PhosphodiesteRasE-5 Inhibition to Improve CLinical Status and EXercise Capacity in Diastolic Heart Failure (RELAX) Trial

Rationale and Design

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Heart Failure With Preserved Ejection Fraction

Heart failure with preserved ejection fraction (HFpEF) or diastolic heart failure (HF) accounts for approximately half of HF cases in the community, and the portion of HF with preserved ejection fraction (EF) is increasing.1 Patients with HFpEF have limited functional capacity and poor prognosis. With ongoing shifts in the age distribution of the population, the burden of HFpEF is projected to increase. To date, there is no proven therapy for HFpEF. There is an urgent need for effective therapies for HFpEF.

Randomized Clinical Trials in HFpEF

To date, 3 randomized control trials in HFpEF have tested the impact of renin-angiotensin-aldosterone antagonists on clinical outcomes in HFpEF. None of these trials demonstrated benefit individually (Figure 1) or in a pooled analysis (n=8021).3 An aldosterone antagonist trial in HFpEF is ongoing (clinical-trials.gov NCT00094302). A trial of the β-blocker nebivolol in HF patients with normal or reduced EF was underpowered but suggested a benefit of β-blocker in the relatively small subset of HFpEF patients (Figure 1).3 The digitalis investigation group (DIG) trial showed no reduction in mortality with digoxin in a small ancillary study of HFpEF patients.4

Although inadequate power or crossover may have contributed to negative findings in these trials, unique pathophysiology in HFpEF may mediate the differential response to neurohumoral antagonists and mandate novel therapies for the treatment of HFpEF. The Phosphodiesterase-5 Inhibition to Improve CLinical Status And EXercise Capacity in Diastolic Heart Failure (RELAX trial; clinicaltrials.gov NCT00763867) trial has been designed by and is being conducted within the National Heart, Lung, and Blood Institute–sponsored HF clinical research network. Herein, we provide the rationale for RELAX by summarizing the unique pathophysiological derangements in HFpEF and the studies suggesting that phosphodiesterase-5 inhibition (PDE5I) may target these derangements. The design of the RELAX trial is described with particular emphasis on the rationale for the primary end point (change in peak oxygen consumption [VO2] with sildenafil versus placebo).

HFpEF Pathophysiology

LV Diastolic Dysfunction

In HFpEF, abnormalities in left ventricular (LV) stiffness and relaxation impair filling and result in the need for elevated filling pressure to achieve adequate LV preload (end-diastolic volume) at rest or during physiological stress. An early study, including patients with hypertrophic and infiltrative cardiomyopathy, identified the inability to enhance end-diastolic volume as a key mechanism limiting exercise capacity in HFpEF.5 However, studies in more typical HFpEF patients have not corroborated this finding.6–8 However, elevation in LV filling pressure at rest or with exertion with normal LV volume is pathognomonic of HFpEF.9

Increases in passive LV diastolic stiffness in HFpEF may be caused by structural abnormalities, including myocyte hypertrophy, matrix deposition, posttranslational oxidative modification of collagen, and altered titin isoform expression. Dynamic perturbations in the phosphorylation of titin or other myofilament proteins, diastolic calcium concentrations, and calcium sensitivity may acutely impair LV stiffness in HFpEF.10,11

Impaired relaxation may contribute to elevated filling pressures during exercise-related tachycardia in HFpEF.12,13 Isovolumic relaxation is an energy-requiring process, and abnormalities in myocardial energetics, which have been demonstrated in HFpEF, may contribute to abnormal relaxation reserve.14

LV Systolic Dysfunction

Although EF is by definition preserved (≥50%) in HFpEF, other measures of resting myocardial systolic function are subtly but
terized by dramatic deficits in systolic reserve capacity during HF symptom severity, and incident pulmonary edema.7,18 Reserve is associated with reduction in overall exercise capacity, extraction of oxygen at the tissue level.8,22 Accumulation of metabolic byproducts in skeletal muscle during exercise may contribute to impaired output limiting exercise capacity and incident pulmonary edema.7,18

