Myocardial perfusion, oxidative metabolism, and fibrosis in HFpEF (HFpEF-PRoF)

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1. Study Rationale

Cardiovascular disease is the leading cause of death in the United States and in most countries around the world.[1] Heart failure, in particular, is increasing in frequency and is associated with high morbidity, mortality, and economic cost.[2] Heart failure with preserved ejection fraction (pump function) (HFpEF) accounts for approximately half of all cases of heart failure and is associated with 35-50% mortality over 5 years.[2, 3] Unlike heart failure with reduced ejection fraction (HFrEF) where several medicines and devices have been demonstrated to reduce mortality, no such therapies have been identified in HFpEF.[4] This may be in part due to incomplete understanding of the underlying mechanisms of HFpEF.

Recently, impaired myocardial blood flow reserve, reduced myocardial energy utilization, and increased myocardial fibrosis have been postulated to play important pathophysiologic roles in HFpEF.[5] We and others have demonstrated that HFrEF may be associated with altered myocardial energy utilization and "energy starvation."[6] However, there is limited data regarding "energy starvation" in HFpEF and the relationships between myocardial blood flow reserve, energy utilization, and fibrosis in HFpEF are largely unknown.[7] Therefore, the purposes of this study are to use non-invasive cardiac imaging techniques to describe cardiac perfusion, energetics, and fibrosis, and their relationships, in order to better understand underlying mechanisms in HFpEF.

Specifically, we hypothesize that HFpEF is associated with reductions in myocardial blood flow reserve and energy utilization and increased myocardial fibrosis as compared to gender matched hypertensive and healthy controls. We will test our hypotheses by comparing measurements of myocardial blood flow reserve, energy utilization, and fibrosis between three subject groups (HFpEF vs hypertension vs healthy). Myocardial blood flow will be quantitated from 13N-Ammonia Positron Emission Tomography (PET) and gadolinium enhanced cardiac magnetic resonance imaging (cMRI) both at rest and stress following coronary vasodilation with regadenoson. Myocardial energy utilization will be quantified with 11C-Acetate PET imaging and myocardial fibrosis will be assessed with gadolinium enhanced MRI. Echocardiography will be utilized to quantify diastolic function and myocardial strain.

It is anticipated that the results of this proposed study will provide a foundation that will inform future studies aimed at identifying novel preventive or therapeutic agents in HFpEF. In addition, the design of the study affords opportunities to compare myocardial perfusion between PET and cMRI across a spectrum of patient populations and obtain valuable normative perfusion data in an age group most likely to undergo stress testing. Importantly, the findings may also help establish a role for regadenoson stress myocardial perfusion imaging in the evaluation of coronary endothelial function and myocardial blood flow in hypertensive and HFpEF patients.

Specific Aims

Heart failure with preserved ejection fraction (HFpEF) is a major public health problem. HFpEF accounts for half of all new cases of heart failure (HF), is increasing in prevalence, and portends a poor prognosis. Unfortunately, medical therapies with proven mortality benefit in HFpEF are lacking. The absence of effective therapies is attributable in part to the poor understanding of the underlying mechanisms of HFpEF.

Coronary endothelial dysfunction and impaired myocardial blood flow reserve are postulated to be important mechanisms in HFpEF. The delivery of oxygen and fuel sources needed for myocardial performance is dependent upon myocardial blood flow. Impaired myocardial blood flow at stress can occur even in the absence of flow limiting epicardial coronary artery disease, may be a marker of coronary endothelial dysfunction, and is associated with adverse cardiovascular outcomes.[8] However, coronary endothelial function and myocardial blood flow reserve have not been well described in HFpEF.

Experimental and clinical evidence suggests that myocardial oxidative metabolism is impaired in HF. Impaired oxidative metabolism has been associated with HF with *reduced* ejection fraction (HFrEF). However, few studies have investigated oxidative metabolism in HFpEF and in particular, whether differences in oxidative metabolism exist between hypertensive patients and those with HFpEF.

Myocardial fibrosis is regarded as an important mechanism for HFpEF. Increased extracellular fibrosis has been observed in animal models of hypertension, human left ventricular hypertrophy, and endomyocardial biopsies from patients with HFpEF. Hypertension is the major risk factor for HFpEF; however, it is unknown why some individuals with hypertension develop progressive myocardial fibrosis.

Experimental evidence suggests that impaired oxidative metabolism contributes to myocardial fibrosis. In animal models of hypertension, therapies targeting oxidative metabolism have been associated with decreased LV fibrosis. Consequently, understanding oxidative metabolism and myocardial fibrosis in HFpEF is of biologic importance and may have therapeutic implications.

In preliminary work, we have shown that we can quantitatively measure myocardial blood flow reserve, oxidative metabolism and myocardial fibrosis through non-invasive cardiac imaging. Myocardial blood flow reserve can be quantitatively measured with cardiac magnetic resonance imaging (cMRI) and positron emission tomography (PET). The mono-exponential

decay rate (Kmono) of 11C-Acetate determined with PET is a validated measure of myocardial oxidative metabolism. Fibrotic myocardium can be quantified using cMRI T1 mapping.

We postulate that reduced myocardial blood flow reserve contributes to impaired oxidative metabolism and increased myocardial fibrosis in HFpEF. We propose to test the following primary hypotheses: 1) Compared to healthy and hypertensive individuals, subjects with HFpEF have reduced myocardial blood flow reserve, 2) Compared to healthy and hypertensive individuals, subjects with HFpEF have reduced oxidative metabolism; 3) Compared to healthy and hypertensive individuals, subjects with HFpEF have increased myocardial fibrosis, and 4) Myocardial blood flow reserve is positively correlated with oxidative metabolism and inversely correlated with myocardial fibrosis.

Aim 1. To compare myocardial blood flow reserve in individuals with HFpEF versus those with hypertension and healthy individuals. We will enroll 60 subjects (20 HFpEF, 20 hypertensive, and 20 healthy) who will undergo cMRI and 13N-Ammonia PET to quantify myocardial blood flow at rest and during pharmacologic stress with regadenoson.

Aim 2. To compare myocardial oxidative metabolism in individuals with HFpEF versus those with hypertension and healthy individuals. Subjects enrolled in aim 1 will also undergo 11C-Acetate PET imaging to quantify oxidative metabolism via the mono-exponential decay rate of 11C-Acetate.

Aim 3. To compare myocardial fibrosis in individuals with HFpEF versus those with hypertension and healthy individuals. Subjects enrolled in aim 1 will also undergo cMRI with T1 mapping to quantify myocardial fibrosis.

Aim 4. To assess the relation between myocardial blood flow reserve, oxidative metabolism and fibrosis. The correlations between myocardial blood flow reserve, oxidative metabolism and fibrosis will be evaluated.

2. Study Design

2.1 Study Design and Hypotheses

We propose a cross sectional study to investigate the hypotheses that HFpEF is associated with reduced myocardial blood flow reserve, reduced oxidative metabolism, and increased myocardial fibrosis, and that myocardial blood flow reserve is positively correlated with oxidative metabolism, while both are inversely correlated with myocardial fibrosis. We will quantify myocardial blood flow reserve, oxidative metabolism and fibrosis using non-invasive imaging in 60 subjects (20 healthy, 20 hypertensive, and 20 HFpEF). In addition, clinical information and circulating biomarkers will be collected during the protocol. All protocols will

take place at Vanderbilt University Medical Center. We will specifically test the following primary hypotheses (Table 1):

1) Compared to healthy and hypertensive individuals, subjects with HFpEF have reduced myocardial blood flow reserve,

2) Compared to healthy and hypertensive individuals, subjects with HFpEF have reduced oxidative metabolism,

3) Compared to healthy and hypertensive individuals, subjects with HFpEF have increased myocardial fibrosis, and

4) Myocardial blood flow reserve is positively correlated with oxidative metabolism and both are inversely correlated with myocardial fibrosis.

