



University of Pittsburgh

School of Medicine
Vascular Medicine Institute

CONSENT TO ACT AS A PARTICIPANT IN A RESEARCH STUDY

TITLE: An Open-Label Study of Oral Nitrite in Adults with Metabolic Syndrome and Hypertension

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SOURCE OF SUPPORT: Clinical and Translational Science Institute, Vascular Medicine Institute of the University of Pittsburgh	



We invite you to take part in a research study. We want you to know that taking part in this research study is entirely voluntary. Before agreeing to participate in this research study, or at any time during your study participation, you may discuss your care with another doctor who is not associated with this research study. You are not under any obligation to participate in any research study offered by the doctor. Please take your time to make your decision about taking part.

A description of this clinical trial will be available on www.clinicaltrials.gov, as required by US Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

One of the investigators conducting this research has a financial interest in or a patent for the development of Sodium Nitrite for cardiovascular indications. This means that it is possible that the results of this study could lead to personal profit for the individual investigator and/or the University of Pittsburgh. The investigator has agreed to not be involved in the recruitment of volunteer subjects, will not administer the informed consent, engage in the recording of research data, be involved in clinical assessments of study eligibility criteria and intervention outcomes, will not participate in data and safety monitoring activities and will not solely be involved in the interpretation of study results. This project has been carefully reviewed to ensure that your well-being holds more importance than any study results. Any questions you might have about this will be answered fully by the Principal Investigator, Dr. Kara Hughan (telephone number: 412-692-5173), who has no financial conflict of interest with this research, or by the Human Subject Protection Advocate of the University of Pittsburgh (866-212-2668).

Why is this research being done?

Metabolic syndrome is a name for a group of risk factors that occur together and increase the risk for type 2 diabetes and cardiovascular disease (CVD) including heart attacks and strokes. Insulin resistance (IR) is a key link in the metabolic syndrome, and IR within muscle is a significant risk factor for the development of type 2 diabetes and CVD. Mitochondria are specialized units within cells that generate energy for the body. The potential link between impaired mitochondria function and muscle IR has been extensively studied and remains controversial. While weight loss and exercise are effective treatments of IR, long term adherence to these treatments are typically poor. While there are effective medication treatments, they have significant side effects.

This research involves the use of an investigational drug called inorganic nitrite (sodium nitrite). It has been shown that various types of nitrite (given intravenously, as a nitrite solution taken by mouth, and dietary sources such as beetroot juice that can be processed in the body to form nitrite) are able to lower blood pressure. More recently, it has also been shown that inorganic nitrite may be involved in the regulation of blood sugar and insulin balance in mice. Inorganic nitrite is currently approved by the Food and Drug Administration (FDA) and given by injection for the treatment of cyanide poisoning. In this research study, nitrite will be given by mouth, in capsule form, which was prepared at the National Institutes of Health Investigational Drug Pharmacy Service. The capsule form is not FDA approved for use in patients with metabolic syndrome and high blood pressure. However, the FDA does permit it to be used in experimental research studies like this, under strict regulations.

The research study is being conducted to examine what effect daily inorganic nitrite study treatment has on high blood pressure and hormonal disturbances in overweight/obese adults with the metabolic syndrome and high blood pressure. Ultimately, oral nitrite therapy may translate to



having an impact on the prevention and treatment of both diabetes and cardiovascular disease.

Who is being asked to take part in this research study?

We are enrolling 30 overweight/obese adults between 18-60 years of age who meet all of the eligibility criteria.

How long will I be in this research study?

Your participation will last for about 4-5 months.

What procedures will be performed for research purposes?

If you decide to take part in this research study, you will receive one study medication, sodium nitrite three times daily, for 12 weeks as part of the research study. You will need to visit the study clinic on schedule, get tests, and tell the study doctor or study staff about any changes in your health or the way you feel. All research study tests or procedures listed below are not part of your routine care. You will make at least 12-18 visits to the study clinic over 4-5 months and have 1 follow-up telephone call from the study staff approximately four weeks after finishing your study medicines. You may also receive reminder calls or emails.

The study visits will take place at the Clinical Translational Research Center (CTRC) and Vascular CTRC, both of which are located on the 6th floor of Montefiore Hospital of UPMC and/or the Endocrinology and Metabolism Research Center (EMRC), which is located on the 8th floor of Montefiore Hospital of UPMC.

SCREENING PROCEDURES:

Procedures to determine if you are eligible to take part in a research study are called “screening” procedures.

Visit 1 (Screening)

This visit will take place either in the CTRC or the EMRC, and may take about 1-1 ½ hours. For this research study, you will be asked to fast for 8 hours before coming in for the screening blood work, if it is safe to do so based upon any medicines you take. The screening procedures include:

1. A review of your medical history, a brief physical exam, height, weight, waist and hip measurements, and temperature, blood pressure, breathing rate and heart rate will be performed. If you are a female, we will also ask your menstrual history.
2. Blood will be drawn from a vein in one of your arms or hands to conduct laboratory tests to help evaluate your overall health (e.g. standard blood counts, fasting blood fats, fasting blood sugar, and thyroid screen). This will involve removal of approximately 1 tablespoon of blood from you.

