PennState

Consent Form

NCT03380000

Acute Blood Pressure Lowering Effects of Beetroot Juice in Post-Menopausal Women with and without Hypertension

July 16, 2018

PENNSTATE

Informed Consent Form for Biomedical Research
The Pennsylvania State University

Title of Project: Acute blood pressure-lowering effects of beetroot juice in postmenopausal women with and without hypertension

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1. Purpose of the study:

Women are more likely to develop high blood pressure (e.g., hypertension) after menopause. Postmenopausal women also experience greater increases in blood pressure in response to physical stressors such as exercise compared with either premenopausal women or men of a similar age. The purpose of this research study is to test the ability of an acute (one-time) dose of beetroot juice to lower resting blood pressure as well as blood pressure responses to exercise in postmenopausal (55-80 years) women with and without hypertension and to compare their results to those obtained from premenopausal women (18-35 years).

2. Procedures to be followed:

The study and its procedures are outlined below

Note: If you are a premenopausal woman, you will not participate in the beetroot juice portion of this study (Postmenopausal visits 3 & 4).

_____ **(your initials)**

If you are a premenopausal woman, you will be asked to complete the following:

Visit 1- Screening/Familiarization Visit.

You should also report to this visit in a fasted state without consuming food (except water) for 12 hours prior to arrival. After you check in, a blood sample from a vein in your arm will be taken by a Clinical Research Center (CRC) nurse to measure your lipid profile, blood chemistry, and the number of cells in your blood. Following the blood sample, you will be given a small snack (e.g., granola bar or other carbohydrate food, with water or juice) to help reduce feelings of hunger

_____ **(your initials)**

You should report to this visit without consuming caffeine for 12 hours prior to arrival. You should not consume any alcohol or dietary supplements for 48 hours prior, or participate in any exercise workouts (e.g., weight lifting or sustained aerobic exercise > 15 minutes) for 24 hours prior to arrival.

_____ **(your initials)**

During this visit, you will complete a health history and physical activity/fitness questionnaire. Your height, weight, as well as resting heart rate and resting blood pressure will be measured by a trained member of the Vascular Aging and Exercise Laboratory. You will then perform a submaximal (less than "all-out" effort) exercise test that will estimate your aerobic fitness. During this test, you will be asked to step on and off of a low (30 cm or approximately 1 foot high) box for 5 minutes. A metronome will help you to step on and off the box at a set pace. Throughout this test, researchers will measure your heart rate with monitor that straps around your chest. After you complete the exercise test, you will be familiarized with the study protocol and the measurements that will be performed during your subsequent study visits.

_____ **(your initials)**

Urine Collection: In order for your menstrual cycle hormones to be measured, you will be asked to collect a urine sample every morning for one complete menstrual cycle. During your screening visit, you will receive written and verbal instructions for collecting, storing, and recording urine samples. You will collect urine every morning using a small urine cup that is provided by the lab. You will store urine samples in a freezer, then transport the urine to the lab using supplies that the lab will provide. You will begin urine collection on the first day of your first period following your general screening visit and collect a sample daily until the first day of the following menstrual period. You will keep track of menses and urine collection times on a daily menstrual and urine calendar provided by the lab. Collection, processing, and recording of urine collection will take about 5 minutes per day.

_____ **(your initials)**

Visit 2 - Baseline Study Visit.

You should report to this visit in a fasted state, without consuming food or caffeine 12 hours prior to arrival. You should not consume any alcohol or dietary supplements for 48 hours prior, or participate in any exercise workouts (e.g., weight lifting or sustained aerobic exercise > 15 minutes) for 24 hours prior to arrival. Upon arrival, you will check in at the CRC in Noll Laboratory, and a CRC nurse will take a 16 mL (~1 tbsp.) blood sample from a vein in your arm. This sample will be used to determine the amount of nitrate and nitrite (by-products of nitric oxide, a naturally occurring substance in your body that causes blood vessels to widen) and estradiol (estrogen) in your blood. Following the blood sample, you will be given a small snack (e.g., granola bar or other carbohydrate food, with water or juice) to help reduce feelings of hunger.

_____ **(your initials)**

A urine pregnancy test will be provided to you by a CRC nurse before the blood sample is taken during this visit. If the pregnancy test reading is **negative**, you will provide the blood sample and continue with the remainder of the experiments performed in this study visit. However, if the pregnancy test is **positive** reading, you **will not** provide a blood sample or continue with the remainder of the study visit, and we will ask that you schedule an appointment with a physician (Ob/Gyn) to confirm the positive test.

