Comprehensive O2 Transfer Analysis From the Lung to Mitochondria of Inhaled Treprostinil in Interstitial Lung Disease Pulmonary Hypertension

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Background and significance

A. Rationale:

Pulmonary arterial hypertension related to Interstitial Lung disease (PAH-ILD) is the second most common cause of pulmonary hypertension in the developed world.[1] The pathogenesis involves progressive lung fibrosis which impacts lung reserve, and may also result in secondary pulmonary vascular remodeling impeding right heart function. The primary symptom PAH-ILD patients suffer from is exercise intolerance, which is heterogenous in origin related to having both lung disease and right heart failure which variably contribute in individual patients. There is also increasing recognition of the role skeletal muscle and peripheral vascular changes play in exercise intolerance in PAH.[2–6] Therefore, mechanisms of exercise intolerance are complex in PAH-ILD and *no study has simultaneously evaluated pulmonary, cardiac and peripheral mechanisms during exertion in PAH-ILD, or the effects of pulmonary vasodilators on these indices.*

Inhaled treprostinil has been recently demonstrated in a randomized trial to result in an improvement in 6-minute walk distance and has now become the standard of care for pulmonary vasodilator therapy in PAH-ILD. However, the mechanisms of improvement with inhaled treprostinil in these patients remains unclear, and patient response remains heterogeneous in real-world clinical practice. Therefore, further clarification of the physiological mechanisms of symptom improvement is important for practicing clinicians and patients to optimally utilize this proven therapy. Understanding mechanisms of benefit will also help guide future investigations into treating earlier disease with primarily exercise pulmonary hypertension which is very common in ILD.

B. Hypotheses:

Hypothesis#1: Patients with ILD-PAH will have impaired cardiac output reserve during exercise, and inhaled treprostinil will acutely increase cardiac output with preserved skeletal muscle O2 uptake efficiency.
Hypothesis#2: Baseline hemodynamic improvement in exertional cardiac output with inhaled treprostinil will predict subsequent NYHA class improvement at 3 months with chronic inhaled treprostinil therapy.

C. Innovation:

1. The combined assessment of ventilatory, central and peripheral hemodynamic impairment in PAH-ILD has never been quantified simultaneously during exercise
2. The use of skeletal muscle biopsy for mitochondrial oxidative capacity, along with physiological measures of peripheral O2 utilization has never been performed in PAH-ILD and will provide comprehensive quantification of peripheral O2 impairments
3. Simultaneous gas exchange analysis along with central and peripheral measures will allow better understanding of the complex interplay between ventilatory and hemodynamic indices in PAH-ILD and their relationship to exercise intolerance
4. The use of acute vasodilator testing with inhaled treprostinil in PAH-ILD will allow paired assessment of improvement in ventilatory, central and peripheral exertional response in the same patient for the first time, with correlation with symptom response with chronic therapy

D. Significance:

1. The mechanisms of improvement in exertional response in PAH-ILD with inhaled treprostinil remain unknown and can probably only partially be explained by reduction in resting pulmonary vascular resistance.
2. Delineating mechanisms of benefit would help explain to patients and providers the benefits of inhaled treprostinil, and potentially extend use to milder severities of PAH-ILD such as those with exercise PAH

E. Brief Synopsis of Methods:

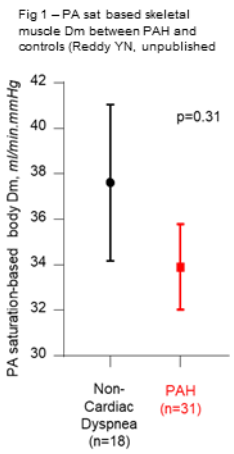
- Recruitment of 23 patients with suspected PAH-ILD undergoing right heart catheterization
- Performance of rest and exercise catheterization with simultaneous gas exchange measures and skeletal muscle biopsy for mitochondrial oxidative capacity to study O2 transfer from the lungs to skeletal muscle during exertion. 6 healthy controls were initially planned to be recruited for comparative hemodynamics and muscle biopsy analyses. They will not be administered inhaled treprostinil. As of protocol v5 dated 05Jan2024, only 1 control patient was enrolled and recruitment of control subjects has been halted due to shifting study priorities of the PI for the healthy control population with other separate ongoing studies for healthy controls. Remaining 5 previously planned subjects will now be enrolled as PAH-ILD subjects to allow further study of treprostinil in the patient population of interest.