An important feature of the proposal is the inclusion of two control groups: healthy individuals and individuals with hypertension. This design not only maximizes contrast by evaluating healthy individuals and HFpEF patients, but also allows us to test whether a "gradient" of abnormal myocardial structure and function exists, by including a group with hypertension but no HF. This is of particular relevance because hypertension is the most common risk factor for the development of HFpEF and may promote reduced myocardial blood flow reserve and oxidative metabolism and increased fibrosis prior to the onset of HF.

Table 1. Summary of study aims and hypotheses

Aim	Trait	Measure	Healthy (n=20)	Hypertensive (n=20)	HFpEF (n=20)
1	Myocardial Fibrosis	Cardiac MRI & ^{13}N-Ammonia PET	Normal	Normal/↑	↑↑
2	Oxidative Metabolism	^{11}C-Acetate PET	Normal	Normal/↓	↓↓
3	Myocardial Fibrosis	Cardiac MRI	Normal	Normal/↑	↑↑
4	Relationships between blood flow reserve, oxidative metabolism & myocardial fibrosis	Cardiac MRI & ^{13}N-Ammonia PET & ^{11}C-Acetate PET	+ blood flow & oxidative metabolism; inverse blood flow & fibrosis		

2.2 Recruitment of Study Sample

2.2.1 Cohorts Defined

Heart Failure Preserved Ejection Fraction (n=20). HFpEF patients will be aged 50 years or older, have a physician confirmed diagnosis of HF, be symptomatic (New York Heart Association class II or III), with a LVEF ≥50% within the prior 6 months, and no prior history of LVEF < 50%. HFpEF subjects will have evidence of elevated LV filling pressures by ≥ 1 of the following within the last 6 months: invasive cardiac catheterization LV end diastolic pressure or pulmonary capillary wedge pressure > 12 mmHg, plasma B-type natriuretic peptide >100 pg/ml, or echocardiographic E/e' > 15. HFpEF patients with known coronary artery disease (CAD) or angina) will be excluded to reduce potential confounding. Patients with significant valvular (≥ moderate stenosis or regurgitation), pericardial, or congenital heart disease, or symptoms due predominantly to right heart failure and pulmonary arterial hypertension will also be excluded, as will those with an estimated glomerular filtration rate (eGFR) < 45 ml/min/1.73m^2. HFpEF patients in acute decompensated heart failure requiring intravenous diuretics, hospitalization, or use of intravenous vasoactive medications will be eligible for screening, but must be compensated at the time of the protocol visit. HFpEF patients will be recruited through the Vanderbilt Heart and Vascular Institute HFpEF program.

Healthy (n=20): We will enroll volunteers matched on gender to the HFpEF group. Healthy subjects will be free of known cardiovascular disease (HF or cardiomyopathy, CAD, valvular heart disease, pericardial disease, arrhythmias, stroke, peripheral arterial disease), cardiovascular risk factors (hypertension, DM, tobacco use, dyslipidemia, family history of cardiovascular disease, CKD, autoimmune disorder), or use of medications for cardiovascular disease or its risk factors. In addition, healthy individuals must have normal cardiac structure and function, to be confirmed by conventional transthoracic echocardiography at the time of the screening visit. We will use the "Research Studies" email sent to accounts at Vanderbilt University Medical Center and the Research Study Volunteer Program, a registry that enables identification of healthy volunteers.

Hypertensive (n=20). We will recruit subjects matched on gender to the HFpEF group. Subjects in the hypertensive group will have a physician confirmed diagnosis of hypertension, but without known cardiovascular disease (as defined above). Hypertension will be defined as a physician confirmed diagnosis of hypertension, history of blood pressure >140/90 mmHg and treatment with ≥ 1 anti-hypertensive medication. At the time of the protocol visit, the hypertensive subjects must have adequate blood pressure control, defined as < 160/100 mmHg. Hypertensive subjects with other cardiovascular risk factors (as previously defined) will be excluded to reduce confounding. In addition, hypertensive individuals must have preserved LVEF, defined as ≥ 50%. We will recruit hypertensive patients through several means, including the "Research Studies" email sent to accounts at Vanderbilt University Medical Center,

identification of patients from the Vanderbilt Heart and Vascular Institute clinics, and Vanderbilt general medicine and Hypertension clinics.

2.2.2 Inclusion/Exclusion Criteria

Subject Selection: Regardless of study group, any potential subjects with contraindications to cardiac imaging or adverse reactions to echocardiographic contrast, 11C Acetate, gadolinium, regadenoson or its reversal agent (aminophylline), or 13N-Ammonia will be excluded. Based upon the number of subjects needed for the study, we expect the number of subjects screened and eligible will exceed the number needed to enroll. An important aspect of the inclusion/exclusion criteria is the age requirement of ≥ 50 years old. The typical HFpEF patient is in the sixth decade of life or older,[9, 10] and therefore, in order to capture a representative HFpEF population, we will specifically target this age range. Recruitment will be monitored on an ongoing basis by the PI. The investigators' research group has substantial experience recruiting participants into clinical studies and trials using the mechanisms outlined above.

Table 2. Key Inclusion and Exclusion Criteria

Study Group	Inclusion	Exclusion
All	• Age ≥ 50 years • Able to provide informed consent • eGFR ≥ 45 ml/min/1.73m^2	• Coronary artery disease • Contraindication to cMRI (e.g. claustrophobia, metal) • Weight > 350 lbs (PET & MRI limits) • Inability to lie flat for imaging • Pregnancy • Anemia, Hgb < 10 • Contraindication to regadenoson or aminophylline
Healthy	• Normal cardiac structure & function • BP < 140/90 mmHg	• Known cardiovascular disease (see text), risk factors (see text), or use of cardiac medications • Diabetes Mellitus
Hypertensive	• History of BP > 140/90 mmHg • ≥1 anti-hypertensive medication • LVEF ≥ 50% • Current BP < 160/100 mmHg	• Known cardiovascular disease or risk factors (other than hypertension) or use of cardiac medications (other than anti-hypertensives or statins) • Diabetes Mellitus

HFpEF	• Physician confirmed Dx of HF • Symptomatic HF (NYHA II or III) • LVEF ≥ 50% • ↑LV filling pressures (see text) • Current BP < 160/100 mmHg	• Prior history of LVEF < 50% • Acute decompensated HF • ≥ Moderate valvular disease • Significant arrhythmias (AF, SVT, VT) • Pericardial disease • Congenital heart disease • 1' Pulmonary Arterial hypertension

HFpEF subjects

Detailed inclusion criteria
1) Age ≥ 50 years old regardless of sex and race
2) Physician confirmed diagnosis of HFpEF, with corroborating evidence of:
a. Left ventricular ejection fraction ≥ 50%, and
b. Symptomatic heart failure based upon Framingham criteria, and
c. Recent heart failure hospitalization within the prior 6 months, or
d. Evidence of elevated left ventricular filling pressures by any of the following (LVEDP or PCWP > 12 mmHg, BNP > 100 pg/ml, echocardiographic E/e' > 15).

