PennState

HRP-591 - Protocol for
Human Subject Research

Protocol Title:
STUDY00010736: Age Comparisons of Exercising Muscle O2 Supply in Healthy Adults: Effects of Esmolol Infusion.

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1.0 Objectives

1.1 Study Objectives

In this study, we will test the central hypothesis that postmenopausal women will demonstrate increased oxygen extraction in active leg muscle during leg cycling exercise while receiving an infusion of Esmolol, a fast-acting β1 selective antagonist, when compared to premenopausal women. β_1 selective antagonists (or "β_1 blockers") are used to lower heart rate and improve O_2 supply-to-demand balance in patients with coronary artery disease[3]. By using esmolol to attenuate the central sympathetic response to exercise (increased heart rate and cardiac output) we can examine peripheral mechanisms of O_2 delivery. The current project will evaluate how older postmenopausal women adjust active muscle O_2 supply to an acute reduction in systemic O_2 delivery during large muscle dynamic exercise when compared to younger premenopausal women.

1.2 Primary Study Endpoints

The primary outcome variable is the difference in the change in skeletal muscle oxygenation ($\%SmO_2$) in the active leg muscle from baseline during recumbent cycling exercise between saline and esmolol ($\Delta\%\Delta SmO_2$). We predict that older women will have a greater $\Delta\%\Delta SmO_2$ than premenopausal women during esmolol infusion.

1.3 Secondary Study Endpoints

Secondary outcome variables will include the effect of concurrent sympathetic stimulation via isometric handgrip exercise on active thigh and inactive forearm muscle $\Delta\%\Delta SmO_2$. We will also include measurements of blood pressure, heart rate, cardiac output, and VO_2.

2.0 Background

2.1 Scientific Background and Gaps

Understanding the mechanisms facilitating the acute adjustments in oxygen uptake (VO_2) during large muscle exercise is biomedically important, as it is a major determinant of exercise tolerance[17,18] and functional quality of life[1] in both health and disease. The physiological adjustments that mediate increases in VO_2 during exercise are highly integrated and involve increases in mean arterial pressure (MAP), systemic and exercising muscle blood flow, and arterial-venous O_2 content (primarily via increased O_2 extraction)[9]. The matching of O_2 delivery-to-utilization/demand during dynamic exercise, and the speed with which these processes occur at exercise onset (kinetics), reflects the integrated functioning of the autonomic, pulmonary, cardiovascular, and muscular metabolic systems which in turn determine the necessity for substrate-level (nonoxidative) energy supply and subsequent exercise tolerance/intolerance[22].

Interventions that alter the normal balance between sympathetic control, active muscle O_2 supply, and extraction have the potential to advance our understanding of the mechanisms regulating kinetic and steady state VO_2 responses to exercise. Studies employing beta-adrenergic blockade, which acutely reduce heart rate, contractility, and systemic O_2 delivery (refs), report compensatory increases in systemic[4,6] and leg[15,16] arterial-venous O_2 content difference during submaximal cycling exercise in healthy younger humans.

Age- and sex-related differences in O_2 utilization. It has been previously shown that older women demonstrate an attenuated ability to increase cardiac output and leg vascular conductance during moderate-intensity (50-60 watts) cycling exercise resulting in reduced leg blood flow and O_2 delivery[19]. However, unlike older men (who displayed no reduction in leg blood flow during exercise), older women did not demonstrate an increase in O_2 extraction compared to their younger counterparts[21]. It is unclear whether these differences represent a better-preserved ability to deliver oxygen to active muscle or an impairment in oxygen extraction. By infusing esmolol during exercise, we will attempt to further reduce oxygen delivery to examine oxygen extraction in young vs older women.

Age-related reductions in Functional Sympatholysis. Previous studies, both from our lab[19,21] and others[8,23], indicate substantial age differences in the ability of metabolically active muscles to blunt sympathetic vasoconstriction (functional sympatholysis). The age-related attenuation of this response is particularly evident in older post-menopausal women and could contribute, along with an accelerated stiffening of central arteries and altered baroreflex function, to the exaggerated systemic blood pressure responses observed in these women when they perform dynamic exercise. Isometric handgrip has been established as a reliable way to acutely augment systemic sympathetic outflow[2,12] and NIRS has been used to assess the resultant functional sympatholysis response in various populations[24] including in post-menopausal women[5]. The present study will assess how functional sympatholysis is affected by an acute esmolol-mediated reduction in cardiac output during dynamic cycling exercise and how this response may differ between younger and older women.

2.2 Previous Data

Because of its rapid onset and offset, Esmolol is used commonly in anesthesia and critical care to treat tachycardia. Our dose-finding studies indicated that infusing of ~125 mg of Esmolol (range 90 to 162 mg) acutely blocks beta-1 adrenergic receptors (β1ARs) in healthy young men and women[14]. β1ARs remain blocked in these subjects if a maintenance infusion of Esmolol continues. When the infusion ends, the β1ARs return to baseline within 45 minutes.

There are minimal published data using Esmolol in the context of exercise physiology experiments. The Proctor lab recently utilized the rapidly acting β_1 selective antagonist esmolol to transiently reduce exercise heart rate in a mixed (age and sex) sample of healthy adults[20]. Larger reductions in near infrared spectroscopy (NIRS)-derived oxyhemoglobin/myoglobin O_2 saturation (an index of O_2 extraction) were observed within the exercising leg muscles (vastus lateralis and medial gastrocnemius) of these subjects during esmolol (vs. saline) infusion[20]. These findings provide strong evidence of the dynamic coupling that exists between systemic O_2 delivery and active muscle O_2 extraction during submaximal exercise in humans.

2.3 Study Rationale

The effects of infused Esmolol on the heart are rapid (observed within 5 min), quickly reversible (9 min half-life), and do not provoke lightheadedness, nausea, or significant hypotension in healthy adults[14]. This is in contrast to non-selective (β_1+β_2) blockers such as Propranolol, which are not rapidly reversible. The use of β_1-selective blockade is also advantageous in these studies because it does not impact β_2-mediated responses in skeletal muscle (as we have shown with Esmolol[14]) and does not appear to suppress skeletal muscle glycolysis or hasten exercising muscle fatigue in the way that non-selective blockade does[11].

3.0 Inclusion and Exclusion Criteria

3.1 Inclusion Criteria

1. Capable of giving informed consent
2. Premenopausal women ages 18-35 years OR post-menopausal women ages 55-70 years
3. Satisfactory medical history and physical exam, as determined by a CRC clinician
4. Not currently taking medications affecting heart rate or contractility
5. Fluent in written and spoken English

3.2 Exclusion Criteria

Participants who will **not** be studied are those who:

1. Are less than 19 years of age or more than 70 years of age
2. Are pregnant or lactating
3. Are prisoners or institutionalized individuals or unable to consent

4. Diagnosed renal failure (Creatinine >2.0 mg/dl)
5. Diagnosed liver disease (ALT and AST 2 times normal)
6. Diagnosed Reynaud's disease
7. Have uncontrolled diabetes
8. Have uncontrolled hypertension
9. Have a left ventricular ejection fraction < 40%
10. Have a recent history of unstable angina or myocardial infarction (<6 months), unstable angina, or use of nitrate medications within past 2 weeks
10. Severe lung disease (i.e., on supplemental oxygen or frequently use rescue inhalers)
11. Diagnosed bleeding or clotting disorder or recent blood transfusion
12. Have asthma, history of thyroid issues or hyperkalemia
13. Known use of recreational drugs
14. Methylphenidate use

3.3 Early Withdrawal of Subjects

3.3.1 Criteria for removal from study

Participation in this study will be discontinued if pre-screening requirements are not met, pregnancy, failure to follow pre-study instructions or main variables cannot be measured. If vital signs fall below or rise above acceptable levels or if the subject does not tolerate a procedure, the procedure will be stopped. The subject may withdraw from the study at any time.

3.3.2 Follow-up for withdrawn subjects

Subjects withdrawn from the study will not return for any more visits. The research nurses will contact subjects after withdrawal to ensure there are no further problems.

4.0 Recruitment Methods

4.1 Identification of subjects

Subjects will be identified from our own subject pool who meet the inclusion/exclusion criteria and previously agreed to be contacted.

Subjects will also be recruited from Penn State campus, State College community, and Centre County area by flyers and advertisements. Flyers will contain basic information about the study, as well as contact information for study personnel in the Integrative Vascular Physiology Laboratory (201 Noll Lab). Potential participants who show interest in the study by contacting study personnel will be sent an informed consent form over email with instructions to contact one of the study investigators if she has any questions or would like to schedule a visit to Noll Lab for the screening and familiarization visit.

In addition to flyers and advertisements, StudyFinder will be used for recruitment purposes.

4.2 Recruitment process

4.2.1 How potential subjects will be recruited.

Subjects will be recruited through the CRC and through study finder. Potential subjects, who are interested in participating, will be informed about the details of the study using the phone script. If they are interested in participating, then they will be informed that a series of pre-screening questions pertaining to their health, medical background and history (Patient Information sheet) will be asked over the phone and that by answering the questions, they will be giving their consent to allow us to use and retain that information. If subjects wish to participate in additional research

studies, they may indicate that they allow their information to be retained and used in the future by the research group to contact them about future studies. If they indicate that they are not interested in future studies, their information will be destroyed at the end of this study or at any future time when they request destruction. The information will not be disclosed to anyone outside of the CRC Research team

No race or ethnicity will be excluded from the study.