EXPERIMENTAL PROCEDURES:

If you qualify to take part in this research study, you will undergo a number of tests and procedures that evaluate how the study drug affects your body and your health.

Visit 2 (First Baseline Assessment Visit):

You will be scheduled for this outpatient fasting visit within 4 weeks of screening. You will be asked



not to drink or eat anything other than plain water for 8 hours prior to your arrival for this visit. Testing will take approximately 3-4 hours in total at the CTRC and EMRC and includes:

1. Your body weight will be measured.
2. Blood vessel stiffness measurement by Pulse Wave Velocity: To test the stiffness of your arteries, blood pressure cuffs are attached to your arms and ankles and are inflated several times for various readings. Two flat probes, which resemble chalk erasers, are moved up and down your neck and upper thigh a few times to obtain special waveforms that will tell the researchers about the stiffness of your arteries. Cool gel might be applied to the neck and upper thigh before some of these readings. Multiple readings are obtained so the results can be averaged. This test takes about 30 minutes.
3. Ultrasound measurement of carotid arterial disease: This is a noninvasive test that uses sound waves to evaluate the carotid arteries. This test will require applying cool, jelly-like, hypoallergenic substance on your neck while a probe is moved up and down your neck (just like a pregnancy ultrasound). Sound waves are bounced off the arteries, creating a picture of what the arteries look like on the inside. This test takes approximately 15 minutes. Blood pressures will be taken during the exam.
4. Endothelial function assessment with flow-mediated dilation (FMD): This is a non-invasive test that evaluates the ability of your blood vessels to relax and expand to allow greater blood flow. For this test you will lie flat for 30 minutes. The test will require placing a blood pressure cuff below your elbow. The blood pressure cuff will be inflated, like when your blood pressure is taken, and will stay inflated for 5 minutes. Blood flow will be detected by an ultrasound probe during inflation and in the 3 minutes after deflation.
The Pulse Wave Velocity, Carotid Ultrasound, FMD and automated blood pressures will be performed in the Vascular Clinical and Translational Research Center (VCTRC) of Montefiore Hospital of UPMC. After your Vascular CTRC testing is complete, you will be given a light breakfast snack.
5. Urine pregnancy test: For females who are able to have children, you will be asked to provide a urine sample for pregnancy testing. The result of the pregnancy test must be negative in order for you to be in this research study.
6. DEXA: Bone density/Body composition - We will measure how many pounds of bone, muscle, and fat tissue that you have in your body, spine and thigh by using a technique called dual-electron x-ray absorptiometry (DEXA). For this test, you will lie flat on a table for around 10 minutes and a scan will be taken. DEXA is a routinely performed procedure for patient care and is very important for overall health. An excessive amount of body fat is a risk factor for heart disease, type II diabetes, and other diseases.
7. Exercise testing: You will perform an exercise test on a stationary bicycle in the Exercise Physiology Laboratory (EMRC) on the 8th floor of Montefiore Hospital. The test will begin with a warm-up of light pedaling. Then you will perform two, 4-minute standardized workloads of 25 and 50 Watts (or very light effort) to provide important information about whole body metabolic efficiency. Watts are the amount of power you produce when pedaling. You will then continue with 2-minute stages of incrementally greater workloads



of 25-50 Watts until a maximal voluntary effort is attained.

8. Blood pressure monitor: You will have a blood pressure cuff placed and worn around the upper part of your non-dominant arm for the next 24 hours. It is attached to a monitor that can fit in your pocket or be worn on your waist/belt. The monitor pumps and then records your blood pressure every 20 minutes during the day and every half hour overnight.
You should only take off the cuff and monitor when showering/bathing or swimming, but then immediately put it back on when finished. After the 24 hours, you will remove the cuff and turn off the monitor. We ask that you return the cuff and monitor to us at Visit 3. We may not be able to give this to you until Visit 3 depending on availability. If this happens, we ask that you return the cuff and monitor at Visit 4.
9. We will assess at baseline for adverse event symptoms (AE) prior to receiving your first dose of study drug at Visit 4.

The above studies will be repeated at Visit 16 (Week 12).

Visit 3 (Second Baseline Assessment Visit)

You will be scheduled to complete this fasting visit at the CTRC 2-7 days after Visit 2. You will be asked not to drink or eat anything other than plain water for 8 hours prior to your arrival for this visit.

1. Interval history and physical exam will be performed by the study doctor. Your temperature, blood pressure, breathing rate, heart rate and body weight will be measured. If you are a female, we will also ask your menstrual history.
2. Urine pregnancy test: For females who are able to have children, you will be asked to provide a urine sample for pregnancy testing. The result of the pregnancy test must be negative in order for you to continue in this research study.
3. Blood samples for blood fats, liver and kidney function, blood count, hemoglobin A1c, hormones and heart disease markers and an extra blood sample will be obtained and stored for an indefinite length of time in the research laboratory of the EMRC for future analyses of hormones if further tests are indicated with the advance of science. The results of any future analyses done on the stored samples will not be shared with you because it will not be clinically relevant. The maximum amount of blood drawn for the above testing is approximately 3 tablespoons.
4. Food record form to take home to complete over 3 different days. We will assess nitrite intake. We ask that you return the forms to us at Visit 4.
5. Hyperinsulinemic euglycemic clamp: An IV (a plastic needle inserted in your vein) will be placed in the bend of your arm or hand to provide a way to infuse insulin, sugar water and a solution called *deuterated glucose*. Deuterated glucose is a tracer solution (a solution of glucose that has heavy atoms) that does not give off radiation. It allows the researchers to better test how your body uses sugar by letting them measure how much sugar your liver is making versus what is given through the vein during the study. Deuterated glucose is found in nature and is called a stable isotope. It is safe, non-radioactive, and has no known harmful effects. This isotope is not required to have Food



