Define in Preclinical Diastolic Dysfunction (PDD) with renal dysfunction, the cardiorenal and humoral actions of chronic PDEV inhibition

NCT02058095

7/29/2016

Version4 – IRB approved 7/29/2016

Define in Preclinical Diastolic Dysfunction (PDD) with renal dysfunction, the cardiorenal and humoral actions of chronic PDEV inhibition

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Summary

Preclinical diastolic dysfunction (PDD) is the initial compensated phase of left ventricular diastolic dysfunction with preserved ejection fraction (EF) and without symptoms of heart failure (HF).

We have previously demonstrated that a common hallmark of preclinical diastolic dysfunction (PDD) was impaired renal function, characterized by decreased glomerular filtration rate (GFR) and renal blood flow (RBF), with an attenuated plasma natriuretic peptide and renal cyclic guanosine 3',5'-monophosphate (cGMP) response to saline volume expansion (VE). The mechanism for the renal dysfunction and attenuated renal cGMP response to VE is undefined.

Type V phosphodiesterase (PDEV) metabolizes cGMP and is abundant in the kidney, vasculature, and has been recently reported in the heart.(2,3) We and others have demonstrated that renal PDEV is up-regulated in experimental HF and may lead to the attenuation of renal cGMP generation in response to both endogenous and exogenous BNP, thus serving as a mechanism for renal resistance to BNP. Furthermore, in experimental overt HF, 10 days of PDEV inhibition treatment resulted in reduction of left ventricular (LV) mass, increased LV fractional shortening and cardiac output. Furthermore, chronic PDEV inhibition did enhance the renal actions of exogenous BNP, specifically improving GFR and renal cGMP generation.(4) PDEV inhibitors are FDA approved for erectile dysfunction and pulmonary hypertension.

Objective: To determine the effect of 12 weeks of chronic PDEV inhibition with Tadalafil versus placebo on basal cardiorenal and humoral function and on the integrated cardiorenal and humoral response to acute sodium loading in subjects with PDD and renal dysfunction.

Hypotheses:

1. Chronic PDEV inhibition with Tadalafil will result in the improvement of renal function measured by glomerular filtration rate (GFR) as compared to placebo in subjects with PDD and renal dysfunction.
2. Chronic PDEV inhibition with Tadalafil will result in the improvement urinary sodium excretion and urinary cGMP excretion in response to acute sodium loading as compared to placebo in subjects with PDD and renal dysfunction.

Study design:

This will be a single center **double blind placebo-controlled design**. Subjects will be randomized in a 2:1 design, to the Tadalafil alone group or placebo.

Primary end points:

1. Change in GFR from baseline after 12 weeks of chronic Tadalafil versus placebo.

2. Change in urinary sodium excretion and urinary cGMP excretion in response to acute sodium loading from baseline after 12 weeks of chronic Tadalafil versus placebo.

Background

In the AHA/ACC classification of HF, stage B is defined as patients with abnormal heart structure/function (systolic or diastolic dysfunction) without symptoms. This concept of preclinical HF is based on the fact that abnormal heart structure/function can be detected by complementary methods before the development of symptoms.(5,8) Patients with those abnormalities may progress to heart failure and are at increased risk of adverse cardiac events. Preclinical diastolic dysfunction (PDD) is the initial compensated phase of left ventricular diastolic dysfunction with preserved EF and without symptoms of HF.

We have previously demonstrated that a common hallmark of preclinical diastolic dysfunction (PDD) was impaired renal function, characterized by decreased glomerular filtration rate (GFR) and renal blood flow (RBF), with an attenuated plasma natriuretic peptide and renal cyclic guanosine 3',5'-monophosphate (cGMP) response to saline volume expansion (VE). The mechanism for the renal dysfunction and attenuated renal cGMP response to VE is undefined.

Cyclic GMP is the second messenger of the natriuretic peptide system (NPS) and the nitric oxide system (NO) and plays an important role in the preservation of myocardial, vascular, and renal function. Hence, disruption of this signal transduction process may contribute to the development of cardiorenal dysfunction(1). Type V phosphodiesterase (PDEV) metabolizes cGMP and is abundant in the kidney, vasculature, and has been recently reported in the heart.(2,3) We and others have demonstrated that renal PDEV is up-regulated in experimental HF and may lead to the attenuation of renal cGMP generation in response to both endogenous and exogenous BNP, thus serving as a mechanism for renal resistance to BNP. Furthermore, in experimental overt HF, 10 days of PDEV inhibition treatment resulted in reduction of left ventricular (LV) mass, increased LV fractional shortening and cardiac output. Furthermore, chronic PDEV inhibition did enhance the renal actions of exogenous BNP, specifically improving GFR and renal cGMP generation.(4) PDEV inhibitors are FDA approved for erectile dysfunction and pulmonary hypertension.

Objective: To determine the effect of 12 weeks of chronic PDEV inhibition with Tadalafil versus placebo on basal cardiorenal and humoral function and on the integrated cardiorenal and humoral response to acute sodium loading in subjects with PDD and renal dysfunction.