Recruitment materials
Invitation to participate/Permission to contact letter
Phone screening questions
StudyFinder
Study Flyers

4.2.2 Where potential subjects will be recruited.

Subjects will be identified from our own subject pool who meet the inclusion/exclusion criteria and previously agreed to be contacted.

Potential subjects will be recruited from State College, PA and the surrounding region (i.e., Centre County).

4.2.3 When potential subjects will be recruited.

Recruitment of potential subjects will begin upon study approval.

4.2.4 Describe the eligibility screening process and indicate whether the screening process will occur before or after obtaining informed consent. Screening begins when the investigator obtains information about or from a prospective participant in order to determine their eligibility. In some studies, these procedures may not take place unless HIPAA Authorization is obtained OR a waiver of HIPAA Authorization when applicable for the screening procedures is approved by the IRB.

Members of the Integrative Vascular Physiology Laboratory will answer phone calls (office phone) or emails from potential participants. During these exchanges, prospective participants may ask any questions regarding the study and/or schedule a visit to complete informed consent and screening process.

After signing the IRB approved consent the subjects will participate in the screening visit a brief medical history, physical exam, resting EKG, and blood draw (CBC and CMP) will be performed to confirm each participant's health status. Women of childbearing potential will have a urine pregnancy test to confirm they are not pregnant. We will also have subjects fill out a physical activity history questionnaire. These questionnaires will not be used to determine study eligibility (inclusion or exclusion), but will be used to describe the study groups in future publications.

5.0 Consent Process and Documentation

5.1 Consent Process:

Check all applicable boxes below:

☒ **Informed consent will be sought and documented with a written consent form**

☒ **Implied or verbal consent will be obtained – subjects will not sign a consent form (waiver of written documentation of consent)**

☐ **Informed consent will be sought but some of the elements of informed consent will be omitted or altered (e.g., deception).**

☐ **Informed consent will not be obtained – request to completely waive the informed consent requirement.**

5.2 Obtaining Informed Consent

5.2.1 Timing and Location of Consent

Prior to coming to the laboratory, subjects will be contacted using the Study Prep phone script. The participants will avoid alcohol and strenuous exercise for 24 hours prior to each study visit. Participants will also refrain from caffeine at least 12-24 hours and NSAIDS/cold prep medications 24 hours prior to each study visit. Participants will be asked to eat a small meal 2 to 3 hours before their scheduled appointment but dietary intake will not be rigorously controlled. ALL participants will review their prescribed medications, with a CRC (Clinical Research Center) clinician.

Once the subject arrives at the CRC, they will be given sufficient time to read the consent, all procedures will be explained in layman's terms and they will be given a copy of the signed consent form.

5.2.2 Coercion or Undue Influence during Consent

The subject will be provided with ample opportunity to have questions answered before participating in the study. All procedures will be explained to them in layman's terms and they will be given a copy of their signed consent form. Subjects will have the right to withdraw from the study at any time.

5.3 Waiver of Written Documentation of Consent

N/A Subjects will sign the IRB-approved consent form.

5.3.1 Indicate which of the following conditions applies to this research:

☐ The research presents no more that minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context.

OR

☐ The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality. Each subject will be asked whether the subject wants documentation linking the subject with the research, and the subject's wishes will govern.

OR

☐ If the subjects or legally authorized representatives are members of a distinct cultural group or community in which signing forms is not the norm, that the research presents no more than minimal risk of harm to subjects and provided there is an appropriate alternative mechanism for documenting that informed consent was obtained.

5.3.2 Indicate what materials, if any, will be used to inform potential subjects about the research (e.g., a letter accompanying a questionnaire, verbal script, implied consent form, or summary explanation of the research)

Subjects will be contacted using the Study Prep phone script to obtain verbal consent to avoid alcohol and strenuous exercise for 24 hours prior to the experiments. This poses no more than minimal risk, so verbal consent is appropriate. Once the subject arrives in the Clinical Research Center (CRC), they will be given sufficient time to read the consent form and have all their questions answered, and to sign the written consent.

5.4 Informed consent will be sought but some of the elements of informed consent will be omitted or altered (e.g., deception).

5.4.1 Indicate the elements of informed consent to be omitted or altered. - Not applicable

5.4.2 Indicate why the research could not practicably be carried out without the omission or alteration of consent elements. - Not applicable

5.4.3 Describe why the research involves no more than minimal risk to subjects. Not applicable

5.4.4 Describe why the alteration/omission will not adversely affect the rights and welfare of subjects. Not applicable

5.4.5 If the research involves using identifiable private information or identifiable biospecimens, describe why the research could not be practicably be carried out without using such information or biospecimens in an identifiable format. Not applicable

5.4.6 Debriefing Not applicable

5.5 Informed consent will not be obtained – request to completely waive the informed consent requirement

5.5.1 Indicate why the research could not practicably be carried out without the waiver of consent- Not applicable

5.5.2 Describe why the research involves no more than minimal risk to subjects.
Not applicable

5.5.3 Describe why the alteration/omission will not adversely affect the rights and welfare of subjects
Not Applicable

5.5.4 If the research involves using identifiable private information or identifiable biospecimens, describe why the research could not be practicably be carried out without using such information or biospecimens in an identifiable format.
Not applicable

5.5.5 Additional pertinent information after participation. - Not applicable

5.6 Consent – Other Considerations

5.6.1 Non-English-Speaking Subjects - Not applicable

5.6.2 Cognitively Impaired Adults.

5.6.2.1 Capability of Providing Consent - Not applicable

5.6.2.2 Adults Unable to Consent - Not applicable

5.6.2.3 Assent of Adults Unable to Consent - Not applicable

5.6.3 Subjects who are not yet adults (infants, children, teenagers)

5.6.3.1 Parental Permission - Not applicable

5.6.3.2 Assent of subjects who are not yet adults - Not applicable

6.0 HIPAA Research Authorization and/or Waiver or Alteration of Authorization

6.1 Authorization and/or Waiver or Alteration of Authorization for the Uses and Disclosures of PHI

Check all that apply:

☒ **Not applicable, no identifiable protected health information (PHI) is accessed, used or disclosed in this study.**

☐ **Authorization will be obtained and documented as part of the consent process.**

☐ **Partial waiver is requested for recruitment purposes only (Check this box if patients' medical records will be accessed to determine eligibility before consent/authorization has been obtained).**

☐ **Full waiver is requested for entire research study (e.g., medical record review studies).**

☐ **Alteration is requested to waive requirement for written documentation of authorization (verbal authorization will be obtained).**

6.2 Waiver or Alteration of Authorization for the Uses and Disclosures of PHI

6.2.1 Access, use or disclosure of PHI representing no more than a minimal risk to the privacy of the individual

6.2.1.1 Plan to protect PHI from improper use or disclosure

Not applicable

6.2.1.2 Plan to destroy identifiers or a justification for retaining identifiers

Not applicable

6.2.2 Explanation for why the research could not practicably be conducted without access to and use of PHI

Not applicable

6.2.3 Explanation for why the research could not practicably be conducted without the waiver or alteration of authorization

Not applicable

6.3 Waiver or alteration of authorization statements of agreement

Protected health information obtained as part of this research will not be reused or disclosed to any other person or entity, except as required by law, for authorized oversight of the research study, or for other permitted uses and disclosures according to federal regulations.

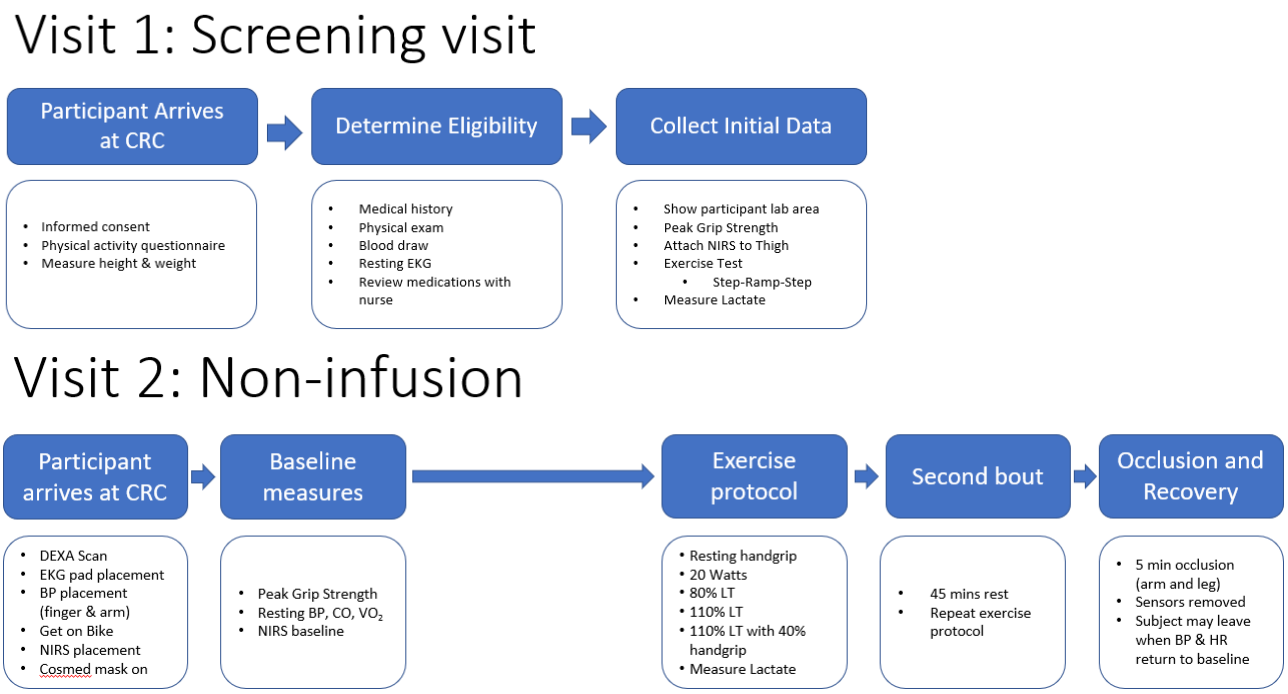
The research team will collect only information essential to the study and in accord with the 'Minimum Necessary' standard (information reasonably necessary to accomplish the objectives of the research) per federal regulations.