and Drug Administration (FDA) approval. Another IV will be placed in the opposite arm, in your hand, to sample blood. This hand IV will be heated with a heating pad to make the blood sampling easier. An insulin infusion, lasting 4 hours, will be given to see how well your body uses sugar. Insulin is the hormone made by your body to control your blood glucose levels. During the insulin infusion, your blood sugar level will be watched closely (every 5 minutes) and kept at a normal level (85–95 mg/dl) with a sugar water infusion (20% dextrose). You will be asked to stay awake during the insulin infusion so that we can be certain that you are not having problems from the infusion. The amount of blood drawn during the insulin infusion part of the study is approximately 22 tablespoons or about 1½ cups. This amount of blood is about 1/2 of what you would give if you made a blood donation. Your blood is checked during the screening visit to make sure that you have enough blood to safely donate this amount.

6. Indirect calorimetry: During two 45-minute periods, one before the start of the insulin infusion and one at the end of the insulin infusion, a clear plastic canopy will be placed over your face and neck to collect the air you breathe out. This measurement is called *indirect calorimetry* (resting energy expenditure) and is done to measure the amount of oxygen your body uses and to calculate how much fat and sugar it is burning.
7. Muscle biopsy: Sometime after the start of the deuterated glucose infusion, you will have a muscle biopsy of your thigh muscle, performed by a physician who has experience doing biopsies. The purpose of the biopsy is to obtain a small piece of your muscle tissue so we can look at the amount of fat, mitochondria (specialized units within cells that generate energy for the body) and protein in your muscle. Lidocaine, an FDA approved medicine that numbs the area (similar to medicine used by dentists) prior to the actual biopsy procedure, is used so that any pain can be minimized. On the skin on one of your thighs, an area the size of a quarter will be numbed by injecting 5-11 mL of lidocaine. After making sure the area is numb, a small incision (about a quarter of an inch long) will be made using a sterile scalpel and a biopsy needle will be passed into the muscle in order to obtain a small piece of muscle (the size of a pencil tip, equal to 100-150 milligrams). If the first pass of the biopsy needle doesn't get an adequate muscle sample (or if a fat sample is obtained), you will be asked if it is okay to make a second pass through the same small incision. You can choose to say no to a second pass if you do not want it. This will not affect your payment or your being in the study.

To decrease the risk of infection and bleeding, we will cover it with steri-strips (or a stitch if you have an allergy to steri-strips), apply a triple antibiotic ointment on the incision and a bandage with elastic wrap. An ice pack will be applied for 20 minutes after the biopsy. You will be given instructions about how to care for the incision site and provided with the telephone numbers of the investigators to call if you have any problems.

The muscle sample will be used to examine the health of the muscle cells in regards to their structure and metabolism (chemical reactions). This will be done with various research techniques including microscopy and laboratory tests. After your muscle sample has been analyzed, and upon your request, the Principal Investigator will discuss your personal results of your muscle biopsy analysis. Please consult your primary care physician regarding the decision whether or not to receive this information. Approximately 100-150mg of your muscle sample will be stored.

8. You will return your blood pressure monitor and cuff today so that we may download the



recorded blood pressure information.

9. After the clamp testing has been completed, you will be given lunch.
10. A meter blood sugar, blood pressure, breathing rate, and heart rate will be performed 30 minutes after completion of the clamp test to make sure they are returning to baseline before discharge home.

The above studies will be repeated at Visit 18 (Week 12) and will occur 2-7 days after Visit 17.

Visit 4 (Day 1: First Drug Doses Visit)

This fasting visit will take place at the CTRC or EMRC within 1-4 weeks following completion of all above baseline assessments. You will be asked not to drink or eat anything other than plain water for 8 hours prior to your arrival for this visit. This visit will take approximately 3 hours.

1. Body weight and temperature will be measured.
2. If you are a female, we will also ask your menstrual history. A repeat urine pregnancy test will be performed for females who are able to have children.
3. You will be given your first dose of study drug- sodium nitrite 40 mg by mouth. Blood pressure, breathing rate and heart rate will be measured after approximately 30 minutes of rest and quiet in the CTRC to ensure blood pressures are at a steady state before taking the medicines and then every 15 minutes for 2 hours after taking the study medication.
4. Safety monitoring for methemoglobin level (methemoglobin is a stable oxidized form of hemoglobin that is unable to release oxygen to the tissues) by a finger probe before, 0.5, 1 and 2 hours after study medication administration. Research blood samples and blood samples for platelet mitochondrial function will be drawn before, 0.5 and 2 hours after study medication administration. The total volume of blood drawn is approximately 3 Tablespoons.
5. You will be provided a meal after completion of testing.
6. You will be provided a dosing diary card to record how much of the study medication you are taking (sodium nitrite) and to help you better understand how it made you feel. The information that needs to be recorded in the diary includes the time and doses of the study medication, new health symptoms, new medications taken, and any emergency department (ER) visits or hospitalizations. You will fill in a new diary card every 7 days. You should have the diary card(s) available with you when you are interviewed at your weekly safety visits and then also the every other week safety study visits.
7. Assess adverse events (AE) and you will be provided a supply of the daily study drug (sodium nitrite 40mg three times daily) to get you through to your next visit (typically an 11-17 day supply).