_____ **(your initials)**

Next you will be escorted to the Vascular Aging and Exercise Laboratory (201 Noll Lab), where the following procedures will be performed:

Heart Rate. Heart rate will be measured by placing three sticky electrodes on your chest and reading the electrocardiogram (ECG) signal. A small-inflatable cuff will also be placed around a finger on your hand to measure changes in your pulse detected at your fingertip. After the electrodes and the finger cuff are applied to you, you will be asked to lie flat on a bed and rest for 20 minutes while your heart rate and finger pulse on recorded.

_____ **(your initials)**

Pulse Wave Velocity Pulse wave velocity is a non-invasive measurement that allows us to estimate the "stiffness" of your blood vessels. You will lay flat on a bed with a blood pressure cuff placed around your femoral artery (a blood vessel in your thigh), while a researcher holds a small (pen-sized) sensor over your carotid artery (a blood vessel in your neck). To make a measurement, the blood pressure cuff placed on your thigh will inflate to a pressure that temporarily prevents blood flow into your leg. The cuff will then deflate over 1-2 minutes, while sensors in the cuff and on your neck measure how fast each pulse of blood travels through your blood vessels. This measurement will be performed 3-4 times.

_____ **(your initials)**

Resting Blood Pressure. Your resting blood pressure will be measured by an automated blood pressure machine. An inflatable cuff will be placed on your upper arm, and you will be asked to sit quietly in a chair for at least 5 minutes. The cuff will then be inflated to a pressure that prevents blood from flowing into your forearm. As soon as flow into your forearm is stopped, the cuff will gradually deflate over 1-2 minutes. As the cuff deflates sensors inside of the cuff will detect the blood pressure in your upper arm, and the machine will use a validated equation to also calculate the blood pressure in the artery that leaves your heart (i.e, aorta). This measurement will be performed 3-4 times.

_____ **(your initials)**

Leg Suction. You will lie on a bed, and your leg will be placed inside of a closed box up to the middle of your thigh. A sleeve made from a stretchy material (i.e., neoprene) will help to seal your leg inside of the box in a way that provides a snug fit, but does not constrict your leg. Once your leg is sealed inside of the box, you will lie quietly for 5 minutes. Next, a vacuum will apply up to 4 different levels of suction to your leg inside the box for one minute at a time. The amounts of suction used will increase the pressure inside the blood vessels of your leg to similar pressures experienced when you stand up. There will be a 2-4 minute rest period between each level of suction. Each level of suction will be applied between 2-4 times. Some of these times we will measure the amount of blood flowing into your leg inside the box. Some of these times we may measure the amount blood flowing into your opposite leg (i.e., the one not inside the box). The amount of blood flowing into your leg(s) will be measured using a Doppler ultrasound machine that produces sound waves to measure the size of your blood vessel and the speed of your blood. Throughout this procedure, your blood pressure will be monitored with a small, inflatable finger cuff. The information collected from this procedure will allow us to estimate how much your vessels constrict (i.e., get smaller) when they are stretched.

_____ **(your initials)**

Static Handgrip Exercise. First, we will place a blood pressure cuff on your upper arm and forearm. The cuff on your upper arm will be inflated to above systolic blood pressure. You will rest with the cuff inflated for a period of 2 minutes, after which the cuff on your forearm will rhythmically inflate and deflate at a rate of 30 inflations/min for a period of 1 minute (upper arm cuff to remain inflated) or your wrist will be flexed and extended by a member of the Vascular Aging lab. After this 3 minute period, the upper arm cuff will be deflated and you will be given a 10-15 minute resting period. Upon completion of the rest period, you will squeeze a handgrip device as hard as you can (for 1-3 seconds) to determine your maximal grip strength. Your maximal grip strength will be determined as the highest value recorded over three such maximal efforts, each separated by a 1-minute rest period. A sticky plastic sensor will be placed over the muscles in your forearm. This sensor will emit light into your forearm muscles, and measure the amount of oxygen and hydrogen ions (H^+, a byproduct of lactic acid) in your muscle tissue. A blood pressure cuff will also be placed on your upper arm and forearm. After sitting quietly for 10 minutes, you will begin to squeeze the

handgrip device intermittently at a rate of 30 contractions per minute at a low force (10-20% of your maximal grip strength). You will perform this intermittent exercise twice. The first trial will be performed without the blood pressure cuff inflated and will continue until you report a rate of perceived exertion value of 19 or 20 (on a scale of 20). During the second course of this intermittent exercise, the blood pressure cuff on your arm will be gradually inflated (tightened by approximately 12 millimeters of cuff pressure each minute). The exercise period will stop when you report a rate of perceived exertion value of 19 or 20 (on a scale of 20) or when there is a >90% reduction in blood flow through your arm. During the last five seconds of exercise in both trials, the blood pressure cuff on your upper arm will be inflated to above systolic blood pressure for 3 minutes. During the last minute of this 3 minute period, the blood pressure cuff on your forearm will be rhythmically inflated and deflated for a 1 minute period or your wrist will be flexed and extended by a member of the Vascular Aging lab. Throughout this procedure, your blood pressure will be monitored with a small, inflatable finger cuff.