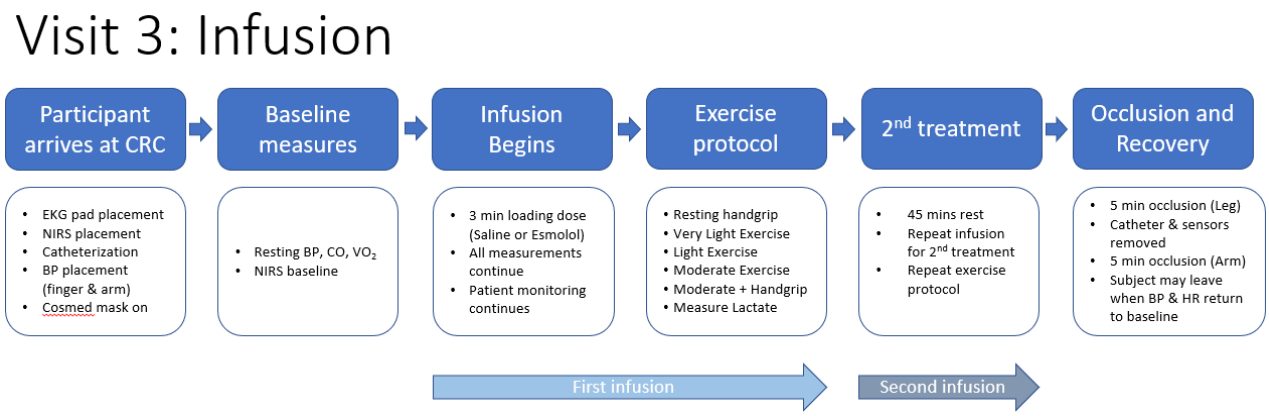
Access to the information will be limited, to the greatest extent possible, within the research team. All disclosures or releases of identifiable information granted under this waiver will be accounted for and documented.

7.0 Study Design and Procedures

7.1 Study Design

Design: single blind, randomized, placebo-controlled, crossover (each person receives both treatments)
Subjects: young and older women (2 groups, n=13 completed subjects each)
Independent variable: β_1 selective blockade with Esmolol vs. saline
Dependent variables: heart rate, cardiac output, arm BP, finger BP, VO_2, forearm, forehead, thigh and calf NIRS
There will be one screening visit and two study visits (one non-infusion and one infusion). The procedures for each visit are outlined in the diagram below:





This is a single-blind study because, although the subjects, data collectors, and data analysts will not be aware of the treatment order, the nurses monitoring the subjects will be aware of the label on the infusion bag (Esmolol or saline). The nurses will be assessing safety (i.e., HR, BP, and symptoms). However, the nurses will not be involved in any data analysis.

Esmolol hydrochloride (2500 mg in 250 ml normal saline) will be purchased from McKesson in pre-mixed bags. The placebo will be a 250 ml, 500 ml, or 1000 ml bag of normal saline. The volume and rate of infusion will be constant for both trials. Once the infusion pump is programmed by the research nurse, an opaque bag will be placed over the fluid bag so that the participants and researchers remain blinded to the treatment order.

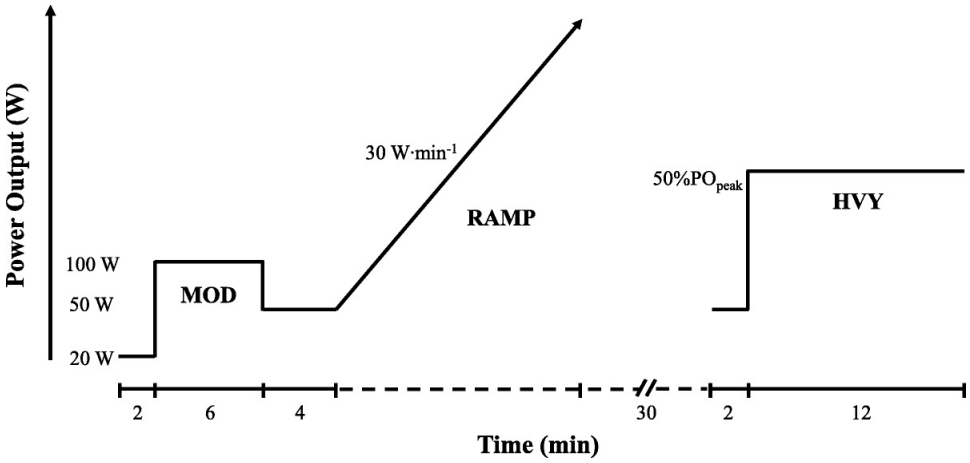
7.2 Study Procedures

7.2.1 Visit 1 (screening visit)

When research staff schedules the subject (over the phone), the subject will be told to avoid alcohol, over the counter cold prep and NSAIDs, and strenuous exercise for 24 hours, and to eat a small meal 2-3 hours prior to coming to the laboratory. They will also be asked to refrain from items containing caffeine 12 hours prior to this visit. All participants will review their medications, including beta-blockers, with one of the research nurses. This information will be shared with the CRC clinician who will then recommend which medications can be taken prior to the patient's scheduled second and third study visit, and which ones should be withheld until after each study visit.

During the first visit, informed consent will be obtained. Women of childbearing potential (pre-menopausal) will take a urine pregnancy test to ensure they are not pregnant. This will be done at all 3 visits before conducting any of the study measurements. The subject will then complete a medical history questionnaire (all subjects) and a physical activity history questionnaire. The subject will also have their height and weight (without shoes) and waist circumference measured. The subject will then undergo a physical exam and a resting EKG (conducted by medical personnel). A venous blood draw will then be performed by a CRC nurse and sent for analysis (CBC and CMP; 7.5 ml total). Lab values will be verified/signed off by a CRC clinician before infusion begins, and will be obtained no more than 6 weeks prior to the infusion. During this first study visit, all subjects will also be asked to squeeze a handgrip device as hard as they can 3 times, with approximately 1 min recovery between each. After a short recovery, the subject will then be asked to squeeze the handgrip device for approximately 1 minute at 40% of their maximum effort. Immediately after, we will ask the subject how they tolerated the 40% grip task (rating of perceived effort scale). This procedure will familiarize the subject with the handgrip before they are asked to perform these tasks (for longer durations) during visits 2 and 3. Analysis of the blood

tests, EKG and medical history/exam results, and the subject's tolerance to the handgrip will collectively determine the final eligibility of the participant.



(Figure from *Iannetta et al., 2020*)[10]

Then the subject will then lay on an exam bed for placement of EKG pads and a doppler ultrasound device will be used to determine skinfold thickness of the thigh. The NIRS sensors will then be placed (one on the forearm, one on the forehead and two on the leg). The subject will then be assisted onto the recumbent bike. The subject will be asked to squeeze a handgrip device as hard as they can with their hand. The maximum force they can exert (highest of 2 to 3 efforts) will be used to determine how hard they will squeeze this device later in the study (40% of this maximum value). A silicone face mask will be placed over the subject's nose and mouth to monitor their oxygen consumption and breathing frequency, and blood pressure devices will be placed over their arm (SunTech Tango) and finger (Finometer). After all instrumentation is in place, baseline measurements of heart rate, cardiac output, oxygen consumption, blood pressure, and NIRS will begin.

The subject will then be asked to complete three exercise bouts on a recumbent bike. The first bout will consist of very light pedaling for 2 minutes (warm-up) followed by approximately 6 minutes of pedaling at what should be a moderate effort for the subject. After a few minutes of rest and/or light pedaling (recovery), the subject will be asked to perform a second bout of exercise, which will consist of a very gradual increase in workload ("ramp") until the subject can no longer maintain the required pedal speed or needs to stop due to fatigue. This second bout will likely last between 6 and 12 minutes. After a few minutes of light pedaling, the face mask will be removed and the subject will recover in a comfortable chair. After 30 minutes of recovery, the subject will be assisted back on the bike and the facemask will be reattached. The subject will then pedal for 2 minutes at a very light workload, followed immediately by up to 12 minutes of pedaling at a moderately heavy workload. After a brief cool-down of light pedaling, the facemask and other sensors (e.g., EKG, NIRS, etc) will be removed. At several points throughout this visit (minimum of 3, maximum of 6) a small drop of blood from your fingertip will be used to measure the subject's blood lactate level. The measurements obtained during this first study visit will determine the exercise workloads that will be individually assigned during study visits 2 and 3.

Women who are currently taking hormone therapy of any kind (oral contraceptives, estrogen replacement, etc) will not be excluded from participation in this study. However, given the well-documented effects of female sex hormones on resting blood pressure and exercise metabolic responses we will ask 1) pre-menopausal women to indicate their current menstrual status and oral contraceptive use and 2) post-menopausal women to self-report their history and duration of sex hormone replacement[13]. This information will be collected at the time of their medical history/physical exam.