There is a rare possibility that you will have a high methemoglobin level or you will have a low blood pressure from your study drug dose during this visit or future study visits. If this happens, your study nitrite drug dose will be lowered so that you receive half the dose of the nitrite (20 mg sodium nitrite three times daily). You will be asked to undergo a repeat of safety monitoring for



blood pressure, breathing rate and heart rate before taking the medicines and then every 15 minutes for 2 hours after taking the study medications, methemoglobin level by a finger probe, research blood and storage samples and blood samples for platelet mitochondrial function that will be drawn before, 0.5 and 2 hours after the lowered doses of study medication are administered (as above in number 4). The total volume of blood drawn is approximately 3 Tablespoons. If you still have a high methemoglobin or low blood pressure on these lower doses, your participation in the study will be discontinued.

8. The forms with the 3 days of food records that you completed will be collected.

Visits 6, 8, 10, 12, 14 (Weeks 2, 4, 6, 8, and 10)

You will return every other week beginning at week 2 \pm 7 days for safety visits that will take place in the Endocrinology and Metabolism Research Center (EMRC) in Montefiore Hospital of UPMC. Each visit will take approximately 1.5 hours.

1. You will be seated in the EMRC and you will be given your daily dose of study drug- sodium nitrite 40 mg (either the first/morning dose or the second/midday dose [depending on the time of the visit] of the three doses taken each day) by mouth at this visit.

2. Interval history and brief physical exam will be performed.

3. Assess adverse events (AE) and review drug diary card(s). We ask that you bring any unused study drug with you to each safety visit so we can assess study medication compliance.

4. You will be provided a supply of the daily study drug (sodium nitrite 40 mg three times daily) to get you through to your next visit (typically an 11-17 day supply).

5. Approximately one half hour after your study medication is taken and a minimum of 15 minutes after sitting calmly and relaxed, we will have you press the button on the blood pressure machine for a blood pressure measurement. You will have a timer and we ask that you wait 5 minutes after the first measurement to take a blood pressure measurement taken again. You will wait another 5 minutes and take a final blood pressure measurement (for a total of 3 measurements).

6. A breathing rate and heart rate measurement, a methemoglobin level by a finger probe and a safety blood draw for research blood and storage samples will be performed by study staff. The total volume of blood drawn is approximately 1 1/3 Tablespoons.

At the Visit 10 (Week 6) visit, you will have a full physical exam and have your body weight measured. Additional menstrual history and a urine pregnancy test will be performed for females who are able to have children.

At the Visit 14 (Week 10) visit, you will have an extra 1 teaspoon of blood drawn to check your blood count for anemia. If your blood count is low, you may be recommended to take an iron supplement until your blood count is rechecked at Visit 16 (Week 12).

Visits 5, 7, 9, 11, 13, 15 (Weeks 1, 3, 5, 7, 9, 11)

You will be contacted by phone every other week \pm 7 days which will last about 10 minutes by the study staff. You will be asked about any side effects, study medication compliance, any changes in medication, and any medical history such as emergency room visits or hospitalizations since your last study visit.



In addition to these formal evaluations, you are encouraged to contact the study doctor or the study staff anytime with questions, concerns, or to report new symptoms that occur during your study participation.

Visit 16 (Week 12: First Completion Assessment Visit):

All studies from Visit 2 (First Baseline Assessment Visit) in the Montefiore Vascular CTRC and EMRC will be repeated at this visit (First Completion Assessment Visit). This fasting visit will take approximately 3 hours in total. You will be asked not to drink or eat anything other than plain water for 8 hours prior to your arrival for this visit. Please bring your completed diary cards and your morning dose of sodium nitrite 40 mg with you as you will take it after arrival. You will have the following tests:

1. Your body weight will be measured.
2. Blood vessel stiffness measurement by Pulse Wave Velocity
3. Ultrasound measurement of carotid arterial disease and automated blood pressures
4. Endothelial function assessment with flow-mediated dilation (FMD)

After your Vascular CTRC testing is complete, you will be given a light breakfast snack.

5. If you are a female, we will also ask your menstrual history. For females who are able to have children, you will be asked to provide a urine sample for pregnancy testing. The result of the pregnancy test must be negative in order for you to continue in this research study visit.

6. DEXA: Bone density/Body composition

7. Waist and hip circumference measurements

8. Exercise testing

9. Blood pressure monitor for 24 hours. We ask that you return the cuff and monitor to us at Visit 17. We may not be able to give this to you until Visit 17 depending on availability. If this happens, we ask that you return the cuff and monitor at Visit 18.