_____ **(your initials)**

Dynamic Leg Exercise. For this measurement, you will be seated in a supine (i.e., reclined) position. Your left leg will be placed inside of a boot that is attached to the pedal arm of a stationary bicycle, and a blood pressure cuff will be placed on your upper thigh. A sticky plastic sensor will also be placed over a muscle in your thigh to measure the amount of oxygen and hydrogen ions in your leg muscle. After a 10-minute rest period, the blood pressure cuff on your upper thigh will be inflated to a pressure that prevents blood from flowing into or out of your leg, and a researcher will move your lower leg for you in a kicking motion (i.e., passive exercise) for up to 3 minutes. The blood pressure cuff will then be deflated, and you will rest for another 10 minutes. You will then perform voluntary (i.e., active) single-leg knee extensions for 9 minutes. For the first 3 minutes you will perform knee extensions with no resistance. For the last 6 minutes, you will perform knee extensions against a moderate resistance. At the end of the 9-minute exercise bout, the cuff on your upper thigh will re-inflate to a pressure that prevents blood from flowing into or out of your leg for 3 minutes. A researcher will move your lower leg for you in a kicking motion during the last 1 minute of leg occlusion. Throughout this procedure, the amount of blood flowing into your leg will be measured with a Doppler ultrasound machine, and your blood pressure will be monitored with a small, inflatable finger cuff.

_____ **(your initials)**

Visit 3 – Estradiol assessment.

Prior to your second experimental visit (Premenopausal visit 4), you will be asked to return to the lab on one day between days 9-11 of your menstrual cycle for a brief blood draw. Approximately 10 ml of blood will be taken from an arm vein during this visit. This blood draw will be used to assess the amount of estrogen present in your blood on that day. The results of this test will be used to schedule your second experimental visit (visit 4).

_____**(your initials)**

Visit 4 – Experimental Study Visit.

You should report to this visit in a fasted state, without consuming food or caffeine for 12 hours prior to arrival. You should not consume any alcohol or dietary supplements for 48 hours prior, or participate in any exercise workouts (e.g., weight lifting or sustained aerobic exercise > 15 minutes) for 24 hours prior to arrival. During days 10-14 of your menstrual cycle, you will return to the CRC to repeat the same procedures as you did during visit 2. Procedures and timeline will be identical to those during visit 2.

_____**(your initials)**

If you are a postmenopausal woman, you will be asked to complete the following:

Visit 1- Screening/Familiarization Visit.

You will bring a copy of your most recent blood test results (lipids, glucose, red blood cell count, etc.) provided to you by your primary care physician. If your most recent blood test indicates a blood glucose level between 110 and 125 mg/dl we will perform a blood draw to determine the average glucose in your blood over a 3 month period (HbA1c).Your willingness to provide this information is voluntary, and all information will be kept confidential.

_____ **(your initials)**

You should report to this visit without consuming caffeine for 12 hours prior to arrival. You should not consume any alcohol or dietary supplements for 48 hours prior, or participate in any exercise workouts (e.g., weight lifting or sustained aerobic exercise > 15 minutes) for 24 hours prior to arrival.

_____ **(your initials)**

During this visit, you will complete a health history and physical activity/fitness questionnaire. Your height, weight, as well as resting heart rate and resting blood pressure will be measured by a trained member of the Vascular Aging and Exercise Laboratory. You will then perform a submaximal (less than "all-out" effort) exercise test that will estimate your aerobic fitness. During this test, you will be asked to step on and off of a low (30 cm or approximately 1 foot high) box for 5 minutes. A metronome will help you to step on and off the box at a set pace. Throughout this test, researchers will measure your heart rate with monitor that straps around your chest. After you complete the exercise test, you will be familiarized with the study protocol and the measurements that will be performed during your subsequent study visits.