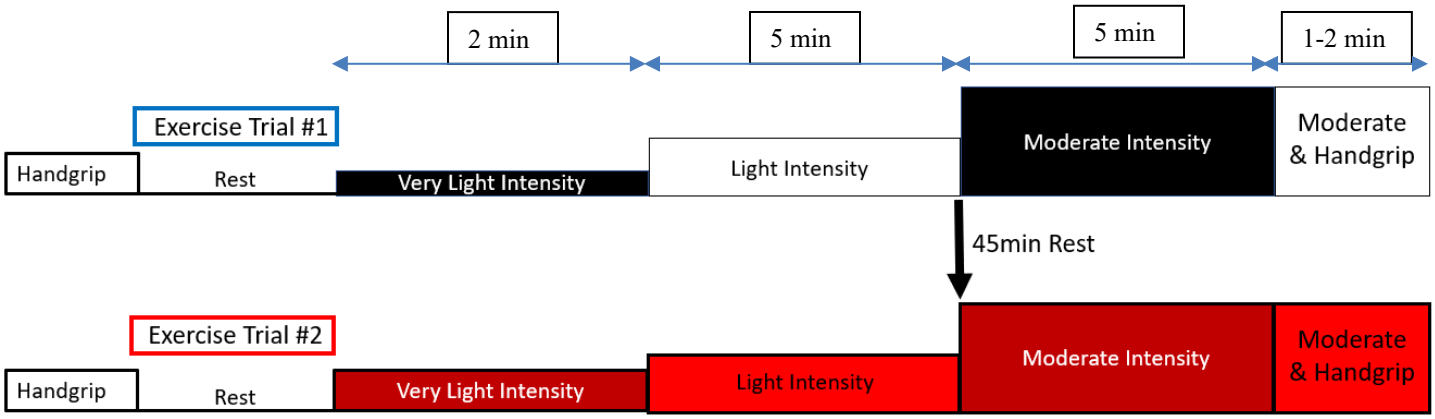
Eligible participants will be asked to return for two experimental visits (non-infusion and infusion) at least 48 hours apart.

7.2.2 Visit 2 (non-infusion)

As with the screening visit, the subject will be told to avoid alcohol, over the counter cold prep and NSAIDs, and strenuous exercise for 24 hours, and to eat a small meal 2-3 hours prior to coming to the laboratory. They will also be asked to refrain from items containing caffeine 12 hours prior to this visit. Women of childbearing potential (pre-menopausal) will take a urine pregnancy test to ensure they are not pregnant.

The subject will then lay supine as a Dual-Energy X-ray Absorptiometry (DEXA) scan is performed to determine fat free mass. Then the subject will then lay on an exam bed for placement of EKG pads as well as NIRS devices (one on the forearm, one on the forehead, and two on the leg) and measurement of vital signs.

The subject will then be assisted onto the recumbent bike. The Subject will be asked to squeeze a handgrip device as hard as they can with their hand. The maximum force they can exert (highest of 2 to 3 efforts) will be used to determine how hard they will squeeze this device later in the study (40% of this maximum value). A silicone face mask will be placed over the subject's nose and mouth to monitor their oxygen consumption and breathing frequency, and blood pressure devices will be placed over their arm (SunTech Tango) and finger (Finometer). After all instrumentation is in place, baseline measurements of heart rate, cardiac output, oxygen consumption, blood pressure, and NIRS will begin.



All measurements (HR, BP, cardiac output, oxygen consumption and NIRS) continue to be collected throughout the exercise protocol (some continuously, others intermittently). Once these measurements have stabilized, the subject will be asked to squeeze the handgrip device (constant tension) at 40% of the force they developed during the maximum grip testing for 1-2 minutes. Once all measurements have returned to baseline, the subjects will then begin the cycling exercise protocol, (see figure above). Subjects will be asked to pedal for 2 minutes at a very light intensity, followed immediately by 5 minutes at a light intensity, and 5 minutes of cycling at a moderate intensity. Finally, the subject will continue cycling at the same moderate intensity for 1-2 minutes while simultaneously squeezing a handgrip device at 40% of their maximal effort. The subject will then rest for 45 mins, after which they will repeat the exercise protocol. Immediately following the second exercise bout, cuffs will be placed on the upper arm and on the upper thigh of the limbs with the NIRS probes attached. The cuffs will then be inflated to ~250 mmHg until the limbs have been completely deoxygenated (~5 minutes as determined by NIRS).

At least three lactate measurements will be collected during each trial (6 total); one during rest, one after 4 mins of light intensity exercise, and one after 4 mins of moderate intensity exercise.

After the recovery period, most instrumentation will be removed (except for the blood pressure cuffs and EKG). The subject will then sit up slowly as we monitor their BP and HR, and offer them juice and crackers. Once the subject's BP and HR have returned to baseline levels, we will remove the remaining monitoring equipment and the subject may leave.

7.2.3 Visit 3 (Infusion)

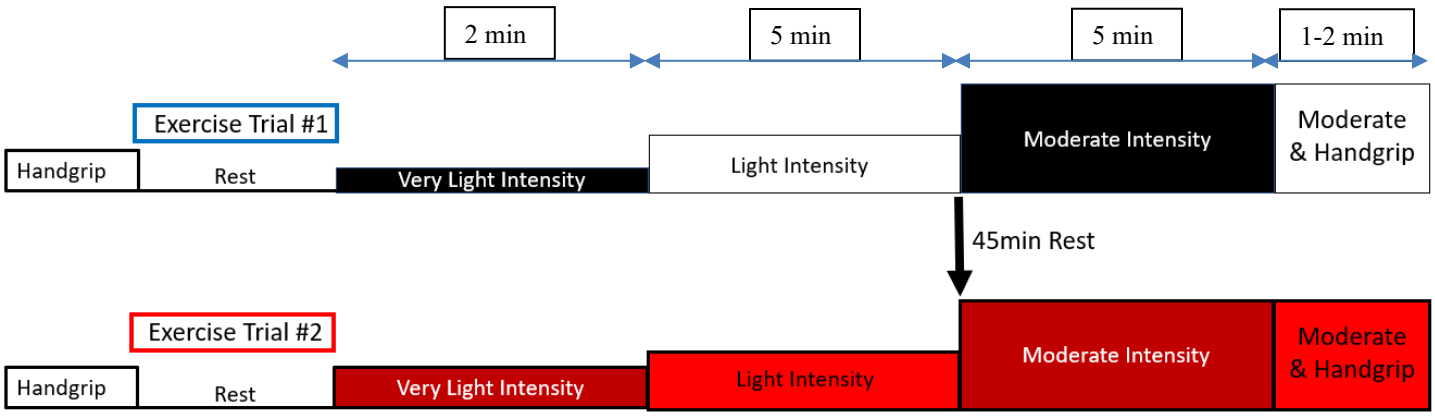
The order of infusion will be randomized and "blinded" such that neither the subject nor the investigators will know what the infusion order is (only the nurses will know the order, for safety reasons).

As with the screening visit, the subject will be told to avoid alcohol, over the counter cold prep and NSAIDs, and strenuous exercise for 24 hours, and to eat a small meal 2-3 hours prior to coming to the laboratory. They will also be asked to refrain from items containing caffeine 12 hours prior to this visit. Women of childbearing potential (pre-menopausal) will take a urine pregnancy test to ensure they are not pregnant.

Then the subject will then lay on an exam bed for placement of EKG pads as well as NIRS devices (one on the forearm, one on the forehead, and two on the leg) and measurement of vital signs. A nurse will then insert a catheter into a vein of the subject's arm (visit 3 only). This catheter will enable us to infuse the drug (Esmolol or saline).

The subject will then be assisted onto the recumbent bike. The subject will be asked to squeeze a handgrip device as hard as they can with their hand. The maximum force they can exert (highest of 2 to 3 efforts) will be used to determine how hard they will squeeze this device later in the study (40% of this maximum value). A silicone face mask will be placed over the subject's nose and mouth to monitor their oxygen consumption and breathing frequency, and blood pressure devices will be placed over their arm (SunTech Tango) and finger (Finometer). After all instrumentation is in place, baseline measurements of heart rate, cardiac output, oxygen consumption, blood pressure, and NIRS will begin. After all instrumentation is in place baseline measurements of heart rate, cardiac output, oxygen consumption, blood pressure, and NIRS will begin.

A CRC clinician will join the research team immediately before it is time to start the infusion. The CRC clinician will be able to review the EKG results at that time if there are any concerns. The subject will then receive an infusion of Esmolol or saline. The Esmolol loading dose will be 0.5 mg/kg fat free mass/min administered over the first 3 minutes (loading dose), followed by a maintenance dose of 0.25 mg/kg fat free mass/min for the remainder of the protocol (maximum of 60 minutes).



All measurements (HR, BP, cardiac output, oxygen consumption and NIRS) will be continue to be collected throughout the exercise protocol (some continuously, others intermittently). Once these measurements have stabilized (i.e., approximately 10 minutes into the maintenance infusion), the subject will be asked to squeeze the handgrip device (constant tension) at 40% of the force they developed during the maximum grip testing for 1-2 minutes. Once all measurements have returned to baseline, the subjects will then begin the cycling exercise protocol. Subjects will be asked to pedal for 2 minutes at a very light intensity, followed immediately by 5 minutes at a light intensity, and 5 minutes of cycling at a moderate intensity. Finally, the subject will continue cycling at the same moderate intensity for 1 to 2 minutes while simultaneously squeezing a handgrip device at 40% of their maximal effort. The subject will then rest for 45 mins, after which they will repeat the exercise protocol. Immediately following the second exercise bout, cuffs will be placed on the upper arm and on the upper thigh of the limbs with the NIRS probes attached. The cuffs will then be inflated to ~250 mmHg until the limbs have been completely deoxygenated (~5 minutes as determined by NIRS).

