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Sex Differences in Chronic and Acute Vascular Responses to Aerobic Exercise in Older Adults

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Sex Differences in Chronic and Acute Vascular Responses to Aerobic Exercise in Older Adults

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3. Abstract

Cardiovascular disease (CVD) is the leading cause of death in the U.S. in both men and women. Although biological sex is an important determinant of CVD pathophysiology, a major knowledge gap remains regarding sex differences in CVD prevention and treatment. A key early event in CVD development is endothelial dysfunction, characterized by impaired flow-mediated dilation (FMD). Aging progressively impairs endothelial function which independently predicts CVD progression and events. Therefore, strategies to optimize endothelial function in aging are clinically important. Regular aerobic exercise ameliorates endothelial dysfunction in healthy older men, but the data in healthy postmenopausal women are inconsistent with many studies showing no effect. The mechanisms underlying these sex disparities are unknown. Higher intensity exercise is thought to be required for chronic endothelial adaptations in postmenopausal women, but this has not been investigated. We have developed a novel exercise strategy by adapting high-intensity interval training (HIIT) on a non-weight-bearing all-extremity (NWA) ergometer. We have demonstrated that our innovative NWA-HIIT is feasible and safe in older adults. The primary objective of this study is to examine sex differences in acute and chronic endothelial responses to NWA-HIIT in older men vs. postmenopausal women. Our overarching hypothesis is that acute and chronic endothelial responsiveness to aerobic exercise is influenced by sex in healthy aging and acute endothelial responses to exercise will be predictive of chronic endothelial adaptations.

4. Background

Cardiovascular disease (CVD) is the leading cause of death in the U.S. in both men and women. Although biological sex is increasingly recognized as an important determinant of CVD development, progression and outcomes, a major gap remains regarding sex differences in CVD pathophysiology, prevention and treatment. A key early event in CVD development is endothelial dysfunction, characterized by impairments in flow-mediated dilation (FMD)[1, 2]. In women, the onset of endothelial dysfunction begins a decade later than in men, but the rate of decline is steeper[3]. Aging progressively impairs endothelial function which independently predicts CVD progression and events[4]. Therefore, strategies to optimize endothelial function in aging are clinically important but should account for the potential impact of sex.

Aerobic exercise is often prescribed to reduce CVD risks; however, controversy exists regarding the optimal exercise prescription for preserving or restoring endothelial function in older adults. Moderate-intensity aerobic exercise ameliorates endothelial dysfunction in older men, but the data in postmenopausal women are inconsistent, with most studies showing no effect (reviewed in Seals et al[5]). The mechanisms underlying these sex disparities in healthy aging are unknown. A recently introduced theory of exercise-induced hormesis proposes that an acute exercise bout that generates an optimal level of stress can stimulate adaptations to increase resistance against subsequent stressors; if the exercise-induced stress does not reach this threshold, then adaptation will not occur. Consistent with this theory, higher exercise intensity is thought to be required to induce chronic endothelial adaptations in postmenopausal women, but this has not been

investigated. High intensity interval training (HIIT) is receiving considerable attention as an innovative strategy for cardiac rehabilitation, but data in sedentary aging are limited [6, 7]. HIIT involves short periods of vigorous exercise, mainly "uphill" treadmill walking, interspersed by periods of moderate-intensity exercise. However, weight-bearing HIIT is often problematic in older adults due to musculoskeletal issues. *We have recently developed a novel alternative by adapting HIIT to a non-weight-bearing all-extremity (NWA) ergometer; our innovative NWA-HIIT allows compensation for lower extremity weakness/fatigue.* We have established in sedentary older adults that NWA-HIIT is feasible, well-tolerated and safe[8]. Our preliminary data suggest that 8-week NWA-HIIT improves endothelial function in older men, but not in postmenopausal women. Our preliminary studies also indicate substantial sex differences in acute endothelial responsiveness to HIIT; there is a transient attenuation in endothelial function in older men following an acute bout of HIIT, but this is absent in postmenopausal women. We postulate that in men, NWA-HIIT presents an optimal challenge to endothelial homeostasis thereby initiating chronic adaptive responses; in contrast, the stimulus is inadequate for chronic adaptations in postmenopausal women. *Accordingly, acute responses to exercise may provide keys to understanding sex-related differences in long-term endothelial adaptive responses to exercise.*

Our overarching hypothesis is that acute and chronic endothelial responsiveness to aerobic exercise is influenced by sex in healthy aging and acute endothelial responses will be predictive of chronic endothelial adaptations. Our experimental model of acute and chronic responses to NWA-HIIT presents a unique tool to test this hypothesis and explore the underlying mechanisms in healthy sedentary older adults. *Our translational approach will examine, for the first time, sex differences in endothelial responses to exercise at multiple levels spanning from the molecular and cellular to the organism level. Identifying the defective adaptive pathways in women may lead to novel therapeutic targets.*

5. Specific Aims

1. To study sex differences in chronic endothelial adaptations to aerobic exercise

2. To study sex differences in acute endothelial responses to aerobic exercise and if acute endothelial responses are predictive of chronic endothelial adaptations.

6. Research Plan

***Study design and timing of measures*.** An equal number of older men and postmenopausal women will be enrolled in this study and the same subjects will complete Aims 1 and 2. For Aim 1, subjects will complete 8-week NWA-HIIT preceded by an 8-week control period of normal lifestyle. Therefore, each subject will serve as his/her own control and the control period of normal lifestyle will also allow determination of potential changes due to measurement error. FMD and other measures associated with Aim 1 will be assessed at baseline, and after the 8-week control and 8-week NWA-HIIT (**Diagram 1**). FMD and other measures associated with Aim 2 will be assessed at pre-exercise, at the end of a single session of NWA-HIIT, and 1-hr and 24-hrs post-exercise (**Diagram 2**) in the untrained and trained state (**Diagram 1)**. Measures in the trained state for Aim 2 will be completed 72-96 hours after the last chronic exercise session.