- Administration of inhaled treprostinil with repeat assessment of central and peripheral measures during exertion following 30 minutes of rest
- Correlation of hemodynamic changes with NYHA class improvement with chronic treprostinil therapy

F. Preliminary studies to support feasibility:

The only proven pharmacological intervention for PAH patients is pulmonary vasodilator therapy. The primary mechanism of symptom improvement with pulmonary vasoactive therapy is believed to be an increase in exertional Q_C [7] to provide improved leg blood flow during exercise but this has never been directly measured in PAH-ILD. A few studies have demonstrated resting skeletal muscle abnormalities in PAH patients, their role in limiting exercise performance and the response to treprostinil in PAH-ILD is unclear.[2–6] Limited studies have evaluated the mechanisms of exercise intolerance in PAH patients following vasodilators, but have consistently shown a higher PA saturation during exercise than baseline suggesting impaired peripheral O_2 uptake.[8–10] But due to limitations in using mixed venous PA blood and Q_C to estimate skeletal muscle O_2 diffusion without accounting for the faster flow rate, these findings are preliminary.[11,12]

I worked with Dr. Borlaug (my primary mentor) to ***estimate*** skeletal muscle Dm in patients with HF with preserved ejection fraction, which although a disease of the left heart is characterized by prominent central and peripheral abnormalities similar to those hypothesized in PAH.[11,13] Using a simplified approach as the quotient of whole body VO_2 and PA pO_2, we demonstrated in 51 patients using a double blind, acute, placebo-controlled design that this *estimate* of Dm improves acutely following nitrite treatment.[16] I have since begun a systematic program of exercise RHC evaluation of PAH, recruiting 31 PAH patients in 1 year, and have calculated estimated skeletal muscle Dm again using the above over-simplified Dm calculation (VO_2/PA pO_2) which demonstrated a trend towards impaired skeletal muscle Dm in PAH (Fig 1). However, these data are associated with substantial systematic error in calculating Dm due to the lack of femoral blood flow, femoral venous blood and leg VO_2 in addition to frequent arterial hypoxemia in PAH confounding Dm calculations without accounting for arterial pO_2. In this study, we will use gold standard peripheral O_2 assessments using direct mitochondria tissue analysis, and will more precisely determine the role of impaired peripheral O_2 handling in PAH-ILD. To our knowledge, <u>*not a single study has directly evaluated the combined central and peripheral O_2 transfer during exertion in patients with PAH-ILD*</u>.



This work will utilize existing investigative collaborations as part of my NIH K23 award application that is under review. Dr. Borlaug has conducted more than 1,000 invasive exercise hemodynamic studies for both clinical and research purposes, and has published extensively on invasive exercise hemodynamics in patients with heart failure and PAH.[14–17] Here, I will apply these same techniques to again characterize convective O_2 transport as in prior studies of HF, but in order to now evaluate peripheral diffusive O_2 transfer, I will work together with Dr. Thomas Olson, who has extensive investigative experience in peripheral O2 transfer.[18–20] To further characterize mitochondria in muscle, I will also work closely with Dr. Nair, who is a world expert in mitochondrial biology and quantifying oxidative capacity.[21,22,22,23] Under the mentorship of this multidisciplinary team, I will be able to comprehensively study O_2 transfer from the lungs to skeletal muscle mitochondria in PAH-ILD and better understand exercise intolerance in these patients and the effects of inhaled treprostinil on exertion.

G. Study design:

<u>Hypothesis #1</u> Patients with ILD-PAH will have impaired cardiac output reserve and leg blood flow during exercise, and inhaled treprostinil will acutely increase exertional cardiac output with preserved skeletal muscle O2 uptake efficiency.