_____ **(your initials)**

Urine Collection: In order for menstrual cycle hormone exposure to be measured, you will be asked to collect a urine sample every morning for one 30-day monitoring period. During your screening visit, you will receive written and verbal instructions for collecting, storing, and recording urine samples. You will collect urine every morning using a small urine cup that is provided by the lab. You will store urine samples in a freezer, then transport the urine to the lab using supplies that the lab will provide. You will begin urine collection on a random day, designated by the lab, and collect daily for 30 days. You will keep track of urine collection times on a daily menstrual and urine calendar provided by the lab. Collection, processing, and recording of urine collection will take about 5 minutes per day.

_____ **(your initials)**

Visit 2 - Baseline Study Visit.

You should report to this visit in a fasted state, without consuming food or caffeine 12 hours prior to arrival. You should not consume any alcohol or dietary supplements for 48 hours prior, or participate in any exercise workouts (e.g., weight lifting or sustained aerobic exercise > 15 minutes) for 24 hours prior to arrival. Upon arrival, you will check in at the CRC in Noll Laboratory, and a CRC nurse will take a 16 mL (~1 tbsp.) blood sample from a vein in your arm. This sample will be used to determine the amount of nitrate and nitrite (by-products of nitric oxide, a naturally occurring substance in your body that causes blood vessels to widen) and estradiol (estrogen) in your blood. Following the blood sample, you will be given a small snack (e.g., granola bar or other carbohydrate food, with water or juice) to help reduce feelings of hunger.

_____ **(your initials)**

Next you will be escorted to the Vascular Aging and Exercise Laboratory (201 Noll Lab), where the following procedures will be performed:

Heart Rate. Heart rate will be measured by placing three sticky electrodes on your chest and reading the electrocardiogram (ECG) signal. A small-inflatable cuff will also be placed around a finger on your hand to measure changes in your pulse detected at your fingertip. After the electrodes and the finger cuff are applied to you, you will be asked to lie flat on a bed and rest for 20 minutes while your heart rate and finger pulse on recorded.

_____ **(your initials)**

Pulse Wave Velocity Pulse wave velocity is a non-invasive measurement that allows us to estimate the "stiffness" of your blood vessels. You will lay flat on a bed with a blood pressure cuff placed around your femoral artery (a blood vessel in your thigh), while a researcher holds a small (pen-sized) sensor over your carotid artery (a blood vessel in your neck). To make a measurement, the blood pressure cuff placed on your thigh will inflate to a pressure that temporarily prevents blood flow into your leg. The cuff will then deflate over 1-2 minutes, while sensors in the cuff and on your neck measure how fast each pulse of blood travels through your blood vessels. This measurement will be performed 3-4 times.

_____ **(your initials)**

Resting Blood Pressure. Your resting blood pressure will be measured by an automated blood pressure machine. An inflatable cuff will be placed on your upper arm, and you will be asked to sit quietly in a chair for at least 5 minutes. The cuff will then be inflated to a pressure that prevents blood from flowing into your forearm. As soon as flow into your forearm is stopped, the cuff will gradually deflate over 1-2 minutes. As the cuff deflates sensors inside of the cuff will detect the blood pressure in your upper arm, and the machine will use a validated equation to also calculate the blood pressure in the artery that leaves your heart (i.e, aorta). This measurement will be performed 3-4 times.

_____ **(your initials)**

Leg Suction. You will lie on a bed, and your leg will be placed inside of a closed box up to the middle of your thigh. A sleeve made from a stretchy material (i.e., neoprene) will help to seal your leg inside of the box in a way that provides a snug fit, but does not constrict your leg. Once your leg is sealed inside of the box, you will lie quietly for 5 minutes. Next, a vacuum will apply up to 4 different levels of suction to your leg inside the box for one minute at a time. The amounts of suction used will increase the pressure inside the blood vessels of your leg to similar pressures experienced when you stand up. There will be a 2-4 minute rest period between each level of suction. Each level of suction will be applied between 2-4 times. Some of these times we will measure the amount of blood flowing into your leg inside the box. Some of these times we may measure the amount blood flowing into your opposite leg (i.e., the one not inside the box). The amount of blood flowing into your leg(s) will be measured using a Doppler ultrasound machine that produces sound waves to measure the size of your blood vessel and the speed of your blood. Throughout this procedure, your blood pressure will be monitored with a small, inflatable finger cuff. The information collected from this procedure will allow us to estimate how much your vessels constrict (i.e., get smaller) when they are stretched.