10. Assess adverse events (AE) and review diary cards. You will be provided the remaining supply of study drug necessary until your scheduled Visit 18 Third Completion Assessment Visit (Week 12).

11. Food record forms to take home to complete over 3 different days. We will assess nitrate and nitrite intake. We ask that you return the forms to us by Visit 18.

Visit 17 (Week 12: Second Completion Assessment Visit)

This fasting visit will take place at the CTRC or EMRC. You will be asked not to drink or eat anything for 8 hours prior to your arrival for this visit. This visit will take approximately 3 hours.

1. Body weight and temperature will be measured.



2. If you are a female, we will also ask your menstrual history. A repeat urine pregnancy test will be performed for females who are able to have children.
3. Please bring your morning dose of sodium nitrite 40 mg. Blood pressure, breathing rate and heart rate will be measured after approximately 30 minutes of rest and quiet in the CTRC to ensure blood pressures are at a steady state before taking the study medication and then every 15 minutes for 2 hours after taking the study medication.
4. Safety monitoring for methemoglobin level by a finger probe before, 0.5, 1 and 2 hours after study medication administration. Research blood samples and blood samples for platelet mitochondrial function will be drawn before, 0.5, and 2 hours after study medication administration. The total volume of blood drawn is approximately 3 Tablespoons.
5. You will be provided a meal after completion of testing.
6. Assess adverse events (AE) after completion of study testing.
7. You will return your food records if you have completed them and your blood pressure monitor and cuff today so that we may download the recorded blood pressure information.

Visit 18 (Week 12: Third Completion Assessment Visit)

You will be scheduled to complete this fasting visit 2-7 days after Visit 17 at the CTRC. All studies from Visit 3 (Second Baseline Assessment Visit) will be repeated at this fasting visit plus you will be given your final home dose of sodium nitrite 40 mg. You will be asked not to drink or eat anything for 8 hours prior to your arrival for this visit. If you have not already returned your food record forms, we will collect these today.

No further study medication will be dispensed. We ask that you bring your drug diary card(s) and any unused study drug with you to this visit so we can assess study medication compliance. Adverse event assessment and review of drug diary card(s) will occur.

Visit 19 (Week 16: Telephone Assessment)

You will be contacted by phone which will last about 10 minutes by the study staff. You will be asked about any side effects, any changes in medication, and any medical history such as emergency room visits or hospitalizations since your last study visit.

In addition to these formal evaluations, you are encouraged to contact the study doctor or the study staff anytime with questions, concerns, or to report new symptoms that occur during your study participation.

What are the possible risks, side effects, and discomforts of this research study?

As with any research study, there may be adverse events or side effects that are currently unknown and it is possible that certain of these unknown risks could be permanent, serious or life-threatening.



Risks of sodium nitrite:

The risks associated with the study drug, sodium nitrite, are rare and include nausea, belly pain, dry mouth, vomiting, flushing, increased heart rate or breathing rate, or low blood pressure. You can also develop an abnormal amount of methemoglobin in the blood, which causes oxygen not to be released into your blood, and you can experience headache, dizziness, shortness of breath, a bluish color of the skin, seizure, or coma.

Risks of blood draw:

The amount of blood to be drawn over the course of this investigation could be a maximum of 64 tablespoons. To minimize the risks of blood tests, a licensed technician or registered nurse will draw your blood. Common risks of blood sampling by venipuncture or intravenous line placement include temporary discomfort, bruising which may last for several days, redness, swelling or lower hemoglobin level (or anemia) A common symptom of anemia is fatigue (feeling tired or weak). Infrequent risks include feeling lightheaded or faint when blood is drawn. This is usually due to nervousness and is not usually serious. Rare risks include infection and bleeding.

Risks of hyperinsulinemic euglycemic clamp:

The clamp study involves likely risk of discomfort associated from two venipunctures. The risk of receiving insulin is hypoglycemia or abnormally low blood sugar (rare). Hypoglycemia may cause you to feel shaky, sweaty, hungry, and dizzy and have a rapid heart rate. If severe, hypoglycemia can cause coma, seizure or even death (rare). It is very unlikely, however, that severe hypoglycemia will occur during the insulin infusion because your blood sugar will be checked every 5 minutes. To decrease the risk of hypoglycemia, you will be given sugar water by IV (a plastic needle inserted in your vein) to keep your blood sugar at a normal, safe level. As an additional safety measure, you will be kept awake during the insulin infusion so that the study team can be certain that you are not having any kind of unusual or unexpected reaction to the insulin infusion. The deuterated glucose is a naturally occurring, non-radioactive labeled isotope that has been carefully tested in a laboratory so we know that it is pure and does not have germs in it that could cause an infection. However, on rare occasions, it can leak outside the vein and cause tissue damage. Rarely people may have reactions to the isotope such as back pain, chills, or nausea. If this happens, the test and the isotope infusion will be stopped immediately.

Risks of indirect calorimetry:

The risk of anxiety due to the canopy being placed over the head during the indirect calorimetry (resting energy expenditure test) test is (rare). We will fully explain this test to you at your scheduled visit. You may stop the study at any time.

