BLOCKade of calcium channels and beta adrenergic receptors for the treatment of hypertension in Heart Failure with Preserved Ejection Fraction (BLOCK HFpEF)

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Protocol

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1. Background and Specific Aims

Heart failure (HF) affects >5 million individuals in the US. Approximately half of individuals with HF have a preserved left ventricular (LV) ejection fraction (EF), termed HF with preserved EF (HFpEF). HFpEF is associated with high morbidity, mortality, and impaired quality of life. While there are several effective pharmacologic therapies for HF with reduced ejection fraction (HFrEF), none have been identified to clearly improve outcomes in HFpEF. Hypertension, which is present in approximately 80% of individuals with HFpEF, is the foremost modifiable risk factor for the development and progression of HFpEF. Current guidelines recommend β-blockers – but not calcium channel blockers (CCBs) – as first-line antihypertensive therapy in HFpEF; this is contrary to hypertension guidelines in the general population and is based on limited evidence. Despite the clinical importance of hypertension in HFpEF, sparse randomized controlled trial (RCT) data exist evaluating the mechanistic role of common antihypertensive agents, particularly CCBs and β-blockers, in the management of HFpEF. **We propose a novel mechanistic investigation regarding the role of dihydropyridine CCBs compared to β-blockers in targeting key physiologic abnormalities in HFpEF.**

HFpEF is characterized by unique physiologic abnormalities that may be differentially impacted by β-blockers and CCBs. HFpEF is associated with a greater blood pressure (BP) response to β-blockers than HFrEF, suggesting beneficial effects of β-blockers in this patient population. Excessive β-adrenergic stimulation may also be a driver of reduced aerobic capacity in HFpEF, which could respond favorably to β-blockade. However, in HFpEF, prolonged diastasis caused by the negative chronotropic effect of β-blockers may reduce cardiac output, particularly during exercise, contributing to impaired cardiac output reserve and aerobic limitations. β-blockers may also have effects on the pattern of ventricular contraction and arterial load, potentially impacting diastolic function. Similarly, CCBs may have beneficial effects related to vasodilation and reduction in late systolic load beyond their BP-lowering effect. However, CCB-induced vasodilation at rest may limit the vasodilatory reserve. Despite the high prevalence of hypertension in HFpEF and the important potential effects of β-blockers and CCBs on determinants of exercise capacity and quality of life, it is unknown which agent class has a more favorable impact in this patient population.

Our study team has combined expertise in antihypertensive physiology and pharmacoepidemiology, HFpEF, arterial hemodynamic measurements, and exercise physiology. **Our goal is to assess the mechanisms by which CCBs and β-blockers (commonly used antihypertensive agents in clinical practice), impact aerobic capacity and quality of life in HFpEF**. We will compare the impact of a dihydropyridine CCB (amlodipine besylate 5-10mg daily) vs. a β1-selective β-blocker (metoprolol succinate 100-200mg daily) on densely measured home BP, physical function, quality of life, arterial function, chronotropic reserve, vasodilatory reserve, and LV function among 50 subjects with HFpEF in a randomized crossover trial design. Participants will receive 4 weeks of each intervention, with a 1-week washout period in-between.

Specific Aim 1: To determine the effect of β-blocker therapy, compared to CCB therapy, on key clinical endpoints: blood pressure (BP), aerobic capacity, and quality of life.

Hypothesis 1a: β-blocker and CCB therapy will differ in their effect on densely measured home BP.

Hypothesis 1b: β-blocker and CCB therapy will differ in their effect on peak aerobic capacity (peak VO_2).

Hypothesis 1c: β-blocker and CCB therapy will differ in their effect on quality of life, as determined using the Kansas City Cardiomyopathy Questionnaire (KCCQ).

Specific Aim 2: To determine the mechanisms by which β-blocker vs CCB therapy may differentially impact aerobic capacity and quality of life.

Hypothesis 2a: β-blocker and CCB therapy will differ in their chronotropic effects during exercise.

Hypothesis 2b: β-blocker and CCB therapy will differ in their impact on vasodilatory reserve.

Hypothesis 2c: β-blocker and CCB therapy will differ in their impact on arterial load, including resistive and pulsatile afterload assessed with gold-standard central pressure-flow analyses.
Hypothesis 2d: β-blocker and CCB therapy will differ in their impact on LV systolic function (LV longitudinal strain) and diastolic function assessed with doppler echocardiography.

Specific Aim 3: To assess if changes in BP and cardiovascular function measured in Aim 2 mediate the effect of CCB and β-blocker therapy on aerobic capacity and quality of life. We will perform formal explanatory analyses to assess which mechanistic factors have differential effects on these clinical endpoints.

Our mechanism-driven approach will provide evidence-based guidance regarding the management of hypertension in HFpEF, enhance our understanding of the pathophysiology of HFpEF, and characterize the physiologic potential of these common antihypertensive agents to reduce progression and improve symptom management in this disease.

A1. Heart failure with preserved ejection fraction (HFpEF) is a critical public health problem. Heart failure (HF) affects over 2% of adults in the United States (US) and is a major source of morbidity, mortality, and impaired quality of life.[1-3] Approximately 10% of adults age 75 years or older have a diagnosis of HF, and HF is the leading cause of hospitalization in adults age 65 years or older.[4] Accordingly, HF is associated with over $21 billion in direct health care costs in the US annually.[2] Among individuals with HF, left ventricular (LV) ejection fraction (EF) provides an important phenotypic distinction with regard to the pathophysiology of HF[5] and response to pharmacologic therapy.[6-8] Between 46 and 54% of individuals with HF have a preserved LVEF, termed HF with preserved EF (HFpEF).[1, 3, 5]

The prevalence of HFpEF has increased disproportionately compared to HF with reduced EF (HFrEF). The incidence and prevalence of HF have risen in recent decades. With the aging of the population, the prevalence of HF is projected to increase by 46% between 2012 and 2030.[2] In 2012, there were an estimated 915,000 new cases of HF in the US,[9] compared to 670,000 cases in 2007.[10] The relative prevalence of HFpEF is increasing compared to that of HFrEF,[1, 11] strongly suggesting that the population-wide burden of HFpEF will exceed that of HFrEF in the coming decades.[3]

HFpEF is a malignant disease associated with premature mortality, high risk of hospitalization, and poor quality of life. Patients with HFpEF have a similarly high incidence of HF hospitalization, mortality, and impaired quality of life as those with HFrEF.[11, 12] Approximately half of individuals who develop HF die within 5 years of diagnosis.[9] Following HF hospitalization, the 5-year survival in HFpEF has been demonstrated to be as low as 35-40%.[1] An estimated 50% of the deaths in HFpEF are attributed to cardiovascular causes.[3]

A2. There is an urgent need to identify therapies that target mechanisms of pathophysiologic progression of HFpEF. Multiple therapies have been identified that provide substantial clinical benefit in HFrEF. In contrast, no interventions currently exist that clearly reduce adverse outcomes in patients with HFpEF.[7, 8] The disproportionate rise in the prevalence of HFpEF relative to HFrEF highlights the growing need for targeted pharmacologic therapy in HFpEF. Our proposal aims to evaluate the effect of commonly used and understudied antihypertensive agents on key physiologic abnormalities in HFpEF, including blood pressure (BP), arterial vasodilator reserve, and late systolic LV load from arterial wave reflections. Altering these mechanisms has the potential for both immediate-term improvements in exercise tolerance and long-term disease-modifying effects. Furthermore, individuals with HFpEF are often older than those with HFrEF,[3, 11] and may benefit more from targeting factors related to disease progression, symptoms, and quality of life than mortality alone. Our proposal will address the potential benefit of CCBs and β-blockers in HFpEF using endpoints with direct clinical relevance. The study will help us to better understand specific physiologic mechanisms involved in the progression of HFpEF and identify potential differential benefits from these common antihypertensive agents.

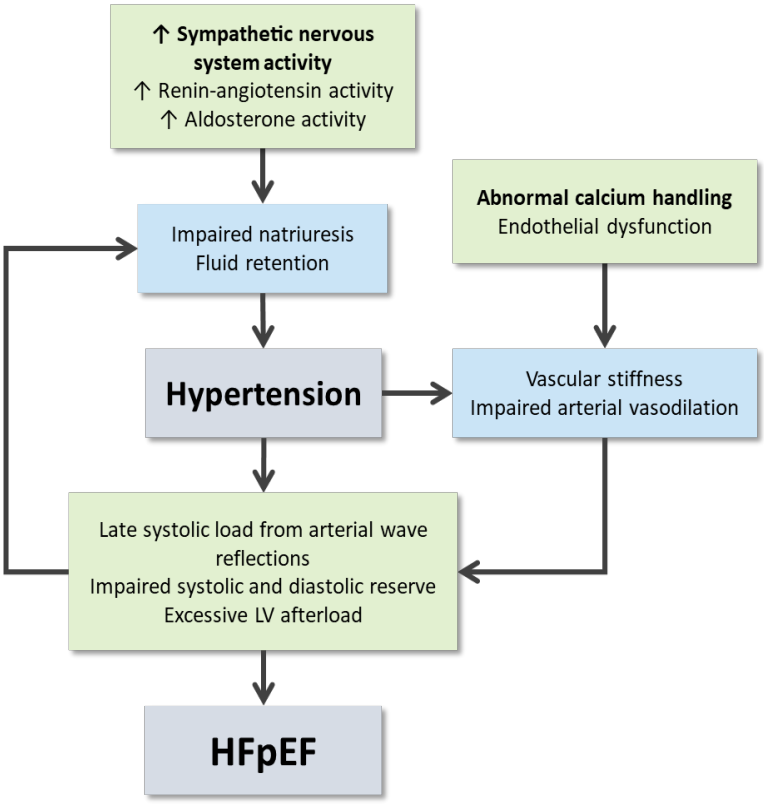
A3. Hypertension is the foremost modifiable risk factor for the development and progression of HFpEF. The increasing prevalence of HFpEF is partly attributed to rising rates of hypertension globally.[11, 13] Based on data from the Framingham

Heart Study, individuals with a systolic BP >160 mmHg have double the lifetime risk of HF compared to those with a systolic BP <140 mmHg.[9, 14] Correspondingly, treatment of hypertension reduces the risk of developing HF.[15, 16] Hypertension is more commonly associated with development of HFpEF than HFrEF.[3, 11] Studies have shown that 80%-90% of individuals with HFpEF have a diagnosis of hypertension.[11, 17] Hypertension is a crucial target for therapeutic interventions in these patients due to its high burden and important pathophysiologic role in disease progression in HFpEF.