_____ **(your initials)**

Static Handgrip Exercise. First, we will place a blood pressure cuff on your upper arm and forearm. The cuff on your upper arm will be inflated to above systolic blood pressure. You will rest with the cuff inflated for a period of 2 minutes, after which the cuff on your forearm will rhythmically inflate and deflate at a rate of 30 inflations/min for a period of 1 minute (upper arm cuff to remain inflated) or your wrist will be flexed and extended by a member of the Vascular Aging lab. After this 3 minute period, the upper arm cuff will be deflated and you will be given a 10-15 minute resting period. Upon completion of the rest period, you will squeeze a handgrip device as hard as you can (for 1-3 seconds) to determine your maximal grip strength. Your maximal grip strength will be determined as the highest value recorded over three such maximal efforts, each separated by a 1-minute rest period. A sticky plastic sensor will be placed over the muscles in your forearm. This sensor will emit light into your forearm muscles, and measure the amount of oxygen and hydrogen ions (H^+, a byproduct of lactic acid) in your muscle tissue. A blood pressure cuff will also be placed on your upper arm and forearm. After sitting quietly for 10 minutes, you will begin to squeeze the handgrip device intermittently at a rate of 30 contractions per minute at a low force (10-20% of your maximal grip strength). You will perform this intermittent exercise twice. The first trial will be performed without the blood pressure cuff inflated and will continue until you report a rate of perceived exertion value of 19 or 20 (on a scale of 20). During the second course of this intermittent exercise, the blood pressure cuff on your arm will be gradually inflated (tightened by approximately 12 millimeters of cuff pressure each minute). The exercise period will stop when you report a rate of perceived exertion value of 19 or 20 (on a scale of 20) or when there is a >90% reduction in blood flow through your arm. During the last five

seconds of exercise in both trials, the blood pressure cuff on your upper arm will be inflated to above systolic blood pressure for 3 minutes. During the last minute of this 3 minute period, the blood pressure cuff on your forearm will be rhythmically inflated and deflated for a 1 minute period or your wrist will be flexed and extended by a member of the Vascular Aging lab. Throughout this procedure, your blood pressure will be monitored with a small, inflatable finger cuff.

_____ **(your initials)**

Dynamic Leg Exercise. For this measurement, you will be seated in a supine (i.e., reclined) position. Your left leg will be placed inside of a boot that is attached to the pedal arm of a stationary bicycle, and a blood pressure cuff will be placed on your upper thigh. A sticky plastic sensor will also be placed over a muscle in your thigh to measure the amount of oxygen and hydrogen ions in your leg muscle. After a 10-minute rest period, the blood pressure cuff on your upper thigh will be inflated to a pressure that prevents blood from flowing into or out of your leg, and a researcher will move your lower leg for you in a kicking motion (i.e., passive exercise) for up to 3 minutes. The blood pressure cuff will then be deflated, and you will rest for another 10 minutes. You will then perform voluntary (i.e., active) single-leg knee extensions for 9 minutes. For the first 3 minutes you will perform knee extensions with no resistance. For the last 6 minutes, you will perform knee extensions against a moderate resistance. At the end of the 9-minute exercise bout, the cuff on your upper thigh will re-inflate to a pressure that prevents blood from flowing into or out of your leg for 3 minutes. A researcher will move your lower leg for you in a kicking motion during the last 1 minute of leg occlusion. Throughout this procedure, the amount of blood flowing into your leg will be measured with a Doppler ultrasound machine, and your blood pressure will be monitored with a small, inflatable finger cuff.

_____ **(your initials)**

Visit 3 - Experimental Study Visit.

You should report to this visit in a fasted state, without consuming food or caffeine for 12 hours prior to arrival. You should not consume any alcohol or dietary supplements for 48 hours prior, or participate in any exercise workouts (e.g., weight lifting or sustained aerobic exercise > 15 minutes) for 24 hours prior to arrival. Upon arrival, you will check in at the CRC in Noll Laboratory, and a CRC nurse will take a 16 mL (~1 tbsp.) blood sample from the vein in your arm. This sample will be used to determine the amount of nitrate and nitrite (by-products of nitric oxide, a naturally occurring substance in your body that causes blood vessels to widen) and estradiol (estrogen) in your blood. You will then be escorted to the Vascular Aging and Exercise Laboratory (201 Noll Lab), where the following procedures will be performed:

1. Heart Rate
2. Pulse Wave Velocity
3. Resting Blood Pressure

Next, you will be asked to consume 140 mL (9.5 tablespoons) of either nitrate-rich beet juice (known as the "active" drink) or beet juice with nitrates removed (known as the "placebo" drink). You will not be able to tell which drink you are consuming. You will also be provided a small snack (e.g., granola bar or other carbohydrate food, with water or juice) to help reduce feelings of hunger. You will then be asked to wait in the CRC or Noll lab for approximately 1.5 hours to allow time for the active (or placebo) drink to be fully digested and absorbed. During these 1.5 hours, you will be able to use a personal laptop computer or other portable electronic device. A second (6 mL) blood sample will then be collected after 1.5 hours, to determine if there is an increase in nitrate or its by-products in your blood after consuming the beet juice. You will then return to the Vascular Aging and Exercise Laboratory where the following procedures will be performed.