Detailed exclusion criteria for HFpEF
1) Age < 50 years old
2) Inability to provide written informed consent
3) Insufficient data to confirm the diagnosis of HFpEF
4) Known coronary artery disease (prior myocardial infarction, coronary revascularization, or stenosis >50%), or subjects with symptoms or signs of acute myocardial ischemia, for example unstable angina or cardiovascular instability, who may be at greater risk
5) HFpEF due to pericardial disease
6) HFpEF due to right ventricular failure and/or pulmonary arterial hypertension alone.
7) Inability to lie flat for non-invasive cardiac imaging procedures
8) Decompensated and/or acute heart failure requiring active therapy with IV diuretics
9) Estimated glomerular filtration rate < 45 ml/min/1.73m^2 and/or on dialysis.
10) Indwelling metal that is unsafe/incompatible with cardiac magnetic resonance imaging.
11) Any history of adverse reactions or contraindications to cardiac imaging, including adverse reactions and/or contraindications to echocardiographic contrast, gadolinium, 11C-Acetate, or 13N-Ammonia.
12) Any history of adverse reactions or contraindications to regadenoson.
13) Any history of adverse reactions or contraindications to aminophylline (the reversal agent for regadenoson).
14) Uncontrolled hypertension (≥160/100mmHg) or symptomatic hypotension (systolic blood pressure <90mmHg)

15) Obesity, with weight or waist circumference greater than the compatible limit of cMRI and/or PET scanners.
16) Active pregnancy.
17) Prior cardiac transplant.
18) Inability to complete cardiac imaging procedures over 2 consecutive days.
19) Inability to fast for 6 hours and abstain from caffeine intake for >24 hours
20) Non-English speaking

Hypertensive subjects without heart failure

Detailed inclusion criteria for hypertensive controls
1) Age ≥ 50 years old regardless of sex and race
2) No known cardiovascular disease, including but not limited to coronary heart disease, heart failure, valvular stenosis or ≥ moderate valvular regurgitation, arrhythmias, pericardial disease, stroke, and/or peripheral arterial disease
3) No known cardiovascular risk factors, other than hypertension. Specifically, no current or prior history of smoking, obesity, chronic kidney disease, or rheumatologic disorders.
4) No known use of cardiovascular medications, other than anti-hypertensives, lipid lowering agents and/or aspirin for primary prevention. Specifically, no current therapy with digoxin and/or anti-arrhythmic medications.

Detailed exclusion criteria for hypertensive controls
1) Age < 50 years old
2) Inability to provide written informed consent
3) Known coronary artery disease (prior myocardial infarction, coronary revascularization, or stenosis >50%), or subjects with symptoms or signs of acute myocardial ischemia, for example unstable angina or cardiovascular instability, who may be at greater risk
4) Diabetes mellitus
5) Valvular heart disease (≥ moderate stenosis or regurgitation)
6) Pericardial disease
7) Right ventricular failure and/or pulmonary arterial hypertension.
8) Inability to lie flat for non-invasive cardiac imaging procedures
9) Estimated glomerular filtration rate < 45 ml/min/1.73m^2 and/or on dialysis.
10) Indwelling metal that is unsafe/incompatible with cardiac magnetic resonance imaging.
11) Any history of adverse reactions or contraindications to cardiac imaging, including adverse reactions and/or contraindications to echocardiographic contrast, gadolinium, 11C-Acetate, or 13N-Ammonia.
12) Any history of adverse reactions or contraindications to regadenoson.
13) Any history of adverse reactions or contraindications to aminophylline (the reversal agent for regadenoson).

14) Uncontrolled hypertension (≥160/100mmHg) or symptomatic hypotension (systolic blood pressure <90mmHg)
15) Obesity, with weight or waist circumference greater than the compatible limit of cMRI and/or PET scanners.
16) Active pregnancy.
17) Prior cardiac transplant.
18) Inability to complete cardiac imaging procedures over 2 consecutive days.
19) Inability to fast for 6 hours and abstain from caffeine intake for >24 hours
20) Non-English speaking

Healthy Controls

Detailed inclusion criteria for healthy controls
1) Age ≥ 50 years old regardless of sex and race
2) No known cardiovascular disease, including but not limited to coronary heart disease, heart failure, valvular stenosis or ≥ moderate valvular regurgitation, arrhythmias, pericardial disease, stroke, and/or peripheral arterial disease
3) No known cardiovascular risk factors, including but not limited to hypertension, diabetes mellitus, smoking, obesity, chronic kidney disease, or rheumatologic disorders.
4) No known use of aspirin, statins, and cardiovascular medications, including but not limited to beta-blockers, inhibitors of the renal-angiotensin-aldosterone axis, diuretics and/or other anti-hypertensive medications, digoxin, and/or anti-arrhythmic medications.

Detailed exclusion criteria for healthy controls
1) Age < 50 years old
2) Inability to provide written informed consent
3) Known coronary artery disease (prior myocardial infarction, coronary revascularization, or stenosis >50%), or subjects with symptoms or signs of acute myocardial ischemia, for example unstable angina or cardiovascular instability, who may be at greater risk
4) Diabetes mellitus
5) Valvular heart disease (≥ moderate stenosis or regurgitation)
6) Pericardial disease
7) Right ventricular failure and/or pulmonary arterial hypertension.
8) Inability to lie flat for non-invasive cardiac imaging procedures
9) Estimated glomerular filtration rate < 45 ml/min/1.73m^2 and/or on dialysis.
10) Indwelling metal that is unsafe/incompatible with cardiac magnetic resonance imaging.
11) Any history of adverse reactions or contraindications to cardiac imaging, including adverse reactions and/or contraindications to echocardiographic contrast, gadolinium, 11C-Acetate, or 13N-Ammonia.
12) Any history of adverse reactions or contraindications to regadenoson.

13) Any history of adverse reactions or contraindications to aminophylline (the reversal agent for regadenoson).
14) Obesity, with weight or waist circumference greater than the compatible limit of cMRI and/or PET scanners.
15) Active pregnancy.
16) Prior cardiac transplant.
17) Inability to complete cardiac imaging procedures over 2 consecutive days.
18) Inability to fast for 6 hours and abstain from caffeine intake for >24 hours
19) Non-English speaking

2.3 Study Procedures

Screening Visit: At the screening visit, which will take place at the Clinical Research Center, a medical history and physical examination will be administered with eligibility for the protocol determined **(Table 2)**. A venous blood draw of will be performed for measurement of the complete blood count and creatinine to assess inclusion and exclusion criteria. For healthy and hypertensive subjects, an echocardiogram will be performed at the screening visit to assess inclusion and exclusion related to cardiac structure and function, if an echocardiogram within the previous 6 months is not available. If the subject fulfills the inclusion criteria without exclusions to participation, a protocol visit will be scheduled through the Clinical Research Center (CRC).

Protocol Visit: There will be one protocol visit during which the subject will undergo testing as outlined in **Table 3**. After arrival at the CRC, the subject will undergo a history and physical examination. If any intervening medical events between the screening and protocol visits occurred that would exclude the subject from participation, then the subject will be withdrawn from the study. A venous blood draw of will be performed for measurement of complete blood count, basic chemistry panel, B-type natriuretic peptide, and collection of plasma and serum for storage at -80^{o}C for future assays of circulating biomarkers. If the subject is anemic (hemoglobin <10g/dL), or has an eGFR < 45ml/min/1.73m2 according to the Modified Diet and Renal Disease equation then the subject will be withdrawn from the study. Women of child bearing potential will also undergo a serum pregnancy test. If the serum pregnancy test is positive, the subject will be withdrawn from the study. Eligible subjects will then undergo cardiac imaging as described below. For all imaging procedures, data will be deidentified with the exception of a randomly generated subject number, such that imaging data cannot be linked directly to subject data, particularly categorization as healthy, hypertensive, or HFpEF. This will ensure that the analyzing investigator will be blinded to study group.