A4. There is insufficient evidence to guide the treatment of hypertension in HFpEF. Diuretics play a central role in the management of symptoms related to volume overload in HFpEF. Guidelines otherwise recommend the use of β-blockers, angiotensin converting enzyme inhibitors (ACE-Is), and angiotensin receptor blockers (ARBs) to control blood pressure in patients with HFpEF, based largely on expert opinion.[18, 19] Most studies evaluating the effectiveness of antihypertensive medications in reducing adverse outcomes in HFpEF have focused on agents that inhibit the renin-angiotensin aldosterone system.[7, 8] Overall, these studies failed to consistently demonstrate meaningful effects of treatment with ACE-Is or ARBs on diastolic function, quality of life, HF hospitalizations, or mortality.[8, 20] Although the prevention of diabetic nephropathy may compel the use of ACE-Is or ARBs in HFpEF, BP control usually required additional antihypertensive medications. Mineralocorticoid antagonists seem to reduce the risk of HF hospitalization; however, mineralocorticoid antagonists are associated with high rates of hyperkalemia and do not have any consistent benefit with regard to exercise tolerance, quality of life, or mortality.[7, 8, 20-22]

Sparse randomized controlled trial (RCT) data exist evaluating the role of β-blockers and CCBs in the management of hypertension in HFpEF.[8] For the management of hypertension in the general population, dihydropyridine CCBs are recommended as first-line agents, while β-blockers are not recommended for first-line therapy.[23] Nonetheless, β-blockers are used more commonly than CCBs for the treatment of hypertension in HFpEF. In the Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist (TOPCAT) trial, 91% of participants had a diagnosis of hypertension; among these, 78% of were on β-blockers at baseline, while only 38% were on CCBs;[7] these patterns of antihypertensive use were similar after excluding individuals with atrial fibrillation. β-blockers have consistently been demonstrated to reduce the risk of mortality in HFrEF.[18, 19] However, few RCTs have evaluated the role of β-blockers for the management of hypertension in HFpEF, with inconclusive findings. Meta-analyses of data from three RCTs suggest that β-blockers may reduce cardiovascular mortality in HFpEF compared to placebo or usual care (hazard ratio [HR] 0.75, 95% confidence interval [CI] 0.60-0.94[20]), with no effect on HF hospitalization or quality of life.[8, 20] These RCTs were limited in quality, and used definitions for HFpEF that are not consistent with current guidelines (HFpEF was defined using an LVEF threshold of ≥35%[24] or ≥40%,[25, 26] compared to the consensus definition of LVEF ≥50%[19]). To the best of our knowledge, no RCTs have evaluated CCBs for the management of hypertension in HFpEF.

Figure 1. Conceptual model: Overview of the complex pathophysiologic relationship between hypertension and HFpEF



A5. The pathophysiologic relationship between hypertension and HFpEF is multifaceted, with

several overlapping cardiac and vascular mechanisms. HFpEF is physiologically complex, with a number of contributing etiologies. HFpEF and hypertension are characterized by several interrelated mechanisms, including chronic activation of the renin-angiotensin aldosterone system[27-29] and sympathetic nervous system,[30] and abnormal calcium handling (**Figure 1**).[31] Hypertension-mediated abnormalities in intrinsic myocardial and arterial mechanics have a major role in the development and progression of HFpEF.[32] Pressure overload due to hypertension promotes LV hypertrophy and cardiac fibrosis.[33] In individuals with hypertension, increased arterial stiffness, endothelial dysfunction, and renally-driven volume overload further drive the development and worsening of HFpEF.[34-38] Targeting hypertension-mediated mechanisms in HFpEF has the potential to improve exercise tolerance and cardiac function, and in turn reduce the long-term risk of adverse outcomes.

A6. Several pathophysiologic factors contribute to exercise intolerance in HFpEF. Exercise intolerance is a salient feature of HFpEF and determines quality of life in these patients.[12, 39, 40] Therefore, enhancing exercise capacity in HFpEF is an important objective with immediate clinical relevance. The underlying mechanisms contributing to exercise intolerance in HFpEF are important to consider when attempting to achieve this unmet goal. The early pathophysiologic paradigm of HFpEF was a failure to recruit the Frank-Starling mechanism to augment stroke volume during exercise due to an inadequate increase in end-diastolic volume in response to increased LV filling pressure.[41] Several studies also reported depressed chronotropic reserve,[42-46] attributed to abnormal responses to adrenergic activity.[44] However, various studies have not demonstrated abnormal end-diastolic LV volume during exercise,[42, 47] stroke volume reserve (i.e., increase during exercise),[42-44] or chronotropic incompetence.[47] Consequently, rather than resulting exclusively from cardiac abnormalities, HFpEF is now seen as a multifaceted disease process with a need to address not only cardiac, but also peripheral abnormalities.[48]

Exercise arterial vasodilatory reserve is abnormal in HFpEF, leading to reduced O_2 utilization and excessive LV afterload. Peak oxygen uptake (VO_2) during exercise, the most widely accepted index of aerobic capacity, is consistently reduced in HFpEF.[37, 41-44] Peripheral oxygen utilization requires adequate cardiac output during exercise in addition to adequate flow distribution. HFpEF is associated with endothelial dysfunction that results in reduced vasodilatory reserve.[36, 49] Impaired vasodilatory reserve plays an important role in oxygen delivery and extraction. Vasodilatory responses during exercise are important for reducing LV afterload. During exercise, LV afterload must decrease to accommodate increases in cardiac output without excessive rise in blood pressure. In several studies, patients with HFpEF had blunted exercise-induced reductions in systemic vascular resistance compared to age-matched hypertensive subjects without HF.[37, 47] This reduced vasodilatory reserve leads to an energetically-inefficient ventricular-arterial coupling state during exercise.[37] In addition, the abnormal vasodilatory reserve has profound implications for peripheral oxygen delivery and utilization. The peripheral ability to redistribute flow to working muscle is a key component of the normal response to exercise.[50] This flow distribution depends on the vasodilatory response in locomotive muscle, allowing it to effectively "compete" for the available cardiac output.[50]

A7. Wave reflections and late systolic load play an important role in the pathophysiology of HFpEF. Late systolic pulsatile load from wave reflections, which are increased in HFpEF,[51] have adverse long-term consequences on LV remodeling and function. Wave reflections increase the late systolic workload of the LV and profoundly impact the LV loading sequence (late relative to early systolic load).[52-56] The pulse wave generated by the LV travels forward in arteries and is partially reflected at sites of impedance mismatch (i.e., bifurcations, points of change in arterial size or wall stiffness, predominantly in middle-sized conduit arteries).[52, 53, 57] Wave reflections travel back to the heart, merging into a discrete reflected wave, and arrive while the LV is still ejecting blood in mid-to-late systole.[53, 57] The magnitude and timing of wave reflections strongly predicts incident HF.[34, 35, 56, 58] Hashimoto et al. demonstrated that changes in reflection magnitude during antihypertensive therapy are associated with regression of LV mass independent of blood pressure reduction.[59] Our group has demonstrated that, for any given level of systolic and diastolic blood pressure, a greater area under the pressure curve in late systole (relative to early systole) is strongly predictive of incident HF.[60] Our findings implicate late systolic load from arterial wave reflections as a novel strong risk factor for HF, supporting animal and human mechanistic findings from previous studies[34, 35, 52-59, 61] and demonstrating the relevance of late systolic load in humans.

All of these pathophysiologic mechanisms may be impacted differentially by CCBs vs. β-blockers in HFpEF. Despite the high clinical importance of this issue and common use of these drugs in HFpEF, no evidence is available comparing the effect of these agents on BP, exercise capacity, and quality of life in this population. Furthermore, no mechanistic data are available regarding the effects of these agents on the various determinants of exercise impairment in HFpEF.

A8. The understudied role of β-blockers and dihydropyridine CCBs in HFpEF. Although β-blockers are recommended as first-line antihypertensive therapy in HFpEF[18, 19] there is inconsistent evidence regarding their potential role in disease progression and symptom management.[62-65] While two RCTs showed mortality benefit from β-blocker use in HFpEF,[26, 65] the Swedish Doppler Echocardiographic Study demonstrated worsening of heart failure symptoms with randomized treatment with carvedilol compared to placebo.[64] β-blockers are highly beneficial in HFrEF because they reduce sympathetic activity, myocardial oxygen demand, and cardiac remodeling.[66] There are no clear data regarding whether chronic sympathetic nervous system activation, which plays a major role in the progression of systolic dysfunction in HFrEF, has a similar role in HFpEF. Some evidence suggests that increased adrenergic activity may have adverse effects on exercise capacity in HFpEF.[67] For example, there is *in vivo* evidence that β-adrenergic receptor stimulation provokes systolic and diastolic dysfunction in HFpEF.[30] Individuals with HFpEF seem to have a greater BP response to β-blockers than individuals with HFrEF.[63] However, the hemodynamic effects of heart rate lowering (i.e. with the use of β-blockade) have not been evaluated in HFpEF.[68] Prolonged diastasis ("stand still" without active filling) caused by slowing the heart rate with a β-blocker may limit cardiac output reserve during exercise, which is an important component of oxygen consumption for any given peripheral oxygen extraction,[69] thus worsening exercise tolerance.[64] Given inconsistent evidence on the clinical effects of β-blockers in HFpEF, there is great need to better understand their mechanistic effects.

The vasodilating effects of CCBs may reduce LV afterload and improve aerobic capacity in HFpEF. Dihydropyridine CCBs are recommended as first-line therapy for hypertension in the general population[23] due to widely demonstrated effectiveness in reducing BP and cardiovascular risk, along with a favorable side effect profile.[70-72] Observational evidence suggests no mortality benefit from treatment with CCBs in HFpEF.[73] However, there are no existing trials evaluating the potential role of CCBs in HFpEF. While CCBs are generally well-tolerated in individuals with HFrEF, they do not improve aerobic capacity, LV function, or mortality risk.[74-76] Nonetheless, in the general population, CCBs have beneficial effects related to vasodilation and arterial function beyond their BP-lowering effect.[77, 78] These vasodilatory properties may reduce LV afterload and improve O_2 utilization in HFpEF. Additionally, HFpEF is characterized by impaired systolic and diastolic reserve, attributed to elevated end-systolic and diastolic volume resulting in limited stroke volume response during exercise. This maladaptive response to exercise is likely in part due to abnormal calcium handling.[31] In animal models, cardiac calcium handling in HFpEF is different than in HFrEF. In contrast to reduced cardiomyocyte calcium availability in HFrEF,[79] Curl et al.'s Hypertrophic Heart Rat model of HFpEF exhibits high calcium availability.[80] These findings suggest that CCBs may improve systolic and diastolic reserve, which could improve exercise tolerance in HFpEF.

In summary, **multiple physiologic considerations suggest potentially beneficial and deleterious effects of β-blockers and CCBs in HFpEF. Despite the common use of these agents, studies comparing their mechanistic and clinically relevant effects are lacking. There is a compelling need for a randomized trial to fill this important knowledge gap.**

2. Trial Summary

Trial Title/Acronym	BLOCKade of calcium channels and beta adrenergic receptors for the treatment of hypertension in Heart Failure with Preserved Ejection Fraction (BLOCK HFpEF)
Funding Opportunity Announcement	PAR-18-406
Proposed Funding Agency	National Heart, Lung, and Blood Institute, National Institutes of Health
Number of Subjects	50
Study Site	University of Pennsylvania
Randomized Intervention	Amlodipine besylate 5mg to 10mg daily for approximately four weeks versus metoprolol succinate 100mg to 200mg daily for approximately four weeks
Primary Aim	To compare the BP-lowering effect of amlodipine besylate and metoprolol succinate therapy by home BP monitoring in HFpEF
Secondary Aims	1. To compare the effect of amlodipine besylate and metoprolol succinate therapy on aerobic capacity 2. To compare the effect of amlodipine besylate and metoprolol succinate therapy on quality of life 3. To compare the effect of amlodipine besylate versus metoprolol succinate therapy on LV diastolic function and arterial load
Exploratory Aims	1. To assess if amlodipine besylate and metoprolol succinate have differential effects in men versus women, African Americans versus non-African Americans, and diabetic versus non-diabetic subjects 2. To compare the effect of amlodipine besylate versus metoprolol succinate therapy on: A) Non-dipping measured using home BP monitoring (novel) B) BP variability using home BP monitoring C) Ventilatory threshold and VO_2 kinetics
Primary Endpoint	Difference in mean home systolic BP after four weeks of amlodipine besylate versus metoprolol succinate
Secondary Endpoints	1. Difference in mean office systolic BP 2. Difference in mean home diastolic and office diastolic BP 2. Difference in mean home pulse pressure and office pulse pressure 3. Total work performed and peak oxygen uptake (VO_2) during a symptom-limited maximal effort exercise test 4. Quality of life score, assessed using the Kansas City Cardiomyopathy Questionnaire

5. Measures of LV diastolic function, including E/e' and brain natriuretic peptide
6. Measures of arterial load, including arterial wave reflection, central systolic blood pressure, augmentation index, pulse pressure amplification, and forward and backward wave amplitudes

3. Study Design and Population

3.1 Overview of the study design

1) This is a randomized double-blind crossover trial in which fifty (50) subjects with HFpEF will be assigned to treatment with:
    A) Amlodipine besylate 5mg to 10mg by mouth daily for approximately four weeks;
    B) Metoprolol succinate 100mg to 200mg by mouth daily for approximately four weeks.
2) The order of the interventions (AB-BA design) will be randomized, with an approximately one-week washout period separating each intervention.
3) The crossover design will expose each subject to both treatments, reducing inter-subject variability and maximizing statistical power to evaluate the comparative effectiveness of amlodipine versus metoprolol in this patient population.
4) The drugs will be prepared at the Investigational Drug Pharmacy at the University of Pennsylvania and dispensed by an investigational drug pharmacist, blinded to both the subjects and investigators.