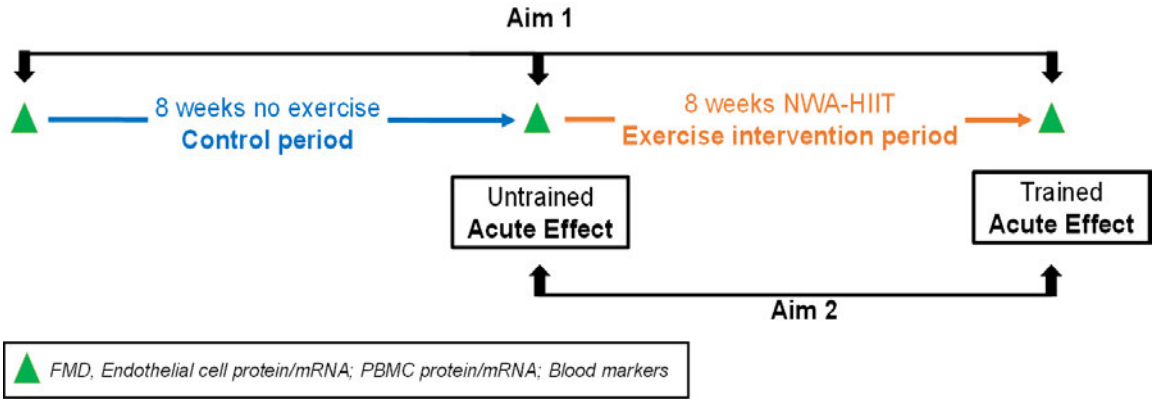

Diagram 1. Study timeline for Aims 1 and 2

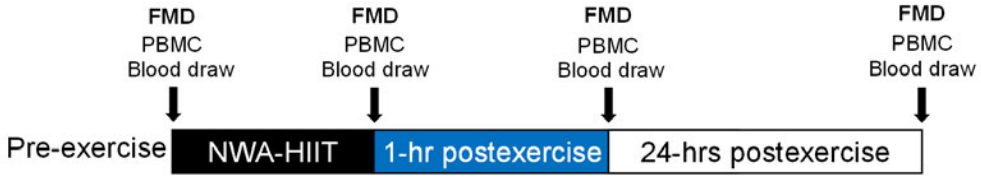

Diagram 2. Study timeline for Aim 2

Study procedures will be performed at the Integrative Cardiovascular Physiology Laboratory, Center for Exercise Science, University of Florida.

Exercise intervention. Our HIIT regimen is based on a treadmill protocol that has been shown to be safe and highly effective in improving CVD risks in patients with cardiometabolic disease[7, 9]. NWA-HIIT will be performed on an air-braked ergometer (AD7, Schwinn) 4 days/week, for 8 weeks, as we have previously published[8]. To mitigate the risk for COVID-19, exercise sessions will be performed at the participant's home and will be remotely supervised live via Zoom by a member of the Integrative Cardiovascular Physiology Laboratory. The participant will be provided the following to be used during the 8-week exercise training: 1) an exercise ergometer; 2) a Zephyr wearable device (Medtronic); 3) a tablet with the OmniSense 5.0 software (Medtronic) and Zoom and WiFi capability. Participants who do not have WiFi available at their home will be provided with a data plan. A familiarization session will be provided for using Zoom and the Zephyr wearable device and Omnisense software. The

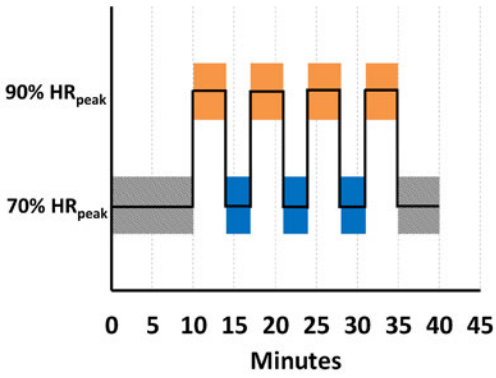

Diagram 3. NWA-HIIT exercise protocol

Zephyr Cloud technology allows real-time monitoring of physiological responses during exercise including heart rate, breathing rate, body temperature, caloric expenditure, and accelerometry. A member of the Integrative Cardiovascular Physiology Laboratory will lead each virtual exercise session and will guide the participants through the setup, warm up, work out and cool down.

NWA-HIIT will consist of 4x4-min bouts at 90% of maximal heart rate (HR_{max}) interspersed by 3x3-min bouts at 70% of HR_{max} (**Diagram 3**)[8]. A 10-min warm-up and 5-minute cool-down at 70% of HR_{max} will be included. HR_{max} will be determined during the baseline maximal exercise test. Chronic NWA-HIIT will be preceded by a period of familiarization as we have previously described[8]. Based on our extensive experience, on average 5±1 session is required in sedentary older adults to be able to complete an entire HIIT session. The total number of completed exercise sessions by each study participant will depend on their ability to perform all-extremity exercise, their initial fitness level and scheduling availability for the post-intervention procedures. The number of sessions will be recorded and incorporated in data analysis and interpretation.

Study Procedures for Aims 1 and 2

- Medical history. Subjects will complete a medical history form.
- Physical exam. A clinician on our team will perform a physical examination. This will consist of review of medical history and check of general physical condition including examination of the head and neck, heart, lungs, abdomen, nerves, muscles, joints and skin. The Allen test which evaluates blood circulation to the hands will be performed to screen out subjects for whom intra-arterial cannulation is contraindicated.
- 24-hour questionnaire. We will ask questions regarding sleep, food, drink, tobacco, alcohol, caffeine and medication intake and physical activity over the last 24 hours. We will also ask if subjects are experiencing any health problems (e.g., sore throat, head cold, upset stomach, etc.).
- Height. We will measure height using a ruler attached to a wall.
- Weight. We will measure body weight using an electronic scale.
- Heart rhythm. We will record heart rhythm.
- Blood pressure. We will measure blood pressure with a cuff around the ankles. We will also measure blood pressure with a cuff around the upper arms.
- Body composition and circumferences. We will measure waist and hip circumferences using a tape measure. We will also measure body composition including body fat and fat free weight, as well as bone density, using a non-invasive dual-energy x-ray absorptiometry (DXA) scan as we have previously published[8]. This test requires that subjects lie very still on a padded table that gives off low-level X-ray radiation. The "arm" of the machine will slowly pass over the body to make the measures. This should take less than 10 min. Female participants who are younger than 62 years of age and who have not had a hysterectomy, will complete a urine pregnancy test prior to the scan. The DXA scan will be prescribed by Dr. Carl Pepine from the Division of Cardiology.

    - To investigate the chronic effects of exercise on body composition and circumferences, these tests will be performed: 1) at baseline; 2) after 8 weeks of normal lifestyle; and 3) after 8 weeks of exercise training.

- Physical activity habits. We will ask questions regarding how hard, how often and for how long subjects perform a variety of physical activities. We will also ask subjects to wear an activity monitor over their hip for 4 days to track their activity.
    - These measures will be performed: 1) at baseline; 2) after 8 weeks of normal lifestyle; and 3) after 8 weeks of exercise training.
- Dietary habits. We will ask questions regarding what subjects ate and drank on the previous day.
    - This information will be collected: 1) at baseline; 2) after 8 weeks of normal lifestyle; and 3) after 8 weeks of exercise training.
- Quality of life questionnaire. We will ask questions about perceptions of health and ability to perform daily activities.
    - This information will be collected: 1) at baseline; 2) after 8 weeks of normal lifestyle; and 3) after 8 weeks of exercise training.
- Maximal exercise test on treadmill. An incremental maximal exercise test will be used to measure maximal oxygen consumption (VO_{2max}) on the treadmill and screen out subjects with cardiovascular problems as we have previously published [8]. ECG and blood pressure responses will be monitored by our experienced study clinicians and oversight will be provided by our cardiologist (Dr. Carl Pepine). VO_{2max} will be measured using open-circuit spirometry[8]. To measure oxygen consumption, subjects will breathe through a mouthpiece while wearing a nose clip. The treadmill will begin slowly and the speed (pace) will gradually increase based on their walking ability and their heart rate. Upon completion of a 6-min warmup, the speed will remain the same while the treadmill will slightly tilt every 2 min to produce the effect of going up a small hill until they reach their maximal exercise capacity (usually 12 to 15 min excluding the warm-up period). At certain times during the test, we will ask them to point to a scale which tells us how hard they feel they are working. If they have a problem walking on the treadmill they may perform the test on a stationary bicycle. If any abnormalities occur during the test, they will be referred to their primary care physician for further testing.
- Maximal exercise test on all-extremity cycle. This test will measure the maximal ability to perform all-extremity cycling. Heart rhythm, blood pressure and oxygen consumption will be measured throughout the test as described above. Subjects will complete a 6-min warmup by performing all-extremity cycling at a moderate pace. Upon completion of the warmup, the pace will gradually increase every 2 min until they reach their maximal exercise capacity (usually about 12 to 15 min excluding the warmup). Prior to this visit they will be given an opportunity to practice all-extremity cycling and breathing through a mouthpiece.

- To investigate the chronic effects of exercise on maximal exercise capacity, this test will be performed: 1) at baseline; 2) after 8 weeks of normal lifestyle; and 3) after 8 weeks of exercise training.

- 6-min walk test. The purpose of this test is to assess the ability to walk for 6 min. The test will be completed at the subject's pace, walking back and forth between 2 cones. The objective is to walk as far as possible in 6 min. They will be permitted to slow down, to stop, and to rest, as necessary.
    - To investigate the chronic effects of exercise on walking ability, this test will be performed: 1) at baseline; 2) after 8 weeks of normal lifestyle; and 3) after 8 weeks of exercise training.

- Blood vessel tests. Our primary outcome is brachial artery FMD, an established non-invasive measure of endothelial function[10]. FMD will be determined via ultrasonography as we have previously described[11, 12] and strictly conforming with established guidelines[13]. Arterial stiffness is known to influence FMD, therefore, it will be non-invasively measured using the SphygmoCor device as we have previously described [14]. Measures will be performed while subjects rest supine in a quiet, semi-darkened, temperature-controlled room. Individual subject data will be coded to ensure blinding during data analysis.
    - To investigate the chronic effects of exercise on blood vessel function, these tests will be performed: 1) at baseline; 2) after 8 weeks of normal lifestyle; and 3) after 8 weeks of exercise training.
    - To investigate the acute effects of HIIT on blood vessel function, these tests will be performed: 1) at rest; 2) at the end of a HIIT session; 3) after 1-hour recovery; and 4) after 24-hours recovery. To examine how chronic exercise training affects the acute responses of exercise, these tests will be performed at the beginning and end of the 8 weeks of exercise training.
    - To investigate the acute effects of maximal exercise on blood vessel function, these tests will be performed: 1) at rest; 2) at the end of the maximal exercise test on treadmill (described above); 3) at the end of the maximal exercise test on all-extremity cycle (described above); and after 1-hour recovery. To examine how chronic all-extremity exercise training affects the acute responses to maximal exercise test on all extremity cycle, these tests will be performed at the beginning and end of the 8 weeks of exercise training.

- Nitroglycerin test. Endothelium-independent dilation, a measure of vascular smooth muscle responsiveness to a nitric oxide donor (0.4 mg nitroglycerin sublingual tablet; an FDA approved drug which causes blood vessels to relax) will be determined as we have previously described [15]. Brachial artery images will be recorded using ultrasonography before nitroglycerin administration and for up to 10 min while subjects lie still on their back. Prior to administering nitroglycerin, an intravenous catheter will be in place and will remain for up to 30 min after taking the nitroglycerin pill.
    - To investigate the chronic effects of exercise on blood vessel function, the nitroglycerin test will be performed: 1) at baseline; 2) after 8 weeks of normal lifestyle; and 3) after 8 weeks of exercise training.

- Blood draw. The amount of blood drawn over study participation (6-7 months) will total 25 TBSP, which is less than a whole blood donation which is typically a pint. Subjects will be informed not to donate blood during study participation or within 2 months of completion.
    - To perform general blood tests including comprehensive metabolic panel, lipid panel, insulin and blood cell count with differential, a TBSP of blood will be collected: 1) at baseline; 2) after 8 weeks of normal lifestyle; and 3) after 8 weeks of exercise training.
    - To investigate the chronic effects of HIIT, 2 TBSP of blood will be collected to measure circulating markers of oxidative stress and inflammation and endothelial function, vasoconstrictor and sex hormones and to isolate peripheral blood mononuclear cells to investigate protein and gene expression of markers of oxidative stress and inflammation and endothelial function: 1) at baseline; 2) after 8 weeks of normal lifestyle; and 3) after 8 weeks of exercise training.
    - To investigate the acute effects of HIIT, 2 TBSP of blood will be collected to measure circulating markers of oxidative stress and inflammation and endothelial function and to isolate peripheral blood mononuclear cells to investigate protein and gene expression of markers of oxidative stress and inflammation and endothelial function: 1) at rest; 2) at the end of a HIIT session; 3) after 1-hour recovery from HIIT; and 4) after 24-hours recovery from HIIT. To examine how chronic exercise training affects the acute responses of exercise, these tests will be performed at the beginning and end of 8 weeks of exercise training.
- Endothelial cell collection and analysis***.*** We have the experience, resources, and access to experts to support the proposed endothelial cell measures. The proposed study will lay the foundation upon which we will base future studies focusing on dissecting *in vivo* the sex-specific effect of exercise on mechanisms of endothelial dysfunction. The procedures for studying endothelial cells have been described in detail by Colombo et al[16-18], and by our group [11, 19-22]. A clinician on our team from the Pulmonary and Critical Care Division, Cesar Trillo-Alvarez, MD, who has extensive experience with intra-arterial catheterizations (~1500 procedures over 15 years) will perform the procedure. He will place a standard 20 gauge arterial catheter with no sheath at the radial artery guided by ultrasonography after numbing the area with local lidocaine injection. No vasodilators will be used. Once the catheter is secure, four small (0.018-inch diameter) sterile J-shaped guidewires will be sequentially advanced (~4 inches) through the radial arterial catheter and retracted to collect a small number of endothelial cells. Following the cell collection and blood draw, the catheter will be removed and hemostasis will be achieved by manual compression of the site. The physician will monitor bleeding at 5, 10 and 15 minutes after removal of the catheter to assure hemostasis and no hematoma formation. Subjects will not be on anticoagulants, therefore normal coagulation time is anticipated. Cells will be recovered by rinsing the wires and centrifugation. Endothelial cell protein levels of key factors associated with oxidative stress, inflammation, and endothelial function and sex hormone receptors will be examined using immunostaining. Detailed procedures for immunofluorescence staining and analysis have been described previously by our

group [11, 19-22]. Endothelial cells will also be analyzed using RNA sequencing to identify new pathways that lead to sex differences in endothelial responses to exercise in older adults.

  - To investigate the chronic effects of HIIT, cells will be collected and analyzed: 1) at baseline; 2) after 8 weeks of normal lifestyle; and 3) after 8 weeks of exercise training.

- Protein and mRNA levels in peripheral blood mononuclear cells (PBMCs). Protein and mRNA levels will be determined for key factors associated with oxidative stress, inflammation, and endothelial function and sex hormone receptors. Ficoll-Paque will be used to isolate the mononuclear cells from whole blood by centrifugation. Protein levels will be analyzed using Western blots. RNA isolation will be performed using the RNAqueous (Ambion) kit and gene expression will be measured using RT-PCR[23].

  - To investigate the acute effects of HIIT on protein and mRNA levels, these tests will be performed: 1) at rest; 2) at the end of a HIIT session; 3) after 1-hour recovery; and 4) after 24-hours recovery. To examine how chronic exercise training affects the acute responses of exercise, these tests will be performed at the beginning and end of the 8 weeks of exercise training.

List of Procedures and Duration by Visit

Consent (1.5 hrs) Informed consent Medical history and activity questionnaire
Visit 1 (1.5 hrs) 24hr questionnaire and activity monitor pick up Height/weight Blood pressure
Visit 2 (1.5 hrs) 24hr questionnaire Weight Physical exam Blood pressure and heart rhythm Maximal exercise test on treadmill Pre-exercise and end of exercise: Blood vessel tests
Visit 3 (1 hr) Questionnaires Pregnancy test Weight, waist/hip circumferences, body composition scan 6-min walk test All-extremity cycling practice
Visit 4 (1 hr) 24-hr questionnaire Weight Blood pressure and heart rhythm Maximal exercise test on all-extremity cycle

Visit 5 (2.5 hrs) 24-hr questionnaire Weight Blood pressure and heart rhythm Blood vessel tests Nitroglycerin test and intravenous catheter Intra-arterial catheter, cell collection and blood draw
8-week control period: normal lifestyle
Visit 6 (1 hr) Questionnaires and activity monitor pick up Pregnancy test Weight, waist/hip circumferences, body composition scan 6-min walk test All-extremity cycling practice
Visit 7 (2.5 hrs) 24-hr questionnaire Weight Blood pressure and heart rhythm Blood vessel tests Nitroglycerin test and intravenous catheter Intra-arterial catheter, cell collection and blood draw
Visit 8 (3 hrs) 24-hr questionnaire Weight Maximal exercise test on all-extremity cycle Pre-exercise, end and 1-hour post-exercise: Blood pressure and heart rhythm Blood vessel tests
All-extremity HIIT Practice Prior to initiating the 8-week training program, a period of familiarization with all-extremity HIIT exercise will be provided. During this time, the exercise duration and pace will gradually increase with each session until subjects are able to complete the HIIT protocol.
Visit 9a (3 hrs) 24-hr questionnaire Weight Exercise session on all-extremity cycle Pre-exercise, end and 1-hour post-exercise: Blood pressure and heart rhythm Blood vessel tests, intravenous catheter and blood draw
Visit 9b (1 hr) 24-hr questionnaire Weight Blood pressure and heart rhythm Blood vessel tests and blood draw
8-week training period:40 min/session scheduled every week on M, T, Th, F

Visit 10 (2.5 hrs) 24-hr questionnaire and activity monitor pick up Weight Blood pressure and heart rhythm Nitroglycerin test and intravenous catheter Intra-arterial catheter, cell collection and blood draw
Visit 11 (1 hr) Questionnaires Pregnancy test Weight, waist/hip circumferences and body composition scan 6-min walk test
Visit 12a (3 hrs) 24-hr questionnaire Weight Exercise session on all-extremity cycle Pre-exercise, end and 1-hour post-exercise: Blood pressure and heart rhythm Blood vessel tests, intravenous catheter and blood draw
Visit 12b (1 hr) 24-hr questionnaire Weight Blood pressure and heart rhythm Blood vessel tests and blood draw
Visit 13 (3 hrs) 24-hr questionnaire Weight Maximal exercise test on all-extremity cycle Pre-exercise, end and 1-hour post-exercise: Blood pressure and heart rhythm Blood vessel tests

These visits will be scheduled over 6-7 months which should alleviate some of the burden:

1) visits 1-5 will be scheduled over 2-3 weeks and will total 7.5 hours;
2) during the 8-week control period of normal lifestyle there will be no scheduled visits;
3) visits 6, 7, 8, 9a and 9b will be scheduled over 2-3 weeks and will total 10.5 hours;
4) during the 8-week exercise training period there will be 4 scheduled exercise sessions/week;
5) visits 10, 11, 12a, 12b and 13 will be scheduled over 2-3 weeks and will total 10.5 hours.

Sample Size, Statistical Analyses, and Anticipated Results

Sample size justification. For power analyses, we set $\alpha=0.05$ and $\beta=0.80$ and used the smallest effect size (ES) based on our preliminary data on FMD, which is our primary outcome. To detect significant sex differences in acute/chronic FMD responses, we will need 11 men and 11 women (ES=1.57 for acute and 1.28 for chronic response). To detect

significant acute and chronic changes in FMD in response to NWA-HIIT in older men we will need 10 men (ES=1.02 for acute and 3.87 for chronic response). We anticipate we will need to consent and screen 165 research participants to meet the study inclusion criteria and obtain complete datasets in 11 men and 11 women.

Statistical analyses. Our primary outcome will be FMD. For both Aim 1 and 2, we will use mixed factorial ANOVA. Post hoc and multiple tests will be corrected using the Bonferroni adjustment. Secondary analyses will be performed using ANCOVA to account for influence of potential confounders. Exploratory analysis will evaluate the mechanisms by which exercise influences FMD and stimulate future mechanistic studies. Using linear regression, we will examine whether responses to acute NWA-HIIT are correlated with responses to chronic 8-week NWA-HIIT. Analysis of the RNA sequencing data will be conducted by the ICBR bioinformatics staff.

HUMAN SUBJECTS

Human Subject Characteristics

Subjects will be sedentary men and postmenopausal women recruited from the community using advertisements. Subjects will be screened by our experienced study clinicians with oversight by our cardiologist (Dr. Pepine) according to the study inclusion/exclusion criteria based on the medical history, physical examination, general blood tests and diagnostic maximal exercise test.

Inclusion criteria

- adults able to give consent
- men and women
- women must be postmenopausal (either natural or surgical)
- 60 to 79 years of age

Exclusion criteria

- any relevant cardiovascular diseases (e.g., history of coronary artery bypass surgery or angioplasty, or heart failure, myocardial infarction, angina pectoris, peripheral arterial disease)
- myocardial ischemia during maximal graded exercise test
- major chronic clinical disease (e.g., diabetes, renal or hepatic disease or infection with hepatitis B, C, or HIV)
- seizures, or other relevant on-going or recurrent illness
- recent (within 3 months) or recurrent hospitalizations
- systolic blood pressure <100mmHg
- body mass index > 35 kg/m^2
- >5% weight change in past 3 months or unwilling to remain weight stable during study participation
- use of tobacco products including smoking traditional or e-cigarettes
- use of hormone replacement therapy in women or men (e.g., estrogen, progesterone or testosterone)

- regular aerobic exercise training (≥30 min/session and ≥ 3 days/week)

Informed Consent

Research volunteers who contact the PI's laboratory in response to our recruitment efforts (advertisements) will be asked to complete a phone screening or an online RedCap screening questionnaire to determine if they qualify to participate in the study. Volunteers who meet the preliminary inclusion criteria and are willing to participate will be given the consent form to read prior to obtaining written consent. Informed consent will be obtained by the PI, Dr. Christou, or her research assistant, Stephanie Lapierre, in a quiet and private area or via a secure password-protected UF Zoom meeting. If consent is obtained via Zoom, then the subject's signature will be obtained using REDCap eConsent. All procedures and risks related with participation in the study will be described and the subject's understanding of the study will be confirmed. Any questions will be answered prior to obtaining the subject's written consent. The consent form will then be signed by both the subject and investigator as documentation of consent. Consent will be documented in the subject's research file. A copy of the signed consent form will be provided to the subject.

Compensation

Subjects will be compensated a total of $360 for completing visits 5-13 in the form of a gift card upon completion of the study. In addition, for every week that a subject completes all 4 supervised exercise sessions s/he will be compensated an additional $5 (e.g., a subject who completes 4 sessions/week for 8 weeks will receive an additional $40, thus increasing the total compensation from $360 to $400). Payment will be given to subjects within a few weeks following completion of their participation or withdrawal from the study.

If subjects do not complete all visits, then compensation will be prorated according to the following plan:

Visits 1-4	Will receive the health screening results at no cost. No financial compensation will be provided.
Visit 5	$45
Visit 6	$10
Visit 7	$45
Visit 8	$25
Visit 9a	$25
Visit 9b	$10
Visit 10	$75

Visit 11	$10
Visit 12a	$45
Visit 12b	$20
Visit 13	$50
Exercise sessions	Will receive supervised exercise training at no cost. An additional $5/week will be provided for each week that all 4 supervised sessions are completed.

Protection of Privacy and Confidentiality

Subject privacy will be protected by performing research procedures in a private room. When use of UF Zoom is needed, a password-protected session will be used and the option to record will be disabled. The collection of sensitive information will be limited to the amount necessary to achieve the aims of the research, so that no unneeded sensitive information is being collected. All subject identities and records will remain strictly confidential and will be accessible only by the research team. Whenever feasible identifiers will be removed from the study-related information. The names of the subjects will not be identified in any publication or presentation arising from these studies. Ultrasound images will be labeled by subject ID and will be stored digitally and backed-up on tapes. Blood, endothelial cell and peripheral blood mononuclear cell samples will also be labeled using subject ID numbers. The samples and tapes will be stored in the PI's laboratory where access is limited to the study personnel. Paper copies of data will be stored in a locked cabinet in a secure location accessible only to members of the investigative team. Electronic data will be kept on the laboratory server, which is accessed by individual user login and passwords. Electronic data will be backed-up on the College server which is also password-protected. Real-time exercise response data collected live by the Zephyr Omnisense wearable device will be stored using subject ID to the Zephyr Omnisense cloud and will be downloaded to the College server; to access the cloud and data on the server will require user login and password.

7. Possible Discomforts and Risks

- Flow mediated dilation- Inflating the blood pressure cuff on the forearm during this procedure may cause a mild to moderate intensity "pins and needles or numbing" sensation that goes away as soon as the cuff is deflated.
- Body composition (DXA) scan- The radiation exposure from each DXA scan is equal to about 3 millirem, which is comparable to about 4 extra days of natural background radiation to which people in the United States are exposed to during their lives. The risk from this radiation exposure is considered to be extremely low when compared to other everyday risks.
- Maximal graded exercise test- Arrhythmia (<1%); minimal risk of myocardial infarction (<0.05%) and death (<0.02%); small risk of syncope and fatigue.

- Exercise training- When one begins a new exercise program there is a risk that it may lead to fatigue or muscle or joint soreness, or injury. There is a small risk of irregular heart rhythm and very rarely heart attack or stroke which could potentially lead to death. In general, exercise does not lead to heart problems or stroke in individuals who have completed health screening and are free of heart disease. To allow monitoring of the exercise responses using the wearable device, the location option on the tablet must be turned on. Neither the study team nor the company that manufactures the wearable device will have access to the location information. But there is a small risk that an unauthorized person may get access to this information, while the tablet is in use.
- Intravenous catheter and blood draw- Discomfort at the site of puncture; possible bruising and swelling around the puncture site; rarely an infection or blood clot; and, uncommonly, faintness from the procedure.
- Intra-arterial catheter and cell and blood collection- Discomfort associated with lidocaine injection. Discomfort from needle stick and placement of catheter at the time it is inserted or after it has been removed. Possible bleeding, bruising, and swelling around the puncture site. Rarely local blood clot, infection, significant blood loss, nerve or artery damage, tissue injury and damage of surrounding tissue and extremity. Pain from compression of puncture site if severe bleeding occurs. The risks related to the cell collection are similar to those associated with an intra-arterial catheter, although the risk of blood clot, infection or blood vessel damage may be slightly greater compared to having intra-arterial catheter placement without cell collection.
- Lidocaine- Lidocaine will be used for numbing the area prior to arterial cannulation. Expected sensation of coolness or numbness at injection site; likely mild temporary skin irritation including burning, redness or swelling at injection site; unlikely but possible reactions include nausea, vomiting, dizziness, ringing in ears, nervousness and blurred or double vision; extremely rare allergic reaction.
- Nitroglycerin test- The oral administration of nitroglycerin (0.4 mg sublingual tablet) is expected to cause a small transient decrease in arterial blood pressure. The subject may experience increased heart rate, lightheadedness, tingling on the tongue and in the arms and legs, light headache, and at the extreme, fainting. In rare cases heart rate may decrease in response to the nitroglycerin pill which can also cause fainting. The dose of nitroglycerin used is considered safe and is commonly used in patient populations to relieve angina. With any drug used in testing, there is a small risk of an unforeseen life-threatening allergic reaction. The on-site study clinician will confirm that there are no contraindications for nitroglycerin administration (e.g., systolic blood pressure <100 mmHg, phosphodiesterase inhibitors including Sildennafil) before administering the drug and will monitor the subject until ready to be released.

Protection Against Risks

In general, risks will be minimized by:

- allowing only individuals who meet the inclusion criteria to participate
- screening for contraindications for nitroglycerin administration, maximal graded exercise testing or intra-arterial catheter placement
- providing clinical supervision, including ECG and blood pressure monitoring, for the maximal graded exercise tests and nitroglycerin administration
- ensuring constant personal monitoring of each experimental session by the investigators
- using only safe, well-established procedures, and having only qualified and experienced personnel perform the procedures
- following UF guidelines for reducing the risk of COVID-19
- having available emergency equipment/medications and well-planned emergency procedures
- providing complete confidentiality

Risks associated with COVID-19: Risks related to COVID-19 will be mitigated by strictly following UF COVID-19 safety guidelines and the advice of Dr. Cesar Trillo-Alvarez, a Co-Investigator and experienced clinician at the UF Health Division of Pulmonary and Critical Care. These include:

- Limiting the number of people in a room and social distancing whenever possible.
- Frequently sanitizing laboratory surfaces and hands.
- Study personnel wearing KN95 masks and shields, disposable gowns and gloves and participants wearing a surgical mask.
- Upon arrival at the Center for Exercise Science for a scheduled visit, participants will be screened for history and symptoms of COVID-19 and body temperature will be taken using a contactless forehead thermometer prior to exiting their car. If they answer yes to any of the screening questions and/or their temperature is greater than 100.4 degrees, then they will need to reschedule the visit. Participants will be given a surgical mask and a 2 oz. bottle of hand sanitizer. They will be asked to wear the mask and use the hand sanitizer prior to entering the building and frequently sanitize their hands during the visit and social distance whenever possible.
- During the pandemic, participants will complete a COVID-19 test 3-5 days prior to VO_2max testing and only those who are confirmed negative will proceed to complete the VO_2max test. The COVID-19 test will be prescribed as long as Dr. Trillo-Alvarez deems it necessary and will be paid by the study.
- Exercise sessions will be performed at home and exercise supervision will be conducted remotely live via UF Zoom using a wearable device which provides real-time heart rate monitoring.

Specific risks associated with nitroglycerin: Risks related to nitroglycerin administration will be minimized by providing adequate clinical supervision and by taking the following safety precautions. An intravenous catheter will be inserted in a peripheral vein prior to nitroglycerin administration, in case the effect of nitroglycerin necessitates the administration of fluids or medication. ECG will be monitored continuously. Blood pressure will be monitored prior to nitroglycerin administration and post-administration as frequently as deemed necessary. For individuals whose systolic blood pressure is <100 mmHg, nitroglycerin will not be administered. Subjects will remain in bed for 30 minutes after nitroglycerin administration to allow blood pressure to return to baseline before ambulating. Nitroglycerin has been administered safely in our laboratory for more than 10 years, and in other laboratories, and clinical situations worldwide.

Risks associated with intra-arterial catheter and cell collection: Risks related to intra-arterial catheter insertion and cell collection will be minimized by having an experienced clinician from the Pulmonary and Critical Care Division, Dr. Cesar Trillo-Alvarez, perform the procedure. Dr. Trillo will use ultrasound imaging to guide the cannulation to avoid injury to nerves and surrounding structures. Subjects will be screened for contraindications including absent pulse at the location of the artery, history of Raynaud's Syndrome, insufficient collateral perfusion based on Allen test, coagulopathy, recent use of thrombolytic, anticoagulant or antiplatelet drugs, and partial or full thickness burns over the proposed cannulation site. Up to two attempts to insert the arterial catheter will be performed; attempts will be aborted if any complications are expected. Dr. Trillo will monitor the patient until the arterial line procedure and cell collection are completed and no complications are noted.

Specific risks and complications will be mitigated and managed as follows:

a. Radial Artery Spasm (RAS)
    i. Subjects will be monitored for RAS clinically by physician inserting arterial catheter
    ii. Initial management:
        1. Subjects scheduled to receive nitroglycerin 0.4 mg sublingual (as per study protocol to assess endothelium-independent dilation) will receive the dose of nitroglycerin as per study protocol and RAS will be reevaluated (at 10-min intervals up to 30 min). If no improvement, then escalation to higher level of care will be initiated.
        2. Subjects not scheduled to receive nitroglycerin will be monitored for severity of RAS. If moderate or severe by clinical judgement, then the patient will receive a single dose of nitroglycerin 0.4 mg sublingual and RAS will be reevaluated (at 10-min intervals up to 30 min). If no improvement, then escalation to higher level of care will be initiated.
b. Thrombosis
    i. Mitigation of risk:
        1. Cancel procedure if subject has history (or present during evaluation) of Raynaud's phenomenon
        2. Catheter will stay inserted for less than 5 min
        3. No use of large bore catheters, only 20G or smaller

4. No insertion if patient has low cardiac output (by history)
5. No insertion if patient has history of severe peripheral artery disease
6. No insertion if blood vessel is considered too small for cannulation

ii. Initial management: If moderate or severe by clinical judgement, then the patient will receive a single dose of nitroglycerin 0.4 mg sublingual and RAS associated with thrombosis will be reevaluated (at 10-min intervals up to 30 min). If no improvement, then escalation to higher level of care will be initiated.

c. Dissection, pseudoaneurysm, AV fistula
   i. Mitigation of risk by using procedures described above and
      1. Continuous clinical monitoring at 10-min intervals up to 30 min.
      2. Local pressure to control bleeding and hematoma
      3. If present and no improvement with initial local pressure, then escalation to higher level of care will be initiated.
d. Infection
   i. Mitigation of risk:
      1. All sterile insertion as per usual guidelines. Use of mask, caps, gowns, sterile gloves, and chlorhexidine (or equivalent if patient allergic) to prepare the skin.
      2. Catheter will be inserted for less than 5 minutes.
e. Blood loss
   i. Mitigation of risk:
      1. No arterial puncture will be attempted in any subject with systemic anticoagulation.
   ii. Initial management:
      1. Bleeding will be controlled by local pressure; monitoring at 5, 10, and 15 min after removal of arterial catheter. If bleeding persists continuous pressure and evaluation at 10-minute intervals up to 30 min.
      2. If bleeding present and no improvement with initial local pressure, then escalation to higher level of care will be initiated.
f. Neuropathy
   i. Mitigation of risk:
      1. Use of ultrasound guided technique to avoid nerve injury.
      2. Abort procedure if producing pain or neurological injury.
   ii. Initial management:
      1. If severe neurological injury noticed, then escalation to higher level of care will be initiated.

Procedure for escalation of care after initial control in research laboratory:

Local initial first aid, including pressure to stop bleeding and vital signs monitoring. The patient will be transferred to University of Florida Hospital for further evaluation and care if complications arise. If emergent we will contact 911 for immediate support and transport. We will immediately refer patient to University of Florida/Shands Medical Center for any complications for further evaluation and therapy.

Risks associated with exercise training:

In general, risks related to exercise training will be minimized by:

- adhering to the exercise training guidelines published by the American Heart Association and the American College of Sports Medicine
- providing thorough health screening to ensure there are no contraindications for exercise training
- providing remote live supervision of exercise training via Zoom

Minimizing specific risks associated with exercise training:

Cardiac risks during exercise will be minimized by completing extensive health screening tests before subject enrollment to the exercise intervention. Subjects with signs of cardiovascular disease or those at elevated risk for cardiac complications will be excluded from the study (see exclusion criteria). A physical examination will be conducted, and medical history and resting ECG will be reviewed for contraindications to exercise. Prior to exercise testing, heart rate and blood pressure will be obtained while the subjects rest supine, sitting and standing to screen for cardiac autonomic dysfunction. Baseline maximal exercise tests will be performed to rule out the presence of silent myocardial ischemia. Because the exercise level during the diagnostic tests will be maximal, it should allow identification/exclusion of individuals who are likely to develop ischemia during the exercise training. In addition, subjects will be educated on how to recognize cardiovascular disease symptoms such as angina, dizziness and excessive shortness of breath. They will be asked to inform the research team immediately if they experience any of these symptoms during the diagnostic exercise test or the exercise sessions. If participants experience a medical emergency during an exercise session, we will call 911 while we continue to monitor them via video conferencing. In summary, cardiac risks associated with exercise training will be minimized, although we recognize they cannot be completely eliminated. Overall, the widely accepted benefits of exercising in older adults should outweigh the risks.

8. Possible Benefits

The risk-to-benefit ratio is relatively small because the risks of participating in this study are relatively small. Subjects may or may not benefit from participating in this research study. The subjects will receive information regarding their health at no cost from the extensive testing performed for screening purposes (i.e., medical information derived from physical examination, diagnostic exercise test, blood pressure measurements, body composition analysis including bone density, aerobic fitness, physical activity and dietary analysis, and general blood tests including comprehensive metabolic panel, lipid panel, insulin, complete blood count with differential). Although individual responses may vary, exercise training has been shown to lead to many health benefits that may improve quality of life or lower the risk for heart disease including higher fitness level, improved heart function, healthier blood vessels, lower blood pressure, lower body fat and blood lipids, reduced stress and depression. Participation in the proposed studies could also contribute to the advancement

of scientific information that may have important implications for exercise prescription for preventing cardiovascular disease in aging. The study will use a translational research approach to gain mechanistic insight, for the first time, into sex differences in *in vivo* systemic/cellular/molecular adaptations to exercise in older adults.

9. Conflict of Interest

There is no conflict of interest to report.

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