Table 3. Protocol Visit	
Day 1	**Day 2**
Arrive at CRC (fasting > 6 hours)	Arrive at Cardiac MRI suite
History and Physical	Peripheral IV
Venous blood draw/Peripheral IV	Cardiac MRI (fibrosis imaging)
Transthoracic Echocardiogram	^{13}N-Ammonia PET (rest, stress)
^{11}C-Acetate PET	
Cardiac MRI (rest, stress)	

Echocardiography: At the start of echocardiography, heart rate and blood pressure will be recorded. A registered cardiac sonographer will perform comprehensive 2D, M-mode, and Doppler transthoracic echocardiography following a pre-specified protocol. A minimum of 3 complete cardiac cycles will be recorded for each image. Images will be acquired using a Phillips IE33 (Phillips Corp, Andover, MD) machine and data will be stored digitally for off-line analysis using commercially available software (HeartLab, AGFA and Cardiac Performance Analysis, TomTec). Quantification of cardiac structure and function will be performed in accordance with guidelines from the American Society of Echocardiography.[11-13] In particular, LV size and wall thicknesses will be measured using the 2D method, while LV volumes will be measured from the apical 4 and 2 chamber views via the Simpson's Biplane method. LV ejection fraction will be calculated as LV stroke volume/end diastolic volume. To optimize assessment of left ventricular volumes and ejection fraction, intravenous echocardiographic contrast (Definity, Lantheus Medical Imaging, N Billerica, MA) will be given. LV mass will be calculated using the Devereux formula as recommended by the American Society of Echocardiography.[14] Diastolic function will be assessed using mitral annular tissue Doppler e' velocities, transmitral spectral Doppler E wave velocity and deceleration time, and the ratio of E and A wave velocities, as well as isovolumic relaxation time, and left atrial pressures (defined as E/e'). LV systolic function will be assessed by LVEF, mitral annular tissue Doppler S' velocities, and speckle tracking derived global longitudinal strain and strain rates, which are less angle and load dependent when compared to LVEF and tissue Doppler analyses. Our group has previous experience in quantitative echocardiography, including speckle tracking.[15-18]

11C-Acetate Positron Emission Tomography for the assessment of oxidative metabolism. We will follow an established protocol for 11C-Acetate PET imaging previously used within our lab.[6] An intravenous line will be placed in an upper extremity by trained personnel. The subject will then lie supine on the PET/CT scanner and heart rate and blood pressure will be recorded at baseline and every 5 minutes. A limited transmission CT scan of the chest will be performed for

attenuation correction. 11C-Acetate (0.286 mCi/Kg) will then be injected intravenously and PET imaging will begin. ECG gated images will be acquired in list mode for 30 minutes and reformatted into images of 12 frames of 10 seconds each, 8 frames of 30 seconds, 4 frames of 60 seconds, 2 frames of 300 seconds, and 1 frame of 600 seconds. The 11C-Acetate images will be processed following CT attenuation correction. If the images are uninterpretable due to factors such as patient motion, attenuation artifacts, and/or inability to accurately gate images with ECG, then the subject will be withdrawn from the study with attempts to replace the subject to maintain sample size. Regions of interest over the LV blood pool and LV myocardium from the last image of the study will be applied to all preceding frames. Counts in the regions of interest will be determined and expressed as counts/pixel. The blood pool and LV myocardium activities will be plotted against time. The mono-exponential clearance rate, defined as Kmono, will be determined by least-squares fitting of the linear portion of the time-activity curve. Kmono has previously been demonstrated to correlate with myocardial oxygen consumption.[19-21] As the rate of cardiac metabolism may be influenced by cardiac demand, LV efficiency will be calculated as the work-metabolic index (WMI) using the equation WMI = (LVSVI x SBP x HR)/Kmono, where LVSVI is the LV stroke volume index (stroke volume/body surface area), SBP is systolic blood pressure, and HR is heart rate.[22] In addition, oxidative metabolism may be influenced by blood supply, and therefore we will also assess myocardial blood flow through cMRI and PET as described below.

Cardiac Magnetic Resonance Imaging of myocardial blood flow and myocardial fibrosis. cMRI will be performed on a 1.5T Siemens Magnetom Avanto scanner (Siemens, Erlangen, Germany). Subjects will be scanned with a phased array torso receiver coil. Following localizer images for identification of the heart within the thorax, cine imaging ("steady state free procession") will be performed to generate conventional 4 and 2 chamber views, as well as short axis images. A short axis "stack" of cine images from the base to apex of the heart will be obtained, from which LV wall thickness, mass, volumes, and ejection fraction will be determined. Typical acquisition parameters will be used: field of view 300x340mm, matrix 156x192, slice thickness 8 mm, flip angle 80^{o} with downward adjustment depending upon the specific absorption rate, echo time 1.1ms, bandwidth 930Hz/pixel, and at least 30 phases per cycle to maintain a repetition time < 50 ms. Parallel imaging will be used with generalized autocalibrating partially parallel acquisition (GRAPPA) using an acceleration factor of 2.6

Following cine imaging, myocardial perfusion imaging will be performed at rest following dual bolus (pre bolus =0.005 mmol/kg; main bolus = 0.05 mmol/kg) intravenous injection of Gadobutrol (Gd-DO3A-butrol: Gadavist, Bayer Healthcare Pharmaceuticals, Wayne, IN), as previously described.[23-27] First pass imaging of the wash in of Gd-DO3A-butrol through the LV myocardium will be obtained in 3 short axis imaging planes centered at the mid LV at the level of the papillary muscles, the basal LV below the mitral valve, and apical LV. T1 weighted saturation-recovery turbo fast low angle shot (FLASH) gradient echo sequences will be used for myocardial perfusion imaging. All short axis images for myocardial perfusion imaging will be

acquired over 50-60 consecutive cardiac cycles beginning with injection of the Gd-DO3-butrolA contrast. Typical acquisition parameters for first pass imaging will be used: field of view 320x400mm, matrix 116x192, slice thickness 8 mm, skip 8 mm, flip angle 12^{o}, echo time 1.1 msec, repetition time 160 msec, bandwidth 930Hz/pixel, and parallel imaging with an acceleration factor of 2. Stress myocardial perfusion imaging will be repeated 15 minutes following the resting gadolinium injection. Regadenoson (Astellas Pharma, Northbrook, IL) 400 µg will be injected intravenously over 5-10 seconds, followed by 5-10 ml saline flush over 5-10 seconds, as previously described.[23, 28] Approximately 60 seconds following regadenoson injection, gadolinium will be injected intravenously using the dual bolus method with imaging performed as described above. Heart rate, rhythm, and blood pressure will be monitored throughout the imaging protocol. The myocardial perfusion index (MPI) will be calculated offline as the maximum slope of the time-intensity curve of myocardial enhancement according to the following equation: MPI = maximum slope (myocardium)/maximum slope (LV cavity).[6] MPI has previously been demonstrated to be a valid tool for quantification of myocardial blood flow.[29, 30] Myocardial perfusion reserve index will then be calculated as the ratio of stress to rest myocardial perfusion indices. Similar analyses will be performed to evaluate myocardial blood flow (MPI and MPRI) in the subendocardial and subepicardial layers of the left ventricle.

Myocardial fibrosis will be non-invasively quantified using cMRI.[31] A short axis image at the mid LV will be obtained pre-contrast using the Modified Look-Locker Inversion recovery (MOLLI) sequence with imaging at the same level repeated at 10 and 15 minutes post intravenous 0.15 mmol/kg Gd-DO3A-butrol contrast injection.[32, 33] The MOLLI sequence consists of 3 consecutive inversion recovery prepared ECG gated Look-Locker trains. Each train begins with an inversion pulse at a specific inversion time (T1= 100, 200, and 350 msec), after which multiple single-shot steady state free procession images will be acquired over consecutive heart beats. All images will be acquired with the same trigger delay time in end diastole. Scanning parameters will be a field of view of 360x360mm, matrix 192x193, slice thickness 8 mm, flip angle 35, echo time 1.1 ms, repetition time 2.2ms, GRAPPA factor of 2. Prior to the delivery of Gd-DO3A-butrol intravenous contrast, the “pre-contrast” T1 relaxation time will be determined. Similarly at 10 and 15 minutes post contrast, T1 relaxation times will be determined. The T1 relaxation time has previously been demonstrated to correlate with the extracellular volume of fibrosis by histology.[31, 34-36] The percent extracellular fibrosis will be quantified using the following previously validated equation: $\lambda \times (1 - \text{hematocrit})$, where $\lambda = (\Delta RI_{myocardium}/\Delta RI_{blood\ pool})$ where RI=1/T1 and Δ corresponds to the change in RI pre and post Gd-DO3A-butrol contrast.[37] In addition to T1 mapping, we will also examine late gadolinium enhancement (LGE) as a measure of regional myocardial fibrosis.[31] At 10-15 minutes post Gd-DO3A-butrol contrast injection, short axis myocardial LGE imaging will be performed using both single-shot inversion recovery and phase-sensitive inversion recovery true FISP imaging acquired every other cardiac cycle. In order to select the optimal inversion time (TI), a TI “scout” will be acquired prior to LGE imaging, using progressively longer TI times. The optimal TI time will be determined by visual estimation of when the myocardium appears the darkest.[6]