3.2 Study sites

The study will take place at the Hospital of the University of Pennsylvania; participants will be recruited from cardiology, primary care, and renal clinics affiliated with the University of Pennsylvania and the surrounding community (PCAM, Penn Presbyterian, and 3701 Market St).

3.3 Study population

We will enroll fifty (50) subjects during the study. Eligibility will be determined by the PIs according to the following criteria.

3.3.1 Inclusion Criteria

1) Adults age 18-90 years
2) Diagnosis of hypertension defined by at least two of the following:
    A) ICD-9 (401.0-404.91) or ICD-10 (I10-I13) codes signifying hypertension
    B) Treatment with antihypertensive medication other than a loop diuretic for at least two months
    C) History of previous blood pressure readings ≥130/80 mmHg at two separate office visits
3) Stable antihypertensive therapy; defined as no changes in antihypertensive medications in the preceding 30 days
4) A diagnosis of heart failure in the medical chart or per the investigators' clinical judgement based on a clinical picture consistent with heart failure (e.g., dyspnea on exertion, paroxysmal nocturnal dyspnea, and/or edema in the setting of echocardiographic or hemodynamic measurements consistent with heart failure; or probability of HFpEF ≥90% according to the H2FpEF score[81] or clinical presentation highly suggestive of HFpEF without a more likely clinically apparent cause for symptoms, as determined by the HFpEF screening working group that includes several cardiologists)
5) LV ejection fraction >50%
6) Elevated filling pressures defined by at least one of the following criteria:
    A) Mitral E/e' ratio (lateral or septal) >8 with low e' velocity (septal e' <7 cm/s or lateral e' <10 cm/s) and at least one of the following:

a. Enlarged left atrium (LA volume index >34 ml/m2)
b. Chronic loop diuretic use for management of symptoms
c. Elevated natriuretic peptides (BNP levels >100 ng/L or NT-proBNP levels >300 ng/L)

B) Mitral E/e' ratio (lateral or septal) >14
C) Previously elevated invasively determined filling pressures based on one of the following criteria:
   a. Resting LVEDP >16 mmHg
   b. Mean PCWP >12 mmHg
   c. PCWP or LVEDP ≥25 mmHg with exercise
D) Previous acutely decompensated heart failure requiring IV diuretics

3.3.2 Exclusion Criteria

1) Systolic BP meeting any of the following criteria:
   A) Current office systolic BP <100 mmHg
   B) Current office systolic BP 100-119 mmHg if <u>not</u> receiving treatment with an antihypertensive agent or if holding antihypertensive medication prior to randomization would be clinically contraindicated, as per the investigator's clinical judgement
   C) Current office systolic BP ≥180 mmHg if <u>not</u> receiving treatment with a CCB or β-blocker, or ≥160 mmHg if already receiving a CCB and/or β-blocker prior to the pre-randomization wash-out period
   D) Orthostatic hypotension defined as >20 mmHg decline in office systolic BP 3-5 minutes following the transition from sitting to standing position
2) Resting heart rate <50 or >100 bpm
3) Contraindication to withholding CCB or β-blocker therapy (e.g. use of non-dihydropyridine CCB [diltiazem or verapamil] or β-blocker for rate control for atrial fibrillation) as per the investigator's clinical judgement
4) Children, fetuses, neonates, prisoners, and pregnant women (women of childbearing age will undergo a pregnancy test during the screening visit) are not included in this research study.
5) Inability/unwillingness to exercise
6) Any the following echocardiographic findings:
   A) LV ejection fraction <45% on any prior echocardiogram, unless it was in the setting of uncontrolled atrial fibrillation/flutter or other arrhythmia, as per investigator judgment
   B) Hypertrophic, infiltrative, or inflammatory cardiomyopathy
   C) Clinically significant pericardial disease, as per investigator judgment
   D) Greater than moderate left-sided valvular disease, any degree of mitral stenosis
   E) Severe right-sided valvular disease
   F) Severe right ventricular dysfunction
7) Active coronary artery disease, defined as any of the following:
   A) Acute coronary syndrome or coronary intervention in the past 2 months
   B) Ischemia on stress testing without either subsequent revascularization or a subsequent angiogram demonstrating the absence of clinically significant epicardial coronary artery disease, as per investigator judgement
8) Clinically significant lung disease, defined as any of the following:
   A) Chronic Obstructive Pulmonary Disease meeting GOLD criteria stage III or greater
   B) Treatment with oral steroids within the past 6 months for an exacerbation of obstructive lung disease
   C) The use of daytime supplemental oxygen
9) Primary pulmonary arteriopathy
10) eGFR <30 mL/min/1.73m^2

11) Any medical condition that, under the investigator's discretion, will interfere with safe completion of the study or validity of the endpoint assessments
12) Known history of an allergy or clinically significant sensitivity (as determined by the investigator) to either amlodipine besylate or metoprolol succinate

3.3.3 Criteria that will prompt discontinuation from trial participation at the four-week visit

1) New onset coronary artery disease defined as any of the following:
    A) New onset angina
    B) Acute coronary syndrome or coronary intervention for unstable coronary artery disease after enrollment
    C) New onset ischemia on a clinically indicated stress test after initial enrollment
2) New clinically indicated treatment with a CCB or β-blocker that cannot be withheld as per the investigator's judgement
3) Any new medical condition that, under the investigator's discretion, will interfere with safe completion of the study or the validity of the endpoint assessments

Subjects who meet the following criteria will not be immediately discontinued, but will be scheduled for an ad-hoc additional visit for reassessment of values prior to initiation of the crossover drug:

1) Office systolic BP <90 mmHg
2) Symptomatic bradycardia (resting heart rate <50 bpm), or asymptomatic resting heart rate <45 bpm
3) Resting heart rate >110 bpm

If these criteria persist during the ad-hoc visit, the subject will be discontinued from the study.

3.4 Randomized intervention

The following interventions will be implemented in random order, separated by an approximately one-week washout period:

1) Amlodipine besylate: Amlodipine besylate tablets will be purchased and placed into oral gelatin capsules with an inert filler (lactose monohydrate). Capsules will be prepared at the University of Pennsylvania Investigational Drug Service. Each capsule will contain 5mg amlodipine besylate. The dose for this trial will be 5mg daily, titrated up to 10mg daily for a home systolic BP ≥130 mmHg and heart rate ≥50 bpm after the first week of use.
2) Metoprolol succinate: Metoprolol succinate tablets will be purchased and placed into identical oral gelatin capsules with an inert filler (lactose monohydrate). The does for this trial will be 100mg daily, titrated up to 200mg daily for a home systolic BP ≥130 mmHg and heart rate ≥50 bpm after the first week of use.

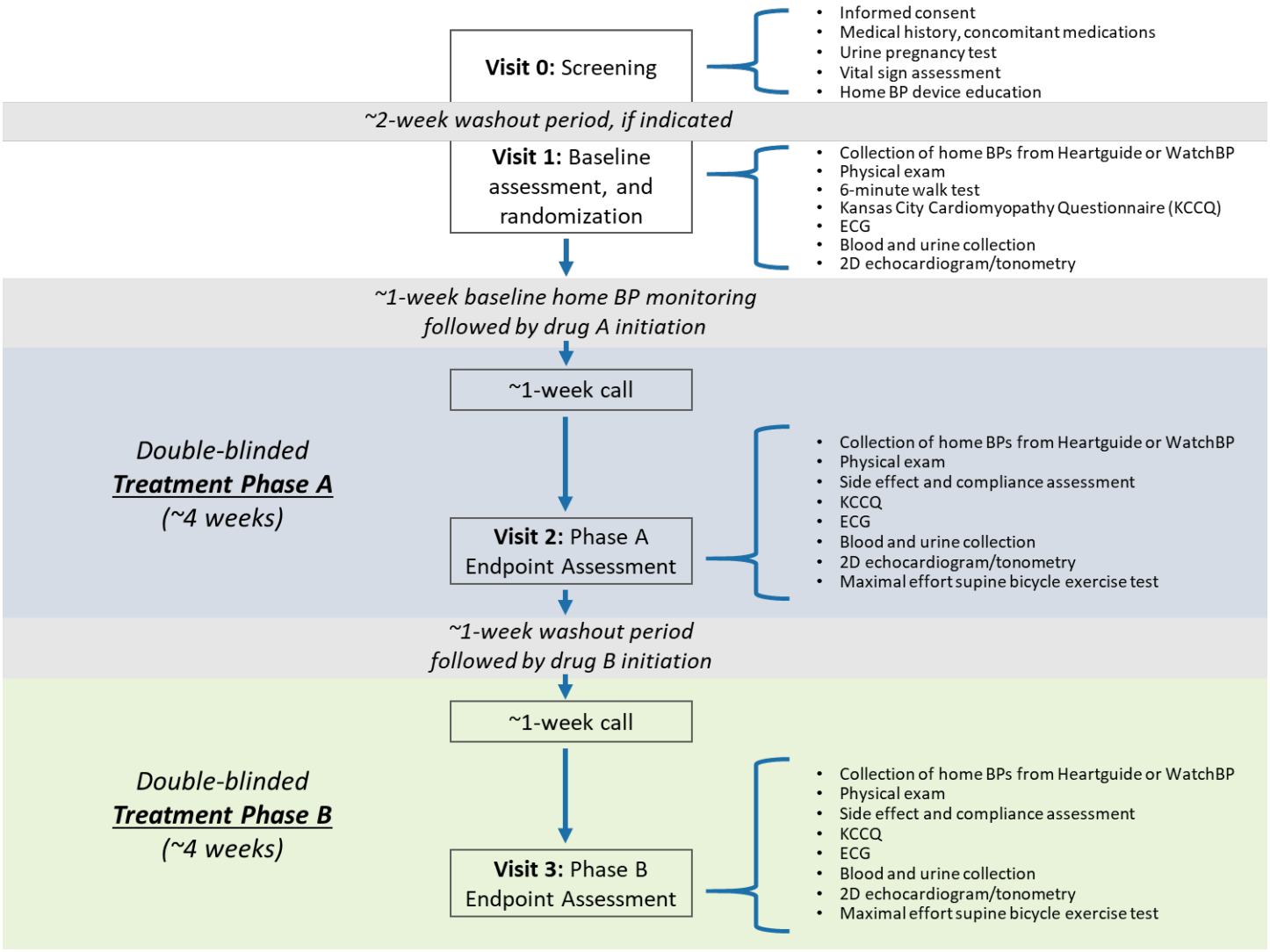
We are randomizing patients because randomization provides a much more robust study design than non-randomized assignment to allow for assessment of differential effectiveness across these two medications. There is also no potential harm in randomizing the order of administration of these medications to the subjects. We selected a crossover study design, with individuals serving as their own controls in a randomized, AB/BA design. Our goal was to maximize the statistical power with an intentionally small sample size to optimize feasibility of recruitment and implementation.[82, 83]

4. Study Visits and Procedures

A letter may be sent to potential subjects or they may be contacted by phone prior to initial in-person contact.

An overview of the study design is presented in Figure 1, below:

Figure 1. Overview of the study design and procedures



4.1 Screening and baseline visits

During the baseline and screening phase, subjects will take part the following steps:

1) Initial screening for eligibility and consent (Visit 0)
2) Approximately two-week washout period (if appropriate)
3) Approximately one week of pre-randomization home BP monitoring, and
4) Baseline study visit (Visit 1)

4.1.1 Visit 0: Screening visit

Screening visit procedures include the following, which are described in greater detail below:

1) Informed consent (obtained prior to any study procedures)
2) Eligibility assessment
3) Urine pregnancy test (if applicable)
4) Medical history and concomitant medications
5) Vital signs assessment
6) Home BP device education

During the screening visit, inclusion and exclusion criteria will be reviewed to ensure subject suitability for the study. Written informed consent will be obtained using Institutional Review Board (IRB) approved documents. Informed consent will be obtained before any study procedures are performed. Subjects will be given the opportunity to have all questions regarding their participation answered in detail in a private setting before entering the study.

Following informed consent, vital signs will be measured including orthostatic blood pressure measurement. A urine pregnancy test will be performed in women of childbearing age. Medical history and concomitant medications will be assessed to determine if a two-week pre-randomization washout period is required.

Subjects will also be educated on the correct use of the home BP monitor (Omron BP8000-M HeartGuide,[84] Omron Healthcare Co., Ltd., Kyoto, Japan or Microlife BP 3MX1-4 WatchBP Home N,[85] Microlife Corporation, Taiwan, ROC).

4.1.1.1 Optional approximate two-week pre-randomization washout period

Discontinuation of the study drugs will be necessary prior to performing the baseline measurements because the study will assess for differences in the outcome measures compared to the absence of either medication, and to enable blind randomization. If individuals are receiving treatment with a CCB or β-blocker prior to the screening visit or if they require discontinuation of another antihypertensive medication to achieve a sufficient BP to tolerate the addition of a new antihypertensive medication (i.e. screening systolic BP 100-119 mmHg), they will enter an approximate two-week washout period during which they will not receive the medication. In individuals who are receiving a fixed-dose combination of antihypertensive medication that includes a CCB or a β-blocker, the non-CCB/non-β-blocker drug(s) in the fixed-dose combination will be provided separately by IDS at the start of the washout period, if appropriate based on the baseline blood pressure (I.e., if it does not also need to be held due to a low systolic BP). In individuals who are receiving a high dose β-blocker (see Table, below, for qualifying doses), we will reduce the dose by ½ of their current dose every other day for one week followed by withholding the medication for approximately one week.[86, 87] This discontinuation of medication is expected to cause an increase in blood pressure by an average of 10 mmHg and no more than 20 mmHg.[88] Given the anticipated increase in blood pressure during the washout period, we will ask participants to contact us immediately by phone with any home BP readings ≥180 mmHg for assessment by one of the investigators to determine if they require referral to the emergency department (including determination if they require treatment for hypertensive emergency). We will also contact participants approximately 2 days after discontinuation or each down-titration step of their antihypertensive to assess the last 48 hours of home BP readings via phone call. Any individuals with a systolic BP ≥180 mmHg will be discontinued from the study.

Table. Total daily dose of β-blocker treatment qualifying for taper

Acebutolol	800 mg
Betaxolol	20 mg
Nebivolol	40 mg
Bisoprolol	10 mg

Pindolol	60 mg
Carvedilol	50 mg
Atenolol	100 mg
Nadolol	120 mg
Metoprolol succinate	200 mg
Metoprolol tartrate	400 mg
Labetalol	800 mg
Carteolol	10 mg
Penbutolol	40 mg
Propranolol IR	480 mg
Propranolol LA	320 mg

4.1.2 Visit 1: Baseline study visit

In individuals who do not require a washout period, the baseline visit will occur on the same day as the screening visit. Baseline visit procedures include the following, which are described in greater detail below:

1) Physical exam (including repeat vital signs assessment if washout was required)
2) KCCQ
3) ECG
4) Blood (CBC, comprehensive metabolic panel, NTproBNP) and urine collection (UPr/UCr)
5) 6-minute walk test
6) 2D echocardiogram
7) Arterial tonometry

A physical examination will be performed, including an ECG (and repeat vital sign assessment if a washout period was necessary). The Kansas City Cardiomyopathy Questionnaire (KCCQ) will be administered. Blood will be collected for measurement of a complete blood count, comprehensive metabolic panel, and NTproBNP. Urine will be collected for measurement of UPr/UCr. A 6-minute walk test will also be performed during this visit. Patients who demonstrate significant desaturation during exercise (≤94% or a fall in SpO_2 ≥5%) will be excluded from the study.

Echocardiography will then be performed using a standardized protocol. Images will be obtained from the parasternal long axis, short axis, apical 5-, 4-, 3-, and 2-chamber, subcostal, and suprasternal views for offline analysis of myocardial strain. Dedicated ventricular chamber images will be obtained in the 4- and 2-chamber apical positions for determination of left ventricular volumes. Mitral inflow velocities, including color M-mode interrogation, will be assessed in the 4-chamber view. Tissue Doppler imaging will be performed at the mitral septal position, approximately 1-cm apical to the mitral valve plane. Additional images will be obtained in the parasternal short axis at the level of the papillary muscles, 2-chamber, and 4-chamber apical views for assessment of myocardial strain. Pulse-wave Doppler interrogation of the left ventricular outflow tract (LVOT) will be performed in the apical 5-chamber view.

Concurrent arterial tonometry will be performed using a high-fidelity tonometer at the carotid, femoral, and radial arteries, using a Sphygmocor device. Waveforms will be calibrated using the brachial artery blood pressures, obtained using a validated oscillometric device. Waveforms will be digitally-stored for off-line analysis. We will also measure BP using a BP+ device (Uscom, Sydney, Australia), which is an FDA-approved device that measures brachial and central blood pressure using a standard brachial blood pressure cuff. Body surface measurements will be made to determine distance between the suprasternal notch to the carotid, radial, and femoral arteries.

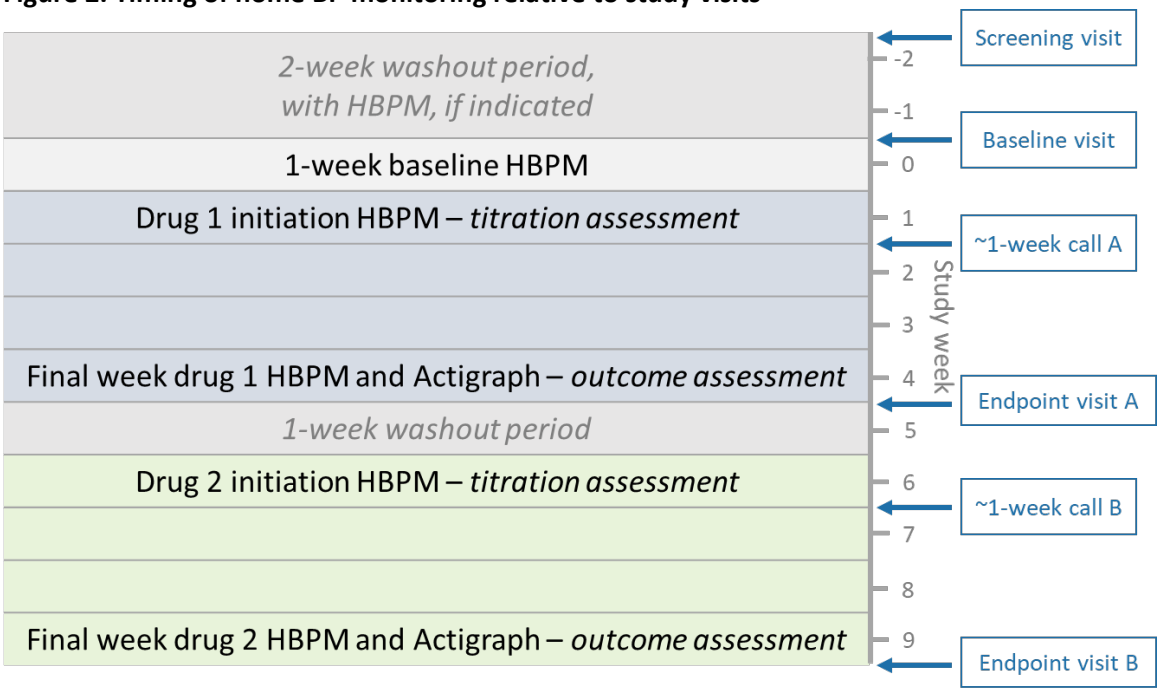
4.2 Randomization procedure

To preserve blinding, the treatment order assignments and the associated study-specific patient identification number will be provided to each study Investigation Drug Service representative. Upon determination of subject eligibility into the trial, the Investigation Drug Service representative will determine the treatment group assignment, and will record the patient identification number for that study subject. Subjects will be randomized at their baseline visit, but will not initiate therapy until after the completion of the approximately one-week baseline pre-treatment home BP assessment.

4.3 One-week (5-7 day) pre-treatment home BP assessment

Immediately following the baseline visit and prior to initiating the drug for phase A, participants will be instructed to perform baseline home BP monitoring using the Omron HeartGuide or Microlife WatchBP Home N. They will receive instructions to strictly perform the BP measurements while sitting with their back supported, following a 5-minute rest period, on a bare/unclothed wrist. Subjects will be asked to obtain two measurements, approximately 1 minute apart, in the morning prior to taking any antihypertensive medications and again in the evening approximately 12 hours later during every day of the baseline home BP assessment. An overview of the timing of the home BP measurements is presented in Figure 2, below:

Figure 2. Timing of home BP monitoring relative to study visits



4.4 Intervention Phase A

Subjects will be randomized to either amlodipine besylate or metoprolol succinate as the first drug for Phase A. The initial dose for amlodipine will be 5mg daily and for metoprolol will be 100mg daily, administered in the morning. We will provide subjects with a 5-week supply of study medication to account for potential delays in follow up.

4.4.1 Post-randomization home BP measurements

Subjects will again be asked to obtain two measurements, approximately 1 minute apart, in the morning prior to taking the medication and again in the evening approximately 12 hours later for 5-7 days prior to the one-week call, and again during the 5-7 days prior to the endpoint assessment.