After the recovery period, most instrumentation will be removed (except for the blood pressure cuffs and EKG). The subject will then sit up slowly as we monitor their BP and HR, and offer them juice and crackers. Once the subject's BP and HR have returned to baseline levels, we will remove the remaining monitoring equipment and the subject may leave. There is no need to send subjects home with a heart rate monitor after the study because the effects of Esmolol are very short (9-minute half-life).

Listed below are all the parameters that will be measured. The risks of each procedure are listed in Section 11.0.

1. **Oxygen consumption (VO_2):** Pulmonary oxygen consumption will be measured using a COSMED Quark breath-by-breath system. A silicone mask will be placed over the subject's nose and mouth to measure breather frequency and volume in addition to the expired gas content.

2. **Cardiac Output and Heart Rate:** Cardiac output and heart rate will be measured non-invasively using a PhysioFlow Enduro device. 6 EKG pads will be placed on the subject (2 on the neck, 2 on the chest, and 2 on the back). This device will determine heart rate using EKG and will use bioimpedance to estimate cardiac output throughout the experiment.

3. **Blood pressure:** Blood pressure and heart rate will be measured non-invasively via an automatic sphygmomanometer (SunTech Tango) and with a photoplethysmographic device (Finometer, FMS, Netherlands). To optimize the accuracy of beat-by-beat data collected by the Finometer, care will be taken to stabilize the device on the hand at heart level, and the device will be turned off between protocols to prevent finger edema, which may interfere with the ability to measure finger blood pressure. Baseline BP will be measured by the SunTech Tango and the Finometer will be adjusted to match that pressure to provide an accurate baseline pressure reading. The SunTech Tango device will not be used during the handgrip exercises because it cannot measure blood pressure under those conditions.

4. **Venous catheter:** An IV will be placed in a peripheral vein to infuse Esmolol and saline, and to withdraw blood for CBC (complete blood count) and CMP (Chem-7). The total volume of blood obtained will not exceed 50 ml. Blood will be sent to HMC Clinical Laboratory for processing.

5. **Skinfold thickness**: Doppler ultrasound will be used to measure the fat thickness at the sites of the NIRS sensors (forearm and calf).

6. **Physical activity questionnaire:** Healthy subjects will complete a physical activity history questionnaire, which is a pen and paper assessment of their estimated amount of leisure time physical activity

7. **Near infrared spectroscopy (NIRS):** We will place a wireless portable near infrared spectroscopy (NIRS) device on the forearm (non-handgrip arm), as well as on the forehead, the vastus lateralis (thigh) and the gastrocnemius (calf) muscle of one leg. These devices are essentially small plastic boxes that measure skeletal muscle oxygenation noninvasively. The system measures the hemoglobin saturation of the investigated tissue, called the 'tissue saturation index' (TSI). In addition to TSI, the system also measures oxygenation changes in terms of oxy-hemoglobin, deoxy-hemoglobin, and total hemoglobin, which is an indication of the blood volume in the tissue. At the end of study visits 2 & 3, a cuff will be placed on the upper arm and upper thigh of the limbs with NIRS attached. The cuff will be inflated to a suprasystolic pressure (~250 mmHg) to occlude blood flow to fully deoxygenate the limb. This allows for calibration of the NIRS data.

8. **Isometric handgrip exercise**: Subjects will perform a maximal voluntary handgrip using a handgrip dynamometer (highest of 2 to 3 maximum efforts). A percent maximum is calculated and set on a visual display so the subject grips at the appropriate intensity. Subjects will be asked to grip at 40% of their maximum and to maintain that grip for 90 seconds.

9. **DEXA**: Subjects will lay supine on a flat, open x-ray table and will be asked to remain very still during the scan. This will provide us with the subject's fat free mass, which will be used to determine esmolol dosing.

10. **Blood Lactate**: Before each sample is collected, the finger will be cleaned with an alcohol wipe and allowed time to dry. The blood sampling procedure will involve a single-use fingerstick device, on the 3rd and 4th fingers of the hand. The first drop will be wiped with gauze and the second drop will collected on the test strip of the lactate analyzer.

7.3 Duration of Participation

The first visit could take up to 3 hours. Visit 2 will take up to 3.5 hours. Visit 3 will take up to 4.5 hours.

7.4 Test Article(s) (Study Drug(s) and/or Study Device(s))

Esmolol hydrochloride will be handled following the Institution's Standard Operating Procedures for Clinical Research Section III, PM 306. Esmolol will be tracked using an accountability record, and dispensed by a physician, pharmacist, PA, or CRNP. The receipt, storage, dispensing and return/destruction will follow the Institution's Standard Operating Procedures for Clinical Research.

- **Description**
  Esmolol hydrochloride (2500 mg in 250 ml normal saline) will be purchased from McKesson in pre-mixed bags. The placebo will be a 250 ml, 500 ml, or 1000 ml bag of normal saline.

- **Treatment Regimen**
  The route of administration of Esmolol in our study is the same as in clinical care (IV pump). The loading dose will be 0.5 mg/kg fat free mass/min administered over the first 3 minutes, followed by a maintenance dose of 0.25 mg/kg fat free mass/min for the remainder of the protocol (maximum of 60 minutes).

- **Method for Assigning Subject to Treatment Groups**
  This study uses a crossover design so all subjects will complete both treatments in random order. Subjects will be randomized to one of two treatment sequences using an equal allocation ratio of 1:1. The two treatment sequences to which subjects will be randomized are 1) Esmolol infusion first and saline infusion second and 2) saline infusion first and Esmolol infusion second. The CRC nurses will generate the randomization that will use variable size, random permuted blocks with block size 2 and 4. CRC staff will maintain the randomization list until data collection has been completed to keep the research staff blinded.

- **Subject Compliance Monitoring**
  Not applicable.

- **Blinding of the Test Article**
  This study is single blind because the subjects, data collectors, and data analysts will not be aware of the treatment. For safety reasons, the CRC nurse will not be blinded. Additionally, once the Esmolol/saline infusion pump is programmed by the research nurse, an opaque bag will be placed over the fluid bag so that the participant and all investigators in the room remain blinded to the treatment order.

- **Receiving, Storage, Dispensing and Return**

7.4.1 Receipt of Test Article

Esmolol hydrochloride will be purchased from McKesson.

7.4.2 Storage

Esmolol is stored at room temperature in a locked cabinet that is temperature-monitored in the CRC. A special thermometer that records high and low temperatures will monitor the temperature in the storage area. The thermometer is checked on a regular basis by staff in the lab. The CRC is locked when unoccupied.

7.4.3 Preparation and Dispensing

A CRC physician or CRNP will calculate the required esmolol dose, configure the IV pump settings and monitor the subject during infusion. A CRC nurse will perform a double check of the calculated esmolol dose and IV pump settings prior to infusion. The amount of Esmolol used for each study/participant will be recorded on the study protocol flow sheet.

7.4.4 Return or Destruction of the Test Article

Any Esmolol bags (empty or partially used) remaining after completion of a study visit will be disposed on site.

7.4.5 Prior and Concomitant Therapy

A list of prior and current medications will be obtained during the screening visit as part of the medical history. Subjects will be asked to withhold all over-the-counter medications (e.g. motrin, cold medicine) for 24 hours prior to each study and during the study procedures**.** ALL participants will review their prescribed medications, including beta-blockers, with one of the research nurses. This information will be shared with the CRC clinician who will then recommend which medications can be taken prior to the patient's scheduled study visit, and which ones should be withheld until after the study. All medications consumed by the participants will be written on the flow sheet.

8.0 Subject Numbers and Statistical Plan

8.1 Number of Subjects

We plan to enroll no more than 40 subjects (20 subjects per group) in this study. We plan to complete data collection on 13 young women (age 21-35) and 13 older postmenopausal women (age 55-70).

8.2 Sample size determination

$\Delta\%\Delta SmO_2$ (difference between $\%\Delta SmO_2$ during saline and esmolol trials) in response to moderate-intensity cycling exercise is our primary outcome. We do not have any pilot data pertaining to Esmolol infusion and exercise in postmenopausal women. However, using data from our recent publication, older adults (65.0 $\pm$ 2.6 years, n=3) displayed a greater $\Delta\%\Delta SmO_2$ compared to their young (25.4 $\pm$ 1.9 years, n=5) counterparts (22.0 $\pm$ 4.0 % vs 16.2

± 4.0 %)[20]. Considering this prior data, we will consider the difference between the pre-to-post Esmolol change and the pre-to-post placebo change (i.e., delta delta) of 5.8% to be clinically relevant[20]. Furthermore, we assume the variability (i.e., standard deviation) will be 4.78%, the standard deviation seen from this previous data[20]. We also assume there will be no carryover effect. Given these assumptions, a sample size of 11 subjects per group will provide 80% power to detect a difference in the change in $\%\Delta SmO_2$ between Esmolol and placebo, assuming a standard deviation of 4.78%, using a two-sided test having a significance level of 0.05. To account for differences in subject population and exercise protocol between that study and this protocol, we plan to complete data collection on 13 subjects per group to ensure sufficient power. However, we expect a small number of individuals may not complete the study. Therefore, we plan to enroll up to 20 subjects per group for a total of no more than 40 subjects so that 13 subjects per group may complete the study.