***Research Design for Hypothesis #1*:** This will be a prospective study 24 p*atients <u>with PAH-ILD</u>* undergoing cardiac catheterization for clinical indications at Mayo Clinic Rochester. PAH-ILD patients referred for catheterization >18 years who can exercise will be enrolled. PAH will be confirmed by standard criteria. We will screen 35 patients to reach target accrual of 24 total subjects. A separate control group of 6 participants was initially planned to be enrolled from the community for comparative analyses to the PAH-ILD group. As

of protocol v5 dated 05Jan2024, only 1 control patient was enrolled and recruitment of control subjects has been halted due to shifting study priorities of the PI for the healthy control population with other separate ongoing studies for healthy controls. Remaining 5 previously planned subjects will now be enrolled as PAH-ILD subjects to allow further study of treprostinil in the patient population of interest.

Exercise Catheterization procedure

Venous access will be obtained in the internal jugular vein and arterial access in the radial artery (Fig 2). Pressures in the right atrium, pulmonary artery (PA), Pulmonary Capillary Wedge pressure, and radial artery will be measured using high fidelity micromanometers. Oxygen consumption (VO_2) will be directly measured using expired gas analysis along with simultaneous ventilation and CO_2 production. Blood gases will be performed to measure arterial (CaO_2) and mixed venous O_2 content drawn from the PA (CvO_2), and thereby calculate whole body arterial-venous O_2 content (A-V O_2) difference. Q_C will be determined by the direct Fick method (=VO_2/A-V O_2 difference). The participants will exercise on a supine cycle ergometer at an initial workload of 20 watts for 5 minutes (submaximal exercise). This workload represents peak exercise capacity for >90% of PAH-ILD patients in our experience and given the need for repeat exercise assessment we will not continue exercise to peak exhaustion. Moreover, hemodynamic changes during early submaximal exercise are much more relevant to patient reported symptoms during activities of daily living and quality of life.[24] Patients will be administered inhaled treprostinil (32 mcg) and allowed to rest for 30 minutes and measurable effects with inhaled treprostinil can be seen in this time.[25] All measurements will be repeated after treprostinil at rest and the matched 20 watt exercise stage. Simultaneous echocardiogram and blood sample storage will be performed at rest (10 ml) and exercise (5 ml) for future cardiac structural and biomarker analyses.

Mitochondrial assessments

Muscle biopsies of the quadriceps muscle (on the same side as the leg blood flow assessment) will be performed at a separate visit at least 3 days remote from the maximal exercise test, using a modified Bergstrom needle as has been extensively utilized by Dr. Nair's group, to directly assess the skeletal muscle mitochondrial oxidative capacity component of peripheral O_2 utilization.[26–28] High-resolution respirometry will be performed on mitochondria freshly isolated from the biopsy sample. Approximately 100 mg of tissue will be homogenized, and mitochondria separated using differential centrifugation. Mitochondria are added to a 2 ml chamber (Oxygraph-2K, Oroboros) and allowed to equilibrate. Glutamate (10 mM) and malate (2 mM) are added to stimulate State 2 respiration specific to Complex I, then ADP is added at saturating concentrations (2.5 mM) to induce State 3 respiration of Complex I. Cytochrome-c is added to verify mitochondrial membrane integrity. Succinate (10 mM) is added to stimulate State 3 respiration through Complex I+II and then rotenone (0.5 µM) is added to inhibit complex I for State 3 respiration specific to Complex II. State 4 respiration (Leak) is induced by addition of oligomycin (2 µg/µl) then the proton gradient is dissipated by sequential titration of 0.05 mM carbonylcyanide-4-(trifluoromethoxy)-phenylhydrazone to induce uncoupled respiration. Protein content of isolated mitochondrial is determined using a commercially available kit (DC Protein Assay, Bio-Rad). Mitochondrial respiration measures will be normalized to tissue wet weight (reflective of mitochondrial content) and mitochondrial protein (reflective of mitochondrial protein quality). *Maximum mitochondrial respiration* for the respiratory complexes (Complex I+II) will be reported in pmol O_2/ml/sec (per tissue content) and in pmol O_2/µg mito/sec (per mitochondrial protein content). *Mitochondrial efficiency* will be calculated as Respiratory Control Ratio (State 3/State 4).[28,29]

Isolated quadriceps muscle mitochondria will be treated with glutamate and malate, and *H_2O_2 emission (ROS)* will be measured using Amplex Red oxidation (p mol/s/µg protein).[30]