Risks of muscle biopsy:

The muscle biopsy with a needle may cause discomfort, bruising, scar and soreness for several days (common), but to further reduce these risks, an elastic wrap and ice bag are applied to the leg post-biopsy to decrease the risk of bruising. There is also the possibility of a vasovagal reaction (reaction of the nervous system due to anxiety) that may cause fainting (common). Bleeding and infection are rare. In addition to using sterile materials, a topical antibiotic is applied to minimize the risk of infection. Additional unusual risks include allergic reactions to the elastic bandage wrap, leg numbness that would indicate the elastic bandage had been applied too tightly; and skin redness, irritation, and chafing from the applied antibiotic ointment and/or steri-strips. An infrequent risk is that there is an allergic reaction to the Lidocaine used to numb the muscle and is given just before the biopsy. Lidocaine has risks which are described next. A rare risk is allergy



to the steri-strip, which would require a stitch.

The local numbing medicine Lidocaine is used by the physician performing the muscle biopsy. This FDA approved numbing medicine, buffered lidocaine, is injected into the site before the biopsy procedure. If you have had prior difficulty with this local anesthetic, the procedure will not be performed and you will be excluded from the study. A possible side-effect is an anaphylactic reaction (an exaggerated sensitivity to the lidocaine) that could result in a severe allergic reaction with symptoms such as severe shortness of breath, swelling of the throat, inflammation of the skin, skin rash, low blood pressure and death (rare). In order to prevent such an occurrence, you will be questioned on screening about prior experiences with the local anesthetic lidocaine. While the risks of having a reaction to buffered lidocaine used for this purpose are rare, it is always possible that you could have an unexpected serious reaction to any medication.

Risks of exercise test:

The exercise test may cause muscle soreness or fatigue (common). Some people get anxious while breathing through a mouthpiece or experience a fall (rare). Another risk is redness, skin chafing or irritation from the EKG electrodes used during exercise testing. If an abnormal rise in blood pressure or changes in the electrical pattern of your heart is noted, or if you develop chest pain, the exercise will be stopped immediately. Rarely, exercise may cause muscle sprains, muscle strains, or broken bones. Other risks include abnormal blood pressure (infrequent), fainting, dizziness (common), disorders of heart rhythm (infrequent), and in very rare instances, heart attack, stroke, or even death (rare). In adults without a known history of heart disease, the risk of heart attack or death from maximal or sub-maximal exercise bouts is rare. The relative risk of exercise testing for obese adults has not been clearly defined. However, a survey of more than 2,000 clinical exercise testing laboratories, in which more than 600,000 tests were performed, showed a death rate of approximately 1 per 20,000.

Risks of DEXA:

The radiation dose of the whole body DEXA is approximately 3 mrems. For comparison, adult radiation workers are permitted, by Federal regulation, to receive a maximum whole body radiation dose of 5,000 mrems/year and a maximum single organ radiation dose of 50,000 mrem/year. There is no minimum level of radiation exposure that is considered totally free of the potential risk of causing genetic changes (cellular abnormalities) or cancer. However, the risk associated with the amount of radiation exposure that the subject will receive from participation in this research study is considered to be low and comparable to everyday risks. Menstruating females in the reproductive age group must have a negative pregnancy test within 24 hours prior to each of these studies in order to reduce the potential for exposing a fetus to radiation.

Risks of FMD test:

During the FMD test, the occlusion cuff will be placed on your arm and will stay inflated for 5 minutes. The cuff could cause minor pain, possible bruising, numbness, tingling and irritation of your skin. You will be asked to lie still on your back for 30 minutes during the test; this could cause stiffness or dizziness.

Risks of Carotid Ultrasound or Pulse Wave Velocity:

The rare risks associated with ultrasound testing of the neck and groin arteries are dizziness, bruising, and a feeling of pressure at the sites on the neck or groin where the ultrasound probe or tonometer are placed during the test. Likewise there is also a rare risk of slight skin irritation from the EKG lead removal and the blood pressure cuffs. If you begin to feel unwell during the testing,



we will stop the test immediately. We will take your blood pressure and pulse. Even if your symptoms go away, we will take your blood pressure and pulse while you are sitting up and keep you at the lab for another 15-30 minutes. You will be able to go back to the CTRC if you are feeling no more symptoms and your blood pressure and pulse are stable. However, if you continue to feel unwell and/or your blood pressure and pulse are not within normal limits, we will contact your physician and/or emergency medical services.

Risks of Ambulatory Blood Pressure Monitoring:

The rare risks associated with arm blood pressure monitoring are a feeling of pressure, bruising, or irritation at the site where the blood pressure cuff is worn.

Reproductive risks:

It is not known if the study drug can affect an unborn baby. Therefore, you should not become pregnant or father a baby while on this study. If you are physically able to father a baby, you must use an effective method of birth control while on this study. If you become aware that you or your sexual partner is pregnant during the course of your participation in this research study, you must contact, as soon as possible, the study doctor listed on the first page of this form.

Risks of breach of confidentiality:

Although we are taking many steps to protect your information, there is always a chance that your information or identity could be disclosed. We will continue to review and improve the ways we keep your information private. To protect the research data, a code will be assigned and the information linking the code to your identity will be stored in a separate secure location. To protect your information, we will keep your name and address separate from our information file.

If I agree to take part in this research study, will I be told of any new risks that may be found during the course of the study?

You will be promptly notified if any new information develops during the conduct of this research study which may cause you to change your mind about continuing to participate.

What are possible benefits from taking part in this study?

There is no guarantee that you will benefit from participating in this research study. Possible benefits to you from participating in this research project are obtaining a complete physical examination and laboratory blood tests including blood glucose and blood cholesterol and fat levels which may identify hidden diabetes or dyslipidemia. You will have your percentage and distribution of body fat assessed by DEXA. This information has been shown to predict cardiac and diabetic risk and may help you if you choose to participate in a weight management program after this study is completed. The information learned from this study may help investigators provide better care of patients with metabolic syndrome, diabetes, and heart disease in the future.

What other choices do I have if I do not take part in this research study?

You may continue to get regular care from your doctor.

Will my insurance provider or I be charged for the costs of any procedures performed as part of this research study?

Neither you, nor your insurance provider, will be charged for the costs of any of the procedures performed for the purpose of this research study (i.e., the Screening Procedures, Experimental Procedures, or Follow-up Procedures described above). If you think that you or your health insurance has been charged, please contact a member of the research team and the UPMC billing



office that sent the bill.

Will I be paid to take part in this research study?

If you decide to participate, we will compensate you for your time: \$200 for the (2) Baseline Assessment visits and \$300 for (2) of the 3 Week 12 Completion Assessment visits. You will also be reimbursed \$25 for the first dose of study drug visit, \$25 for the week 12 dose of study drug visit and \$10 for each of the (5) in person safety visits. The total compensation will be received when participation in all aspects of this study are completed and will be \$600.00. For all visits except the screening visit, we will pay for your parking in the Montefiore Hospital parking garage. In the event that you drop out due to an adverse event, you will be reimbursed for the individual study visits that you completed to date per the above reimbursement schedule.

Your biological sample may lead, in the future, to new inventions or products. If the investigators are able to develop new products from the research use of your biological sample, there are currently no plans to share with you any money or other rewards that may result from the development of these new products.

Who will pay if I am injured as a result of taking part in this study?

If you believe that you are injured as a result of the research procedures being performed, please contact immediately the Principal Investigator or one of the co-investigators listed on the first page of this form. Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of UPMC. It is possible that UPMC may bill your insurance provider for the costs of this emergency treatment, but none of these costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care unless otherwise specifically stated. There is no plan for monetary compensation. You do not, however, waive any legal rights by signing this form. University of Pittsburgh researchers and their associates who provide services at UPMC recognize the importance of your voluntary participation in their research studies. These individuals and their staffs will make reasonable efforts to minimize, control, and treat any injuries that may arise as a result of this research.

Who will know about my participation in this research study?

Any information about you obtained from this research will be kept as confidential (private) as possible. All records related to your involvement in this research study will be stored in a locked file cabinet. Your identity on some of your records will contain your name when necessary (such as your consent form or lab results) and others will be indicated by a case number rather than by your name. The information linking these case numbers with your identity will be kept separate from the research records. Your biologic specimens will remain de-identified. You will not be identified by name in any publication of the research results unless you sign a separate consent form giving your permission (release).

Will this research study involve the use or disclosure of my identifiable medical information?

This research study will involve the recording of current and/or future identifiable medical information from your hospital and/or other (e.g., physician office) records. The information that will be recorded will be limited to information concerning your screening laboratory work and study participation. This information will be used for the purpose of determining whether you qualify, based on study specific eligibility criteria, for study participation. This information will be placed into your medical records held at the University of Pittsburgh Medical Center (UPMC). The nature



of the identifiable information resulting from your participation in this research study that will be recorded in your medical record possibly includes your screening laboratory work, and information related to the muscle biopsy.

Will my information be kept private?

A portion of your biological samples may be stored away indefinitely and will be used for research pertaining to diabetes and heart and blood vessel diseases. Use of your biological samples, and information about your clinical illness obtained from your medical records will be under the control of the principal investigator of this research project. Your biological samples will be stored in the Endocrinology and Metabolism Research Center. Your stored samples may be shared without identifiers with other researchers. In other words, these researchers will not be able to identify you from the specimens or from information extracted from the medical record.

We will do our best to protect your privacy and keep your information safe by:

- Using a number code to label your samples and other information.
- Keeping your number code separate from your name, address, and other personal information. We will look at your information using the number code and not your personal information.
- Keeping your test results and other information in a secure computer database.
- Storing samples and other information in a secure place. We will limit and keep track of access to your samples to make sure they are safe.

Who will have access to identifiable information related to my participation in this research study?

In addition to the investigators listed on the first page of this authorization (consent) form and their research staff, the following individuals will or may have access to identifiable information (which may include your identifiable medical information) related to your participation in this research study:

Authorized representatives of the University of Pittsburgh Research Conduct and Compliance Office may review your identifiable research information (which may include your identifiable medical information) for the purpose of monitoring the appropriate conduct of this research study.