4.4.2 One-week call

Subjects will be called by telephone approximately 7 days after initiating the drug to assess their home BPs in the 48 hours prior to the call. If their home systolic BP is ≥130 mmHg and their heart rate is ≥50 bpm, the drug dose will be doubled (up to 10mg daily for amlodipine and 200mg daily for metoprolol). The presence of orthostatic symptoms (i.e. sustained lightheadedness upon standing) will prompt an ad hoc visit to assess for orthostatic vital signs. The presence of symptomatic orthostatic hypotension (20 mmHg reduction in systolic blood pressure with associated symptoms such as dizziness) at this visit will prompt discontinuation of participants from the trial.

Subjects may experience symptoms after dose uptitration that do not prompt exclusion from the trial, as per the criteria noted above (for example, but not limited to, mild dizziness, fatigue). In these instances, the investigator can reduce the dose back to one capsule daily for the remainder of the treatment phase.

If a participant reports worsening heart failure symptoms or blood pressures ≥180/100, we will perform twice weekly calls to obtain a symptom check and repeat blood pressure values until the participant is determined to be stable by the investigators. Three or more blood pressure values above this threshold or progression of symptoms will trigger a symptom visit and assessment for early termination from the study, per the investigators' discretion.

At approximately 3 weeks into each interventional phase, the Actigraph devices will be given to the subjects. The goal will be for the subjects to wear these devices during the final week of each interventional phase to look for differences with each therapy. The devices will be brought back by the subject at his/her next visit.

4.4.3 Visit 2: Intervention Phase A Endpoint Assessment

Following approximately four weeks of therapy with amlodipine or metoprolol, participants will return for endpoint assessments. Endpoint assessments will include the following:

1) Collection of home BPs
2) Physical exam
3) Side effect and compliance assessment
4) KCCQ
5) ECG
6) Blood and urine collection
7) 2D echocardiogram
8) Arterial tonometry
9) Actigraphy
10) Maximal effort supine bicycle exercise test
11) Continuous wave near infrared spectroscopy (NIRS) to measure oxygenation of muscle tissue (optional)

Physical examination with measurement of orthostatic blood pressure, pill count, side effect assessments, and KCCQ will be performed. A urine pregnancy test will be performed again in women of childbearing age. Subjects will then undergo repeat echocardiography, arterial tonometry, and blood pressure measurements (including BP+ central blood pressure measurements). Subjects will then perform a maximal-effort peak oxygen consumption (VO_2) test using a supine bicycle exercise test with expired gas analysis.

4.5 One-week washout period

Following the endpoint assessment, subjects will enter an approximately one-week washout period during which they will not receive any study medications.

4.6 Intervention Phase B

Following the washout period, subjects will receive either amlodipine besylate or metoprolol succinate during Phase B of the trial. Subjects will receive the intervention (amlodipine or metoprolol) that was not administered in Phase A, such that each subject will receive both study interventions in this crossover design. The one-week call and Endpoint Assessment Visit will be repeated, as above. We will provide subjects with a 21-day supply of study medication to account for potential delays in the shipment of the remainder of their medication after the one-week call.

4.6.1 Post-randomization home BP measurements

Subjects will again be asked to obtain two measurements, approximately 1 minute apart, in the morning prior to taking the medication and again in the evening approximately 12 hours later for 5-7 days prior to the one-week call, and again during the 5-7 days prior to the endpoint assessment.

4.6.2 One-week call

Subjects will again be called by telephone approximately 7 days later to assess their home BPs in the 48 hours prior to the call. If their home systolic BP is ≥130 mmHg and their heart rate is ≥50 bpm, the drug dose will be doubled (up to 10mg daily for amlodipine and 200mg daily for metoprolol). The presence of orthostatic symptoms (i.e. sustained lightheadedness upon standing) will prompt a visit to assess for orthostatic vital signs. The presence of symptomatic orthostatic hypotension (20 mmHg reduction in systolic blood pressure with associated symptoms such as dizziness) will prompt exclusion of patients from the trial.

Subjects may experience symptoms after dose uptitration that do not prompt exclusion from the trial, as per the criteria noted above (for example, but not limited to, mild dizziness, fatigue). In these instances, the investigator can reduce the dose back to one capsule daily for the remainder of the treatment phase.

If a participant reports worsening heart failure symptoms or blood pressures ≥180/100, we will perform twice weekly calls to obtain a symptom check and repeat blood pressure values until the participant is determined to be stable by the investigators. Three or more blood pressure values above this threshold or progression of symptoms will trigger a symptom visit and assessment for early termination from the study, per the investigators' discretion.

At approximately 3 weeks into each interventional phase, the Actigraph devices will be given to the subjects. The goal will be for the subjects to wear these devices during the final week of each interventional phase to look for differences with each therapy. The devices will be brought back by the subject at his/her next visit.

4.6.3 Visit 3: Intervention Phase B Endpoint Assessment

Following approximately four weeks of therapy with amlodipine or metoprolol, participants will return for endpoint assessments. Endpoint assessments will include repeat measurements of the following:

1) Collection of home BPs
2) Physical exam

3) Side effect and compliance assessment
4) KCCQ
5) ECG
6) Blood and urine collection
7) 2D echocardiogram
8) Arterial tonometry
9) Actigraphy
10) Maximal effort supine bicycle exercise test
11) Continuous wave near infrared spectroscopy (NIRS) to measure oxygenation of muscle tissue (optional)

Physical examination with measurement of orthostatic blood pressure, pill count, side effect assessments, and KCCQ will be performed. A urine pregnancy test will be performed again in women of childbearing age. Subjects will then undergo repeat echocardiography, arterial tonometry, and blood pressure measurements (including BP+ central blood pressure measurements). Subjects will then perform a maximal-effort peak oxygen consumption (VO_2) test using a supine bicycle exercise test with expired gas analysis.

4.7 Subject withdrawal/early termination

Subjects may voluntarily withdraw from the study at any time and for any reason. Subjects may also be withdrawn at the investigator's discretion. The investigator may withdraw a patient from the study due to protocol non-compliance, incorrect enrollment or randomization, or for any other reasons related to subject safety. The reason for study discontinuation will be recorded on the appropriate case report form and all such subjects will be asked to complete an early termination visit.

During the early termination visit, we will document: 1) vital signs; 2) compliance with the medications, including pill count; 3) adverse effects; 4) specific reason for withdrawal. We will also collect any remaining study medications.

4.8 Concomitant medication

Subjects should be treated with standard of care medications for HFpEF or associated comorbidities. As per the inclusion criteria, subjects should be on a stable medical regimen for HFpEF prior to entry. Further adjustment of diuretics or blood pressure medications during the study period is discouraged and should only be performed according to new and clinically compelling worsening of clinical status. Subjects will be withdrawn from the study if a CCB or β-blocker is initiated during routine clinical care that cannot be withheld.

5. Additional details regarding study measurements and data collection

5.1 Assessment of exercise capacity

We will use a supine bicycle exercise protocol in conjunction with expired gas analysis to assess oxygen consumption (VO_2) during exercise. Subjects will perform a maximal exertion limited exercise test using a graded-exercise protocol. We will use a supine cycle ergometer designed for stress echocardiography (Stress Echo Ergometer 1505, Medical Positioning, Inc, Kansas City, MO). Subjects will undergo expired gas analysis with a Parvo Medics True One 2400 device (Parvo Medics, Sandy, UT), an Innocor device (Innovision Inc) or equivalent. Resistance began at 15 W for 3 minutes, increasing to 25 W for 3 minutes, and then increasing by 25 W every 3 minutes thereafter. Breath-by-breath information will be recorded. We will use custom-designed software already developed in Matlab (MathWorks, Natick, MA) at our

lab for offline processing and quantification of all exercise data.[89] All data quantification will be blinded to treatment. Total work performed will be computed and exercise efficiency will be defined as (total work/total oxygen consumed). Sensitivity analyses will be performed excluding those individuals with a respiratory exchange ratio below a reasonable threshold at baseline.

5.2 Quality of life assessment

This will be assessed with the Kansas City Cardiomyopathy Questionnaire (KCCQ).[90] We will administer the KCCQ to subjects prior to randomization and at the end of each intervention phase (4-week time point in both phases). This is a validated 23-item questionnaire that assess physical function, symptoms, social function, self-efficacy and knowledge, and quality of life. It has been used extensively in heart failure studies (http://cvoutcomes.org/pages/3214).

5.3. Measurement of late systolic load and arterial wave reflections

We will use a high-fidelity Millar applanation tonometer to record carotid pressure waveforms, which will be calibrated using brachial artery pressures. Central arterial tonometric recordings and Doppler flow velocity files will be processed off-line using custom-designed software written in Matlab (The Mathworks, Natick, MA) as previously described.[34, 91] We have successfully implemented this method in multiple previous studies[34, 53, 92] and have published a tutorial detailing our analysis methods.[52, 53] After signal-averaging of pressure and flow, time alignment of carotid pressure and LV outflow curves will be performed to maximize concordance of the rapid systolic upstroke of pressure and flow, concordance of the dicrotic notch and cessation of flow, zero value of the phase angle of higher-frequency harmonics (7^{th} to 10^{th}) of input impedance, and linearity of the early systolic pressure-flow relationship.[91] After computation of aortic input impedance, proximal aortic characteristic impedance (Zc) will be computed in the frequency domain as previously described.[91] Pressure and flow harmonics were separated into forward and backward components using standard wave separation analysis.[52, 53, 91] The sum of forward and backward pressure harmonics yields the forward and backward waves, respectively. We will assess the reflection coefficient in the first 3 harmonics. As reflection coefficient is derived from the ratio of two sine waves, it is a complex number with an amplitude and phase-angle, which can correspond to different degrees of destructive or constructive interference between forward and backward waves. Therefore, the net-effect of reflections will be expressed as the real part of Γ, which becomes increasingly positive as pressure from wave reflections increases (constructive interference), and negative when destructive interference leads to a net decrease in pressure by wave reflections at a given harmonic.[93] Since all harmonics of wave reflection contribute variably to systolic LV load, our primary measure of late systolic load will be the net pressure related to wave reflections during ejection in the time domain, which better represents the impact of reflections on LV afterload. We will first compute the product of flow and aortic Zc (QZc product), which represents the pressure resulting from the interaction of blood flow with aortic root Zc.[53] The relation between QZc and measured pressure reveals the direct effect of wave reflections on the arterial system.[94] We will therefore quantify the additional ejection-phase pressure load from wave reflections arising distal to the root (i.e., reflection-related pressure time integral during ejection, as the difference between measured pressure and the QZc product). Carotid-femoral pulse wave velocity (PWV), an index of large artery stiffness,[95, 96] will also be measured[97] using a Sphygmocor device (Atcor Medical).[95] We will also measure carotid-radial PWV using the Sphygmocor device.

5.4 Physical Activity

We will measure activity level in all subjects with the Actigraph device, which has been previously validated. This device can be worn on the wrist, and measures acceleration each minute in the anterior-posterior, mediolateral, and vertical axis, and summarizes that information as a vector magnitude. The software will use this data to calculate physical activity in METS and steps. The accelerometer will be provided during both phase 1 and phase 2. We will give the

Actigraph device to the subjects during approximately the final week of each interventional phase. We will ask the subjects to put the device on and will provide informational materials to assist with device placement. We will also discuss device placement with the subjects at the study visits as well as over the phone as needed. Subjects will bring the devices to the endpoint assessment visits. Subjects will be fully oriented in the use of this device. The device may be removed for showering or swimming but otherwise should be worn continuously. The specific details in the use of this device will be provided to study subjects. We will use the average METS of activity over this time period as an estimate of physical activity. We will also records steps taken.