1. Heart Rate
2. Pulse Wave Velocity
3. Resting Blood Pressure
4. Leg Suction
5. Static Handgrip Exercise
6. Dynamic Leg Exercise

When you complete these procedures, a third (6 mL) blood sample will be taken to determine how well the nitrates and nitrites remain elevated in your blood.

_____ **(your initials)**

Visit 4 - Experimental Study Visit.

You should report to this visit in a fasted state, without consuming food or caffeine for 12 hours prior to arrival. You should not consume any alcohol or dietary supplements for 48 hours prior, or participate in any exercise workouts (e.g., weight lifting or sustained aerobic exercise > 15 minutes) for 24 hours prior to arrival. At least 5 days after visit 3, you will return to the CRC to repeat the same procedures as you did during visit 3. Procedures and timeline will be identical to those during visit 3 except that you will consume whichever drink supplement you did not receive during visit 3 i.e., the active (nitrate-containing) drink or the placebo (nitrate-removed) drink. The order of these two drink supplements will be randomly determined for each participant.

_____ **(your initials)**

3. Discomforts and risks:

Blood Sampling. You may experience some mild discomfort and bruising where the needle is inserted into your arm. This discomfort often goes away once the needle is securely placed in your vein. There is also a very small risk of developing an infection or a clot in your arm. However, these risks are minimized by having a trained nurse perform all blood samples using sterile equipment and techniques.

_____ **(your initials)**

Step-based Fitness Test. This step-based test is designed to provide an exercise intensity of sub-maximal (i.e., less than an "all-out") effort. However, you may still experience some discomfort with the exercise test such as muscle fatigue, shortness of breath, or get a muscle cramp. You may also experience lightheadedness, chest discomfort, or irregular heartbeats during this test. There is also a small risk of stumbling and/or falling during the test. To minimize this risk, researchers will monitor you closely throughout the test, and will stand nearby to provide support if you lose your balance.

_____ **(your initials)**

Heart Rate. It is possible that the adhesive on the sticky electrodes may irritate your skin. There is also a minimal risk that an allergic reaction could occur from the adhesive.

_____ **(your initials)**

Blood Pressure/Pulse Wave Velocity. There is a risk of temporary discomfort at the sites where blood pressure cuffs are inflated (upper arm/upper thigh). The discomfort might be greater the longer the cuffs are inflated. In addition, you may feel a numb and/or tingling sensation in your hands/feet while the cuff is inflated; however, these feelings go away quickly after the cuff is deflated. During the pulse wave velocity test, you may also experience some temporary discomfort while the researcher holds a small sensor over an artery in your neck. However, this feeling will also go away quickly after the sensor is removed.

_____ **(your initials)**

Leg Suction. You may feel some mild discomfort associated with the leg suction procedure. A vacuum applied to the sealed box will cause blood vessels in your leg to fill with more blood than usual. Thus, you may feel temporary sensations of swelling in your leg. There is also a small risk that you may experience temporary feelings of lightheadedness. The vacuum will tend to pull your leg into the box, and as a result you may feel a moderate pressure pushing against your upper thigh. Any of these feelings will go away immediately after the vacuum is turned off. Moreover, the potential for these risks or discomforts to occur will be minimized by limiting bouts of suction to a short duration (1 min each).

_____ **(your initials)**

Doppler Ultrasound. There is a minimal risk that the ultrasound probe and/or gel will irritate your skin. You may feel minor discomfort (pressure) when the researcher is pressing the ultrasound sensor against your skin (upper arm and upper thigh/groin) to allow the researcher locate a good image of the underlying artery.

_____ **(your initials)**

Near-Infrared Spectroscopy. There are no known risks associated with use of the near-infrared device. However, it is possible that the adhesive on the sticky plastic sensor may irritate your skin.