Authorized representatives of the UPMC hospitals or other affiliated health care providers may have access to identifiable information (which may include your identifiable medical information) related to your participation in this research study for the purpose of (1) fulfilling orders, made by the investigators, for hospital and health care services (e.g., laboratory tests, diagnostic procedures) associated with research study participation; (2) addressing correct payment for tests and procedures ordered by the investigators; and/or (3) for internal hospital operations (i.e. quality assurance).

Authorized representatives of the Food and Drug Administration and the study sponsor (National Institute of Health), who may need to review the records for accuracy and completeness. Representatives of the study sponsor may also be present during your participation in the research study. While the study sponsor understands the importance of maintaining the confidentiality of your identifiable research and medical record



information, UPMC and the University of Pittsburgh cannot guarantee the confidentiality of this information after it has been obtained by the study sponsor.

In unusual cases, the investigators may be required to release identifiable information (which may include your identifiable medical information) related to your participation in this research study in response to an order from a court of law. If the investigators learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform, as required by Pennsylvania law, the appropriate agencies.

For how long will the investigators be permitted to use and disclose identifiable information related to my participation in this research study? The investigators may continue to use and disclose, for the purposes described above, identifiable information (which may include your identifiable medical information) related to your participation in this research study for a minimum of 7 years and for as long (indefinite) as it may take to complete this research study. It is University Policy to maintain research data for 7 years after final reporting and publication of a project.

May I have access to my medical information that results from my participation in this research study?

In accordance with the UPMC Notices of Privacy Practices document that you have been provided, you are permitted access to information (including information resulting from your participation in this research study) contained within your medical records filed with your health care provider.

Is my participation in this research study voluntary?

Your participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above, is completely voluntary. (Note, however, that if you do not provide your consent for the use and disclosure of your identifiable information for the purposes described above, you will not be allowed, in general, to participate in the research study.) Whether or not you provide your consent for participation in this research study will have no effect on your current or future relationship with the University of Pittsburgh, current or future medical care at a UPMC hospital or affiliated health care provider, or current or future relationship with a health care insurance provider.

Your doctor may be involved as an investigator in this research study. As both your doctor and a research investigator, s/he is interested both in your medical care and the conduct of this research study. Before agreeing to participate in this research study, or at any time during your study participation, you may discuss your care with another doctor who is not associated with this research study. You are not under any obligation to participate in any research study offered by your doctor.

May I withdraw, at a future date, my consent for participation in this research study?

You may withdraw, at any time, your consent for participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above (Note, however, that if you withdraw your consent for the use and disclosure of your identifiable medical record information for the purposes described above, you will also be withdrawn, in general, from further participation in this research study). Any identifiable research or medical information recorded from your participation in this research study prior to the date that you formally withdrew your participation may continue to be used and disclosed by the investigators for the purposes



described above. Your biological samples stored for future research analyses will be kept unless you request for the sample to be destroyed. Your samples will not be labeled with direct identifiers. Your identity on these samples and records will be indicated by a case number rather than by your name or social security number.

To formally withdraw your consent for participation in this research study, you should provide a written and dated notice of this decision to the principal investigator of this research study at the address listed on the first page of this form.

Your decision to withdraw your consent for participation in this research study will have no effect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

If I agree to take part in this research study, can I be removed from the study without my consent?

You may be removed from the study by the investigators in the event that the investigators feel that the study may adversely influence your health, if you don't comply with study requirements, if your pregnancy test proves positive, or you develop a severe, acute illness during the study period. Should any of these occur, your participation in the study will be terminated, and you will continue to receive appropriate care as necessary.

Any identifiable research or medical information recorded for, or resulting from your participation in this research study prior to the date that you were withdrawn from participation may continue to be used and disclosed by the investigators for the purposes described.

What are my rights if I take part in this study?

Taking part in this study is your choice. You may choose either to take part or not to take part in the study. If you decide to take part in this study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your regular benefits.

VOLUNTARY CONSENT

The above information has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions, voice concerns or complaints about any aspect of this research study during the course of this study, and that such future questions, concerns or complaints will be answered by a qualified individual or by the investigator(s) listed on the first page of this consent document at the telephone number(s) given. I understand that I may always request that my questions, concerns or complaints be addressed by a listed investigator.

I understand that I may contact the Human Subjects Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668) to discuss problems, concerns, and questions; obtain



information; offer input; or discuss situations in the event that the research team is unavailable.

By signing this form, I agree to participate in this research study. A copy of this consent form will be given to me.

My biological samples, or the information about my illness obtained from my medical or research records, may be combined together without personal identifiers with other research projects pertaining to diabetes and heart and blood vessel diseases to learn even more about nitrite effects on mitochondrial function.

YES NO Initials: _____

Printed Name of Participant

Signature of Participant

Date

CERTIFICATION OF INFORMED CONSENT:

I certify that I have explained the nature and purpose of this research study to the above-named individual(s), and I have discussed the potential benefits and possible risks of study participation. Any questions the individual(s) have about this study have been answered, and we will always be available to address future questions as they arise. I further certify that no research component of this protocol was begun until after this consent form was signed.

Printed Name of Person Obtaining Consent

Role in Research Study

Signature of Person Obtaining Consent

Date