8.3 Statistical methods

Changes in outcomes will be calculated per patient prior to analyses. The data will be analyzed using repeated measures ANOVA, to account for the crossover design, with appropriate contrasts constructed to test hypotheses of interest (e.g., premenopausal vs postmenopausal). Additionally, where applicable, ANOVA will be used to assess differences between the groups for continuous outcomes (e.g., $\mathbf{\Delta\%\Delta SmO_2}$). As this is a relatively small-scale exploratory study, no corrections for multiple hypothesis testing will be employed.

9.0 Data and Safety Monitoring Plan

9.1 Periodic evaluation of data

Dr. Proctor will discuss the study with the research team at regularly scheduled lab meetings. If necessary, he will conduct additional special meetings with relevant personnel if there is an immediate, specific issue that must be addressed (e.g. untoward events). It should also be noted that safety is constantly monitored in our laboratory because our studies involve beat-by-beat monitoring of physiological variables. Any concerns are immediately reviewed by the PI. Adverse events are not anticipated, but any occurring will be documented and reported according to HSPO policies and procedures. The PI will be solely responsible for data collection and verification, and review of cumulative adverse events.

9.2 Data that are reviewed

Relevant data safety and management procedures, interim data evaluation, untoward events (rare in this research), new developments in related research, problems with workflow, and quality control issues will be discussed.

9.3 Method of collection of safety information

Lab members keep lab notebooks into which they note relevant safety issues and data that they identify for discussion. In the case of untoward events, those lab members present during the event are included in a debriefing and notes of the meeting are taken. The participant involved in an untoward event is interviewed in person or via telephone concerning the event and the responses are included into the notes for that event.

9.4 Frequency of data collection

Data collection and note taking starts when the subject arrives at the laboratory for their study visit and continues until the subject leaves the laboratory. Safety is constantly monitored during every laboratory visit and is documented in the subject's chart.

9.5 Individuals reviewing the data

The PI and research team will review the data on a regular basis. The Data and Safety Monitoring Board (DSMB), composed of 3 independent investigators, will meet as required. The PI is not a member of the DSMB but will be present at the meetings. The DSMB Chair is Dr. Gilchrist.

9.6 Frequency of review of cumulative data

Subject data are analyzed by Dr. Proctor's graduate student within two weeks of each study visit. Summary data are reviewed at least once per month to ensure the protocol and measurements are appropriate. Safety is

constantly monitored during the trials and any concerns are brought to the attention of the PI immediately. We do not wait for a cumulative review to assess safety.

9.7 Statistical tests

Subject data are analyzed by the PI within one week of the experimental visit. Summary data are reviewed at least once per month to ensure the protocol and measurements are appropriate. Safety is constantly monitored during the trials and any concerns are brought to the attention of the PI immediately. We do not wait for a cumulative review to assess safety.

9.8 Suspension of research

In the case of an adverse event, research is suspended while evaluation, reporting, and rectification proceeds. Research is restored when the IRB and the DSMB determine that a rectification of the problem is satisfactory.

10.0 Risks

1. **Confidentiality**: There is a risk of loss of confidentiality if medical information or identity are obtained by someone other than the investigators, but precautions will be taken to prevent this from happening.

2. **Pregnancy**: It is important that a fetus not be exposed to any unnecessary risks; therefore, pregnant women will not be studied. A pregnancy test will be administered at the beginning of the study visit to all women of childbearing potential to ensure they are not pregnant.

3. **Blood pressure, heart rate:** Minimal risk of skin irritation from EKG patches.

4. **PhysioFlow**:

5. **Oxygen Consumption:** No Risk.

6. **IV placement:** The discomfort associated with a blood draw/IV placement is a slight pinch or pinprick when the sterile needle enters the skin. The risks of a blood draw include mild discomfort and/or a black and blue mark at the puncture site. Less common risks include infection or bleeding at the puncture site, or on rare occasions, dizziness, light-headedness, nausea or fainting during the procedure. To minimize the risk of infection, aseptic techniques will be used.
7. **Esmolol infusion:** The risks stated by the manufacturer include heart block, hypotension, injection site pain, nausea, seizure, bronchospasm, allergic reaction, infusion site reaction, or extravasation (leaking of infusion into tissue around the IV site which may cause tissue death). However, in our experience these symptoms have never occurred in our research participants. The infusion will end if HR falls by more than 20 beats/min or below 40 beats/min or symptoms occur, or if symptomatic hypotension occurs (mean blood pressure drop of more than 20mmHg and/or symptoms).
   If extravasation would occur, we would proceed as follows:
   1. Stop infusion
   2. Apply warm compress over the catheter site
   3. Elevate the arm
   4. Observe/monitor for at least 45 minutes (i.e. 5 half-lives for Esmolol)
   5. Call subject within 24 hours

8. **Saline infusion:** no risk

9. **Skinfold of the forearm and leg to measure fat thickness**: No risk

10. **Recumbent cycling exercise:** Cycling exercise will cause fatigue, shortness of breath, elevated body temperature and muscle fatigue. Could also cause chest discomfort and/or dehydration.

11. **Isometric handgrip exercise**: Handgrip exercise will cause fatigue in the forearm. This may be associated with a burning sensation in the forearm and possible muscle soreness after the test.

12. **Post exercise occlusion:** The upper arm and upper leg cuff occlusions may result in a tingling or burning sensation in the limbs. This will subside once the cuffs are released.

13. **NIRS**: No risk

14. **DEXA**: There is a very small chance of cancer from excessive exposure to radiation. However, given the small amount of radiation used, this is very unlikely.

15. **Blood Lactate:** A slight pinch or pinprick when the sterile needle enters the skin. The risks include mild discomfort and/or a black and blue mark at the puncture site. Less common risks include infection or bleeding at the puncture site, or on rare occasions, dizziness, light-headedness, nausea or fainting during the procedure. To minimize the risk of infection, aseptic techniques will be used.

16. **PhysioFlow:** Preparation for the PhysioFlow device requires rubbing the skin where patches will be applied with an abrasive gel (Nuprep) that will cause some skin irritation and may cause mild inflammation.

17. **Order of measurements:** There are no additional risks to omitting or changing the order of the hemodynamic measurements in this study.

18. **Risk of incidental finding:** There is a rare risk of an incidental finding. The testing performed during this study is intended solely for research purposes and will not be utilized to detect any medical condition. However, if we notice something unusual, we will consult one of the cardiologists on the research team to determine if it merits follow-up. If so, we will contact the subject within 48 hours and suggest that the subject follow up with their private medical provider. To facilitate follow-up care, a copy of the data may be provided upon request. The subject will be monitored continuously for adverse effects and the medical personnel will remain in the room during the study.

19. There may be risks that are currently unknown.

11.0 Potential Benefits to Subjects and Others

11.1 Potential Benefits to Subjects

None.

11.2 Potential Benefits to Others

The knowledge gained in this project is likely to have important scientific and clinical implications. We expect these studies will provide important insight into blood pressure regulation and exercise tolerance in women.

12.0 Sharing Results with Subjects

Our studies are not intended to diagnose or treat a medical condition. If any of the results from the study are concerning, one of the research nurses will consult one of the CRC clinicians and then advise the subject to consult their own physician and take along a copy of the results.

13.0 Subject Payment and/or Travel Reimbursements

Visit 1) None
Visit 2) $50

Visit 3) $100

14.0 Economic Burden to Subjects

14.1 Costs

The subjects will not be charged for any of the research procedures included in this study.

14.2 Compensation for research-related injury

It is the policy of the institution to provide neither financial compensation nor free medical treatment for research-related injury. In the event of injury resulting from this research, medical treatment is available but will be provided at the usual charge. Costs for the treatment of research-related injuries will be charged to subjects or their insurance carriers.

15.0 Resources Available

15.1 Facilities and locations

201 Noll Laboratory and the Clinical Research Center (Located in Noll Laboratory, Elmore Wing).

124 Noll Laboratory (Biological Specimen Processing and Storage Laboratory): This lab is divided into several workstations for processing and long-term storage of blood samples and includes: six -20 freezers, 1 refrigerator, 1 Forma Scientific Centrifuge, 1 IEC Centra CL2 centrifuge, and a temperature-controlled Eppendorf 5804-R centrifuge.

All screening/familiarization and study visits will take place in Noll Laboratory's Integrative Vascular Physiology Lab (201 Noll Laboratory) and Clinical Research Center (CRC). All blood sampling (except lactate analysis) will be performed at the CRC. All other experimental procedures will be performed in the Vascular Aging and Exercise Lab.

15.2 Feasibility of recruiting the required number of subjects

Dr. Proctor has more than 20 years of experience screening and recruiting younger and older female subjects for cardiovascular exercise studies including invasive catheterization. Our lab has successfully recruited the required number of premenopausal and postmenopausal women utilizing the same recruitment methods.

15.3 PI Time devoted to conducting the research

The PI will devote approximately 20% effort to this project.

15.4 Availability of medical or psychological resources

These studies will be conducted on the 2nd floor of Noll laboratory. A CRC clinician will be present during infusion and the CRC is located nearby should medical attention be required.

15.5 Process for informing Study Team

The PI will review the protocol with all members of the study team and will communicate regularly in person and through email.

16.0 Other Approvals

16.1 Other Approvals from External Entities

We have received an IND exemption for Esmolol infusion from the FDA.