13N-Ammonia Positron Emission Tomography for the assessment of myocardial blood flow. 13N-Ammonia studies will be performed at rest and stress on a whole body PET/CT scanner as previously described.[38] An initial transmission scan will be obtained for the purpose of attenuation correction. Next, rest imaging will be initiated in list mode upon injection of 25mCi of intravenous 13N-Ammonia with serial images acquired over approximately 15-20 minutes. Approximately 50 minutes after the initial 13N-Ammonia injection (5 half-lives), imaging will be repeated following pharmacologic vasodilation with regadenoson (Astellas Pharma, Northbrook, IL). As with the cMRI protocol, 400 µg of regadenoson will be injected intravenously over 5-10 seconds, followed by a 5-10 ml saline flush. Approximately 60 seconds after the regadenoson injection, 25mCi of 13N-Ammonia will be injected with PET imaging in list mode. Heart rate, rhythm, and blood pressure will be monitored throughout the imaging protocol. Myocardial blood flow and coronary flow reserve will be quantified off-line using FlowQuant software (Ottawa Heart Institute, Ottawa, Canada).

Table 3a. Schedule of Events and Research Procedures for Participants	
Procedure/Activity	**Frequency**
Informed Consent	**Once (at time of screening in clinic)**
Blood draw	**Once (at beginning of study visit – day 1)**
Peripheral IV placement	**Twice (at study visit on days 1 & 2)**
Transthoracic Echocardiogram with contrast	**Once (at study visit – day 1)**
^{11}C-Acetate PET	**Once (at study visit – day 1)**
Cardiac MRI (rest, stress)	**Once (at study visit – day 1)**
Cardiac MRI (fibrosis imaging)	**Once (at study visit – day 2)**
^{13}N-Ammonia PET (rest, stress)	**Once (at study visit – day 2)**

Reporting of clinical findings. As the imaging studies are being performed for research purposes, clinical reports will not be generated. However, if unexpected or previously unknown abnormalities of clinical importance are identified or urgent/emergent conditions are found, these will be clinically reported to both the patient and the patient's physicians.

3. Statistical Methods

Aim 1. To compare myocardial blood flow reserve in individuals with HFpEF versus those with hypertension and healthy individuals.

Primary Hypothesis: Compared to healthy and hypertensive individuals, subjects with HFpEF have reduced myocardial blood flow reserve.

Secondary Hypotheses:
A) Myocardial blood flow is progressively impaired from healthy, to hypertensive, to HFpEF.
B) Myocardial blood flow reserve by Gd enhanced cMRI (MPRI) is correlated with CFR by ^{13}N-Ammonia PET.
C) Subendocardial MPRI is lower than subepicardial MPRI in HFpEF.
D) Subendocardial MPRI is progressively impaired from healthy to hypertensive to HFpEF.
E) Myocardial blood flow reserve is positively correlated with LV diastolic function.
F) Myocardial blood flow reserve is positively correlated with LV systolic function.

Analysis for aim 1. Primary outcome: Myocardial blood flow reserve, as measured by MPRI from contrast enhanced cMRI first pass perfusion imaging and CFR from ^{13}N-Ammonia PET perfusion imaging will be compared between the three study groups: healthy, hypertensives, and HFpEF. The distributions of each within each study group will be assessed using histograms, Q norm plots, and the Shapiro Wilk test. If normally distributed then analysis of variance will be used to test for differences in myocardial blood flow reserve between groups. If the p-value from the ANOVA is < 0.05, then pairwise comparisons using unpaired t-tests with unequal variances will be made between groups using a Bonferroni correction. If MPRI or CFR is not normally distributed, then a Kruskal-Wallis test and Wilcoxon rank sum tests will be used in place of ANOVA and t-tests. The null hypothesis is that myocardial blood flow reserve does not differ among HFpEF patients, healthy individuals, and hypertensive individuals. We will also assess correlations (Spearman) between myocardial blood flow reserve, age, gender, blood pressure, heart rate, and body mass index. To account for potential confounders, multivariable linear regression models will be constructed with myocardial blood flow reserve as the dependent variable, study group as the primary independent variable, with adjustments for age, gender, race/ethnicity, anti-hypertensive medications, systolic blood pressure, heart rate, body mass index, LVEF, LV end diastolic volume, and LV mass. Prior to entry into models, variables will be log transformed, as appropriate. We will check model fit by residual plots. Missing data will not be imputed as we will attempt to replace subjects with significant amounts of missing data due to uninterpretable images, as detailed above.

Statistical power for Aim 1: Using a one-way ANOVA at p = 0.05, we present between group differences in myocardial blood flow reserve as assessed by CFR under different sample size and power scenarios in **Table 4**. We anticipate having 80% power to detect a between group difference (largest to smallest) in mean values of CFR of 0.87, using a conservative estimate for the standard deviation of 0.90 when compared to those reported for normal controls and patients with hypertension, 0.68 and 0.88, respectively.[39] The between group difference in CFR is within the range of difference detected between normals (3.17 ± 0.68) and patients with hypertension (2.18 ± 0.88), previously reported.[39] Of note even if sample size is reduced by 25%, we still anticipate having good power to detect a between group difference of 1.05. Therefore, using conservative sample sizes that account for potential dropout or uninterpretable images, as well as larger estimates for standard deviation than previously reported, we should have good power to detect plausible differences in myocardial blood flow reserve between HFpEF, healthy, and hypertensive subjects.

Table 4. Between group differences in myocardial blood flow measured by coronary flow reserve (CFR) for varying samples sizes and powers, at α = 0.050 using ANOVA

Sample size			Power			SD
Healthy	HTN	HFpEF	0.90	0.80	0.70	
20	20	20	1.00	0.87	0.75	0.90
15	15	15	1.20	1.05	0.92	0.90
25	25	25	0.80	0.75	0.60	0.90

ANOVA calculations based upon mean estimated CFR in normals of 3.17 ± 0.88.[39]

Secondary analyses: The secondary hypotheses are presented above. In addition to testing the primary hypothesis for between group differences in myocardial blood flow reserve, we will also evaluate for a significant trend across the three study groups using Spearman correlation. The relationships between myocardial blood flow reserve derived from cMRI (MPRI) and PET (CFR) will be compared by Spearman correlation as well as intraclass correlation in all subjects and by subject group. Using ANOVA, we will also assess whether subendocardial MPRI is lower in HFpEF as compared to healthy and hypertensive controls. Similarly we will assess whether subendocardial MPRI is lower than subepicardial MPRI among HFpEF subjects using paired t-tests. The relationships between myocardial blood flow reserve and cardiac function will also be evaluated. Scatterplots and Spearman correlations will be performed with myocardial blood flow reserve as the independent variable and measures of diastolic function as the dependent variables (mitral annular tissue Doppler e' velocities, isovolumic relaxation time, transmitral spectral Doppler E wave velocity, and E/e'). A nominal p-value threshold of < 0.05 will be used for all correlations and results meeting significance at the nominal threshold will be considered for follow up study. Similarly, we all also examine scatterplots and Spearman correlations between myocardial blood flow reserve and systolic function (LVEF, mitral annular tissue Doppler s' velocities, and global longitudinal strain). For both systolic and diastolic parameters, we will perform multivariable linear regression analyses adjusting for age, gender, race/ethnicity, systolic blood pressure, heart rate, body mass index, LVEF, LV end diastolic volume, and LV mass. We will also test for effect modification on the relationships between myocardial blood flow reserve and diastolic and systolic function by including multiplicative interaction terms in the linear regression models. Prior to entry into regression models non-normally distributed variables will be appropriately transformed.

Aim 2. To compare myocardial oxidative metabolism in individuals with HFpEF versus those with hypertension and healthy individuals.

Primary Hypothesis: Compared to healthy and hypertensive individuals, subjects with HFpEF have reduced oxidative metabolism.

Secondary Hypotheses:
A) Oxidative metabolism is progressively impaired from healthy, to hypertensive, to HFpEF.
B) Oxidative metabolism is positively correlated with LV diastolic function.
C) Oxidative metabolism is positively correlated with LV systolic function.

Analysis for aim 2. Primary outcome: Oxidative metabolism, quantified by K_{mono} from ^{11}C-Acetate PET imaging, will be compared between the three study groups: healthy, hypertensives, and HFpEF. The distribution of K_{mono} within each study group will be assessed using histograms, Q norm plots, and the Shapiro Wilk test. If normally distributed then analysis of variance will be used to test for differences in K_{mono} between groups. If the p-value from the ANOVA is < 0.05, then pairwise comparisons using unpaired t-tests with unequal variances will be made between groups using a Bonferroni correction. If K_{mono} is not normally distributed, then a Kruskal-Wallis test and Wilcoxon rank sum tests will be used in place of ANOVA and t-tests. The null hypothesis is that oxidative metabolism (K_{mono}), does not differ among HFpEF patients, healthy individuals, and hypertensive individuals. We will also check for correlations (Spearman) between K_{mono}, age, gender, blood pressure, heart rate, body mass index, and resting myocardial blood flow. To account for potential confounders, multivariable linear regression models will be constructed with oxidative metabolism (K_{mono}) as the dependent variable, study group as the primary independent variable, with adjustments for age, gender, race/ethnicity, anti-hypertensive medications, systolic blood pressure, heart rate, body mass index, LVEF, LV end diastolic volume, LV mass, and resting myocardial blood flow. Prior to entry into models, variables will be log transformed, as appropriate. We will check model fit by residual plots. Missing data will not be imputed as we will attempt to replace subjects with significant amounts of missing data due to uninterpretable images, as detailed above.

Statistical power for Aim 2: Using a one-way ANOVA at p = 0.05, we present between group differences in K_{mono} under different sample size and power scenarios in **Table 5**. We anticipate having 80% power to detect a between group difference (largest to smallest) in mean values of K_{mono} of 0.010, using a conservative estimate for the standard deviation of 0.010 when compared to those reported for normal controls and patients with HFrEF, 0.006 and 0.001, respectively. The between group

Table 5. Between group differences in oxidative metabolism (K_{mono}) for varying samples sizes and powers, at α = 0.050 using ANOVA

Sample size			Power			SD
Healthy	HTN	HFpEF	0.90	0.80	0.70	
20	20	20	0.012	0.010	0.009	0.01
15	15	15	0.014	0.012	0.011	0.01
25	25	25	0.010	0.009	0.008	0.01

ANOVA calculations based upon mean estimated K_{mono} in normals of 0.060 ± 0.006.[6]

difference in K_{mono} is well within the range of difference detected between normals (0.060 ± 0.006) and patients with HFrEF (0.041 ± 0.001), previously reported by our group.[6, 40] Of note even if sample size is reduced by 25%, we still anticipate having good power to detect a between group difference of 0.012. Therefore, using conservative sample sizes that account for potential dropout or uninterpretable images, as well as larger estimates for standard deviation than previously reported, we should have good power to detect plausible differences in oxidative metabolism between HFpEF, healthy, and hypertensive subjects.

Secondary analyses: The secondary hypotheses are presented above. In addition to testing the primary hypothesis for between group differences in K_{mono}, we will also evaluate for a significant trend across the three study groups using Spearman correlation. The relationships between oxidative metabolism and cardiac function will also be evaluated. Scatterplots and Spearman correlations will be performed with oxidative metabolism as the independent variable and measures of diastolic function as the dependent variables (mitral annular tissue Doppler e' velocities, isovolumic relaxation time, transmitral spectral Doppler E wave velocity, and E/e'). A nominal p-value threshold of < 0.05 will be used for all correlations and results meeting significance at the nominal threshold will be considered for follow up study. Similarly, we all also examine scatterplots and Spearman correlations between oxidative metabolism and systolic function (LVEF, mitral annular tissue Doppler s' velocities, and global longitudinal strain). For both systolic and diastolic parameters, we will perform multivariable linear regression analyses adjusting for age, gender, race/ethnicity, systolic blood pressure, heart rate, body mass index, LVEF, LV end diastolic volume, LV mass, and resting myocardial blood flow. We will also test for effect modification on the relationships between oxidative metabolism and diastolic and systolic function by including multiplicative interaction terms in the linear regression models. Prior to entry into regression models non-normally distributed variables will be appropriately transformed.

Aim 3. To compare myocardial fibrosis in individuals with HFpEF versus healthy individuals and those with hypertension.

Primary Hypothesis: Compared with healthy and hypertensive individuals, subjects with HFpEF have increased myocardial fibrosis.

Secondary Hypotheses:

A) Myocardial fibrosis progressively increases from healthy, to hypertensive, to HFpEF.
B) Myocardial fibrosis is inversely correlated with LV diastolic function.
C) Myocardial fibrosis is inversely correlated with LV systolic function.

Analysis for aim 3. Primary outcome: myocardial fibrosis assessed by T1 relaxation times by cMRI will be compared between the three study groups. The analyses for aim 3 will be the same as for aim 2, except that myocardial fibrosis, instead of oxidative metabolism, will be

compared. The distribution of T1 relaxation times, as a measure of myocardial fibrosis, within each study group will be assessed. Based upon distributions, ANOVA or Kruskal-Wallis tests will be used to identify differences between study groups and if significant $p < 0.05$, then pairwise comparisons with T-tests or Wilcoxon rank sum tests will be performed with Bonferroni correction. The null hypothesis is that T1 relaxation times do not differ among HFpEF, healthy, and hypertensive individuals. We will also check for correlations (Spearman) between myocardial fibrosis, age, gender, blood pressure, heart rate, body mass index, and resting myocardial blood flow. To account for potential confounders, multivariable linear regression models will be constructed with myocardial fibrosis as the dependent variable, with adjustments for the same covariates described for oxidative metabolism above.

Statistical power for Aim 3: Using an ANOVA at $p = 0.05$, we present between group differences in T1 relaxation times (msec) under different sample size and power scenarios in **Table 6**. We anticipate having 80% power to detect a between groups difference (largest to smallest) in T1 relaxation times of 60 msec, using a conservative estimate for the standard deviation of 60 msec, which was based on a 51 msec SD observed in a HFpEF population (mean T1 time 381msec).[41] The difference in T1 relaxation times is well within the range of expected difference based upon reported values for normals (459 ± 38) in the MESA study and patients with HFpEF (381 ± 51).[32, 41] Of note, even if sample size is reduced by 25%, we still anticipate having good power to detect a between group difference of 70 msec, which is still less than the clinical relevant difference of ~80 msec. Therefore, using conservative sample sizes, accounting for potential dropout or uninterpretable images, as well as a larger standard deviation estimate than previously reported, we should have good to excellent power to detect plausible differences in myocardial fibrosis between HFpEF, healthy, and hypertensive subjects.

Table 6. Between group differences in myocardial fibrosis (T1 relaxation times, msec) for varying samples sizes and powers, at α = 0.050 using ANOVA

Sample size			Power			SD
Healthy	HTN	HFpEF	0.90	0.80	0.70	
20	20	20	70	60	54	60
15	15	15	80	70	64	60
25	25	25	60	54	48	60

ANOVA calculations based upon mean estimated T1 relaxation time in HFpEF of 381 ± 51msec.[41]

Secondary analyses: The secondary hypotheses are detailed above and evaluate for progressive increases in myocardial fibrosis across study groups as well as the relationships between myocardial fibrosis and cardiac function. The analyses for myocardial fibrosis will follow the same statistical methods to those described for oxidative metabolism. Myocardial fibrosis, as assessed by T1 relaxation times, will be the independent variable and assessed in relation to features of diastolic and systolic function as previously defined above.

Aim 4. To evaluate the relationships between myocardial blood flow reserve, oxidative metabolism and fibrosis.

Primary Hypothesis: Myocardial blood flow reserve is positively correlated with oxidative metabolism and inversely correlated with myocardial fibrosis.

Analysis for Aim 4: For all subjects completing gadolinium enhanced cMRI myocardial perfusion imaging, ^{13}N-Ammonia PET perfusion imaging, ^{11}C-Acetate PET imaging and cMRI fibrosis imaging, the relationships between myocardial blood flow reserve, oxidative metabolism, and fibrosis will be evaluated using Spearman correlation. To account for potential confounders, multivariable linear regression models will be constructed with myocardial fibrosis or oxidative metabolism as the dependent variable, myocardial blood flow reserve as the primary independent variable with adjustments for age, gender, race/ethnicity, anti-hypertensive medications, systolic blood pressure, heart rate, body mass index, LVEF, LV end diastolic volume, LV mass, and study group. We will check model fit by residual plots. Prior to entry into regression models non-normally distributed variables will be appropriately transformed.

Statistical power for Aim 4: We present different margins of error for the Spearman correlation coefficient based upon different sample sizes and correlation coefficients in **Table 7**. Based upon a sample size of 60 subjects, modest correlation coefficients of |0.40| to |0.50| would have 0.24 and 0.22 margins of error, respectively.

Table 7. Margins of error for the Spearman correlation between myocardial blood flow, oxidative metabolism and myocardial fibrosis.

	Correlation coefficient (absolute value)			
Sample Size	|0.2|	|0.3|	|0.4|	|0.5|
40	0.32	0.31	0.30	0.28
50	0.28	0.28	0.26	0.24
60	0.26	0.25	0.24	0.22

4. Data Management

Patient clinical data is stored in Vanderbilt's electronic medical record. The hospital database is password protected and accessible only to the appropriate personnel. Research data will be labeled with a patient study code and stored in a separate research database. The code key connecting names to specific information will be available only to members of the research staff and will be kept in a separate, secure location.

The research data will be stored in a HIPPA-compliant manner in a REDCap database accessible to only the PI and key study personnel. Only those personnel that are directly involved in the study will have access to this database.

5. Safety Reporting

5.1 Serious Adverse Events

Definitions of Serious Adverse Events
A serious adverse event or reaction is any untoward medical occurrence that at any dose:

- Results in death;
- Is life-threatening
  (Defined as an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.);
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity;
- Results in a congenital anomaly or birth defect;
- Results in the development of drug dependency or drug abuse;
- Is an important medical event-
  (Defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the patient/subject or may require intervention (e.g., medical, surgical) to prevent one of the other serious events or reactions listed above. Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm and blood dyscrasias or convulsions that do not result in hospitalization. For reporting purposes, Lantheus also considers the occurrences of pregnancy, cancer or overdose (regardless of adverse outcome) as events which must be reported as important medical events.)

SAE Reporting to Regulatory Bodies

Regardless of causality, all SAEs will be documented and reported to regulatory bodies, including the IRB as required by the International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice (GCP). Follow-up information for SAEs and information for non-serious AEs that become serious will also be reported in the same manner.

SAE's will be reported to the Vanderbilt Human Research Protection Program according to institutional policies and procedures.

Study Withdraw/Discontinuation
Patients who wish to be withdrawn from the study will notify the PI who will have any of their data that has not been used or analyzed removed from the REDCap database.

Privacy/Confidentiality Issues

Patient privacy and confidentiality will be protected by the usual practices of the echocardiography laboratory. The confidentiality of patient data will be protected by

maintaining a single master list linking Study ID (from the REDCap database) with StarPanel Medical Record Number, which will be stored in the REDCap file repository.

REFERENCES

1. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Huffman MD, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Magid D, Marcus GM, Marelli A, Matchar DB, McGuire DK, Mohler ER, Moy CS, Mussolino ME, Nichol G, Paynter NP, Schreiner PJ, Sorlie PD, Stein J, Turan TN, Virani SS, Wong ND, Woo D, Turner MB, American Heart Association Statistics C and Stroke Statistics S. Heart disease and stroke statistics--2013 update: a report from the American Heart Association. *Circulation*. 2013;127:e6-e245.
2. Roger VL. Epidemiology of heart failure. *Circ Res*. 2013;113:646-59.
3. Meta-analysis Global Group in Chronic Heart F. The survival of patients with heart failure with preserved or reduced left ventricular ejection fraction: an individual patient data meta-analysis. *Eur Heart J*. 2012;33:1750-7.
4. Borlaug BA and Redfield MM. Diastolic and systolic heart failure are distinct phenotypes within the heart failure spectrum. *Circulation*. 2011;123:2006-13; discussion 2014.
5. Paulus WJ and Tschope C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. *J Am Coll Cardiol*. 2013;62:263-71.
6. Bell SP, Adkisson DW, Ooi H, Sawyer DB, Lawson MA and Kronenberg MW. Impairment of subendocardial perfusion reserve and oxidative metabolism in nonischemic dilated cardiomyopathy. *J Card Fail*. 2013;19:802-10.
7. Doenst T, Nguyen TD and Abel ED. Cardiac metabolism in heart failure: implications beyond ATP production. *Circ Res*. 2013;113:709-24.
8. Murthy VL, Naya M, Foster CR, Hainer J, Gaber M, Di Carli G, Blankstein R, Dorbala S, Sitek A, Pencina MJ and Di Carli MF. Improved cardiac risk assessment with noninvasive measures of coronary flow reserve. *Circulation*. 2011;124:2215-24.
9. Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL and Redfield MM. Trends in prevalence and outcome of heart failure with preserved ejection fraction. *N Engl J Med*. 2006;355:251-9.

10. Bhatia RS, Tu JV, Lee DS, Austin PC, Fang J, Haouzi A, Gong Y and Liu PP. Outcome of heart failure with preserved ejection fraction in a population-based study. *N Engl J Med*. 2006;355:260-9.
11. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ, Chamber Quantification Writing G, American Society of Echocardiography's G, Standards C and European Association of E. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr*. 2005;18:1440-63.
12. Nagueh SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, Waggoner AD, Flachskampf FA, Pellikka PA and Evangelista A. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. *J Am Soc Echocardiogr*. 2009;22:107-33.
13. Rudski LG, Lai WW, Afilalo J, Hua L, Handschumacher MD, Chandrasekaran K, Solomon SD, Louie EK and Schiller NB. Guidelines for the echocardiographic assessment of the right heart in adults: a report from the American Society of Echocardiography endorsed by the European Association of Echocardiography, a registered branch of the European Society of Cardiology, and the Canadian Society of Echocardiography. *J Am Soc Echocardiogr*. 2010;23:685-713; quiz 786-8.
14. Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I and Reichek N. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol*. 1986;57:450-8.
15. Gupta DK, Shah AM, Castagno D, Takeuchi M, Loehr LR, Fox ER, Butler KR, Mosley TH, Kitzman DW and Solomon SD. Heart Failure with Preserved Ejection Fraction in African-Americans - The Atherosclerosis Risk in Communities (ARIC) Study. *JACC Heart failure*. 2013;1:156-163.
16. Kraigher-Krainer E, Shah AM, Gupta DK, Santos A, Claggett B, Pieske B, Zile MR, Voors AA, Lefkowitz MP, Packer M, McMurray JJ, Solomon SD and Investigators P. Impaired systolic function by strain imaging in heart failure with preserved ejection fraction. *J Am Coll Cardiol*. 2014;63:447-56.
17. Shah SJ, Aistrup GL, Gupta DK, O'Toole MJ, Nahhas AF, Schuster D, Chirayil N, Bassi N, Ramakrishna S, Beussink L, Misener S, Kane B, Wang D, Randolph B, Ito A, Wu M, Akintilo L, Mongkolrattanothai T, Reddy M, Kumar M, Arora R, Ng J and Wasserstrom JA. Ultrastructural and cellular basis for the development of abnormal myocardial mechanics during the transition from hypertension to heart failure. *Am J Physiol Heart Circ Physiol*. 2014;306:H88-H100.
18. Burke MA, Katz DH, Beussink L, Selvaraj S, Gupta DK, Fox J, Chakrabarti S, Sauer AJ, Rich JD, Freed BH and Shah SJ. Prognostic importance of pathophysiologic markers in patients with heart failure and preserved ejection fraction. *Circ Heart Fail*. 2014;7:288-99.
19. Brown MA, Myears DW and Bergmann SR. Validity of estimates of myocardial oxidative metabolism with carbon-11 acetate and positron emission tomography despite altered patterns of substrate utilization. *J Nucl Med*. 1989;30:187-93.

20. Henes CG, Bergmann SR, Walsh MN, Sobel BE and Geltman EM. Assessment of myocardial oxidative metabolic reserve with positron emission tomography and carbon-11 acetate. *J Nucl Med*. 1989;30:1489-99.
21. Brown M, Marshall DR, Sobel BE and Bergmann SR. Delineation of myocardial oxygen utilization with carbon-11-labeled acetate. *Circulation*. 1987;76:687-96.
22. Knaapen P, Germans T, Knuuti J, Paulus WJ, Dijkmans PA, Allaart CP, Lammertsma AA and Visser FC. Myocardial energetics and efficiency: current status of the noninvasive approach. *Circulation*. 2007;115:918-27.
23. Hsu LY, Groves DW, Aletras AH, Kellman P and Arai AE. A quantitative pixel-wise measurement of myocardial blood flow by contrast-enhanced first-pass CMR perfusion imaging: microsphere validation in dogs and feasibility study in humans. *JACC Cardiovasc Imaging*. 2012;5:154-66.
24. Christian TF, Aletras AH and Arai AE. Estimation of absolute myocardial blood flow during first-pass MR perfusion imaging using a dual-bolus injection technique: comparison to single-bolus injection method. *J Magn Reson Imaging*. 2008;27:1271-7.
25. Jerosch-Herold M. Quantification of myocardial perfusion by cardiovascular magnetic resonance. *J Cardiovasc Magn Reson*. 2010;12:57.
26. Lee DC and Johnson NP. Quantification of absolute myocardial blood flow by magnetic resonance perfusion imaging. *JACC Cardiovasc Imaging*. 2009;2:761-70.
27. Utz W, Greiser A, Niendorf T, Dietz R and Schulz-Menger J. Single- or dual-bolus approach for the assessment of myocardial perfusion reserve in quantitative MR perfusion imaging. *Magn Reson Med*. 2008;59:1373-7.
28. Bhave NM, Freed BH, Yodwut C, Kolanczyk D, Dill K, Lang RM, Mor-Avi V and Patel AR. Considerations when measuring myocardial perfusion reserve by cardiovascular magnetic resonance using regadenoson. *J Cardiovasc Magn Reson*. 2012;14:89.
29. Klocke FJ, Simonetti OP, Judd RM, Kim RJ, Harris KR, Hedjbeli S, Fieno DS, Miller S, Chen V and Parker MA. Limits of detection of regional differences in vasodilated flow in viable myocardium by first-pass magnetic resonance perfusion imaging. *Circulation*. 2001;104:2412-6.
30. Jerosch-Herold M, Vazquez G, Wang L, Jacobs DR, Jr. and Folsom AR. Variability of myocardial blood flow measurements by magnetic resonance imaging in the multi-ethnic study of atherosclerosis. *Invest Radiol*. 2008;43:155-61.
31. Mewton N, Liu CY, Croisille P, Bluemke D and Lima JA. Assessment of myocardial fibrosis with cardiovascular magnetic resonance. *J Am Coll Cardiol*. 2011;57:891-903.
32. Liu CY, Liu YC, Wu C, Armstrong A, Volpe GJ, van der Geest RJ, Liu Y, Hundley WG, Gomes AS, Liu S, Nacif M, Bluemke DA and Lima JA. Evaluation of age-related interstitial myocardial fibrosis with cardiac magnetic resonance contrast-enhanced T1 mapping: MESA (Multi-Ethnic Study of Atherosclerosis). *J Am Coll Cardiol*. 2013;62:1280-7.
33. Bradham W, Bell SP, Smith H, Adkisson D, Lawson MA, Ooi H, Sawyer DB and Kronenberg MW. Myocardial T1 in Nonischemic Dilated Cardiomyopathy is Related to Circulating Procollagen Type 1 Carboxyterminal Peptide and Predicts Reverse Remodeling by Spironolactone Therapy. *Circulation*. 2013;128:A10179.

34. Sibley CT, Noureldin RA, Gai N, Nacif MS, Liu S, Turkbey EB, Mudd JO, van der Geest RJ, Lima JA, Halushka MK and Bluemke DA. T1 Mapping in cardiomyopathy at cardiac MR: comparison with endomyocardial biopsy. *Radiology*. 2012;265:724-32.
35. White SK, Sado DM, Flett AS and Moon JC. Characterising the myocardial interstitial space: the clinical relevance of non-invasive imaging. *Heart*. 2012;98:773-9.
36. Iles L, Pfluger H, Phrommintikul A, Cherayath J, Aksit P, Gupta SN, Kaye DM and Taylor AJ. Evaluation of diffuse myocardial fibrosis in heart failure with cardiac magnetic resonance contrast-enhanced T1 mapping. *J Am Coll Cardiol*. 2008;52:1574-80.
37. Wong TC, Piehler K, Meier CG, Testa SM, Klock AM, Aneizi AA, Shakesprere J, Kellman P, Shroff SG, Schwartzman DS, Mulukutla SR, Simon MA and Schelbert EB. Association between extracellular matrix expansion quantified by cardiovascular magnetic resonance and short-term mortality. *Circulation*. 2012;126:1206-16.
38. El Fakhri G, Kardan A, Sitek A, Dorbala S, Abi-Hatem N, Lahoud Y, Fischman A, Coughlan M, Yasuda T and Di Carli MF. Reproducibility and accuracy of quantitative myocardial blood flow assessment with (82)Rb PET: comparison with (13)N-ammonia PET. *J Nucl Med*. 2009;50:1062-71.
39. Alexanderson E, Jacome R, Jimenez-Santos M, Ochoa JM, Romero E, Cabral MA, Ricalde A, Inarra F, Meave A and Alexanderson G. Evaluation of the endothelial function in hypertensive patients with 13N-ammonia PET. *J Nucl Cardiol*. 2012;19:979-86.
40. Kronenberg MW, Cohen GI, Leonen MF, Mladsi TA and Di Carli MF. Myocardial oxidative metabolic supply-demand relationships in patients with nonischemic dilated cardiomyopathy. *J Nucl Cardiol*. 2006;13:544-53.
41. Mascherbauer J, Marzluf BA, Tufaro C, Pfaffenberger S, Graf A, Wexberg P, Panzenbock A, Jakowitsch J, Bangert C, Laimer D, Schreiber C, Karakus G, Hulsmann M, Pacher R, Lang IM, Maurer G and Bonderman D. Cardiac magnetic resonance postcontrast t1 time is associated with outcome in patients with heart failure and preserved ejection fraction. *Circ Cardiovasc Imaging*. 2013;6:1056-65.