Mitochondrial ATP production rate will be measured using a Fluorolog 3 (Horiba Jobin Yvon) spectrofluorometer. 10 µl of isolated mitochondria suspension is added to 2 ml of buffer Z and 2.5 mM D-Glucose. *ATP production* (p mol/s/µg protein) is measured using an enzymatic system containing hexokinase and glucose-6-phosphate dehydrogenase to convert ATP to NADPH.[21,23]

Expected Outcomes and Interpretation: We anticipate impaired Q_C reserve (<80% predicted based on expected ΔQ_c /ΔVO_2 >6)[31] in PAH-ILD during exercise on average. This will be associated with lactic acidosis and abnormal VE/VCO_2 ratios. Following inhaled treprostinil therapy, there will be an acute improvement in exertional Q_c, lactate levels and VE/VCO_2 ratios. Patients with values of skeletal muscle Dm and mitochondrial indices >median will have less peripheral impairment and a greater improvement in exercise Q_c with inhaled treprostinil.

Hypothesis #2 Baseline hemodynamic improvement in exertional cardiac output with inhaled treprostinil will predict subsequent NYHA class improvement at 3 months with chronic inhaled treprostinil therapy.

Research Design for Aim #2: Following Aim #1 patients with confirmed PAH will be initiated on outpatient inhaled treprostinil therapy with gradual uptitration to target dose as per standard clinical practice. Patients are typically re-evaluated within 3 months in our clinical practice with repeat comprehensive assessment including 6 minute walk distance, echocardiogram, NTproBNP and clinical assessment. Patient improvement will be documented by the patient and practicing providers by NYHA class (provider) and self-reported improvement (yes/no by the patient). More objective quantification of quality of life will be performed by the PAH-SYMPACT score and SF-36 scores. For patients who do not return for follow up assessment within the study time frame of 3 months, they will be remotely contacted with assessment of NYHA class, patient reported improvement and quality of life provided remotely.

Expected Outcomes and Interpretation: We anticipate that the change in CO during exercise following inhaled treprostinil will predict NYHA class improvement with chronic inhaled treprostinil therapy by logistic regression. Exploratory analyses of baseline central and peripheral hemodynamic response to treprostinil stratified by improvement vs no improvement in NYHA class will also be performed. Changes in quality of life will be correlated with acute hemodynamic exercise response. Analyses will also be performed stratified by median skeletal muscle exercise Dm and mitochondrial oxidative capacity to determine the relationship of peripheral abnormalities with symptom response to chronic treprostinil.

Statistical analysis and power considerations: Sample size estimates were performed based on feasibility of recruitment within 1 year, and based on our current lab volumes and interest in PAH-ILD in our practice we anticipate being able to recruit 1 patient a month for a total of 23 patients with follow up clinical evaluations completed 3 months after last patient has been recruited. Prior exercise catheterization studies have shown improvements in PVR and PA pressure/CO slope ratios with acute vasodilator testing with as few as 10 patients.[33] Accordingly with 22 patients with paired measures, we anticipate having adequate power to assess for a within patient increase in exertional CO with inhaled treprostinil therapy by paired T test, and also to explore paired changes in the other novel indices in exertional ventilatory, peripheral and skeletal muscle O_2 transfer.

H. Drug supply

Inhaled treprostinil for acute vasodilator testing in our hemodynamic exercise catheterization laboratory will be provided at no cost by United pharmaceuticals for the 22 patients. Patients will then be prescribed chronic inhaled treprostinil therapy for chronic use as per clinical practice which will be billed and supplied clinically.

I. Anticipated results and potential pitfalls

As described above we anticipate that patients with PAH-ILD will have impaired Q_C reserve with activity (exercise-rest Q_C <80% predicted based on normal measured change in VO_2 - the normal increase in Q_C being 6 ml/min per 1 ml/min in VO_2)[31] during exercise at 20 watts for 5 minutes, which will improve following inhaled treprostinil. The degree of Q_C improvement during exertion with inhaled treprostinil will likely predict an improvement in NYHA class with 3 months of inhaled treprostinil as chronic therapy. Exploratory analyses of changes in other exertional pulmonary, central and peripheral indices with symptom improvement will also be performed.