_____ **(your initials)**

Static Arm Exercise. You may experience temporary fatigue in the muscles of the exercising arm. It is also possible to experience soreness in these muscles within 24-48 hours following this study visit. There is a small risk that you may develop a bruise from the inflation of the cuff that prevents blood from flowing into or out of your forearm. You may also experience some discomfort and/or a numb, tingling sensation in your arm while the cuff is inflated. These sensations go away quickly after the cuff is deflated. There is also a small risk of the appearance of petechia (small pink blotches on the skin) as a result of the increased venous blood pressure in the occluded forearm during exercise. If present, these small pink dots may persist for several days but are not associated with any pain or long term adverse effects.

_____ **(your initials)**

Dynamic Leg Exercise. You may experience temporary muscle fatigue in the thigh muscles of your exercising leg. It is also possible to experience soreness in these muscles within 24-48 hours following this study visit. There is a small risk that you may develop a bruise from the inflation of the cuff that prevents blood from flowing into or out of your leg. You may also experience some discomfort and/or a numb, tingling sensation in your leg while the cuff is inflated. These sensations go away quickly after the cuff is deflated.

_____ **(your initials)**

Consumption of beet juice: There are no known health risks associated with consumption of nitrate-rich beet juice, a commonly sold health drink/supplement in Europe. The most common side-effect of beet juice consumption is pinkish-colored urine (known as "beeturia") and/or stool. This can occur after consuming either the nitrate-rich or placebo version of the drink.

_____ **(your initials)**

Urine Collection: There are no known risks associated with the self-collection of one's urine, Volunteers will be provided screw top and airtight containers to store their urine, and coolers to use for storing and carrying urine samples to the laboratory for analysis.

_____ **(your initials)**

4. Benefits to Participants/Society:

Possible Benefits to participants:
Postmenopausal participants will receive more regular monitoring of their resting blood pressure. Throughout the duration of the study protocol, you will have your untreated blood pressure measured by trained research personnel on 3 separate occasions, and these measurements will be provided at no cost you and/or your insurance provider. You will also be provided information about their aortic blood pressure, a measurement which may have greater predictive value for cardiovascular risk than arm blood pressure measurements alone. With your permission, all blood pressure-related information will be shared with your primary care provider.

For younger women, there are no direct benefits to you for participating in this study other than knowing your cardiovascular risk factors (i.e., blood pressure, blood cholesterol, fitness level, etc.).

Possible benefits to society:
This research may further our understanding about the potential for nitrate-rich food/dietary supplements to help control blood pressure without (or with less) medication(s) in a population with increased blood pressure-related cardiovascular risk.

5. Duration/time of the procedures and study:

The initial screening/familiarization visit (Visit 1) will take approximately 1.5 hours. The baseline study visit (Visit 2) will take approximately 4 hours. The premenopausal estradiol assessment visit (Premenopausal visit 3) will take approximately 30 minutes. The second premenopausal experimental visit (Premenopausal visit 4) will take approximately 4 hours. The following two postmenopausal experimental visits (Postmenopausal Visits 3 & 4) will each take approximately 6 hours.

If you are a **pre**menopausal woman the total duration of all the research sessions will be approximately 10 hours.

If you are a **post**menopausal woman the total duration of all the research sessions will be approximately 17.5 hours. All study visits (Visits 2-4) will be separated by no less than 5 days each. Therefore, it is expected that all postmenopausal participants will be able to complete all 3 study visits over 2-6 weeks following their screening visit.

6. Alternative procedures that could be utilized:

There are alternative procedures available that could be used to measure your aerobic fitness level (e.g., maximal graded exercise test), aortic blood pressure (e.g., central artery catheter), limb blood flow (e.g., limb vein catheter), and muscle metabolite concentration (e.g., microdialysis; placing thin fibers through your skin and into your muscle tissue). However, most of these methods are either invasive (catheters, microdialysis) and/or could present additional risk and discomforts to participants. The procedures we have selected to use in this research study present less risk and potential discomfort to you as a participant, while still providing us with reliable research data and information.

Blood and Urine Sampling: We will utilize a combination of measurements made from blood and urine to study hormones. The collection of daily urine samples eliminates the need for daily blood samples for monitoring reproductive hormones over the course of the menstrual cycle. The fasting blood samples are necessary to track changes in some hormones that cannot be measured in the urine.