16.2 Internal PSU Committee Approvals

Check all that apply:

☐ Anatomic Pathology – Hershey only – Research involves the collection of tissues or use of pathologic specimens. Upload a copy of HRP-902 - Human Tissue For Research Form on the "Supporting Documents" page in CATS IRB. This form is available in the CATS IRB Library.

☐ Animal Care and Use – All campuses – Human research involves animals and humans or the use of human tissues in animals

☒ Biosafety – All campuses – Research involves biohazardous materials (human biological specimens in a PSU research lab, biological toxins, carcinogens, infectious agents, recombinant viruses or DNA or gene therapy).

☐ Clinical Laboratories – Hershey only – Collection, processing and/or storage of extra tubes of body fluid specimens for research purposes by the Clinical Laboratories; and/or use of body fluids that had been collected for clinical purposes, but are no longer needed for clinical use. Upload a copy of HRP-901 - Human Body Fluids for Research Form on the "Supporting Documents" page in CATS IRB. This form is available in the CATS IRB Library.

☒ Clinical Research Center (CRC) Advisory Committee – All campuses – Research involves the use of CRC services in any way.

☐ Conflict of Interest Review – All campuses – Research has one or more of study team members indicated as having a financial interest.

☐ Radiation Safety – Hershey only – Research involves research-related radiation procedures. All research involving radiation procedures (standard of care and/or research-related) must upload a copy of HRP-903 - Radiation Review Form on the "Supporting Documents" page in CATS IRB. This form is available in the CATS IRB Library.

☐ IND/IDE Audit – All campuses – Research in which the PSU researcher holds the IND or IDE or intends to hold the IND or IDE.

☐ Scientific Review – Hershey only – All investigator-written research studies requiring review by the convened IRB must provide documentation of scientific review with the IRB submission. The scientific review requirement may be fulfilled by one of the following: (1) external peer-review process; (2) department/institute scientific review committee; or (3) scientific review by the Clinical Research Center Advisory committee. NOTE: Review by the Penn State Hershey Cancer Institute Scientific Review Committee is required if the study involves cancer prevention studies or cancer patients, records and/or tissues. For more information about this requirement see the IRB website at: http://www.pennstatehershey.org/web/irb/home/resources/investigator

17.0 Multi-Site Research

N/A

18.0 Adverse Event Reporting

18.1 Reporting Adverse Reactions and Unanticipated Problems to the Responsible IRB

In accordance with applicable policies of The Pennsylvania State University Institutional Review Board (IRB), the investigator will report, to the IRB, any observed or reported harm (adverse event) experienced by a subject or other individual, which in the opinion of the investigator is determined to be (1) unexpected; and (2) probably related to the research procedures. Harms (adverse events) will be submitted to the IRB in accordance with the IRB policies and procedures.

19.0 Study Monitoring, Auditing and Inspecting

19.1 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the Penn State quality assurance program office(s), IRB, the sponsor, and government regulatory bodies, of all study related documents (e.g., source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g., pharmacy, diagnostic laboratory, etc.).

20.0 Future Undetermined Research: Data and Specimen Banking

Not applicable. All specimens will be discarded after assay.

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22.0 Confidentiality, Privacy and Data Management

22.1 Which of the following identifiers will be recorded for the research project? Check all that apply. If none of the following identifiers will be recorded, do not check any of the boxes.

	Hard Copy Data	Electronic Stored Data
Names and/or initials (including on signed consent documents)	☒	☒
All geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code, and their equivalent geocodes,	☒	☒
All elements of dates (except year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death; and all ages over 89 and all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 or older	☒	☒
Telephone numbers	☒	☒
Fax numbers	☐	☐
Electronic mail addresses	☒	☒
Social security numbers	☒	☒
Medical record numbers	☐	☐
Health plan beneficiary numbers	☐	☐
Account numbers	☐	☐
Certificate/license numbers	☐	☐
Vehicle identifiers and serial numbers, including license plate numbers	☐	☐
Device identifiers and serial numbers	☐	☐
Web Universal Resource Locators (URLs)	☐	☐
Internet Protocol (IP) address numbers	☐	☐
Biometric identifiers, including finger and voice prints	☐	☐
Full face photographic images and any comparable images	☐	☐
Any other unique identifying number, characteristic, or code (such as the pathology number)	☒	☒
Study code number with linking list	☒	☒
Genomic sequence data	☐	☐
State ID numbers	☐	☐
Passport numbers	☐	☐
Driver's license numbers	☐	☐

22.2 If storing paper records of research data, answer the following questions:

22.2.1 Where will the paper records, including copies of signed consent forms, associated with this research study will be stored?

Noll 201

22.2.2 How will the paper records be secured?

Locked in filing cabinets

22.2.3 How will access to the paper records be restricted to authorized project personnel?

Only member of the Integrative and Vascular Physiology Laboratory will have access to the key for the locked filing cabinets.

22.3 If storing electronic records of research data, indicate where the electronic data associated with this research study will be stored. Check all that apply.

☒ Penn State-provided database application. Check which of the following database applications are being used (check all that apply):

☐ Penn State REDCap
☐ Other – Specify - provided and approved database application:

☒ Penn State, College, or Department IT file server
☐ Box.psu.edu (To be retired Sept. 2021; see https://storage.psu.edu/)
☐ Web-based system provided by the sponsor or cooperative group - Specify URL and contact information:

☐ Other – Specify the database application or server:

Provide details about the data security features or attach security documentation provided by sponsor or group:

22.4 Is there a list/key that links code numbers to identifiers?

☒ Yes - explain how the list that links the code to identifiers is stored separately from coded data:
The list linking the code to identifiers is maintained by the CRC.

☐ Not applicable, there is no list that links code numbers to identifiers. Skip to section 22.6.

22.5 Is there a list of people who have access to the list/key?

☒ Yes – explain how access to that list is restricted and why certain persons require access.
Access to the list is maintained by the CRC

☐ No – explain why not:

22.6 Describe the mechanism in place to ensure only approved research personnel have access to the stored research data (electronic and paper).

☒ Password-protected files
☒ Role-based security
☐ Specify all other mechanisms used to ensure only permitted users have access to the stored research data.

22.7 Will any research data (such as survey data) be collected on a mobile device, such as an electronic tablet, cell phone, or wireless activity tracker?

☒ No
☐ Yes - answer the following questions:

22.7.1 Specify the provider of the mobile devices(s)

☐ Supplied by the sponsor
☐ Penn State owned device
☐ A personal device
☐ Other – Please specify source:

22.7.2 Specify the type(s) of mobile device(s) that will be used to capture data and all identifiers captured on the mobile device(s). Please list all devices, and if more than one, the identifiers to be collected on each.

22.7.3 Specify the type of data collected on the mobile devices(s).

22.7.4 Specify the application or website used to collect the data from the mobile device, if applicable.

22.7.5 Describe the measures taken to protect the confidentiality of the data collected on mobile device(s). Please address physical security of the device(s), electronic security, and secure transfer of data from device(s) to the previously indicated data/file storage location provided in section 22.3.

22.8 Will any research data be directly entered/sent by subjects over the internet or via email (e.g., data capture using on-line surveys/questionnaires, surveys via email, observation of chat rooms or blogs)?

☒ No
☐ Yes - answer the following questions:

22.8.1 Specify the identifiers collected over the internet or via email (Including IP addresses if IP addresses will be collected).

22.8.2 Specify the type of data collected over the internet or via email.

22.8.3 Describe the measures taken to protect the confidentiality of the data collected?

22.8.4 Describe how the research team will access the data once data collection is complete.

22.8.5 If the research involves online surveys, list the name(s) of the service provider(s) that will be used for the survey(s) (e.g., REDCap, Penn State licensed Qualtrics, Survey Monkey, Zoomerang)? (Note: The IRB strongly recommends the use of REDCap for online surveys that obtain sensitive identifiable human subjects data.)

☐ Penn State REDCap
☐ Penn State Qualtrics (de-identified data only)
☐ Other - Please specify:
Application:
URL (If applicable):

22.8.6 If the answer above is "Other" contact security@psu.edu for approval of an alternative data capture method

22.9 Will any type of recordings (e.g., audio or video) or photographs of the subjects be made during this study?

☒ No - skip to section 22.10
☐ Yes - answer the following questions:

22.9.1 What will be used to capture the audio/video/images? Give a brief description of content.

☐ Audio – Describe the intended content of the audio recording:

☐ Video – Describe the intended content of the video recording:

☐ Photographs of the subjects – Describe the intended content of the photographs:

☐ 3-D Images – Describe the intended content of the of 3-D images:

☐ Other - Specify:

22.9.2 How will the recordings/photographs/images be stored (electronically or physically)?

22.9.3 Where will the recordings/photographs/images be stored?

22.9.4 Who will have access to the recordings/photographs/images?

22.9.5 Will any of the recordings be transcribed?

☐ Not applicable
☐ No
☐ Yes – indicate who will be doing the transcribing?

22.9.6 Will the recordings/photographs be used for purposes other than this research study?

☐ No
☐ Yes - specify purpose(s) (e.g., publication, presentations, educational training, future undetermined research):

22.10 Certificate of Confidentiality (COC) - Is the research biomedical, behavioral, clinical or other research that is funded by the National Institutes of Health (NIH)?

☒ Yes - check one of the following:
- ☒ The research involves human subjects as defined by the DHHS regulations (See Worksheet HRP-310).
- ☐ The research involves collecting or using biospecimens that are identifiable to an individual.
- ☐ If collecting or using biospecimens as part of the research, there is a small risk that some combination of the biospecimen, a request for the biospecimen, and other available data sources could be used to deduce the identity of an individual.
- ☐ The research involves the generation of individual level, human genomic data.