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1. Chronic PDEV inhibition with Tadalafil will result in the improvement of renal function measured by glomerular filtration rate (GFR) as compared to placebo in subjects with PDD and renal dysfunction.
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Primary end points:

1. Change in GFR from baseline after 12 weeks of chronic Tadalafil versus placebo.
2. Change in urinary sodium excretion and urinary cGMP excretion in response to acute sodium loading from baseline after 12 weeks of chronic Tadalafil versus placebo.

Secondary end point:

1. Change in LVEF, dimensions and diastolic function from baseline after 12 weeks of chronic Tadalafil versus placebo.
2. Change in blood pressure (BP), heart rate, right ventricular (RV) end-systolic pressure and RV functions.

Study design:

This will be a single center **double blind placebo-controlled design**. Subjects will be randomized in a 2:1 design, to the Tadalafil alone group or placebo .

Study population

A total of 45 patients with PDD as defined by ejection fraction of equal too or greater than 50% with moderate or severe diastolic (grade 2 or greater) dysfunction as assessed by Doppler echocardiography, **assessed within 36 months of enrollment),)** If patient's EF was assessed greater than 36 months, the PI will performed an assessment of the LV EF during the screening visit using an hand-held echo machine. If EF is equal or greater than 50% and a grade 2 or greater , the patient will be enrolled. The patient will receive compressive echo assessment during the study.

Also having no clinical signs or symptoms of congestive heart failure, a minimal distance on 6-minute walk of equal or >450 meters will be recruited and calculated creatinine clearance of equal or less than 90 ml/min and greater than 30 ml/min, using the MDRD formula assessed within the past 24 months. If the subject is not able to walk 450 meters due to pain in hips and knees and not fatigue or shortness of breath than they will still qualify for the protocol.

Subjects who enroll that had previously participated in in our other PDD protocol IRB 11-004257, we will be able to use the data from the screening visit such as labs, cardiac exam, 6 minute walk and dietician visit

Exclusion criteria:

- Current or anticipated future need for nitrate therapy
- Systolic blood pressure < 90 mmHg or > 180 mm Hg
- Diastolic blood pressure < 40 mmHg or > 100 mmHg
- Patients taking alpha antagonists or cytochrome P450 3A4 inhibitors (ketoconazole, itraconazole, erythromycin, saquinavir, cimetidine or serum proteases inhibitors for HIV) who cannot be taken off these medications for the duration of the study.

- Patients taking the following selective alpha blockers and who are unable to stop for the duration of the study:
    - Alfuzosin
    - Prazosin
    - Doxazosin
    - Tamsulosin
    - Terazosin
    - Silodosin

- Also guanylate cyclase stimulators (Riociguat)

- Patients with retinitis pigmentosa, previous diagnosis of nonischemic optic neuropathy, untreated proliferative retinopathy or unexplained visual disturbance
- Patients with sickle cell anemia, multiple myeloma, leukemia or penile deformities placing them at risk for priapism (angulation, cavernosal fibrosis or Peyronie's disease)
- Patients with an allergy to iodine.
- Patients on PDEV inhibition for pulmonary hypertension
- Patients on PDEV inhibition for erectile dysfunction who are not willing to stop the medication for the duration of the study
- Valve disease (> moderate aortic or mitral stenosis; > moderate aortic or mitral regurgitation)
- Obstructive Hypertrophic cardiomyopathy
- Infiltrative or inflammatory myocardial disease (amyloid, sarcoid)
- Pericardial disease
- Have experienced a myocardial infarction or unstable angina, or have undergone percutaneous transluminal coronary angiography (PTCA) or coronary artery bypass grafting (CABG) within ***60 days*** prior to consent, or requires either PTCA or CABG at the time of consent
- Severe congenital heart diseases
- Sustained ventricular tachycardia or ventricular fibrillation within 14 days of screening
- Second or third degree heart block without a permanent cardiac pacemaker
- Stroke within 3 months of screening or other evidence of significantly compromised CNS perfusion
- Hemoglobin <9 g/dL
- Patients with severe liver disease (AST > 3x normal, alkaline phosphatase or bilirubin > 2x normal)
- Serum sodium of < 125 mEq/dL or > 150 mEq/dL
- Serum potassium of < 3.2 mEq/dL or > 5.9 mEq/dL
- Prior diagnosis of intrinsic renal diseases including renal artery stenosis of > 50%
- Peritoneal or hemodialysis within 90 days or anticipation that dialysis or ultrafiltration of any form will be required during the study period
- Less than 21 years of age and over 90 years old.
- Pregnant or nursing women.
- Women of child bearing potential who do not have a negative pregnancy test at study entry and who are not using effective contraception

- Non-cardiac condition limiting life expectancy to less than one year, per physician judgment
- Other acute or chronic medical conditions or laboratory abnormality which may increase the risks associated with study participation or may interfere with interpretation of the data
- Received an investigational drug within 1 month prior to dosing
- In the opinion of the investigator is unlikely to comply with the study protocol or is unsuitable for any reasons

Study protocol

At the consent visit, diet instructions will be given by study personnel and baseline whole blood count, Hgb A1C, electrolyte panel, AST and Bilirubin will be obtained. Prior to the first overnight stay the study, subjects will be given instructions on (120 mEq Na/day) which will be maintained throughout the study period.

If subjects calculated creatinine clearance is not between 30 to 90 ml/min using the MDRD formula the subject will be excluded from the study. Also at consent visit the 6 minute walk will be done to determine eligibility and a physical exam along with vital signs, height and weight will be done. Twenty-four hour urine collection will be obtained one day prior to the active study day for assessment of baseline sodium excretion, creatinine clearance and microalbuminuria. . For instances where subjects are not able to collect urine for 24 hours prior a 12 hr collection will be done.

Therapy with ACE inhibitor or Angiotensin receptor blockers or other vasodilators, beta-receptor antagonists, Digoxin and antiarrhythmic medications will be allowed, however, at stable doses for two weeks prior to the study date.

Subjects will be admitted to the CRU on the evening before the active study day. They will be able to order a no-added salt meal and will be NPO after midnight until the last renal clearance blood draw the next day. Bladder scan will be carried out to assess for urine retention. On the active study day, subjects take their medications upon awakening, however, diabetics will hold their diabetic medications until after the last renal clearance test then they will be able to order a regular diet meal and take their diabetic medications. During the first 15 minutes, two standard intravenous (IV) catheters will be placed (one in each arm). One catheter will be used for infusion and the other (in the contralateral arm) for blood sampling. Subjects will be asked to drink 5ml/Kg of water to insure sufficient urinary flow. A priming dose (calculated according to body size) of Iothalamate, to measure glomerular filtration rate (GFR) followed by a constant rate IV sustaining dose (calculated according to estimated kidney function) of Iothalamate. The subjects will be asked to empty their bladder spontaneously every thirty minutes (if subjects are unable to void every thirty minutes, a urinary catheter can be used upon consent). Extra fluids may be given if subjects are not able to void and the amount recorded in the total of fluids given. Throughout the study, at the end of each 30-minute clearance period, subjects will be asked to drink an amount of water equivalent to the sum of the blood losses and the urinary flow. After an equilibration period of 45 minutes, a 30-minute baseline renal clearance will be carried out. Urinary samples for determination of volume, urinary sodium excretion (UNaV), cGMP, BNP, Iothalmate, , will be obtained at the end of the clearance period. Venous blood samples for Iothalmate, , sodium, ANP, BNP, cGMP, renin, angiotensin II, Lipid profile, glycerol, adiponectin, and aldosterone will be obtained at the middle of the clearance period.

Collection of blood at baseline, during and after sodium load will be stored for future DNA/Protein analysis.

Blood pressure will be measured at 20-minute intervals by using automatic blood pressure cuff, and heart rate will be continuously monitored by electrocardiography.

Echocardiography will be performed during these baseline clearances to determine left atrial (LA) and left ventricular (LV) volumes, systolic and diastolic function, right ventricular end systolic pressure and function according to the American Society of Echocardiography guidelines.

After the baseline clearance the acute saline load will be administered (normal saline 0.9% 0.25 ml/kg/min for 1 hour). During the 1 hour saline load, one 30-minute clearance (as outlined above) will be repeated with the subjects in supine position after which a second 30-minute clearance will be repeated with the subject sitting or the head of the bed up. As above, blood samples are collected midway during each clearance and urine samples are obtained every 30 minutes. Echocardiography will be repeated immediately after the end of the saline infusion.

At the completion of the baseline renal clearance periods and response to acute sodium load, subjects will be randomized to **Tadalafil or placebo.** Subjects will be randomized in a 2:1 fashion. The randomization schedule will be provided by the Department of Biostatistics, implemented by the Mayo Pharmacy and administered by the CRU staff.

All subjects will initially take 1 tab Tadalafil (5mg) or Placebo. The blood pressure will be checked prior to administering the drug. Thereafter, both blood pressure and heart rate will continue to be monitored every 20 minutes for the next 4 hours. If after the first dose of study drug if patient's systolic blood pressure is < 85 mmHg systolic and has symptoms of hypotension e.g. lightheadedness, dizziness, feeling faint, blurred vision, the study drug will be stopped however the subject will continue in the study. After 2 hours if blood pressure is >95 systolic then give 1 more (5 mg) of Tadalafil or placebo and monitor blood pressure for 2 hours. If blood pressure is >95 then dismiss subject on 2 (5 mg) tabs of tadalafil or placebo. If blood pressure is between 90 – 95 mmHg systolic, then dismiss on 1 (5 mg) tab of Tadalafil or placebo to be maintained on this dose for the remainder of the study and not be increased

Subjects will be instructed to take their blood pressure daily for 12 weeks. Subjects will also have access to a 24-hour phone number should they have any questions or develop any side effects.

At 2 weeks (± 5 days) from dismissal if blood pressure is> 100 than add 1 (5 mg) tab of Tadalafil or placebo to make a total of 3 (5mg) tabs of Tadalafil or placebo.

At 4 weeks(± 5 days) if blood pressure is > 100 add 1 (5 mg) tab to make a total of 4 (5 mg Tadalafil or placebo.

The PI can reduce the dose of Tadalafil or placebo if subjects blood pressure is < 90 and/or has symptoms of hypotension e.g. lightheadedness, dizziness, feeling faint, blurred vision.

Subjects will return after one week (± 4 days) for electrolyte check. For patients who live more than 25 miles away we will try to arrange this visit with the patient's local physician. They will also receive a weekly phone call to review status.

After six weeks (± 5 days) , subjects will repeat blood draw for safety labs (electrolytes). For patients who do not live more than 25 miles away we will try to arrange this visit with the patient's local physician.

At the end of the twelve-week study period ($+$ or $-$ 8 days), subjects will be admitted to the CRU the afternoon prior to the renal clearance study. We will get an hgb A1C with the evening blood draw for the creatinine. Echocardiography, renal clearance, humoral determination and acute saline load will be performed in the same manner as the baseline study. Subjects will also perform a 24-hour urine collection the day prior to their return visit for determination of sodium excretion and creatinine clearance. Subjects will be dismissed after the renal clearance study.

Sample size and statistical analysis

The sample size calculation and statistical analysis have been designed in collaboration with the Division of Biomedical Statistics and Informatics, (Kent Bailey, Ph.D) .

The **primary analyses** will be a couple of 2-sample comparisons (2-sample t-test) of the effects of interest: Changes in glomerular filtration rate, sodium excretion, urinary cGMP excretion, left ventricular end systolic and diastolic volume. To further investigate this relationship we will also do an analysis of covariance to look at the group differences on the final effects of the drug after adjusting for the before drug measurements. Values will be expressed as the mean $\pm$ SD. A statistically significant difference will be considered to be present when $p<0.017$.

Sample size calculation: Based on our completed specific aims 1 of the current funding cycle, we were able to construct the magnitude of difference that could be detected for each of the seven parameters. With 26 subjects in the active treatment group and 13 subjects in the placebo group, we have 80% power to detect the differences as reported in the second table.

The table below summarizes preliminary data on these within subject changes:

Comparison Between Treatment Groups Change From Baseline to After Volume Expansion			
Variable	**Placebo (N=18)**	**BNP (N=18)**	**P-value**
GFR (U1-U3 Avg to U4)	-1.2 ± 12.6	5.1 ± 16.0	0.20
Sodium Excretion (U1-U3 Avg to U4)	-17.4 ± 63.1	84.1 ± 133.2	0.007
Renal Plasma Flow (U1-U3 Avg to U4)	5.7 ± 52.1	34.5 ± 79.1	0.21
LV Ejection Frac. (Base to Aft Vol Exp)	3.3 ± 3.1	4.1 ± 2.3	0.40
LV Diastolic Index (Base to Aft Vol Exp)	-2.2 ± 6.4	-7.3 ± 10.1	0.08
LV Systolic Index (Base to Aft Vol Exp)	-3.4 ± 3.0	-5.8 ± 3.4	0.036
Urinary cGMP (Baseline to 30 Min)	57.1 ± 104.3	268.7 ± 282.3	0.003

Based on these estimates, we obtain the following detectable differences between 2 groups:

	Detectable difference between the PDEV inhibition and placebo group for the same parameter(80% power, n=26, alpha=0.25);Two-sample t-test
GFR	15.1
Sodium Excretion	125.4
Renal Plasma Flow	74.5
LV EF	2.2
LV Diastolic Index	9.5
LV Systolic Index	2.1
Urinary cGMP	265.7

It is the primary goal of the study to detect an improvement of GFR of at least **15 ml/min,** which is considered a clinically meaningful improvement of GFR. The detectable differences in the other 6 parameters are plausible and of interest as well.

Recruitment of subjects:
Subjects will be recruited from the echo database and will receive a reimbursement of *$300* for participation in the study.

Assessment of systolic and diastolic function by echocardiography: Transthoracic echocardiography will be performed according to the standard clinical Amercian Society of Echocardiography methods.

Analytic methods
Both plasma and urine concentration of Iothalmate and PAH will be determined by the Mayo Core Renal lab. Glomerular filtration rate (GFR) will be calculated using Iothalmate clearance

$$GFR\ (ml/min) = \frac{U \times V}{P}$$

Where: U = urine concentration; P = plasma concentration; V = urine flow (mL/ min)

Plasma and urine will be analyzed for sodium with the Beckman ion-selective analyzer. Urine creatinine will be measured by the Mayo Core Renal Laboratory. Venous blood for hormone analysis will be collected in heparin and EDTA tubes and immediately placed on ice. After centrifugation at 2,500 rpm at 4C, the plasma will be decanted and stored at -80C until analysis. Specific plasma radioimmunoassays include renin, aldosterone, angiotensin II, ANP, BNP and cGMP. Urine for cGMP and CNP radioimmunoassays will also be collected on ice as previously described.

PROTECTION OF HUMAN SUBJECTS

a) Human Subject Involvement and Characteristics

These protocols will not involve special classes of subjects, such as fetus, neonates, pregnant women, children, prisoners, institutionalized individuals, or others who may be considered vulnerable populations.

The specific subject population is as follows:

PDD-will consist of 45 subjects with an ejection fraction of greater than 50% with moderate or severe diastolic (grade 2 or greater) dysfunction as assessed by Doppler echocardiography with no clinical signs or symptoms of congestive heart failure; a minimal distance on 6-minute walk of >450 meters and calculated creatinine clearance of equal or less than 60 ml/min and greater than 30 ml/min, using the MDRD formula assessed within the past 24 months. A 6-minute walk distance of 450 meters was the average distance achieved by NYHA class I patients in the SOLVD trial and is an objective marker of clinical status. If the subject is not able to walk 450 meters due to pain in hips and knees and not fatigue or shortness of breath than they will still qualify for the protocol.

b) Source of Materials

Plasma samples, urine samples echocardiographic data and clinical data will be used entirely for research purposes. Patient identifiers will not be released outside of Mayo and any publications will exclude any kind of patient identifiers that could be correlated with the specific patient. Limited access data set will be made available according the NHLBI guidelines .

c) Potential Risks and Adequacy of Protection against Risks

The **overall risk** for participation in the protocol is small and the main concern is that the addition of PDEV inhibition (Tadalafil) may result in hypotension. The medication will be started in the CTSA CRU under close monitoring; hence, the study has extremely little risk of causing irreversible consequence on the patients.
In addition the subjects will measure their blood pressure daily at home and will be instructed to report a blood pressure below 95 and or hypotension symptoms e.g. lightheadedness, dizziness, feeling faint, blurred vision. They will receive a weekly phone call and safety labs at week 1 and 6.

The PI can reduce the dose if subject has a blood pressure < 90 and or has symptoms of hypotension e.g. lightheadedness, dizziness, feeling faint, blurred vision.

Tadalafil is a PDEV inhibitor which is FDA approved for the management of erectile dysfunction and pulmonary hypertension. The recommended dose for erectile dysfunction is 5 mg/day and for pulmonary hypertension in patients with renal dysfunction (eGFR<30 <80 ml/min) is 20 mg once a day.

Common side effects include; Flushing (1% to 13%),Indigestion (4% to 13%), Nausea (up to 11%); Backache (3% to 12%);Myalgia (1% to 14%); Headache (3% to 42%); Nasopharyngitis (2% to 13%) and Respiratory tract infection (7% to 13%).

Rare side effects include: Angina (less than 2%), Chest pain (less than 2%), Heart failure, Myocardial infarction (less than 2%), Tachycardia (less than 2%, Stevens-Johnson syndrome, Cerebral hemorrhage, Cerebrovascular accident, Seizure, Non-arteritic ischemic optic neuropathy, Retinal artery occlusion, Thrombosis of retinal vein,

Decreased hearing, Sudden onset (less than 2%), Sudden hearing loss (less than 2%). Priapism.

Acute saline loading: These studies require acute saline loading with normal saline 0.9% 0.25 ml/kg/min for 1 hour. Previous studies by Volpe and others have shown that this is tolerated well. In the current funding cycle, we have completed studies in approximately 120 patients with no adverse event of fluid overload. There is small risk of causing fluid overload, however, the subjects will be closely monitored closely in the CRU and the study will be terminated if the subject develops and signs or symptoms of fluid overload and will be treated appropriately with IV diuretics.

To protect the patients against the risk of hypotension, the following measure are incorporated in the protocol:

- Screening and application of exclusion criteria will minimize risks
- Medication will be initiated in the CTSA CRU under close monitoring.

The following stopping rule will be applied to ensure patient safety:

The blood pressure will be checked prior to giving the drug.

- If after the first dose of study drug if patient's systolic blood pressure is < 85 mmHg systolic and has symptoms of hypotension e.g. lightheadedness, dizziness, feeling faint, blurred vision, then the study drug will be stopped however the subject will continue in the study.
- Any patient that experiences any adverse events as defined below during the study the study drug will be reduced or stopped. The adverse events include: hypotension as defined by a) any clinical symptoms of hypotension e.g. lightheadedness, dizziness, feeling faint, blurred vision and b) if systolic blood pressure is < 85 mmHg; b) symptoms of heart failure for > 45 minutes; c) allergic reaction to the drug and others unexpected adverse events which is deemed to be clinically significant by the investigator.

Plan of intervention in the event of adverse effects to the subjects.

- In the CRU, if the patient does develop hypotension, as defined by a) any clinical symptoms of hypotension e.g. lightheadedness, dizziness, feeling faint, blurred vision and b) if systolic blood pressure is < 85 mmHg; the clinical care team will manage that according to the standard resuscitative procedure: Trendelenburg position, give fluid IV, use of vasopressor if necessary

Other potential minor risk includes:

The risks of blood drawing include bleeding at the puncture site, bruising and pain. These risks occur in a very small portion of the population.

This protocol may be hazardous to an unborn child. There is no medical information to determine whether there are significant risks to a fetus carried by a mother who is participating in this study. Therefore, female participants must be postmenopausal or have been surgically sterilized or have a negative pregnancy test and must agree to use a reliable method of contraception until study completion. Methods of contraception include: birth control pill/implants/injections, intrauterine devices, spermicide, diaphragm or condoms.

Iothalamate contains a small amount of iodine and for persons allergic to iodine, this could pose a higher risk for an allergic reaction. To date in more than 5,000 patients, there have been only rare allergic reactions (one or two) when the solution has been used for this type of test. The amount of the material injected for this test will be approximately the same as that used in a similar kidney function test performed on a routine basis (approximately 250 tests per month) in the Renal Function Laboratory. However, patients with a history of an allergic reaction to iodine should not participate in this study.

d) Recruitment and Informed Consent

Subjects will be recruited at the Mayo Clinic from the Heart Failure Clinic, Community Internal Medicine, and General Internal Medicine and referrals from physicians in the Mayo Health care system. In addition, we also now have in place a new web-based patient identification system developed at Mayo by the Cardiovascular Research Program. This system can be cross-referenced with the Mayo Echocardiography laboratory database and can identify patients with left ventricular systolic or diastolic dysfunction without clinical diagnosis of CHF throughout the entire Mayo system.

Patients will be approached for participation in the study in one the following ways:

- Approached at the clinic
- Letters of invitation or phone calls to patients that we have identified through our web-based patient identification system and colleague's referrals. Both the letters of invitation and phone scripts will be reviewed and approved by Mayo IRB.
- We will also call or send a letter to a list of participants who enrolled in our previous PPG studies

Upon identification of a subject, the study will be explained in detail by the investigators or study co-coordinator, the consent form reviewed questions answered and the consent form signed. The information in the consent form that will be reviewed is as follows:

- *Why is this research study being done?*
- *How many people will take part in this research study?*
- *What will happen in this research study?*
- *How long will I be in this research study?*
- *Are there reasons I might leave this research study early?*
- *Will any biological sample(s) be stored and used for research in the future?*
- *How do researchers from other institutions get the sample?*
- *What are the risks of this research study?*
- *Are there benefits to taking part in this research study?*

- *Will I be compensated for the study?*
- *What other choices do I have if I don't take part in this research study?*
- *Will I need to pay for the tests and procedures?*
- *What happens if I am injured because I took part in this research study?*
- *What are my rights if I take part in this research study?*
- *Who can answer my questions?*

e) Potential Benefits of the Proposed Research to the Subjects and Others

Subjects participating will not benefit from this study.. More importantly, this study will provide us new insights into the human cardiorenal syndrome that may allow us to develop new therapeutic strategies, which may benefit others with the same problem.

f) Importance of Knowledge to be Gained

This research will provide us new insights into the human preclinical left ventricular and renal dysfunction that may allow us to develop new therapeutic strategies, which may benefit others with the same problem.

g) Data and Safety Monitoring Plan

In accordance with the NHLBI requirements the following is the data and safety monitoring plan (DSMP):

Data and Safety Monitoring Board

The data and safety monitoring board (DSMB) will include Dr Richard Rodeheffer and Dr Lyle Olson. One of the members will be appointed the executive secretary (ES).

DSMB ES is responsible for setting up DSMB meetings, and for generating official, written minutes.

The DSMB will review all the Unanticipated Problem Involving Risk to Subjects or Others (UPIRTSO). The DSMB will also review all the adverse events at 3 time points: a) after 10 subjects; b) after 20 subjects; c) after 30 patients have completed the protocol and every 6 months after that.

Stopping Rules

The study sample size is too small for the application of traditional stopping rules. However, due to the potential for increased risk of hypotension, the DSMB will evaluate the number of patients who develop hypotension. If the DSMB is concerned about the number of these adverse clinical events, the DSMB will inform the NHLBI and Mayo IRB either that the protocol needs to be modified or enrollment should be suspended.

Adverse Event and Serious Adverse Event Reporting and Follow-up

The following are in accordance with the NHLBI, Office for Human Research Protections (OHRP) and Mayo Clinic IRB policies.

Definitions:

1) ***Adverse Event:*** As defined by **OHRP**, an adverse event is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research. Adverse events encompass both physical and psychological harms.

2) ***Serious Adverse Events*** include those which:

- Are fatal or life threatening;
- Result in significant or persistent disability;
- Require or prolong hospitalization;
- Result in a congenital anomaly/birth defect;
- Represent other significant hazards or potentially serious harm to research subjects or others, in the opinion of the investigators.

3) **Unanticipated Problem Involving Risk to Subjects or Others (UPIRTSO):**

Mayo Clinic's IRB and OHRP defines unanticipated problem/event involving risk to subjects or others (UPIRTSO) as any problem or event that was 1) serious; 2) unanticipated; AND 3) at least possibly related to the research procedures.

Serious: Serious problems/events can be well defined and include death; life threatening adverse experience; hospitalization: inpatient, new, or prolonged; disability/incapacity: persistent or significant; birth defect/anomaly; and/or per protocol OR may be problems/events that in the opinion of the local Investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

Unanticipated: (unexpected) problems/events are those that are not already described as potential risks in the consent form, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem/event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem/event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected.

Related: A problem/event is "related" if it is possibly, probably or definitely related to the research study

Adverse Event Reporting

All non-serious adverse events (AEs) will include those that occur from randomization through 7 days after the completion of the second study day at the CTSA CRU.

a) Report to DSMB

As stated above, the DSMB will also review all the AEs at 3 time points: a) after 10 subjects; b) after 20 subjects; c) after 30 patients have completed the protocol and every 6 months after that.

b) Report Mayo IRB

All AEs will be reported to the Mayo IRB during the yearly progress report for IRB approval.

c) Report NHLIBI

The AEs will be reported to the NHLBI during the yearly non-competitive renewal.

Serious Adverse Event Reporting

All serious adverse events (SAEs) will include those that occur from randomization through 7 days after the second study day

a) Report to DSMB

If the Serious AE involves death or a life-threatening event the PI will a contact a member of the DSMB within 5 days to determine the relationship between the event and the study intervention. If the event was definitely related, probably related or possibly related to the study intervention, the PI will call the PO at NHLBI and submit the UPIRTSO form to Mayo IRB within 5 days

Regardless of causality, the investigator will report Unanticipated SAE to DSMB within 5 days of knowledge of the event to determine the relationship between the event and the study intervention and include any relevant documents such as medical record notations or reports with the name and medical record number of the individual removed. Assess whether protocol modifications are required as a result of the UPIRTSO. The Investigator must provide an explanation on the UPIRTSO form explaining what corrective actions have already been taken.

If the event was definitely related, probably related or possibly related to the study intervention, the PI will inform the PO at NHLBI and submit the UPIRTSO form to Mayo IRB within 5 days. The investigator will complete and submit a follow-up report when additional relevant information (final diagnosis, outcome, results of specific investigations, etc.) becomes available. The investigator will follow all reportable events until resolution or stabilization. The DSMB will follow all SAEs until resolution, stabilization, or last patient completes follow up, whichever occurs first. Any serious adverse event that is ongoing when a patient completes his/her participation in the trial will be followed by the investigator until any of the following occurs:

• The event resolves or stabilizes.
• The event returns to baseline condition or value (if a baseline value is available).

b) Mayo IRB

Regardless of causality, the investigator will report Unanticipated SAE to DSMB within 5 days of knowledge of the event to determine the relationship between the event and the study intervention. If the event was definitely related, probably related or possibly related to the study intervention, the PI will submit the UPIRTSO form to Mayo IRB within 5 days

The following section is the Mayo IRB's policy for handing a UPIRTSO report:

Mayo IRB Specialist Responsibilities

Upon receipt of a UPIRTSO, the Specialist shall review the report within one working day.

1. Problems/Events that meet the definition of UPIRTSO are referred to a Chair or Vice Chair for determining whether an Administrative Hold is necessary as set forth in IRB Policy II.B, and assigned to the next available Full Committee.

2. Problems/Events that do not clearly meet the definition of UPIRTSO shall be referred to a Chair or Vice Chair to make the determination for appropriate triage and whether an Administrative Hold is necessary as set forth in IRB Policy II.B. [1]
3. Problems/Events that are determined to be Non-UPIRTSOs will be returned to Investigator with instructions to submit in accordance with Section I.C of this procedure.

For those problems/events determined to be UPIRTSOs, the Specialist shall ensure all IRB Committee Members have access to the following documents for review at the next available meeting:

- The UPIRTSO form;
- The DSMB or safety report, if applicable;
- Supplemental material submitted with the report;
- A modification request, if applicable;
- The current IRB approved application and consent document;
- The study protocol;
- The Investigator's Brochure, if applicable; and
- Any other pertinent materials.

The Specialist will generate a minute item describing the Full Committee's decision regarding the UPIRTSO. The minute item will be reviewed and approved by the Full Committee and sent to the Investigator.

The Specialist will complete all appropriate IRB database entries.

Mayo IRB Committee Responsibilities

A. The Chair or Vice Chair will receive UPIRTSO reports from the Specialist in accordance with Section II.A.1, 2 of this procedure.
B. Problems/Events that are determined by the Chair or Vice Chair to be Non-UPIRTSOs will be returned to Investigator with instructions to submit in accordance with Section II.A.3 of this procedure.
C. If the Chair or Vice Chair determines the problem/event is a UPIRTSO or cannot make a determination, the report will be forwarded to the Full IRB Committee to make these decisions.
D. A primary reviewer will be assigned. All IRB Committee Members will receive a copy of the UPIRTSO form and supporting documents provided by the Investigator to assist in determining whether the problem/event meets the applicable criteria.
E. If the IRB determines that more information is needed, it may postpone a decision while awaiting the requested information. In such cases, the IRB will consider the appropriateness of an "Administrative Hold" on the research until a final determination is made.
F. Problems/events that are determined by the IRB to be Non-UPIRTSOs will be documented as set forth in IRB Policy III.G.
G. If the IRB determines that the problem/event meets all three criteria, (serious, unanticipated, and related), the problem/event will be considered a UPIRTSO and the following actions may take place:

The IRB may:

a. Approve the report with no changes;
b. Approve the report with changes to the protocol requested in the modification;
c. Request a meeting with the Investigator;
d. Request further information from the Investigator, Data Safety Monitoring Board (DSMB) or other monitoring bodies;
e. Increase the frequency of continuing progress report review;
f. Impose additional monitoring;
g. Ask the Investigator to place the study on "Administrative Hold" pending receipt of further information;
h. Suspend the study for cause as set forth in IRB Policy II.B with:
    I. Suspension of recruitment;
    II. Suspension of screening and enrollment;
    III. Suspension of intervention and interaction; or
    IV. Suspension of follow-up.

i. Terminate the study for cause as set forth in IRB Policy II.B.

- The problem/event will be reported as set forth in IRB Policy II.C.
- The IRB will consider whether the problem represents serious or continuing non-compliance as set forth in IRB Policy II.C.[3]
- In the case of changes in the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject, the IRB will consider whether the changes were consistent with the protection of the rights and welfare of subjects.

Reporting to NHLBI

In accordance with the NHLBI policy, all investigators conducting clinical studies supported by the NHLBI must report expected and unexpected serious adverse events to the NHLBI. The investigator must forward copies of all reports of **serious adverse events (expected and unexpected)** when they are submitted to the IRB and DSMB. Adverse events will be reported in annual reports.

Expedited reporting is required for serious adverse events that are unexpected.

- If the unexpected events are life-threatening or fatal, they should be reported within 7 calendar days.
- All other serious adverse events that are unexpected should be reported within 15 calendar days.

Copies of reports are sent to the **NHLBI Program or Project Officer** responsible for the study.

INCLUSION OF WOMEN

It is important to define and understand the difference in the pathophysiology of PSD and PDD between women and men. Every effort will be made to include equal number

of male and females in the proposed protocols, with a focus to recruit women from the Mayo Women's Heart Clinic. Experience form the Mayo CRU in 1996-97 showed that 50% of the 2320 patients enrolled in the CRU protocols were women. Separate analysis will be performed on the above data to compare the effect of gender on the integrated cardiorenal and humoral function in all the protocols.

INCLUSION OF MINORITIES

Studies have demonstrated a high prevalence of preclinical LV dysfunction in minority populations. Every effort will be made to recruit minorities in the proposed studies. Subjects will not be excluded from participating in the studies based on race or ethnicity. While the vast majority of residents in Rochester, Minnesota and the surrounding communities are White, population demographics have changed over the past decade due to emigrants from Somalia and from Southeast Asia.

Olmsted County census data from 2000 and American Community Survey for Rochester MN from 2005-2007, are listed below. Rochester city data from July 2007 shows that the African American, non-Hispanic population has increased to 4.9% and that the Hispanic population has increased to 3.5%.

Overall Local Figures for Olmsted County Population (percent)-2000 Census

Experience in Mayo CRU-based protocols has shown that minority patient's recruitment increased from 2.8% in 1992-93 to 15.2 % in 1997. This is in part due to the special initiative undertaken to help ensure the participation of underrepresented populations in clinical research. Miriam A. Marquez, Ph.D., Director of the Mayo Clinic office of Diversity in Clinical Research, and her staff have focused on improving communication with local minority groups and on the continuous education and involvement of medical and paramedical staff on issues regarding diversity. This has provided measurable success in the local community.

Our target enrollment is to recruit approximately 42 minority subjects for the protocols to ensure a minority representation of at least 25% of the study population. We anticipated that 50% of the participants would be female, and 50% male. 7% will be Hispanic, 10% Asian, 8% Black not of Hispanic origin and 75% Caucasian.

Our primary strategy to achieve our target minority recruitment is to work with the medical and paramedical staff of the Diabetes Clinic, Cardiology Clinic, General Internal Medicine Clinic and Echocardiography Laboratory to recruit minority subjects. My clinical study coordinator and research assistant with the help of the Mayo Clinic language interpreters will facilitate these efforts. With the help of Dr Miriam A. Marquez, we have developed a complementary community-based educational strategy for recruitment of local minority study subjects. We will also work to establish community liaisons with the Multicultural Alliance Network in Olmsted County Minnesota and the Rochester Chinese Cultural Association, to provide connection between the Mayo Clinic and minority communities.

INCLUSION OF CHILDREN

	American Indian or Alaskan Native	Asians or Pacific Islander	Black, not of Hispanic Origin	Hispanic	White, not of Hispanic Origin	Total
Male	0.2%	2.4%	0.7%	0.8%	44.6%	51.4%
Female	0.1%	2.4%	0.4%	0.7%	47.7%	48.6%
Total	0.3%	4.9%	1.1%	1.5%	92.3%	100%

The current application is focused on subjects with PSD and PDD which is usually consequent to prolonged exposure to cardiovascular risk factors, such a hypertension, hypercholestrolemia, smoking, diabetes, coronary artery diseases. We have excluded subjects with significant valvular and congenital heart diseases, which is the most common cause of heart failure in children. Thus we believe that this research topic is not relevant to children and justify the exclusion of children form the current application.

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