Vascular Dysfunction
HFpEF is characterized by vascular-ventricular stiffening, with deleterious effects on LV ejection capacity, blood pressure regulation, and LV relaxation.19,20 Conversely, vascular-ventricular stiffening also accentuates the hypotensive effects of preload or afterload reduction, potentially limiting the efficacy of vasodilators or diuretics in HFpEF.21 Vasodilation in skeletal muscle beds during exercise is, in part, endothelium dependent and is impaired in HFpEF.6,7,16 Limiting delivery and extraction of oxygen at the tissue level.22 Accumulation of metabolic byproducts in skeletal muscle during exercise may also contribute to dyspnea via ergoreflex activation,23 which is tightly correlated with impaired endothelium-dependent vasodilation in HF with reduced EF (HFrEF).23

PH and RV Dysfunction
Pulmonary hypertension (PH) is extremely common in HFpEF and associated with increased mortality.24 In HF, PH may be related to both pulmonary venous and reactive pulmonary arterial hypertension, which are both equally severe in HFpEF and HFrEF.21 As the right ventricle (RV) is exquisitely sensitive to afterload, resting and exercise-induced PH may contribute to impaired output limiting exercise capacity and leading to progressive RV dysfunction, as is well described in HFrEF.25-28

Neurohormonal and Renal Abnormalities
Chronic orthostatic incompetence is common in HFpEF and may relate to autonomic dysfunction, because heart rate recovery (a marker of vagal tone), arterial baroreflex sensitivity, and cardiac β-adrenergoreceptor sensitivity are all reduced in HFpEF.6,14,17,29

Renal dysfunction is as common in HFpEF as HFrEF, and its presence is associated with increased morbidity and mortality.1 Natriuretic peptide (NP) levels are less elevated in HFpEF compared with HFrEF,30 despite similar elevation in filling pressures because of lower wall stress, the stimulus for NP production. This NP deficiency may have adverse effects on renal sodium handling.

PDE-5 in Cardiac, Vascular, and Renal Physiology
Both the NP (via particulate guanylyl cyclase) and NO (via soluble guanylyl cyclase) stimulate cyclic guanosine monophosphate (cGMP), an intracellular second messenger whose effector proteins include cGMP-dependent protein kinase.31 The bioactivity of cGMP/cGMP-dependent protein kinase is regulated via cGMP catabolism by phosphodiesterase 5 (PDE5). Although PDE5 expression is low in normal myocardium, compartmentalization to subcellular locations regulates key cGMP pools32 and modulates β-adrenergic responsiveness.33 Furthermore, PDE5 is markedly upregulated with oxidative stress and pressure overload hypertrophy.34-36 Both common in HFpEF.

PDE5 is expressed in vascular smooth muscle cells where cGMP/cGMP-dependent protein kinase causes vasorelaxation. In pulmonary arterial hypertension, PDE5 expression and activity are increased in pulmonary vascular smooth muscle cells, promoting vasoconstriction.35 In experimental HF, PDE5 is upregulated in the kidney, where it may contribute to renal NP hyporesponsiveness and impaired sodium excretion.36,41

Rational for RELAX: Evidence That PDE51 Targets HFpEF Pathophysiology
Based on the complex pathophysiological derangements present in HFpEF, upregulation of PDE5 in stress states typical of HFpEF, and the pleiotropic effects of PDE5 on cardiovascular function, there is abundant evidence to suggest PDE51 will have beneficial effects in HFpEF (Figure 2).

Cardiac Effects
In experimental HF, chronic pharmacological PDE51 attenuates and reverses maladaptive hypertrophy, fibrosis, and contractile dysfunction,34,42 mitigates deleterious effects of cardiac sympathetic excitation,6,43 and improves cell survival.44 In failing RV cardiac myocytes, PDE51 has positive inotropic effects, possibly because of cGMP inhibition of PDE3 with increased cAMP/protein kinase A.35,36

In HFrEF patients, PDE51 increases RV systolic28 and LV diastolic and systolic functions, coupled with reductions in LV size, LV mass, and left atrial size.45 A recent small, single-center trial in HFpEF (n=44) also reported improvements in lung function, RV systolic function, and LV and RV diastolic functions with PDE51.46 Although chronic PDE51 may cause reverse remodeling, acute administration may also improve diastolic function via cGMP/cGMP-dependent protein kinase–mediated phosphorylation of titin.47

Vascular Effects
PDE51 prevents the development of endothelial dysfunction and pulmonary vascular remodeling, coupled with improvements
Neurohormonal and Renal Effects

PDE5I acutely reduces cardiac-specific norepinephrine spillover,43 which may restore normal adrenergic sensitivity and diminish catecholamine-mediated concentric remodeling.55 Postsynaptic attenuation of excessive adrenergic stimulation has been demonstrated with PDE5I in animal models32 and humans,33 and chronically, this effect may help restore normal β-adrenergic responsiveness and improve cardiac reserve function in HFrEF. In the kidney, PDE5I restores NP responsiveness to enhance sodium excretion in HF.40,41

in alveolar capillary membrane structure and RV geometry in experimental HFrEF.48 In humans, PDE5I reduces pulmonary vascular resistance in non-HF and HF states, both at rest and during stress.27,28,39,46,49 Exertional PH in HFrEF is reduced with acute or chronic PDE5I,27,28 in association with improvements in exercise capacity and quality of life. Systemic vascular resistance,27,51 aortic stiffness, and wave reflection,52,53 endothelial function,54 and ergoreflex-related hyperventilation have all been shown to improve with PDE5I in HFrEF.23

Figure 2. Potential beneficial effects of phosphodiesterase-5 inhibition in heart failure with preserved ejection fraction: See text for discussion. CCA indicates catecholamine; F, function; PVR, pulmonary vascular resistance; PVC, pulmonary vascular compliance; Phos, phosphorylation; LV, left ventricular; RV, right ventricular; SVR, systemic vascular resistance; NP, natriuretic peptide; LVH, LV hypertrophy.

RELAX Design

RELAX is a randomized (1:1), double-blind, placebo-controlled treatment study designed to test the hypothesis that chronic PDE5I (sildenafil 20 mg TID for 12 weeks followed by 60 mg TID for 12 weeks) improves exercise capacity and clinical status in patients with HFrEF.

Study procedures include collection of blood for biomarkers, Minnesota Living with Heart Failure Questionnaire, cardiopulmonary exercise testing (CPXT), and 6-minute walk distance at baseline, 12 weeks, and 24 weeks. Doppler echocardiography and cardiac magnetic resonance imaging (CMR; in CMR-eligible patients) are obtained at baseline and 24 weeks. All study parameters are analyzed by the HF clinical research network CORE laboratories, which include the Biomarker CORE (University of Vermont), CPXT CORE (Massachusetts General Hospital, Harvard University), CMR CORE (Duke University), and Echocardiography CORE (Mayo Clinic, Rochester, MN).

The primary end point is the change in VO₂ from baseline to 24 weeks. Secondary outcomes include change in peak VO₂ at 12 weeks, change in 6-minute walk distance at 12 and 24 weeks, and the change in a composite clinical score at 24 weeks. The composite score is a hierarchical rank score based on time to death (tier 1), time to hospitalization for cardiovascular or cardiorenal causes (tier 2), and change in Minnesota Living with Heart Failure Questionnaire from baseline (tier 3).

Prespecified subgroup analysis includes comparison of efficacy in patients according to LV mass index, N-terminal prohormone of brain natriuretic peptide, estimated pulmonary artery systolic pressure, study medication dose tolerated, atrial fibrillation, and HF medication use.

Tertiary end points include additional exercise and clinical parameters, change in LV mass (by CMR and echo), serological markers of extracellular matrix metabolism, LV diastolic dysfunction, peripheral vascular function, aortic thickness and distensibility, pulmonary artery systolic pressure, and neuroendocrine and renal function biomarkers.

Study Population

Specific inclusion criteria are listed in the Table. Evidence of resting or exercise-induced elevation in filling pressures (N-terminal prohormone of brain natriuretic peptide or hemodynamic data if N-terminal prohormone of brain natriuretic peptide is <400 pg/mL) is required for inclusion in HFrEF.

Table. RELAX Inclusion Criteria

| 1. Age >18 years |
| 2. Previous clinical diagnosis of HF with current NYHA Class II–IV symptoms |
| 3. At least one of the following within 12 months before consent: |
| • Hospitalization for decompensated HF |
| • Acute treatment for HF with intravenous loop diuretic or hemofiltration |
| • Chronic treatment with a loop diuretic for control of HF symptoms+chronic diastolic dysfunction on echocardiography as evidenced by left atrial enlargement |
| • Mean PCWP >15 mm Hg or LV end-diastolic pressure >18 mm Hg at catheterization for dyspnea |
| 4. EF ≥50% within 12 months with clinical stability |
| 5. Stable medical therapy for 30 days: |
| • No addition or removal or major (>100%) dose change of RAAS antagonists, BB, or calcium channel blockers |
| 6. Meet both screening criteria |
| • Peak VO₂ ≤60% age/sex-adjusted normal value+ respiratory exchange ratio ≥1.0 |
| • One of the following: |
| (i) NT-proBNP <400 pg/mL or BNP ≥200 pg/mL |
| (ii) NT-proBNP <400 pg/mL/BNP <200 pg/mL, with mean PCWP >20 mm Hg at rest or >25 mm Hg with exercise |

HF indicates heart failure; NYHA, New York Heart Association; EF, ejection fraction; BB, β-blocker; VO₂, peak oxygen consumption; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; BNP, brain natriuretic peptide; RAAS, renin angiotensin aldosterone system; PCWP, pulmonary capillary wedge pressure.
for study entry. Randomization is stratified by site and by the presence of atrial fibrillation (to ensure equal treatment assignment in the CMR cohort). Exclusion criteria (online-only Data Supplement Table) and safety considerations are detailed in the online-only Data Supplement.

**Statistical Considerations**

Power calculations were based on the SD for change in peak VO$_2$ observed in randomized control trials in HFrEF and on limited randomized control trial data in HFrEF. We estimated a 20% rate of incomplete primary end point data because of death, withdrawal, or incidence of new factors limiting ability to exercise. The expected effect size was extrapolated from studies of chronic PDE5I in HFrEF.$^{23,28}$ Using a 2-sample $t$ test and a 2-sided $\alpha$ of 0.05, a sample size of 190 patients would have 85% power to detect a difference of 1.2 mL/kg per min in change in peak VO$_2$, assuming 20% missing data and an SD of change in peak VO$_2$ of 2.5 mL/kg per min. Because an early blinded interim analysis of primary end point completeness indicated that the missingness rate approached 20% (this decreased dramatically as enrollment proceeded), the data and safety monitoring board (DSMB) recommended increasing the sample size to 215 and 216 patients were ultimately enrolled. Although the primary statistical approach to missing primary end point data will be to exclude patients without 24-week primary end point data, a variety of sensitivity analyses are planned, including changes in peak VO$_2$ at 12 weeks and carry forward of 12-week data.

**Peak VO$_2$ at CPXT as the RELAX Primary End Point**

Exercise intolerance is the cardinal manifestation of HFpEF and can be quantified objectively by measurement of peak VO$_2$. The multifactorial pathogenesis of exercise intolerance in HFpEF coupled with the numerous mechanisms by which sildenafil may ameliorate HFpEF pathophysiology (as above) strongly argues for assessment of peak VO$_2$ as a global indicator of exercise capacity that integrates the physiological consequences of intervention on multiple mechanisms in HFpEF.

CPXT, unlike other measurements of functional status such as 6-minute walk distance, permits assessment of the organ system limiting gas exchange. This is crucial in HFpEF, which tends to occur in older individuals with comorbidities that can result in primary pulmonary, mechanical, or orthopedic limitations to exercise that obscure ascertainment of a treatment effect from a cardiovascular intervention. CPXT also permits precise assessment of volitional effort by determining whether the respiratory exchange ratio (VCO$_2$/VO$_2$) exceeds 1.0 during exercise, indicating that a subject has surpassed their anaerobic threshold.$^{58}$ Finally, in a study of patients with HFpEF and HFrEF, CPXT variables predicted survival whereas 6-minute walk distance did not.$^{59}$

Another advantage of CPXT is that easily derived variables other than peak VO$_2$ reflect distinct aspects of HFpEF pathophysiology. For example, CPXT includes assessment of heart rate and blood pressure augmentation and recovery patterns that are known to be abnormal in HFpEF.$^{6,60}$ CPXT also permits measurement of ventilatory efficiency (V$\_E$/VCO$_2$ slope), which is closely related to pulmonary vascular function during exercise,$^{78}$ and exercise oscillatory ventilation (EOV), which has been shown to signal reduced exercise cardiac index in HFrEF.$^{61}$ Elevated V$\_E$/VCO$_2$ slope and EOV are present in a subset of patients with HFpEF and confer a poor prognosis.$^{62,63}$ Both these CPXT variables have been shown to improve with sildenafil in HFpEF.$^{26,64}$ Finally, CPXT is conduite to being integrated with other forms of physiological testing during exercise as will be assessed in RELAX ancillary studies (see below).

Importantly, unlike changes in alternative trial end points, such as circulating biomarkers or echo parameters, there is significant intrinsic value to patients associated with improving exercise capacity.

Although a recent meta-analysis found that therapy-induced changes in peak VO$_2$ in HF clinical trials did not uniformly predict the corresponding intervention’s effect on mortality in larger phase 3 trials, the reviewed trials often included <50 individuals.$^{65}$ In adequately powered studies of peak VO$_2$ of similar size to RELAX (ie, >200 subjects), concordant changes in peak VO$_2$ and mortality are apparent for interventions, such as cardiac resynchronization therapy (+/− for change in VO$_2$ and improvement in mortality, respectively),$^{6,66}$ isosorbide/hydralazine (+/−)$^{64}$ prazosin (−/−),$^{68}$ and calcium channel blockade(−/−).$^{69}$ A notable exception is that small trials with β-blockers in HFrEF (−/+) showed neutral effects on peak VO$_2$,$^{70,71}$ yet β-blockers clearly prolong survival in HFrEF.

Like any measurement, CPXT necessitates attention to detail with metabolic cart testing uniformity across sites, and willingness of subjects to comply with testing. Compliance with repeated maximum exercise testing is of potential concern in an HFpEF population, because these patients are typically sedentary and sometimes frail and may have an aver-sion to repeated maximum exercise testing.

A detailed description of the RELAX CPXT Protocol Design and the RELAX CPXT Core Laboratory methods is provided in the on-line Data Supplement and details the tailoring of the CPXT protocol to the HFpEF population, harmonization of bike and treadmill protocols to allow paired use of either exercise mode, the rigorous site certification process, secure electronic breath-by-breath data transmission, standardized encouragement scripts, pre-CPXT spirometry and hemoglobin data collection, and rigorous end point measurement methodologies.

**Ancillary Studies**

Several ancillary studies have been approved before completion of enrollment in RELAX.

**Mechanisms Mediating the Effects of PDE5I on Exercise Capacity in HFpEF: Ventricular-Vascular Reserve and Ergoreflex Control.** This prospective, 2-center study seeks to define the effect of PDE-5 inhibition on LV and RV contractile reserve, pulmonary and systemic arterial vasodilator reserve, vascular stiffening, endothelial function, and ergoreflex function in RELAX.

**Effect of PDE5I on $V\_E$/CO$_2$ and EOV in HF With Preserved EF.** Breath-by-breath gas exchange data collected during CPET testing in all subjects will establish the incidence,
severity, reproducibility, and phenotypic correlates of heightened \( V_e/V_{CO_2} \) slope and EOV in HFP EF. The effect of PDE5I on \( V_e/V_{CO_2} \) slope and EOV will also be assessed.

**Oxygen Kinetics Characterization in RELAX.** This study tests the hypotheses that HFP EF patients display delayed O\(_2\) kinetics and reduced aerobic efficiency, that impaired O\(_2\) kinetics are related to perceived dyspnea during exercise and quality of life measures, and that O\(_2\) kinetics and the VO\(_2\)/work rate relationship will improve with chronic PDE5I.

**Resting Ventricular-Vascular Function and Exercise Capacity in HFP EF.** This study will test the hypothesis that resting aortic stiffness and LV systolic and diastolic dysfunctions predict exercise capacity in HFP EF.

**Impact of Atrial Fibrillation on Exercise Capacity in HF With Preserved EF.** This study will characterize the clinical, echocardiographic, and neurohumoral phenotype associated with AF in the setting of HFP EF and will determine whether AF influences exercise capacity compared with sinus rhythm.

**Discussion**

The RELAX trial design has many strengths, including the multicenter design, rigorous entry criteria, novel therapeutic intervention, and extensive phenotyping, which will provide insight into mechanisms responsible for the outcome of the study and through ancillary analyses, the pathophysiology of HFP EF. The primary end point is well suited to the enrollment capacity of the HF clinical research network, the pathophysiology of HFP EF, and the biological actions of PDE5I, but its utilization precludes enrollment of frailer patients and may limit generalization of study results to all HFP EF patients.

Although the RELAX trial will determine whether chronic PDE5I improves exercise capacity in HFP EF patients, it is not considered a pivotal trial, and thus the RELAX trial will not result in labeling of sildenafil for the treatment of HFP EF. However, the results from RELAX could lead to guideline recommendations for use of PDE5I to improve symptoms in HFP EF and to an outcome-based study of PDE5I in HFP EF.

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SUPPLEMENTAL MATERIAL

Phosphodiesterase-5 Inhibition in Diastolic Heart Failure: The RELAX Trial Rationale and Design

Redfield et al: RELAX Trial Design

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Supplemental methods

RELAX exclusion criteria:

The RELAX exclusion criteria are outlined in the Supplemental Table.

CPXT Protocol Design Considerations:

Several considerations related to the HFpEF population were taken into account when designing the CPXT testing protocol for RELAX. Low initial work rate is critical to avoid rapid cessation of exercise in patients with HFpEF. Because the linearity of oxygen uptake during exercise is used to ascertain VAT\(^1\) and to determine a cardiovascular basis for exercise intolerance,\(^2\)-\(^4\) it is important to be confident of the linearity of the work rate profile that yielded the response. Targeting a test duration of 8-12 minutes has been shown to maximize achieved peak VO\(_2\)\(^5\).

In order to address these considerations, the RELAX Trial CPXT protocol consists of a 5-min rest period during which gas exchange data is collected followed by a 3-min period of low-level “unloaded exercise” and a 10W/min symptom-limited incremental ramp. The 10W/min increment was implemented based on previous studies in HFpEF demonstrating achieved workloads of 50-80 Watts\(^6,7\) which translates to the desired duration of testing (8-11min)\(^5\). To harmonize testing by cycle and treadmill ergometry, a custom HFpEF treadmill protocol was designed with a linear increase in walking speed coupled with a curvilinear increase in treadmill grade to yield a linear increase in work rate\(^8\) of 10W/min (Supplemental Figure I). Two treadmill protocols were designed to account for the influence of weight on treadmill work rate (< or ≥80kg).

RELAX CPXT Core Laboratory Features

Multicenter trials evaluating exercise gas exchange have been confounded by methodological differences in exercise protocols and lack of uniformity in interpretation of gas exchange data.\(^9,10\)
The CPXT Core Laboratory provides a written manual of operations which contains detailed instructions for site qualification and testing. A rigorous certification process is implemented to ensure uniformity of data collected and validity of derived results. Each of the 37 certified CPXT sites was required to submit two incremental symptom-limited CPXTs on a standard normal subject. Prospective CPXT laboratories were evaluated on their ability to: (1) follow the CPXT qualification protocol, (2) generate reproducible CPXT data within the expected normal physiologic range, and (3) transmit appropriate electronic breath-by-breath data to the core laboratory.

Sites were required to perform both ergometer and metabolic cart calibrations according to published guidelines prior to initiation of testing. Results of 2-point O₂ and CO₂ calibration and 3L-flow meter calibration prior to each test were required to be recorded in a calibration log. The CPXT Core Lab also assessed the remarkably well-conserved VO₂ to work rate relationship of 10ml/W in normal individuals to further determine the adequacy of ergometer and metabolic cart calibration and performance. Of the 37 sites qualified, 32% required repeat qualification testing due to need for equipment recalibration (n=6), faulty O₂ cell (n=1), and inability to follow the protocol (n=5). Examples of test results that prompted corrective action (i.e. the need for an oxygen cell replacement and cycle re-calibration with a torque meter) are provided in supplemental Figure II.

The RELAX CPXT Core Laboratory requires completely electronic breath-by-breath data capture and secure transfer in order to permit uniform processing and interpretation of gas exchange data from different metabolic carts. As a result, the core laboratory has access to breath-by-breath data that can be compartmentalized into uniform time intervals for VO₂ measurement across all studies. Similarly, uniform procedures are applied for outlier breath detection as well as graphical representation of data to facilitate interpretation of secondary endpoints such as VO₂ at the VAT. In addition to being cost-effective, the 100% electronic core lab represents an important advance in
quality control for multi-center CPXT trials because differences of over 15% in VO$_2$ measurements have been reported with different choices of time intervals for assessment of VO$_2$ data from various metabolic carts.$^{12,14}$

Additional steps taken to maximize CPXT data completeness and validity include the option to have a RELAX-protocol CPXT used in initial evaluation (screening) of a patient to serve as their baseline CPXT for the trial. This approach promotes compliance with study procedures by minimizing the number of total CPXTs required for the trial. Additional quality control approaches include specification of standardized gentle encouragement during the protocol to promote achievement of a RER $\geq$ 1.0, recording of Borg Dyspnea Scores (0-10 scale) every 2 minutes, and assessment of spirometry (FEV1, FVC) to permit determination of whether there was a pulmonary mechanical limit to exercise (V$_E$/maximum voluntary V$_E$ >0.7 prior to AT).$^{15,16}$ Current hemoglobin values were also recorded because hemoglobin is a variable that may be subject to change during the course of a 24-week trial that can influence exercise performance.

**Endpoint Measurement Procedures:** Peak VO$_2$ is determined by the highest 30-second median value of breath-by-breath VO$_2$ measurements during the final minute of incremental exercise. Ventilatory anaerobic threshold (VAT) is determined by the modified V-slope method$^1$ in conjunction with assessment of ventilatory equivalents.$^{12,17}$ Because of the inherent challenges of assessing VAT in HF, two independent observers assess VAT and values differing by more than 100ml prompt evaluation by a third reader to adjudicate the final reported value. Peak workload is determined by the highest achieved workload (Watts) on the cycle ergometer. For treadmill testing, Watts are derived according to the following equation WR(t)=m x g x v(t) x sin($\alpha$).$^8$ WR is work rate, m is body mass in kg, g is gravitational acceleration (9.81m·s$^{-2}$), v is velocity in meters per second, and $\alpha$ is
the angle of inclination. Duration of exercise is recorded from the time of initiation of unloaded exercise until the initiation of recovery.

**Safety and tolerability of PDE-5 inhibitors in Heart Failure:** In the limited studies of acute or chronic administration of PDE-5 inhibitors in HF patients for treatment of erectile dysfunction or as HF therapy, the safety and tolerability profile has been excellent. Specifically, systemic hypotension is rarely observed. Use of drugs which potentiate drug levels or hemodynamic effects of sildenafil® are precluded in RELAX. These studies provide assurance that the administration of sildenafil at the planned dose is safe in patients with HF. In DHF, there is less concern regarding hypotension as average blood pressure in DHF is higher and while patients are older, a meta-analysis of sildenafil for erectile dysfunction did not report significant increases in side effect profile according to subgroups such as age, diabetes, hypertension. Rare reports of sudden decrease or loss of vision or hearing have been reported in men taking PDE-5 inhibitors for erectile dysfunction. However, the FDA has stated that, “it is difficult to determine whether these reports are directly related to the use of one of these drugs, an underlying medical condition, or other risk factors for hearing loss, a combination of these factors, or other factors that the direct relationship of these events to drug administration.”
Supplemental Table – RELAX Exclusion Criteria

1. Non-cardiac condition that precludes exercise testing
2. Non-cardiac condition limiting life expectancy to less than one year
3. Current or anticipated future need for nitrate therapy
4. Significant left sided structural valve disease
5. Hypertrophic cardiomyopathy
6. Infiltrative or inflammatory myocardial disease (amyloid, sarcoid)
7. Pericardial disease
8. Primary pulmonary arteriopathy
9. Myocardial infarction or unstable angina, or revascularization within 60 days
10. Other clinically important causes of dyspnea
11. Systolic blood pressure < 110 mmHg or > 180 mm Hg
12. Diastolic blood pressure < 40 mmHg or > 100 mmHg
13. Resting heart rate (HR) > 100 bpm
14. A history of reduced ejection fraction (EF<50%)
15. Implanted metallic device precluding CMR (in patients without atrial fibrillation)
16. Estimated GFR < 20 ml/min/1.73m² (modified MDRD equation)
17. Women of child bearing potential without negative pregnancy test and effective contraception
18. Hemoglobin <10 g/dL
19. Other medications with potential sildenafil interactions (alpha antagonists or cytochrome P450 3A4 inhibitors).
20. Patients with retinitis pigmentosa, previous diagnosis of non-arteritic ischemic optic neuropathy, untreated proliferative retinopathy or unexplained visual disturbance
21. Sickle cell anemia, multiple myeloma, leukemia or penile deformities
22. Severe liver disease
23. Listed for cardiac transplantation
**Supplemental Figure I. CPXT protocols in RELAX:** Top panels are schematic representations of the similar linear increments in work rate during the cycle (Panel A) and treadmill ergometry (Panel B) ramp protocols used for cardiopulmonary exercise testing. In order to appropriately match work increment to exercise capacity, a 10W/min ramp was used for RELAX subjects (red line) and a 20W/min ramp for qualification testing in normal individuals (blue line). The bottom panels are representative data from qualification studies. The expected relationship between VO₂ and work rate (10ml/Watt) is demonstrated for the individual who performed repeated tests on the cycle ergometer (Panel C) and for the individual who performed qualification testing with treadmill ergometry (Panel D).

**Supplemental Figure II.** Panel A illustrates initial qualification tests submitted that indicate a reduced VO₂/W relationship (<10ml/W) as well as a reduction in VO₂/W slope from test 1 to test 2, which was due to a faulty oxygen sensing cell in the metabolic cart. Upon instruction from the core laboratory to replace the oxygen cell, repeat testing on the same subject yielded a normal VO₂/W relationship (Panel B). Panel C illustrates initial qualification testing with an abnormally steep VO₂/W slope (13ml/W) implicating an inappropriately calibrated cycle ergometer. Repeat testing on a second subject (Panel D) revealed the same steep relationship in VO₂/W which made subject-specific causes of an abnormal VO₂/W relationship unlikely. Recalibration of the cycle ergometer with a torque meter resulted in a normal VO₂/W relationship for repeat testing on subject 2 after cycle calibration.
Supplemental Figure I.

A Cycle Ergometry

B Treadmill Ergometry

C

D

E

F
Supplemental Figure II.

A

B

C

D

\[ \text{VO2 (ml/min)} \]

\[ \text{Work (Watts)} \]

\[ \text{Slope: 5.5} \quad R=0.99 \]

\[ \text{Slope = 5.1} \quad R=0.99 \]

\[ \text{Slope 10.4} \quad R=0.99 \]

\[ \text{Slope 10.7} \quad R=0.99 \]

\[ \text{Slope 13.0} \quad R=0.98 \]

\[ \text{Slope 12.8} \quad R=0.98 \]

\[ \text{Slope 13.3} \quad R=0.99 \]

\[ \text{Slope 10.3} \quad R=0.99 \]
Supplemental References