Note: If <u>any</u> of the 4 items above are checked, a COC is automatically issued by NIH and applies to the research. Information about the COC must be included in the consent form.

☐ No - answer the following question.

If the research is not funded by NIH, will the investigator apply for a COC for this research study?

☐ No
☐ Yes

Note: For research not funded by NIH, the IRB may require a COC if the research is collecting personally identifiable information and the information is sensitive and/or the research is collecting information that if disclosed could significantly harm or damage the subject.

22.11 What steps will be taken to protect subjects' privacy interests? (Check all that apply.)

☒ Identification and recruitment of potential subjects follows procedures consistent with privacy standards
☒ Consent discussion and research interventions will take place in a private setting
☒ Limiting the information being collected to only the minimum amount of data necessary to accomplish the research purposes
☒ Limiting the people with access to the identifiable research data to the minimum necessary as specified in the application and consent process
☐ Other – Specify:

22.12 What is the process for ensuring correctness of data entry?

☐ Double data entry to reduce risk of errors
☒ Electronic edit checks to ensure data being entered are not obviously incorrect
☐ Random internal quality and assurance checking of research data
☐ Direct entry by subjects
☐ Other - Specify:

22.13 Does this research involve the generation of large-scale human genomic data as defined in NIH Genomic Data Sharing Policy (http://gds.nih.gov)?

☒ No
☐ Yes – If Yes, describe the plan for de-identifying the dataset before sharing it with NIH-designated data repositories.

22.14 The European Union (EU) General Data Protection Regulation (GDPR)

22.14.1 To determine if the research is subject to the GDPR answer the following questions:

22.14.1.1 Will the Penn State principal investigator, or another entity under the Penn State principal investigator's direction, be collecting, recording, storing, using, any personal data* of any subjects physically located in the European Economic Area (EEA) at the time of data collection (even if the subject is NOT an EEA resident) or any EEA citizens? (This includes recruitment through social media sites, use of third party internet sites,**

mobile devices or apps to collect data, and/or direct receipt of data from subjects.)

☒ No
☐ Yes (This research may be subject to the GDPR)

22.14.1.2 Does this research involve the transfer of personal data collected under the GDPR from an EEA country? (This includes direct transfer of data from research collaborators.)

☒ No
☐ Yes (This research may be subject to the GDPR)

22.14.2 If the research may be subject to the GDPR as indicated in the answers to the questions above, answer the following:

22.14.2.1 Will any of the data fall into one of the following categories: health data, racial or ethnic origin, political opinions, religious or philosophical beliefs, trade union membership, genetic data, biometric data used for purpose of identifying an individual, sex life or sexual orientation?

☐ No
☐ Yes

22.14.2.2 Will any of the data be related to criminal convictions or offenses?

☐ No
☐ Yes

Comments on any of the above responses:

* "Personal data" means any information relating to an identified or identifiable natural person; an identifiable natural person is one who can be identified, directly or indirectly, by reference to an identifier such as a name, an identification number, location data, an online identifier or to one or more factors specific to the physical, physiological, genetic, mental, economic, cultural or social identity of that natural person.

** European Economic Area (EEA) – Includes the 28-member states of the European Union (Austria, Belgium, Bulgaria, Croatia, Republic of Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia Spain, Sweden, UK) and Norway, Iceland, Lichtenstein.

22.15 Does this research involve transfer or disclosure of data and/or specimens to and/or from Penn State?

☒ No - skip the rest of section 22.15.
☐ Yes - answer the following questions.

Check all that apply:

☐ **Data** are being transferred or disclosed **to** Penn State

What is the name of the third party(ies) (the institution, sponsor, etc.) sending or providing the data?

Is the third party requiring us to sign a contract regarding the data?

☐ Yes - If Yes, this contract must go through the Office of Sponsored Programs
https://www.research.psu.edu/osp/overview-pages/data-use-agreements

☐ No

☐ **Data** are being transferred or disclosed **from** Penn State

What is the name(s) of the third party(ies) (the institution, sponsor, etc.) receiving or accessing the data?

Note: Data transfers or disclosures may require a Data Use Agreement (DUA).

☐ **Specimens** are being transferred **to** Penn State

What is the name(s) of the third party(ies) (the institution, sponsor, etc.) sending the specimens?

☐ **Specimens** are being transferred **from** Penn State

What is the name(s) of the third party(ies) (the institution, sponsor, etc.) receiving the specimens?

Note: All material transfers, either sending or receiving, require a Material Transfer Agreement (MTA). Please contact the Office of Technology Management for more information.

22.15.1 Describe how the data/specimens will be securely transferred or disclosed to/from the third party(ies).

22.15.2 How are the research data/specimens being transferred from and/or sent to the third party(ies)? Complete the appropriate section(s) and check all that apply within each completed section.

22.15.2.1 Data being transferred or disclosed **to** Penn State**:**

☐ Data are being received in aggregate/metrics (just counts, no individual data)

☐ De-identified individual data are being received and there is no linking list at either institution (no identifiers, or links to identifiers, such as code numbers)

☐ Coded research data without any identifiers are being received and the linking list remains with the entity sending the data; the recipient of the data will not have access to the linking list

☐ Coded research data with identifiers (such as dates and/or any of the identifiers listed in section 22.15.3 aside from Study Code) are being received and the linking list remains with the entity sending the data; the recipient of the data will not have access to the linking list

☐ Data with identifiers (such as dates and/or any of the identifiers listed in section 22.15.3) are being received and the linking list remains with the entity sending the data; the recipient of the data will have access to the linking list

☐ Data with identifiers along with the linking list are being received

☐ Other – Specify:

22.15.2.2 Data being transferred or disclosed **from** Penn State**:**

- ☐ Data are being sent in aggregate/metrics (just counts, no individual data)
- ☐ De-identified individual data are being sent and there is no linking list at either institution (no identifiers, or links to identifiers, such as code numbers)
- ☐ Coded research data without any identifiers are being sent and the linking list remains with the entity sending the data; the recipient of the data will not have access to the linking list
- ☐ Coded research data with identifiers (such as dates and/or any of the identifiers listed in section 22.15.3 aside from Study Code) are being sent and the linking list remains with the entity sending the data; the recipient of the data will not have access to the linking list
- ☐ Data with identifiers (such as dates and/or any of the identifiers listed in section 22.15.3) are being sent and the linking list remains with the entity sending the data; the recipient of the data will have access to the linking list
- ☐ Data with identifiers along with the linking list are being sent
- ☐ Other – Specify:

22.15.2.3 Specimens being transferred or disclosed **to** Penn State**:**

- ☐ De-identified specimens are being received and there is no linking list at either institution (no identifiers, or links to identifiers, such as code numbers)
- ☐ Coded specimens without any identifiers are being received and the linking list remains with the entity sending the specimens; the recipient of the specimens will not have access to the linking list
- ☐ Coded specimens with identifiers (such as dates and/or any of the identifiers listed in section 22.15.3 aside from Study Code) are being received and the linking list remains with the entity sending the specimens; the recipient of the specimens will not have access to the linking list
- ☐ Coded specimens with identifiers (such as dates and/or any of the identifiers listed in section 22.15.3) are being received and the linking list remains with the entity sending the specimens; the recipient of the specimens will have access to the linking list
- ☐ Coded specimens with identifiers along with the linking list are being received
- ☐ Other – Specify:

22.15.2.4 Specimens being transferred or disclosed **from** Penn State**:**

- ☐ De-identified specimens are being sent and there is no linking list at either institution (no identifiers, or links to identifiers, such as code numbers)
- ☐ Coded specimens without any identifiers are being sent and the linking list remains with the entity sending the specimens; the recipient of the specimens will not have access to the linking list
- ☐ Coded specimens with identifiers (such as dates and/or any of the identifiers listed in section 22.15.3 aside from Study Code) are being

sent and the linking list remains with the entity sending the specimens; the recipient of the specimens will not have access to the linking list

☐ Coded specimens with identifiers (such as dates and/or any of the identifiers listed in section 22.15.3) are being sent and the linking list remains with the entity sending the specimens; the recipient of the specimens will have access to the linking list

☐ Coded specimens with identifiers along with the linking list are being sent

☐ Other – Specify:

22.15.3 If transferring data/specimens with identifiers to or from Penn State, which of the following identifiers will be included with the data/specimens? Check all that apply:

☐ Names	☐ Medical record numbers
☐ Initials	☐ Health plan beneficiary numbers
☐ Street address	☐ Account numbers
☐ City	☐ Certificate/license numbers
☐ Driver's License numbers	☐ Passport numbers
☐ State	☐ State ID numbers
☐ Zip Codes	☐ Vehicle identifiers and serial numbers, including license plate numbers
☐ County	☐ Device identifiers and serial numbers
☐ Geocodes	☐ Web Universal Resource Locators (URLs)
☐ Precincts	☐ Internet Protocol (IP) address numbers
☐ All elements of dates (except year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death	☐ Biometric identifiers, including finger and voice prints
☐ Ages > 89 and all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 or older	☐ Full face photographic images and any comparable images
☐ Telephone numbers	☐ Any other unique identifying number, characteristic, or code (such as the pathology number) Specify:
☐ Fax numbers	☐ Study code numbers
☐ Electronic mail addresses	☐ Master list linking study code numbers to subject(s)
☐ Social security numbers	☐ Genomic sequence data
	☐ Other – specify: