Blood volume redistribution in HFpEF through LBNP/LBPP and the impact on exercise performance

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Purpose of the Study

The primary objective for Aim 1 is to test the impact of LBNP/LBPP on exercise functions, symptoms, blood volume distribution and non-invasive central cardiac hemodynamics in patients with HFpEF as compared to healthy controls.

The primary objective for Aim 2 is to investigate the effect of LBNP/LBPP on central and peripheral hemodynamics during an invasive cardiopulmonary exercise test (CPET) of patients with diagnosed HFpEF.

The key item differentiating Aim 2 from Aim 1 is the ability to obtain invasive arterial hemodynamics, intra-cardiac pressures, and cardiac output. Invasive hemodynamic testing will us allow to differentiate volume distribution effects on right and left sided hemodynamics, ventriculo-pulmonary and ventriculo-arterial coupling.

Exploratory objectives are to determine if measurements of cardiovascular, respiratory, and muscle parameters by non-invasive techniques can provide additional information related to hemodynamic response and blood volume distribution.

The investigators hypothesize that selective reduction or increase in cardiac preload via the lower body negative pressure (LBNP)/ lower body positive pressure (LBPP) will demonstrate the central contribution of the splanchnic vascular compartment to exercise hemodynamics and function in HFpEF.

Background & Significance

HF is a leading cause of morbidity and mortality, affecting > 6 million adults in the US alone. Half of the HF population has HFpEF, which affects patients across the age spectrum but predominantly the older population. HFpEF is among the top two causes of hospitalization in patients over the age of 60 and is characterized by symptoms such as shortness of breath, fatigue or edema, yet with preserved systolic function . HFpEF is a heterogeneous disease and, to date, therapies seeking to improve the neurohormonal balance or cardiovascular performance have failed to improve clinical outcomes. The central problem in HFpEF is cardiovascular congestion with resultant limitation in physical activity, and effective measures to decrease vascular congestion acutely or chronically remain an unmet clinical need. The current guideline-recommended strategy to manage patients with chronic HFpEF is to alleviate signs and symptoms of congestion with diuretics without guidance by specific physiologic phenotypes. Yet, potentially due to an untargeted approach of decongestive therapies, common therapeutic approaches to reduce congestion via diuresis have failed to show a significant benefit in outcomes. Since HF-related symptoms and the process of decompensation

from chronic to acute HF are the result of multiple cardiovascular abnormalities and overlapping extra-cardiac comorbidities, clinicians and researchers now recognize the need to move towards identifying congestion phenotypes and deliver phenotype-specific therapies instead of taking a broad “one-size-fits-all” therapeutic approach 8. The proposed research will provide the fundamental evidence to support the paradigm-shifting hypothesis of volume distribution induced cardiac decompensation in HFpEF. The application of LBNP and LBPP will allow for reversible blood volume redistribution to assess the potential symptom reduction associated with cardiovascular congestion in HFpEF and will provide clinicians insight into the benefit of targeted therapies (such as splanchnic nerve block) to regulate blood volume shifts and prevent inappropriate blood volume redistribution.

The two complimentary aims will utilize the LBNP/LBPP chamber. Together, the aims will investigate volume redistribution as a driver of cardiovascular congestion and functional limitations and will pave the way for the development of Splanchnic Nerve Block (SNB) as a novel therapeutic approach to HF. The aims will run sequentially and learnings from Aim 1 will be incorporated in Aim 2. The LBNP/LBPP chamber is a key diagnostic element of this study and presents the first time LBNP/LBPP is used for the investigation of HFpEF. LBNP is a well-established research technique. LBNP is most often used as a perturbation to the cardiovascular system and has been applied to simulate gravitational stress and hemorrhage, alter preload (via central blood volume), and manipulate baroreceptors. LBNP moves fluid from the chest into the abdominal cavity and the legs. In healthy adults LBNP results in a higher heart rate and lower stroke volume but with no significant change in blood pressure. LBNP that includes the abdomen (instead of legs/pelvis alone) results in greater amount of shifted blood volume and a higher heart rate than the legs/pelvis alone. LBPP increases thoracic blood volume which results in healthy adults in an increase in central venous pressure and mean arterial pressure. Despite these physiological changes, cardiac output and heart rate remain relatively unchanged during LBPP. The hemodynamics of both men and women respond similarly to LBNP and LBPP. LBNP was previously tested in HFrEF without an effect on systolic blood pressure or baroreflex function. LBNP decreases cardiac filling pressure with associated decreases in left atrial dimension but not in calculated left ventricular volumes. Notably, the effect on preload drop (LBNP) or preload increase (LBPP) peaks and plateaus around +/- 40mmHg. LBNP of -40mmHg equates to an estimated blood volume displacement away from the chest of 10ml/kg, so ~700ml for a 70kg human.

The LBNP/LBPP chamber is located in room 4215 in the Dept of Anesthesiology within the Duke Clinics (Orange Zone). The chamber was constructed by personnel who had received training in the operation of LBNP and completed research studies with LBNP, performed at the Centre for Heart, Lung, and Vascular Health, School of Health and Exercise Science at the University of British Columbia – Kelowna for clinical research use. The LBNP chamber is a minimal risk device that has been used in research settings for decades; and though the use of LBPP has only more

recently been utilized, both the application of LBNP and LBPP are safe, non-invasive ways to evaluate blood volume translocation.

Design & Procedures

Following enrollment, subjects will undergo the following tests/measurements during exposure to standard room pressure, negative pressure, and positive pressure. A majority of the clinical testing occurs as part of the clinical study (CS) however the RHC in Aim 2 is standard of care (SOC). The following information will be collected from the medical record per standard of care (SOC). Study specific tests/recordings are also listed below.

Aim 1

If the subject is female and childbearing potential then a urine pregnancy test will be done. If positive the subject will be excluded from the study.

Blood and Urine samples: This will require an extra lab draw. Expected blood loss is <25ml.:
CMP - 4.5mls
NTproBNP - 4mls
CA125 - 4.5mls
Urine sodium
Urine chloride
Blood volume analysis: using radiolabeled albumin I-131. Each blood volume analysis will take about 90 minutes to complete. The agent will be delivered to the radiopharmacy and prepared by the radiopharmacy. The agent will be then transported and stored at the DMP/ Radiology. On the study day the agent will be taken from the DMP to Duke South, Rm 4215 (Aim 1) or cath lab holding area/ patient room (Aim 2) for the injection. An intravenous line connected to a 30 ml saline solution will be inserted in an arm. This line will be used to inject a small amount of diagnostic radioactive material into the circulatory system. An IV line will be used to collect small samples of blood at timed intervals 12 min after injection of drug, then minute 18, 24, 30, 36 and 42. Blood collection timeframes do not have to be exact but time of blood draw will be documented. Approximately 16 ml of blood will be drawn total. Blood samples will be processed by Duke staff in a Duke lab. Blood volume analysis will be completed prior to the start of exercise. Blood samples will be analyzed for hematocrit prior to BVA analysis; this is required for accurate BVA results. This will not require extra blood to be drawn. Completed one time, prior to exercise. (CS) (This is not a mandatory part of the protocol.)
Blood volume analysis (optional) - completed once prior at ambient pressure, prior to exercise
Initial/pre-albumin I-131 injection - 4mls
12 mins post injection - 4mls
18 mins post - 4mls

24 mins post - 4mls
30 mins post - 4mls
36 mins post - 4mls
42 mins post - 4mls

Note: The radiation effect dose from a single administration of I-131 albumin for blood volume measurement is 0.60 mSv which is equal to three chest x-ray exams. It is the equivalent of 2 months of natural background exposure.

Prophylaxis prednisone may be prescribed by the PI, if there is concern for an adverse reaction of Albumin I-131. If prednisone is needed the subject will take a total of 60mg of prednisone 3 times over the 24h, 12h and 0h timepoint prior to administration of Albumin I-131.

An example for this would be if a subject has opted to receive the dose of Albumin I-131 and has a documented allergy/reaction to contrasts and dyes then the PI would prescribe a prophylactic dose of prednisone prior to the injection of Albumin I-131 to make sure there is no adverse reaction to the Albumin I-131.

Vital Signs: Recorded vital signs including weight, blood pressure, heart rate, respiratory rate, SpO2. Completed one time pre-exercise and then recorded intermittently throughout each exercise session.

Dyspnea questionnaire: using a 7-point Likert scale and Visual Analog Scale Recorded once at baseline, then intermittently throughout each exercise session. (CS)

VO2Master Pro: The VO2master pro is a compact, mobile and wireless (bluetooth) breath-by-breath metabolic cart to assess the cardio-metabolic and cardio-respiratory capacity /effort during training (Rehab). The device is directly mounted on the Hans Rudolf mask. The device measured the oxygen consumption just like traditional metabolic carts Measured during each exercise session. (CS)

TestRig: The TestRig is a sensor module used to measure oxygen consumtion just like the traditional metabolic carts Measured during each exercise session. (CS). This device may be used in place of the VO2Master Pro.
Bioelectrical Impedance Spectroscopy (BIS): Multifrequency Bioelectrical impedance Spectroscopy (BIS): Segmental bioelectrical impedance spectroscopy (BIS) is another non-invasive way of assessing muscle quality assessment, but with a different approach. Bioelectrical impedance analysis (BIA) equipment does not measure muscle mass directly, but instead derives an estimate of muscle mass based on whole-body electrical conductivity. Skeletal muscle has a large amount of water, and segmental BIS can separately assess intracellular water (ICW) and extracellular water (ECW), which are divided by the muscle cell

membrane. Segmental BIS can distinguish ICW and ECW from the total water in a particular segment. Segmental BIS is advantageous for assessments of a localized (left /right) region (arm, leg and trunk) instead of only the whole-body ICW reflects muscle cell mass, whereas ECW represents the sum of interstitial fluid and blood plasma in extracellular space. The calculations of ICW and ECW are only affected by segmental volume, so the ECW /ICW ratio could indicate the ratio of non-contractile tissue to contractile tissue regardless of assessed somatotype (age, gender, disease state). InBody S10 will be the BIS device used to obtain BIA. InBody is a FDA approved device, and DUH-approved used in Standard of Care Recorded one time prior to exercise. (CS).

Cheetah Medical Starling SV Noninvasive Hemodynamic Monitoring (Cheetah-Medical): This system gives dynamic and real-time fluid monitoring by providing a full hemodynamic profile via a series of 4 electrodes connected to the subject's torso. A small electrical current is applied between the electrodes on the torso and a voltage signal is recorded. THe flow of blood in torso causes a phase shift in the signal which can then be recorded and displayed on the monitor screen to give cardiovascular parameters such as cardiac output, cardiac index, heart rate, stroke volume, total peripheral resistance, total peripheral resistance index, blood pressure, and thoracic fluid content. Measured during each exercise session. (CS).

PhysioFlow: is a noninvasive cardiac output measurement system which provides hemodynamic parameters using analysis of bioimpedance signals. The system can be used at rest or while exercising.

Artinis (Portamon/Portalite)/ MOXY NIRS: The near-infrared spectroscopy (NIRS) devices that will be used in this study are PortaLite (Artinis, Netherlands) and MOXY (MOXY Monitor, USA); that is a research device ideally suited for assessment of muscle- and brain mitochondrial function during cardiopulomonary exercise testing. The device is developed to measure concentration changes of oxyhemoglobin and deoxyhemoglobin with near infrared light. NIRS uses two low power LED emitters at wavelengths of 760 nm and 850 nm. The device consists of a small and flexible probe, embedding near infrared light sources and detectors, which is connected with one wire to the portable battery housing. The probe, which is made with biocompatible materials, is the only part of the device in contact with the patient's skin Measured during each exercise session.(CS).

Musculoskeletal (MSK) Ultrasound: Muscle metabolic imaging derived from POC MSK ultrasound for muscle mass, intramuscular glycogen content and intramuscular adipose tissue content (IMAT) will be obtained pre exercise assessment. A portable ultrasound device is used (Phillips Lumify, Eindhoven, The Netherlands) with an L12-4 broadband linear array transducer for research purposes. The same make and model is currently being used at Duke Hospital (CS). Device settings are kept on the factory preset ‘MSK’ (musculoskeletal) with a standard gain of 50 and a default depth of 3.5 cm. Four images are taken of each muscle (left and right) in long-

axis plane and in the shortaxis plane in a supine position Ultrasound images are made of Rectus femoris muscle and Temporalis /Styloglossus muscle. Recorded one time prior to exercise.(CS) CipherOx CRI M1: is indicated for continuous noninvasive monitoring of functional oxygen saturation of arterial hemoglobin (SpO2), pulse rate, and the compensatory reserve index (CRI), which trends changes in intravascular volume relative to the individual patient's response to hypovolemia. Raw data from 2-4 subjects may be shared with the vendor of this device to ensure the algorithm for CRI is valid or to make improvements to the algorithm for future subjects. This tablet is owned by Impact Vitals and they created the Compensatory Reserve Index algorithm.

Heart Rate Variability: We will collect data on subject's heart rate variability during a five-minute period using a chest band and POLAR V800 watch. Measured during each exercise session. (CS)

Corsano Cardiowatch (287): This wrist watch captures continuous heart rate, respiration, SpO2, ECG tracing, activity data, sleep, and body temperature.

Maxim Wrist Sensor: This wrist watch displays data for heart rate, SpO2, heart rate variability, respiration rate, and sleep quality.

Negative/Positive Pressure Chamber: **Image of chamber in "Background/Significance" section. The LBNP/LBPP chamber is a closed system which applies a graded vacuum (-20, -40mmHg) or graded positive pressure (+20, +40mmHg) to the abdomen and legs. The inner chamber is isolated from the external ambient air. Suction/pressure is provided by a vacuum pump controlled with a variable autotransformer and calibrated against a mercury manometer. Ambient pressure is restored immediately when vacuum pump is turned off. The pump must remain on to maintain desired pressure (negative or positive) within the chamber. Lower Body Negative Pressure / Lower Body Positive Pressure Chamber is a large plastic chamber that has a stationary bicycle inside. The subject will lower themselves down into the box to waist level so that they are seated on the bike seat but lying down initially with their back against a supportive mat. A neoprene belt will be fastened around the subject's waist to make sure the unit is airtight. The entire chamber will then be tilted up about 45 degrees so that during exercise the subject will be in a more upright position. Changes in pressure inside the box around the legs and abdomen can be controlled from a control unit outside the box. (CS) Cardiopulmonary Exercise Test (CPET): A graded exercise test consisting of a submaximal ramped cycling protocol will be conducted while clinical CRF–related variables such as peak heart rate (HRpeak), and VO2peak are recorded. The ramped cycling protocol will consist of 2 stages of exercise, each lasting no more than 2 minutes (maximum of 4 mins exercise). Stage 1 will include cycling at an incline of 45 degrees at 0 watts at 60 revolutions per minute (RPMs). Stage 2 will be 20 watts at 60 RPMs. Since wattage is a form of power output by the cycle based on speed and resistance there is no way to cycle at 0W. Which that being said to obtain 20W

we will use a delta of 20W. After stage 1 is complete we will add 20W in addition to the wattage achieved in stage 1 to create a delta of 20W. A baseline CPET following this exercise protocol will occur at ambient pressure and will be repeated for both negative and positive pressure application within the chamber. (CS)

Echocardiogram: an ultrasound of the heart may be utilized at the discretion of the PI in order to visualize size and structure of cardiac anatomy. Measured during each exercise session. (CS)
A pressure transducer will be connected to the subject's existing IV and we will record the venous pressure from the peripheral IV catheter (e.g. hand or forearm), which has previously been shown to correlate with right atrial heart pressure.

Any of the above listed devices may be deferred at the discretion of the PIs.

Aim 2 will comprise of the same tests/recordings but with the inclusion of the following
Arterial line: will be placed as part of the RHC (SOC).
Central Venous Catheter (Jugular Vein): will be placed as part of the RHC (SOC).
Valsalva maneuver: During the patient's RHC we will continuously record blood pressure during a Valsalva maneuver. This will be performed with the patient in a supine position (CS).
Transport: Following the patient's SOC RHC, the patient will be transported to Human Pharmacology and Physiology Lab to perform the study procedure. Central Venous catheter will remain in for invasive intracardiac pressures and patient safety monitoring.
Central Venous Catheter (Jugular Vein) : will be removed following the research procedure per SOC precautions.

Randomization: For both Aim 1 and Aim 2, subjects will be randomly assigned to whether they will complete the CPET with LBNP or LBPP first after completing the CPET at ambient pressure. A random number generator with values of either 1 or 2 will be used with "1" representing LBNP and "2" representing LBPP. In the instance a "1" is generated then a subject will complete the CPET at ambient pressure, then the CPET with LBNP, and lastly the CPET with LBPP. In the instance a "2" is generated then a subject will complete the CPET at ambient pressure, then the CPET with LBPP, and lastly the CPET with LBNP.

On the day of the scheduled visit, the follow sequence of events will occur:
1. If subject has not already completed eConsent over the phone, then subject will be consented
2. Preliminary vitals will be measured (heart rate, blood pressure, respiratory rate, SpO2, height, weight, baseline dyspnea)
3. Blood draw for the labs outlined above will be drawn
4. Urine pregnancy test will be completed for women of childbearing potential for Aim 1. Serum pregnancy test will be completed for women of childbearing potential as part of SOC for Aim 2
5. Perform BIS with InBody 10

6. Perform MSK ultrasound
7. Blood volume analysis using Albumin I-131, if subject has consented
8. Set up devices listed above and place on subject (Polar V800 watch, Artinis (Portamon/Portalite)/ MOXY NIRS, Cheetah-Medical, VO2master, and venous pressure transducer)
9. CPET at ambient pressure performed, then period of rest for subject
10. CPET at negative pressure performed, then period of rest for subject
11. CPET at positive pressure performed, then period of rest for subject
12. Ensure vitals recover to baseline values and discharge subject

A follow up call will occur 48hours post study participation.

Selection of Subjects

HFpEF patients will be required to meet additional inclusion/exclusion criteria listed below

Inclusion Criteria (Aim 1):
Age greater than or equal to 30 yrs
Able to speak English
Ambulatory [assistive devices ok]
Able to provide informed consent
Additional Inclusion Criteria for HFpEF Only (Aim 1 and 2):

Established diagnosis of HFpEF with left ventricular ejection fraction greater than or equal to 50%
NYHA II-III symptoms
Stable HF drug regimen for the preceding 1 month
(For Aim 2 only) Wedge pressure greater than or equal to 15 mmHg at rest or greater than or equal to 25 mmHg with peak exercise
(For Aim 2 only) Scheduled for an elective right heart catherization at Duke University Hospital.

Exclusion Criteria (Aim 1):
Acute myocardial infarction (3-5 days)
Unstable angina
Uncontrolled arrhythmia causing symptoms or haemodynamic compromise
Syncope
Active endocarditis
Acute myocarditis or pericarditis
Symptomatic severe aortic aneurysm
Uncontrolled asthma

Arterial desaturation at rest on room air <85%
Untreated left main stem coronary stenosis
Asymptomatic severe aortic stenosis
Severe untreated arterial hypertension at rest (>200 mm Hg systolic, >120 mm Hg diastolic)
Tachyarrhythmias or bradyarrhythmias
Hypertropic cardiomyopathy
Significant pulmonary hypertension
Thrombosis of the lower extremity until treated for a minimum of 2 weeks
Within 2 weeks of acute symptomatic pulmonary embolus
Abdominal aortic aneurysm >8.0 cm
Electrolyte abnormalities
Pregnancy
Inmate of correctional facility (i.e. prisoner)
Diagnosed history of dementia
Inability to ambulate independently
Known active and/or symptomatic thyroid disease
Considered inappropriate to participate by Principal Investigator

Additional Exclusion Criteria for HFpEF Only (Aim 1 and 2):

HF hospitalization for type I myocardial infarction within 3 months
Infiltrative (ie amyloid) or hypertrophic cardiomyopathy
Uncontrolled atrial or ventricular arrhythmia
Chronic oxygen use
History of vasovagal syncope

Subject Recruitment and Compensation

Potential subjects will be identified via five potential pathways.

1. (Aim 1) Healthy volunteers will be invited to participate through a posting on the Clinical Trials section of DukeList and the Clinical Trials Directory which will direct potential participants to fill out a survey on RedCap. Potential participants will then be contacted by CRC via phone for further screening. For participants interested in the study, meet all inclusion, and none of the exclusion criteria a study visit will be scheduled and written consent will be obtained day of the study visit. Or if the potential participant is interested and willing to have a review of the consent process over the phone, an eConsent will be sent to the potential subject. The signed eConsent will be printed and given to the subject on the day of the procedure.

2.(Aim 1) Patients with diagnosed HFpEF will first be approached by a healthcare professional known to the patient. Patients will be informed that their participation is strictly voluntary. They will be assured that the decision to decline participation will have no impact on their care or the perceptions of their providers. For patients interested in the study, meet all inclusion, and none of the exclusion criteria receive a call from the study PI or CRC regarding interest in this research study. If the potential subject is interested and willing to have a review of the consent process over the phone, an eConsent will be sent to the potential subject, prior to coming in for the scheduled catheterization procedure. The study PI/CRC will review the consent over the phone. The signed eConsent will be printed and given to the subject on the day of the procedure.

3.(Aim 2)Outpatients will be pre-screened via the (Cardiac Cath Lab) CCL scheduler. The subjects who meet all the inclusion and none of the exclusion criteria (from the medical charts in Maestro Care) who are identified, the potential subject will receive a call from the study PI or CRC regarding interest in this research study. If the potential subject is interested and willing to have a review of the consent process over the phone, an eConsent will be sent to the potential subject, prior to coming in for the scheduled catheterization procedure. The study PI/CRC will review the consent over the phone. The signed eConsent will be printed and given to the subject on the day of the procedure.

4. (Aim 2) Inpatients will be identified the day before or the day of the procedure, we (HPPL staff) will approach the primary team to do the first contact. Consent process will occur in the subjects' hospital rooms on Duke Hospital Divisions 7700/7100/7300/7200/3100/3300/7 East.

5. (Aim 2) For patients identified the day before or the same day by a healthcare professional, then we (HPPL staff) will consent the patients in the morning of the study if we are given permission by the CCL attending.

Participants will be compensated $200 USD and will be given paid parking passes for use within DUH patient parking garages.

Risk/Benefit Assessment

There is no direct benefit to subjects from participating in the study, since the total amount of physical exercise is limited to less than a total of 30 minutes (4 mins x 3 bouts of exercise via submaximal CPET).

Exercise Risks: The risks of exercise are minimal since moderate amounts of physical activity generally promote good health. Potential adverse events for participants in this project are expected to be relatively few (muscle strain, fatigue, fall, and in very rare instances,

cardiovascular events such as heart attack or death) during the training period. Recent data from the UK suggests that preoperative CPET testing carries a mortality risk of 2 per 100,000 patients, a number mitigated by adhering to guidance from the American College of Sports Medicine's (ACSM) contraindications to exercise testing. These published contraindications to exercise testing are reflected in the exclusion criteria as they pertain to the study. Risk for adverse events is attenuated through the use of experienced staff to conduct the CPET evaluation.

SAFETY ISSUES:
1. Testing should be performed in a location where a rapid, appropriate response to an emergency is possible. The appropriate location of a crash cart should be determined by the physician supervising the study.
2. Supplies that must be available include oxygen, sublingual nitroglycerine, aspirin, and albuterol (metered dose inhaler or nebulizer). A telephone or other means should be in place to enable a call for help.
3. The technician should be certified in cardiopulmonary resuscitation with a minimum of Basic Life Support by an American Health Association–approved cardiopulmonary resuscitation course. Advanced cardiac life support certification is desirable.
4. Physicians are required to be present during all tests (Aim 1 and Aim 2). The physician ordering the test or a supervising laboratory physician may decide whether physician attendance at a specific test is required.

Physiological Parameter Monitor Risks:
All devices will be inspected and approved by Duke Clinical Engineering.

Ultrasound assessment of muscle (MSK POCUS):
Ultrasound assessment has minimal, if any, physical risk. The examination is non-invasive (does not puncture the skin or any other body cavity). Mild pressure discomfort may be experienced from the probe.

ECG Risks:
Possible side effects of the ECG are skin irritation, itching and redness from the ECG electrode pads.

LBNP/LBPP:
Application of negative and positive pressure to the abdomen and legs has minimal risk when applied under the supervision of a physician. Potential adverse events for participants in this project include sensations of pressure on the legs and abdomen and lightheadedness. In healthy adults LBNP results in a higher heart rate and lower stroke volume but with no significant change in blood pressure. LBNP that includes the abdomen (instead of legs/pelvis alone) results in greater amount of shifted blood volume and a higher heart rate than the

legs/pelvis alone. LBPP increases thoracic blood volume which results in healthy adults in an increase in central venous pressure and mean arterial pressure. Despite these physiological changes, cardiac output and heart rate remain relatively unchanged during LBPP. The hemodynamics of both men and women respond similarly to LBNP and LBPP. LBNP was previously tested in HFrEF without an effect on systolic blood pressure or baroreflex function. LBNP decreases cardiac filling pressure with associated decreases in left atrial dimension but not in calculated left ventricular volumes. Notably, the effect on preload drop (LBNP) or preload increase (LBPP) peaks and plateaus around +/- 40mmHg. LBNP of -40mmHg equates to an estimated blood volume displacement away from the chest of 10ml/kg, so ~700ml for a 70kg human.

Radiation:
The radiation effect dose from a single administration of I-131 albumin for blood volume measurement is 0.60 mSv which is equal to three chest x-ray exams. It is also the equivalent of 2 months of natural background exposure and is determined to post a minimal extra cancer risk to subjects. This portion of the study is optional, and subjects may decline participation in this portion with no negative repercussions.

Allergic Reaction Risk:
Occasionally, people have allergic reactions to medications which may require medical treatment. A severe allergic reaction could be life-threatening. Examples of an allergic reaction include: a rash; shortness of breath; wheezing; difficulty breathing; sudden drop in blood pressure; swelling around the mouth, throat, or eye; fast pulse; and sweating. You should get immediate medical help and contact the study doctor if you have any of these or any other side effects during the study. Prednisone will be administered orally prior to the administration of Albumin I-131 if there is a concern that the subject may have an allergic reaction to the injection.

Data Management, Analysis and Statistical Considerations

It is proposed that 60 subjects be recruited to this study and this number will allow for potential screen failures or withdrawals.

Aim 1 will include 30 subjects (15 healthy volunteers and 15 HFpEF patients).
Aim 2 will include 30 subjects (all 30 will be HFpEF patients).

Descriptive statistics will be used for participant characteristics and variables measured during each test: heart rate, oxygen consumption (VO2), respiratory rate and respiratory ventilation. Continuous variables will be reported as mean (range), mean (SD) or median and inter-quartile range (IQR), depending on distribution, and categorical variables as frequency (%). Concurrent

analysis between ambient pressure, LBNP, and LBPP will be established with Pearson correlation coefficients between peak VO2 and other cardiovascular variables.

Raw data from 2-4 subjects from the CipherOx CRI MI may be shared with the vendor of this device to ensure the algorithm for CRI is valid or to make improvements to the algorithm for future subjects.

Data & Safety Monitoring

The physical safety concerns are limited to the conduct of the exercise assessments. The study team will adhere to the published CPET guidelines to exclude appropriate subjects. The study data will be stored in the Duke REDCap database and will conform will the institutional policy on IT safety. Given the low risk of study participation there is no plan for a formal data monitoring committee.