J. Description of potential challenges and possible approaches to address them

Some patients with PAH-ILD may be O_2 dependent which precludes a face mask to directly measure gas exchange and VO_2 at the mouth. In these patients we will measure Q_C using the thermodilution method during rest and exercise which is the most accurate method in patients on supplemental O_2 therapy.[34]. Another potential problem is failure to enroll the target number of participants for the study. This is an unlikely problem because the Mayo Clinic Pulmonary Hypertension program follows more than 300 adult PAH patients who are evaluated in the outpatient clinic annually. For the approximately 50 PAH patients a year who are newly initiating or intensifying therapy, cardiac catheterization is the standard of care, and ILD-PAH is one of the fastest growing areas in our clinical practice with our large ILD practice. In our laboratory, exercise RHC is routinely used in the evaluation of PAH and will provide no additional cost to this proposal. I also practice primarily in the PH clinic and cardiac catheterization hemodynamic lab which will maximize patient recruitment and feasibility. Importantly we have a successful track record of recruiting more than 15 patients a year for acute hemodynamic exercise studies in four completed[14,15,35,36] and three ongoing studies of patients with pulmonary hypertension during exercise which do not overlap with this proposal. We will additionally provide remuneration to patients for their travel expenses and time to participate. Additional challenges relate to the acute effects of exercise on mitochondrial respiration that may confound the biopsy results. To mitigate this, the biopsy will be performed at

least 3 days after the initial exercise study. All baseline hemodynamic and peripheral data will be interpreted and analyzed blinded to subsequent symptom response

Inclusion Criteria for PAH

- PAH due to Interstitial Lung disease being considered for inhaled treprostinil
- Resting right heart catheterization with mean pulmonary artery pressure>20 mmHg and PVR> 3 Wood units
- >=18 years of age

Exclusion criteria

- Inability to exercise
- Females who are pregnant

Inclusion Criteria for controls

- >=18 years of age
- No known heart failure, pulmonary hypertension, lung disease or muscular disease
- Ability to exercise
- No bleeding abnormalities

Schedule of assessments:

For PAH ILD patients

Visit 1

- Baseline assessments of PAH SYMPACT and SF-36 for quality of life will be performed
- Patients will undergo clinically indicated right heart catheterization with exercise as per clinical practice with internal jugular approach and right radial arterial line. Exercise will be performed up to 20 watts for 5 minutes.
- After exercising to 20 watts for 5 minutes, patients will be administered 32 mcg of inhaled treprostinil followed by 30 minutes of rest in the catheterization laboratory
- Repeat exercise up to 20 watts for 5 minutes will be performed after 30 minutes of rest.
- Simultaneous echocardiogram will be performed at rest and exercise.
- 10 ml of blood at rest and 5 ml with exercise will be stored at rest and exercise from the baseline phase. In addition high sensitivity troponin levels will be measured at rest and exercise before and after inhaled treprostinil.
- Following the second exercise phase Visit 1 will be completed

Visit 2 (optional muscle biopsy)

- Patients will be offered an optional muscle biopsy component with percutaneous needle biopsy in Dr Nair's laboratory for skeletal muscle mitochondrial assessment. Remuneration of $200 will be provided for completion of the muscle biopsy component

Virtual Visit 3 (end point assessment)

- Patients will be contacted remotely at 4 months to mail back repeat PAH SYMPACT and SF-36 questionnaires for end point assessment of quality of life. Patients will be contacted by phone to assess NYHA class by Principal Investigator.

PAH-ILD subjects

Study Activity	Visit 1 Day 0	Visit 2 Day 3-14 (+/-)	Virtual Visit 3 Month 4 (+/-14 days)
Informed Consent[1]	X		
Right Heart Catheterization	X		
Treprostinil Test dose	X		

Echocardiogram[2]	X		
Troponin[3]	X		
Research blood[4]	X		
Questionnaires[5]	X		X
Muscle Biopsy[6]		X	

[1]Informed consent will be obtained before research activities are conducted
[2]Echocardiogram at rest and during exercise during RHC
[3]Troponin to be drawn at rest and exercise before inhaled treprostinil during RHC
[4]10 ml of blood at rest and 5 ml with exercise will be stored at rest and exercise from the baseline phase
[5] PAH SYMPACT and SF-36 questionnaires will be mailed. Phone call by PI to assess NYHA class.
[6]optional