5.5. Assessment of the systemic vasodilatory response to exercise

Blood pressure will be measured with a validated oscillometric device at rest, at each stage of exercise and after exercise. Cardiac output will be measured at rest and at each stage of exercise using echocardiography. Systemic vascular resistance (SVR) will be calculated at rest and at peak exercise as mean arterial pressure / cardiac output. Systemic vasodilatory reserve will be measured as the reduction in SVR during exercise, relative to SVR at rest ([rest SVR– peak exercise SVR] / rest SVR). Depending on equipment availability and functionality at each site, we may also monitor and record muscle oxygenation in the calf (lateral aspect of left gastrocnemius) and forearm (flexor digitorum superficialis) during exercise non-invasively using a PortaMon[98] or PortaLite near-infrared spectroscopy device (Artinis Medical Systems; The Netherlands).[99] Both the PortaMon and PortaLite are investigational devices and not currently approved medical devices in the U.S. This is an exploratory metric and is optional.

6. Statistical considerations

6.1 Power calculations

We will randomize 50 subjects to one of 2 sequences, each of which consists of 2 periods (AB/BA design). We considered a mean within-subject difference in daytime systolic BP of 5 mmHg between the therapies to be the minimum clinically significant difference.[100] A previously published double-blind crossover trial randomized subjects with a diagnosis of hypertension, who were otherwise healthy, to treatment with amlodipine besylate 5-10mg daily versus metoprolol succinate 100-200mg daily.[88] The trial demonstrated a slightly greater decline in awake ambulatory BP with metoprolol succinate compared to amlodipine, which did not reach statistical significance. Using standard deviations from this study (the most conservative standard deviation was 10.6 mmHg for the mean baseline ambulatory SBP of 148 mmHg) and assuming a retention rate of 85% and a within-subject correlation of 0.8, we will have 90% power at an alpha of 0.05 to detect a within-subject difference in mean daytime systolic BP of 5 mmHg between the different therapies and to assess for effect modification by sex.[101] Based on the preliminary findings presented using TOPCAT data, we will also have 90% power to detect a 5 point difference in KCCQ score between therapies, which is considered clinically significant.[102] Power calculations were performed using PASS16.[103]

6.2. Data Analysis Plan

The primary outcome variable will be the within-subject difference in mean daytime home SBP between the two therapies. All secondary outcome measures are continuous variables. The predictor of interest for all aims will be the intervention (amlodipine vs. metoprolol), with analyses based upon the total number of subjects randomized. Initial descriptive estimates of all measures will be generated for study participants at each time point by treatment group. Statistics will include estimates of central tendency and measures of variability, and will account for presence of skewness and kurtosis. Analyses of distributional properties will be performed to determine if variance stabilizing or

normalizing transformations should be applied. Outliers will be assessed via visual inspection of distributions and checked for accuracy.

Aim 1: Our primary aim will assess the effects of amlodipine versus metoprolol on home systolic BP, aerobic capacity, and quality of life determined by KCCQ. An initial assessment of the treatment effect will be performed using the paired t-test and the non-parametric Wilcoxon sign-rank test on the difference between the paired within-subject outcome measures across the two therapies. This will be followed by exploratory analyses using more comprehensive linear mixed effects model analyses[104] allowing for assessments of the treatment effect on each continuous outcome of interest while accounting for effects of other covariates including period, sequence, and a random subject effect nested within sequence. For non-normal distributed outcomes, we will use non-parametric methods or consider distribution-stabilizing transformations.

Aim 2 and 3: Our secondary outcomes involve exploratory analyses investigating mechanisms of action. In addition to assessing the effect of our randomized intervention on each mechanistic endpoint (Aim 2) in the same manner described for Aim 1, exploratory structural equation modeling will be used to evaluate associations between biologic mechanistic pathways and the clinical outcomes (Aim 3). The modeling will be carried out in three sequential steps: 1) exploratory factor analysis, 2) confirmatory factor analysis, and 3) structural equation modeling. The exploratory factor analysis will be based on principal axis factoring to decrease the number of variables. For both theoretical and empirical reasons, it will be assumed that retained factors are correlated and thus an oblique rotation method will be used. Measured and latent variables will be examined for co-linearity, and related variables will be combined into single factors with individual factor loadings. To determine the number of factors, the model will be evaluated against the following four rules: 1) eigenvalues greater than 1.0[105]; 2) Glorfeld's[106] extension of parallel analysis, where a large number of random correlation matrices are generated to compare the number of eigenvalues that are significant by chance[107]; 3) high internal consistency (an $\alpha \geq 0.70$) for unit-weighted factors,[108] and 4) interpretability.[109] The heaviest weight will be placed on the minimum average partial and parallel analysis methods, with the scree test as a visual adjunct.[110] The next step involves incorporating the factors into a model using confirmatory factor analysis. The model will be tested using goodness of fit tests to assess the overall fit of the model to the data. Various models will be tested and compared prior to arriving at the best fitting model. And finally, the best fitting model obtained from confirmatory factor analysis will incorporate a structural equation model designed to examine the links between the randomized intervention, the mechanistic variables, and the clinical variables assessed in the trial using mediation analysis.[111, 112]

We are comparing multiple overlapping hypotheses related to cardiac and vascular mechanisms of HFpEF progression. To minimize the likelihood of Type 1 errors in our analyses (i.e. to account for multiple comparisons), we will employ a conservative Bonferroni approach for the comparisons of interventions.[113]

Addressing potential confounders and mediators: Randomization should balance measured and unmeasured confounders across study arms. However, we will measure potential confounders, including participant demographics and comorbidities, and compare their distributions across arms and perform adjustments if necessary. The intervention groups will initially be compared within each period (time-invariant covariates will only be compared for period A) using ANOVA or Kruskal-Wallis analyses, depending upon whether the variables are parametric, and Fisher's Exact tests for categorical variables. Significant differences between groups in these variables will result in their respective adjustment in the modeling of the outcome. The linear mixed-effects models will incorporate adjustments for any period effect or crossover effect and will include data from dropouts.[114, 115] The model will include subject-specific intercepts as random effects, and will assume independent and identically distributed random errors within-subject. Restricted maximum likelihood estimation will be used, and an appropriate covariance matrix will be specified. Model assumptions will be

examined (e.g., QQ plots to assess normally distributed residuals for valid Wald tests). All mixed effects models will be analyzed using STATA (Statacorp LP, College Station, TX).

Stratified analyses and effect modification: As demonstrated in our previous work, there are important differences in response to therapy and outcomes of HFpEF between men and women.[116] It is important to better understand underlying mechanisms contributing to these observed differences across sexes. Accordingly, we will perform stratified analyses and will assess for effect modification by sex. We expect our study cohort to reflect the gender distribution of HFpEF in the general population, in which approximately 60% of patients with HFpEF are women.[1-3, 101] Based on the anticipated distribution of men and women, we will have 90% statistical power to detect a difference in within-subject difference in mean daytime systolic BP of 6.9 mmHg between men and women, and 80% power to detect a 6 mmHg difference.[101] Additional pre-specified stratified analyses will include African American vs. non-African American and diabetes vs. no diabetes. Exploratory analyses will evaluate effect modification by clinical HFpEF phenotypes.[116, 117] We will also assess for effect modification by chronotropic incompetence,[118] which will be determined using the heart rate data collected at the end of stage 1 and/or first 30 seconds of stage 2 (20 or 40 watts) of exercise testing while on beta-blocker therapy.

6.3 Timing and Rationale for Unblinding

Unblinding will either occur after all subjects have been randomized and completed follow-up, or if indicated because the subject requires evaluation at an ad-hoc visit for potential discontinuation from the study, and the investigator needs to be aware of the study medication (at the investigator's discretion)

7. Adverse Events

7.1. Key definitions

7.1.1 Adverse Event

An adverse event (AE) is any untoward medical occurrence associated with the use of a drug in a subject whether or not considered drug or biologic related. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of the pharmaceutical product.

7.1.2 Suspected Adverse Reaction

A suspected adverse reaction (SAR) is any adverse event for which there is a reasonable possibility that the drug caused the event. "Reasonable possibility" suggests there is a causal relationship between the drug and the adverse event. "Suspected adverse reaction" implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Serious Adverse Events (SAE)

An adverse event or suspected adverse reaction is considered serious if the investigator believes any of the following outcomes may occur:

- Death
- Life-threatening AE: Places the subject at immediate risk of death at the time of the event as it occurred. It does not include an AE that, had it occurred in a more severe form, might have caused death.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- Inpatient hospitalization or prolongation of hospitalization.
- Congenital anomaly or birth defect.
- Important medical events that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition above.

This determination is based on the opinion of the investigator

7.2. Classification of AE/ADEs

A medically qualified investigator must assess all AEs in terms of causal relationship to intervention, severity, and "expectedness" using the following guidelines.

7.2.1 Classification of Adverse Events for Causal Relationship to Study Interventions

Not related	There is not a reasonable causal relationship to the investigational product and the adverse event.
Unlikely related	No temporal association or the cause of the event has been identified, or the drug or device is unlikely to be implicated, but there is a low likelihood that a causal relationship exists.
Possibly related	There is reasonable evidence to suggest a causal relationship between the drug and adverse event.
Related	There is evidence to suggest a causal relationship, and the influence of other factors is unlikely.

7.2.2 Classification of Adverse Events Regarding Severity Scale

1	Mild AE: Awareness of sign, symptom, or event, but easily tolerated; no treatment required
2	Moderate AE: Discomfort enough to cause interference with usual activity and may warrant intervention. In the latter scenario, AE responds to treatment

3	Severe AE: Incapacitating, limiting usual/normal activities or significantly affects clinical status requiring hospitalization or prolongation of hospitalization.
4	Life-threatening or disabling
5	Fatal AE

7.2.3 Expectedness

The expectedness of an AE/ADE or SAR shall be determined according to the specified reference document containing safety information (e.g., most current product label). Any AE/ADE that is not identified in nature, severity, or specificity in the current study reference document(s) (e.g. protocol or investigator's brochure) is considered unexpected.

The following AEs are expected, disease-related events in patients with HF with preserved ejection fraction (HFpEF) and hypertension.

1. Unplanned hospitalization, ER visit, or clinic visit for worsening HF
2. Arrhythmias, particularly atrial fibrillation
3. Sudden cardiac death
4. Acute coronary syndrome
5. Cerebrovascular event
6. Venous thromboembolism
7. Lightheadedness
8. Worsening renal function (i.e. rising creatinine)
9. Shortness of breath at rest or during/after exercise
10. Fatigue at rest or during/after exercise
11. Elevated bicarbonate (from diuresis)
12. Low or high potassium levels (0.5 meq/L above or below the expected range; due to diuretics and angiotensin receptor blockers or angiotensin converting enzyme inhibitors, which are widely used in these patients)
13. Rising liver enzymes
14. Rising natriuretic peptide levels
15. Anemia (among individuals with chronic kidney disease at baseline)

The following are potential expected side effects of amlodipine besylate:

1. Dizziness or lightheadedness
2. Fatigue
3. Rash
4. Leg swelling
5. Flushing
6. Low blood pressure
7. Shortness of breath or wheezing
8. Abdominal pain

9. Nausea

The following are potential expected side effects of metoprolol succinate:

1. Dizziness or lightheadedness
2. Fatigue
3. Rash
4. Leg swelling
5. Low blood pressure
6. Slow heart rate
7. Shortness of breath or wheezing
8. Visual changes

Amlodipine and metoprolol are commonly used antihypertensive agents; adverse events are expected to be extremely rare given the well-described tolerability of these medications and their widespread use in routine clinical practice.

