive blood pressure increase and heart arrhythmias. We will monitor blood pressure and heart rate.

Breath Hold
You may feel slight discomfort while holding your breath. You also may feel dizziness right after the test.

WHAT IF YOU ARE INJURED DURING YOUR PARTICIPATION IN THE STUDY?
If you are injured during research procedures, you will be offered first aid at no cost to you. If you need additional medical treatment, the cost of this treatment will be your responsibility or that of your third-party payer (for example, your health insurance). By signing this document you are not waiving any rights that you may have if injury was the result of negligence of the university or its investigators.

WHAT ARE THE POTENTIAL BENEFITS?
You will not directly benefit from taking part in this research. However, the knowledge gained from this study may contribute to our understanding of fluid and electrolyte balance in aging.

NEW INFORMATION THAT COULD AFFECT YOUR PARTICIPATION:
During the course of this study, we may learn new information that could be important to you. This may include information that could cause you to change your mind about participating in the study. We will notify you as soon as possible if any new information becomes available.

HOW WILL CONFIDENTIALITY BE MAINTAINED? WHO MAY KNOW THAT YOU PARTICIPATED IN THIS RESEARCH?
Your identity will be known only to the investigators. You will not be individually identified, except by participant number. All screening data collected will be de-identified. All identifying information will be separated into a different locked filing cabinet. A participant number provided to you 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. The paper files are stored in a locked cabinet. Digital files containing only coded data are stored on a password-protected computer or password protected encrypted file. While the results of the research may be published, your name and identity will not be revealed.

The confidentiality of your records will be protected to the extent permitted by law. Your research records may be viewed by the University of Delaware Institutional Review Board, which is a committee formally designated to approve, monitor, and review biomedical and behavioral research involving humans.

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USE OF DATA COLLECTED FROM YOU IN FUTURE RESEARCH:
The research data we will be collecting from you during your participation in this study may be useful in other research studies in the future. Your choice about future use of your data will have no impact on your participation in this research study. Do we have your permission to use in future studies data collected from you? Please write your initials next to your preferred choice.

_____ **YES** _____ **NO**

WILL THERE BE ANY COSTS TO YOU FOR PARTICIPATING IN THIS RESEARCH?
There are no costs associated with participating in the study.

WILL YOU RECEIVE ANY COMPENSATION FOR PARTICIPATION?
You will be compensated $80.00 following the full completion of the study for your time. If you only complete one of the two three-hour experimental visits, and not physical activity monitoring, you will be compensated $30.00.

DO YOU HAVE TO TAKE PART IN THIS STUDY?
Taking part in this research study is entirely voluntary. You do not have to participate in this research. If you choose to take part, you have the right to stop at any time. If you decide not to participate or if you decide to stop taking part in the research at a later date, there will be no penalty or loss of benefits to which you are otherwise entitled.

Your decision to stop participation, or not to participate, will not influence current or future relationships with the University of Delaware.

As a student, if you decide not to take part in this research, your choice will have no effect on your academic status or your grade in the class.

WHO SHOULD YOU CALL IF YOU HAVE QUESTIONS OR CONCERNS?
If you have any questions about this study, please contact the Principal Investigator, Joseph Watso at (631) 413-1944 or jwatso@udel.edu; or William Farquhar, Ph.D at (302) 831-6178 or wbf@udel.edu
If you have any questions or concerns about your rights as a research participant, you may contact the University of Delaware Institutional Review Board at hsrb-research@udel.edu or (302) 831-2137

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Your signature on this form means that: 1) you are at least 18 years old; 2) you have read and understand the information given in this form; 3) you have asked any questions you have about the research and those questions have been answered to your satisfaction; 4) you accept the terms in the form and volunteer to participate in the study. You will be given a copy of this form to keep.

_____	_____	_____
Printed Name of Participant	Signature of Participant	Date
_____	_____	_____
Person Obtaining Consent (PRINTED NAME)	Person Obtaining Consent (SIGNATURE)	Date

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<u>OPTIONAL</u> CONSENT TO BE CONTACTED FOR FUTURE STUDIES:

Do we have your permission to contact you regarding participation in future studies? Please write your initials next to your preferred choice.

_____ YES _____ NO

Participant's Initials _____