7. Statement of confidentiality:

Your participation in this research is confidential. All records associated with your participation in the study will be subject to the usual confidentiality standards applicable to medical records (e.g., such as records maintained by physicians, hospitals, etc.). Moreover, data will be stored and secured in the Vascular Aging and Exercise Laboratory (201 Noll Laboratory) in password protected computer files. Any hard copies of data will be stored in locked filing cabinets. In the event of any publication resulting from the research, no personally identifiable information will be disclosed. Penn State's Office for Research Protections, the Institutional Review Board, and the Office for Human Research Protections as well as the Food and Drug Administration in the U.S. Department of Health and Human Services may review records related to this project. A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

8. Right to ask questions:

Please contact David Proctor at 814-863-0724 (office) or 814-571-5234 (cell) with questions, complaints or concerns about the research. You can also call these numbers if you feel this study has harmed you. If you have any questions,

concerns, problems about your rights as a research participant or would like to offer input, please contact The Pennsylvania State University's Office for Research Protections (ORP) at (814) 865-1775. The ORP cannot answer questions about research procedures. Questions about research procedures can be answered by the research team.

9. Payment for participation:

You will not be paid for the initial screening/familiarization visit (Visit 1).

If you are a **pre**menopausal woman, you will be paid a total of $40 for completion of all 4 visits. You will be paid for your study visits as outlined below:
Visit 2: $20
Visit 3 and 4: $20

If you are a **post**menopausal woman, you will be paid a total of $80 for completing all of your visits (Visits 1-4). You will be paid for your study visits as outlined below:
Visit 2: $20
Visit 3: $30
Visit 4: $30

Total payments within one calendar year that exceed $600 will require the University to annually report these payments to the IRS. This may require you to claim the compensation that you receive for participation in this study as taxable income.

10. Voluntary participation:

Your decision to be in this research is voluntary. You can stop at any time. You do not have to answer any questions you do not want to answer. Refusal to take part in or withdrawing from this study will involve no penalty or loss of benefits you would receive otherwise.

Additionally, if you do not comply with the study protocol (e.g., you skip/miss an excessive number of study visits or fail to follow pre-visit instructions) we may not seek your continued participation in this study.

If you stop being in the research, already collected data may not be removed from the study database

11. Injury Clause:

In the unlikely event you become injured as a result of your participation in this study, medical care is available. It is the policy of this institution to provide neither financial compensation nor free medical treatment for research-related injury. By signing this document, you are not waiving any rights that you have against The Pennsylvania State University for injury resulting from negligence of the University or its investigators.

12. Abnormal Test Results:

In the event that abnormal lab test results are obtained during initial screening or subsequently throughout this study, you will be informed as quickly as possible of these results and instructed to contact your private physician for further assessment. The lab test results will be made available to your private physician at your request.

You must be 18 years of age or older to take part in this research study. If you agree to take part in this research study and the information outlined above, please sign your name and indicate the date below.

You will be given a copy of this signed and dated consent form for your records.

_____ _____

Participant Signature Date

Person Obtaining Consent _____ Date _____

OPTIONAL BLOOD AND URINE STORAGE: In addition to the main part of the research study, there is an optional part of the research. You can participate in the main part of the research without agreeing to take part in this optional part.

Storage of Leftover Blood and Urine Samples for Future Research Studies
As part of this study, we are obtaining blood and urine from you. If you agree, the research team would like to store leftover samples of your blood and urine that is collected so that your blood and urine can be studied in the future after this study is over. These future studies may provide additional information that will be helpful, but it is unlikely that these studies will have a direct benefit to you. Neither your doctor nor you will receive results of these future research tests, nor will the results be put in your health record. If you have any questions, you should contact David Proctor at dnp3@psu.edu; 863-0724.

Your leftover samples will be labeled with a code number and stored in a locked laboratory. If you consent to have samples of your blood and urine saved for future research, the period for the use of the samples is unknown but you will be free to change your mind at any time. You should contact David Proctor at dnp3@psu.edu; 863-0724 and let him know you wish to withdraw your permission for your blood to be used for future research. If you do this, any unused blood and urine will be destroyed and not used for future research studies.

Please initial below to indicate your preferences regarding the optional storage of your leftover blood and urine for future research studies.

a. Your samples may be stored and used for future research studies performed in our laboratory to learn about the effects of beetroot juice on blood or urine markers of vascular health.

_____ Yes _____ No

b. Your samples may be stored and used for research about other health problems.
_____ Yes _____ No

c. Your samples may be shared with other investigator/groups as long as any identifying information (name, birthdate, etc.) is removed.

_____ Yes _____ No

Participant: By signing below, you indicate that you are voluntarily choosing to take part in this optional part of the research.

Signature of Participant	Date	Printed Name

Person Explaining the Research: Your signature below means that you have explained the optional part of the research to the participant/participant representative and have answered any questions he/she has about the research.

Signature of Research Personnel	Date	Printed Name