7.3 Recording and Reporting of Adverse Events

The investigators will continuously supervise all aspects of the trial and review the records of the study subjects following each visit and at the end of their participation. The investigators will be responsible for ensuring that all adverse events are noted, followed and reported to the IRB.
SAEs occurring from the time of *signed informed consent* to the end of Phase B will be captured on a CIOMS form. AEs will be classified according to the guidelines/definitions specified in section 7 of the protocol. Any AE rated >=3 in severity and all SAEs must be reported by the investigator or qualified designee to the IRB. The investigator will make an immediate determination about the necessity to modify the protocol, include additional information in the consent form, inform previous participants, temporarily hold enrollment of patients, or terminate the study.

The investigator or qualified designee will enter the required information regarding the AE into the appropriate module of the eCRF. All study procedures and cumulative adverse events are subject to full IRB review at least yearly.

7.4 Follow-up

The investigators will record follow-up safety information according to the same process used for reporting the initial event as described above. The investigators will follow all safety events until resolution, stabilization, or the event is otherwise explained.

7.5 Management of Suspected Unexpected Serious Adverse Reaction

The investigators will assess all SAEs and evaluate for “unexpectedness” and relationship to study drug. The investigators are required to complete a report for any event identified as serious, study drug related and unexpected, using the CIOMS format. A copy of the report will be kept in the Regulatory Binder

7.6 Pregnancy

Pregnancy is a contraindication to enrollment in the study. Pregnancy occurring during the study period, although not considered an SAE, must be reported. The pregnancy will be recorded on the appropriate note to file. The drug will be discontinued immediately and subject terminated from the trial, but the pregnancy will be followed until final outcome. Although the study drugs are not known to be unsafe in pregnancy, risk for the fetus has not been ruled out (Pregnancy Category C), and any associated AEs or SAEs that occur to the mother or fetus/child will be recorded in the AE or SAE case report form.

8. Protection of Human Subjects

8.1 Potential benefits of the proposed research, importance of the knowledge to be gained, and risk/benefit ratio

8.1.1 Potential benefits

There are no anticipated direct benefits to the subjects as a result of their participation in this study nor will this be implied when obtaining consent. However, if our hypothesis is correct, subjects receiving amlodipine may experience improvements in their functional class and quality of life, although this will not be implied in any way during informed consent or enrollment.

8.1.2 Importance of the knowledge to be gained

If our hypothesis is correct, this study may identify an effective specific intervention to treat hypertension and peripheral mechanistic abnormalities in HFpEF patients. Furthermore, if improving these factors leads to an enhanced exercise capacity in this patient population, this would lead to a new standard of care in the field and a better understanding of the mechanisms that lead or contribute to HFpEF.

8.1.3 Risk/benefit ratio

The results of this study may ultimately lead to an effective treatment of hypertension in HFpEF. Since there is minimal risk, with potential benefits to medical knowledge and society, the risk / benefit ratio is acceptable.

8.2 Risks to study subjects

This study will involve recruitment of fifty (50) human subjects, each exposed to both antihypertensive medications (amlodipine besylate and metoprolol succinate, in a cross-over design), and therefore individuals will be studied as their own controls. All subjects will be adults able to give informed consent.

For the trial, we will aim to achieve adequate representation of women and African Americans. Children will not be enrolled. Participants will be recruited from the Hospital of the University of Pennsylvania and associated outpatient clinics. The planned gender and ethnic distribution will be facilitated by the diverse clinical population in the enrolling medical center.

The study involves various tests (arterial tonometry, echocardiography, exercise testing) and the administration of randomized therapies (amlodipine besylate versus metoprolol succinate).

8.2.1 Potential Risks of Study Interventions (Amlodipine Besylate and Metoprolol Succinate):

8.2.1.1 Amlodipine besylate

The main potential risks of amlodipine administration are related to its potential effect on blood pressure. Many studies have demonstrated a reduction in blood pressure in both hypertensive and normal subjects following amlodipine administration. Therefore, we will not start the interventions in individuals below the blood pressure threshold, and we will implement exclusion criteria and safety blood pressure checks throughout the conduct of the study to ensure subject safety. Common side effects are listed in section 7.2.

8.2.1.1 Metoprolol succinate

The main potential risks of metoprolol administration are related to its potential effect on 1) blood pressure and 2) heart rate. Many studies have demonstrated a reduction in blood pressure and heart rate in both hypertensive and normal subjects following amlodipine administration. Therefore, we will not start the interventions in individuals below the blood pressure and heart rate threshold, and we will implement exclusion criteria and safety blood pressure and heart rate checks throughout the conduct of the study to ensure subject safety. Individuals are not eligible for this study if they are on medications that slow their heart rate (including β-blockers such as carvedilol, bisoprolol, and propranolol and calcium channel blockers such as verapamil and diltiazem), and have a contraindication to stopping these medications during the pre-randomization washout period. Common side effects are listed in section 7.2.

8.2.2. Potential Risks of Study Procedures

Potential risks are associated with the study tests, the study interventions, and potential breach of confidentiality.

8.2.2.1 Cardiopulmonary stress test

This test is used extensively for research purposes with minimal risk to subjects. The most significant risks of the test are dysrhythmias or other cardiovascular complications, which are extremely rare. These procedures will be performed by qualified personnel according to established American Heart Association Guidelines.[119, 120] Non-revascularized myocardial ischemia, which may increase the risk of complications during exercise testing, is an exclusion criterion for the study.

Subjects may feel uncomfortable as a result of pushing themselves during the maximal effort exercise test. Subjects will likely feel short of breath and fatigued as a result of the exercise test. Various other complaints, such as nausea, lightheadedness, and other aches and pains are also possible as a result of the maximal effort exercise study. Although exercise testing may result in exhaustion, rarely do people develop abnormal heart rate or heart complications during exercise tests. The risk of this happening is the same as if the participant would exert themselves during stressful situations or during exercise elsewhere.

We will perform ECG, heart rate, and blood pressures monitoring during our exercise test. In addition to the blood pressure (generally increases) and heart rate (generally increases) changes during exercise, we will also monitor arterial saturation. This will be done non-invasively using a pulse oximeter. Of note, oxygen levels can decrease with exercise, even in individuals without significant cardiopulmonary disease.[121, 122] If the arterial saturation falls to below 88% ("severe exercise induced hypoxemia"[122]), we will alert the patient's primary care provider as this may prompt consideration for additional or alternative causes for arterial hypoxemia.

8.2.2.2 Venipuncture and IV placement

According to the 2010 WHO guidelines on phlebotomy, major risks associated with blood donations include hematoma at the site of venipuncture in 23%, and vasovagal reactions and fainting in 1%. The placement of an intravenous catheter would be anticipated to increase the risk of hematoma and discomfort slightly. Given that the catheter will be in place for a short-period of time, infection is an unlikely complication.

8.2.2.3 Arterial tonometry, central blood pressure measurement, and echocardiography

Tonometry and assessments of oscillometric arterial pressure waveforms are noninvasive procedures and do not have any known risks. Minor discomfort may occur when the tonometer is placed against the neck, arm, and groin.

The PortaMon and PortaLite (near infrared spectroscopy, or NIRS) devices have no known risks.[99] They are non-invasive devices applied on top of the skin to measure the oxygenation of underlying tissue.

During various procedures (echocardiography, arterial tonometry), we will use adhesive electrodes attached to the participant's skin to record the electrical signal from the heart. These may occasionally cause skin itching and irritation.

8.2.3 Pregnancy Risks

We will not be enrolling subjects who are pregnant or lactating in this study. Amlodipine and metoprolol are not absolutely contraindicated in pregnancy and breast-feeding, however risk to the fetus has not been ruled out (Pregnancy Category C). Additionally, the blood pressure thresholds in this study differ from the goal blood pressures during pregnancy, and the study may result in changes in blood pressure and heart rate that could be dangerous to a developing fetus. A pregnancy test will be given to women of child-bearing potential prior to enrollment in the study and administration of the supplement. The pregnancy test will be repeated at Visit 2 (prior to initiation of the second treatment period). If a woman is enrolled of child-bearing potential, we will ask that they use a medically accepted method of birth control (such as an IUD, birth control combination pill, patch, ring, progestin-only pills, Depo Provera Shot, Implanon, complete abstinence, or condoms) while they participate in the study. As subjects with HFpEF are generally older (>55 years old) we do not anticipate this concern to occur with our study population.

8.3 Adequacy of Protection Against Risks

8.3.1 Recruitment and Informed Consent

Written informed consent will be obtained from the subjects by the investigators prior to entry into the research study. This will be performed in accordance with the guidelines and under the supervision of the University of Pennsylvania

Institutional Review Board. The study procedures and interventions and the associated risks will be explained to the subjects during the informed consent process. Only IRB-approved consent forms and related materials will be used.

8.3.2 Protection Against Risks Associated with Cardiopulmonary Exercise Tests

These tests will be performed by qualified personnel according to established American Heart Association Guidelines,[119] under ECG monitoring. Personnel with adequate cardiopulmonary resuscitation training and resuscitation equipment (crash cart) will be available during these tests. Similarly, these tests will be performed in a hospital setting where a full code team can be deployed immediately should complications occur.

8.3.3 Protection Against Risks from Amlodipine

Subjects will be thoroughly advised regarding the potential risks of the study medication and precautions needed during its administration. As noted in the exclusion criteria, due to the anticipated blood pressure-lowering effect of the medication, individuals will be excluded if their office systolic blood pressure is 1) <100 mmHg, 2) <120 mmHg if holding antihypertensive medication prior to randomization would be clinically contraindicated, or 3) significant for >20 mmHg decline 3-5 minutes following the transition from sitting to standing.

8.3.3 Protection Against Risks from Metoprolol

Subjects will be thoroughly advised regarding the potential risks of the study medication and precautions needed during its administration. As noted in the exclusion criteria, due to the anticipated blood pressure-lowering effect of the medication, individuals will be excluded if their office systolic blood pressure is 1) <100 mmHg, 2) <120 mmHg if holding antihypertensive medication prior to randomization would be clinically contraindicated, or 3) significant for >20 mmHg decline 3-5 minutes following the transition from sitting to standing. They will also be excluded if their resting heart rate is <50, or if they are on a medication that slows their heart rate (including β-blockers such as carvedilol, bisoprolol, and propranolol and calcium channel blockers such as verapamil and diltiazem) with a contraindication to stopping these medications prior to randomization.

8.3.4 Supine and Orthostatic Blood Pressure Measurements

Supine vital signs will be measured at the screening visit. The subject will rest in a supine position for a minimum of 3 minutes prior to obtaining vital sign measurements. The subject will then assume a standing position for 3-5 minutes. Vital signs (BP and pulse rate) will then be measured while the subject is standing. A reduction in systolic blood pressure >20 mmHg will be considered an exclusion criterion for the study. Blood pressure measurements will be performed at home by the participants throughout the study, and will be repeated after approximately four weeks of randomized therapy. Measurements will also be prompted by reports by study subjects of 1) systolic blood pressure <90 mmHg at home, 2) heart rate <50 bpm at home, and 3) orthostatic symptoms at any point during the trial. The presence of symptomatic orthostatic hypotension (20 mmHg reduction in systolic blood pressure with associated symptom such as dizziness) will prompt exclusion of patients from the trial.

8.3.5 Safety Clinical Laboratory Tests

Laboratory evaluations will be collected prior to study drug initiation. Among women who are not surgically sterilized or post-menopausal, a urine pregnancy test will be performed at screening (prior to all baseline studies and drug initiation) and at the end of the first treatment phase. Complete blood count, liver enzymes, a basic metabolic panel (including serum creatinine for assessments of renal function) will also be performed prior to randomization and approximately four weeks after initiation of randomized therapy.

8.3.6 Side Effect Management Plan

Both intervention medications are widely used in clinical practice, and are known to have minimal side effects. However, a plan for management of side effects will be in place. The use of acetaminophen may be used for headaches during the study. Although hypotension is not expected based on stringent exclusion criteria, it should be managed as per standard clinical practice. We will also collect information on the frequency and type of expected and unexpected side effects reported by participants. We will ask participants to contact us immediately via phone if they develop any intolerable side effects, at which time the investigator will assess the safety of continuation with the study, if a dose reduction may be appropriate, or if the study drug should be discontinued and the participant should be removed from the study for their safety.

8.3.7 Other Measures to Minimize Risk

Phlebotomy, arterial tonometry, and echocardiographic examinations will only be performed by appropriately trained personnel as per our institutional standards.

9. Study Administration, Data Handling, Record Keeping

9.1 Confidentiality

All information obtained as part of protocol will be maintained in locked research files. Electronic data will be stored in a password secured database (REDCap). This database will be available only with secure high-level password protection to safeguard the privacy of the participants. Any source documents for each patient involved in the protocol, other than the patient's electronic medical chart, will be maintained in a centralized, secured location. Source documentation is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the study. Examples of source documents include demographic information, medical information, visit records and questionnaires. All information in the database will be traceable to the source documents in the patient's file. Any information that is not already clinically available in the subject's medical record will be directly entered into REDCap by the study team. No data containing any form of patient identifier will be provided for any purpose, including publication and collaborations with other investigators.

9.2 Subject Privacy

Privacy of subjects will be maintained as the subject will interact with the investigators in the setting of a room designated for private patient care. Any written communication (e.g. questionnaires) will be kept confidential and private as outlined above.

Data collected will contain PHI including: name, date of birth, and medical record number. Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study;
- Who will have access to that information and why;
- Who will use or disclose that information;
- The rights of a research subject to revoke their authorization for use of their PHI.

Research data will be stored in a centralized, secure location (locked office of the PI). The consent document asks for specific permission to analyze genomic/proteomic data.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

Research results and are kept confidential and no one will have access to research results without permission from the participant.

9.3. Data Disclosure and Protection

Data disclosed to collaborating sites will not be identifiable and will be assigned a tracking number. If research results are confirmed clinically this information may be made part of a participant's medical record as the information is expected to alter medical management and health outcomes. Research results will not be made part of a participant's medical record.

Although a master database will be kept with the subjects' names and research lab numbers to allow for retrospective correlation of results with clinical features, the subjects' names will not be available to anyone other than the immediate study team and will not be used for identification of the subject in the laboratory or in reports of the research. The database will be kept in a secured computer and file.

9.4 Data Collection and Management

Before entering any data into the database, staff will be trained regarding the protocol and data entry specific to this protocol and database. Throughout the protocol, the Principal Investigator will regularly meet with the study staff to discuss any problems or concerns, as well as study enrollment.

Information will be stored in a research file identified only by a code number. The key connecting the participants' names to their code number will be stored in a separate, secure electronic folder only accessible to study investigators.

Information used for scientific publications will not contain any identifying information. All testing results will be considered confidential and will not be placed in the patients' medical records. They will be kept in locked research files in a separate location. Test results that are not felt to be clinically relevant - have no proven medical management to reduce risk for development of disease - or are of unknown clinical significance will not be disclosed.

9.5 Records Retention

Research records will be retained for at least 7 years after the completion of this study. All study electronic files will be kept for at least 6 years after IRB acknowledgement of study termination.

10. Regulatory Standards

10.1 Informed Consent/HIPAA Authorization

The site investigator, or a person designated by the site investigator, will fully inform the subject of all pertinent aspects of the clinical trial including the review of the informed consent form approved by the IRB. Prior to a subject's participation in the clinical trial, the Informed Consent Form will be signed and personally dated by the subject or by the subject's legally acceptable representative. All subjects will receive a copy of the informed consent form.

10.2 Institutional Review Board (IRB)

The investigators will submit this protocol to the University of Pennsylvania IRB. During the study, any amendment or modification to the protocol will be sent to the IRB. The IRB will also be informed of any event likely to affect the safety of subjects or the continued conduct of the study, in particular any change in safety and all updates to the protocol will be sent to IRB.

10.3 Investigational New Drug (IND) Application

An IND exemption has been issued by the Office of Clinical Research.

10.4 Auditing and Inspecting

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

11. Study Finance

11.1 Funding Source

The trial is supported by the National Institutes of Health, National Heart Lung and Blood Institute R01 HL153646-01

11.2 Conflict of Interest

All University of Pennsylvania Investigators will follow the University of Pennsylvania Policy of Conflicts of Interest Related to Research.

11.3 Subject Stipends or Payments

Subject will receive financial compensation for their participation in this study in the form of a check. We will follow the following reimbursement scheme:

- Completion of Visit #1: $150
- Completion of Visit #2: $250
- Completion of Visit #3: $250

The maximum total amount (if a subject completes all study visits) will be $650. Subjects will be paid for each visit that they complete, including completion of the corresponding home blood pressure monitoring.

We recognize that travel may present a hardship for some participants who have difficulty with transportation. For these individuals, and until our budget prevents us from being able to do so, we may be willing to help arrange for transportation through the university (up to $100 per trip).

12. Publication and Dissemination Plan

The analyzed results of the trial will be submitted for publication to a peer-reviewed journal within six months following the completion of the final trial visit. The project will be registered with clinicaltrials.gov, and the results will be submitted to clinicaltrials.gov no later than one year following the completion date of the project.

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Appendix 1. Anticipated Study Timeline and Key Dates

Study Timeline

The anticipated time of study duration, beginning with the grant award through study completion is 5 years. The study timelines and milestones are detailed below.

Overall Study Timeline

Study Timeline (start date 09/01/2020)	Pre-Funding Period	Year 1				Year 2				Year 3				Year 4				Year 5			
		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Protocol, ICF, DSMB creation, IRB submission	X																				
IRB approval, manuals, CRFs, database, hiring	X																				
Protocol/database training, study drug packaging, initiation mtg	X																				
Report generation, quality control, DSMB approval/initial meeting, clinicaltrials.gov registration		X																			
Open for recruitment, Active enrollment		X																			
Recruitment and Enrollment		X	X	X	X	25%	X	X	X	50%	X	X	X	75%	X	X	100%				
Follow up visits					X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Database lock and analysis																		X	X	X	X
Manuscript preparation and revision; submit clinicaltrials.gov																			X	X	X

Key Milestones

The dates below are deadlines by which key milestones will be completed using an R01 award start date of 9/1/2020.

Year 1 milestones

- Study drug received by investigational pharmacy and packaged for distribution (8/1/19)
- Trial registration in ClinicalTrials.gov complete (8/1/20)
- DSMB membership finalized (9/1/20)
- Database testing; report generation (9/20/20)
- First DSMB meeting (9/20/20)
- Database set up complete (10/1/20)
- Manuals and case report forms finalized (10/1/20)
- GCP, protocol, and database training complete (10/1/20)
- Open for enrollment (11/1/20)
- Protocol implementation and data monitoring; DSMB meetings every 6 months (4/1/21)

Years 2-5 milestones

- 25% enrollment (12/1/21)
- Protocol implementation and data monitoring; DSMB meetings every 6 months (10/1/21)
- Protocol implementation and data monitoring; DSMB meetings every 6 months (4/1/22)
- Protocol implementation and data monitoring; DSMB meetings every 6 months (10/1/22)
- 50% enrollment (12/1/22)
- Protocol implementation and data monitoring; DSMB meetings every 6 months (4/1/23)
- Protocol implementation and data monitoring; DSMB meetings every 6 months (10/1/23)
- 75% enrollment (12/1/23)
- Protocol implementation and data monitoring; DSMB meetings every 6 months (4/1/24)
- 100% enrollment (9/1/24)
- Final follow-up visit (11/1/24)
- Final monitoring (11/15/24)
- Database lock including batched laboratory analysis (12/1/24)
- Submission of primary manuscript (3/1/25)
- Submission of study results to ClinicalTrials.gov (9/31/25)

Appendix 2. History of Protocol Changes

Version 1 (June 2019) was the protocol for the 4-patient trial pilot, which was halted due to restrictions in research during the initial surge of the coronavirus disease 2019 pandemic in March 2020. The protocol was subsequently transitioned to the full 50-subject protocol and was IRB approved in June 2020 (with continuing review approved in July 2020). The only change was the difference in sample size from 4 to 50, and power calculations from the R01 submission.

Version 2 (November 2020) of the protocol was updated with the funding sponsor information, study timeline, and change in threshold for uptitration of study medication from 135 mmHg systolic blood pressure to 130 mmHg systolic blood pressure, consistent with recent hypertension guideline thresholds for blood pressure control. Version 2 was finalized after receiving input from all DSMB members, and before any subjects were enrolled.

Version 3 (March 2022) of the protocol was updated to clarify the exclusion criteria for recovered EF to permit enrollment if prior reduced EF was in the setting of atrial fibrillation/flutter or other arrhythmia, as per investigator judgment. The analytic plan was updated to include assessment for chronotropic incompetence as an effect modifier.

Version 4 (June 2022) of the protocol was updated to clarify the inclusion criteria to permit use of the H2FPEF score and investigator judgement for assessment of the diagnosis of HFpEF, and to expand the exclusion criteria to exclude greater than moderate left-sided valvular disease and mitral valve replacement.

Version 5 (March 2023) of the protocol was updated to clarify the inclusion criteria for the diagnosis of heart failure, which typically needs to be made by the investigators' clinical discretion based on participant signs and symptoms in combination with echocardiographic or hemodynamic measurements, given that the diagnosis is often missed by patients' providers and the diagnostic code, accordingly, is frequently not included in the medical record.[123] This version of the protocol also formalized the frequency of monitoring of participants who develop expected adverse events including worsening heart failure symptoms and elevated blood pressures to require twice weekly monitoring of symptoms/blood pressures by phone and the addition of a symptom visit and formal assessment for possible early withdrawal, if determined to be clinically appropriate by the investigators.

Version 6 (August 2023) of the protocol was updated to add coverage for transportation for participants in whom transportation to appointments creates excessive hardship.

Version 7 (February 2024) of the protocol was updated to add the scenario of fixed-dose combination antihypertensive medications combined with the study drugs, in which case the non-study drug component(s) of the fixed-dose combination will be prescribed and provided by IDS at the start of the pre-treatment washout period. The protocol wording was also updated to ensure that it is clear that NIRS testing is an exploratory metric that is optional based on device availability and functionality.

Version 8 (November 2024) of the protocol was updated to remove arterial tonometry during exercise. It had been included in the protocol in error. The data is not required for the study, is not listed as part of any of the endpoint measurements described in the protocol, and this is not in any way required to determine systemic vasodilatory response to exercise. The change does not impact participants' risk or data in any way.