For healthy controls

Visit 1

- Healthy controls will be recruited by advertisement and will undergo right heart catheterization with exercise with internal jugular approach and right radial arterial line as per standard clinical practice. Exercise will be performed up to 20 watts for 5 minutes, followed by 3 minute stages to peak exhaustion.
- A 5 french femoral venous flush catheter will be placed through which cold saline will be infused with sampling of temperature using a very small .025" Physitemp temperature wire (https://physitemp.com/flexible-implantable-probes_p101) placed parallel in the femoral vein through an additional venous puncture. This will allow direct measurement of leg blood flow during rest and exercise, and also allow femoral venous blood gas sampling during rest and exercise.
- Simultaneous echocardiogram will be performed at rest and exercise. Coronary sinus blood gases will also be collected at rest and during 20 watts of exercise through right heart catheterization.
- 10 ml of blood at rest and 5 ml with exercise will be stored at rest and exercise from the baseline phase. In addition, high sensitivity troponin levels will be measured at rest, 20 watts and peak exercise
- Following exercise to complete exhaustion, and a rest period of 30 minutes, healthy controls will then undergo knee extensor exercise to exhaustion with ankle weights attached in the seated position in the catheterization laboratory to isolate quadriceps muscle function with measurement of leg blood flow, blood gas sampling and central hemodynamics. Following this, the catheterization procedure will be complete.
- Remuneration of up to $200 will be provided for Visit 1
- Screening for pregnancy will be performed prior to catheterization as per standard clinical practice.

Visit 2 (optional muscle biopsy)

- Patients will be offered an optional muscle biopsy component with percutaneous needle biopsy in Dr Nair's laboratory for skeletal muscle mitochondrial assessment
- Remuneration of up to $200 will be provided for completion of the muscle biopsy component

Healthy subjects

Study Activity	Visit 1 Day 0	Visit 2 Day 3-14
Informed Consent**	X	
Right Heart Catheterization	X	
Echocardiogram[1]	X	
Troponin[2]	X	
Research Blood[3]	X	
Pregnancy test	X	
Muscle Biopsy[4]		X

**Informed consent will be obtained before research activities are conducted
[1]Echocardiogram at rest and during exercise during RHC

[2]Troponin to be drawn at rest, 20 watts and peak exercise
[3]10 ml of blood at rest and 5 ml with 20 watt exercise will be stored at rest and exercise from the baseline phase
[4] optional

Risk Analysis
Major procedural complications will be reported through the data collection form and will be monitored and adjudicated by the PI. Adverse events will not be separately reported to the IRB. The drug inhaled treprostinil represents standard of care therapy for PAH-ILD and the acute administration should post no additional risk compared to the chronic therapy that will be subsequently administered at an even higher dose.

Baseline RHC will be performed as per standard clinical practice with simultaneous gas exchange measurement using a metabolic cart. This involves venous access in the jugular vein and placement of an arterial line in the radial artery with a small <0.5% risk of major bleeding or infection. Patients will qualify for the study if PAH is diagnosed on the resting RHC. The femoral venous sheath is of the smallest possible diameter and will be anchored in place by sutures and the catheter introduced through the lumen for the temperature probe minimizing the risk of venous injury and bleeding.

Percutaneous skeletal muscle biopsy of the vastus lateralis muscle will be performed at a separate visit1-14 days after the baseline qualifying RHC. Risks include pain, bleeding/bruising, infection, scarring. Patients will hold anticoagulation for this procedure to minimize the risk of bleeding. The procedure will be completed by a trained RN, MD or PhD. The participant is given complete instructions and expectations prior to the procedure both on the consent form and verbally. To ease pain, 2% Lidocaine is buffered with 8.4% sodium bicarbonate to decrease acidity of the lidocaine. Tylenol is ordered as needed for pain. The participant is instructed to gently flex leg at least every 30 minutes to decrease stiffness in the leg. To minimize bleeding/bruising, the participant is asked not to use aspirin or ibuprofen for 3 days prior to and post biopsy. Careful choice of biopsy site avoids veins and arteries. Pressure is held at least 10 minutes after the biopsy or until hemostasis occurs. An ace wrap applied. An ice pack is advised if bruising occurs. Patient education on post biopsy care is given verbally along with written instructions. To minimize the risk of infection, biopsies are performed using aseptic technique. The participant is educated on signs and symptoms of infection with instructions to call the study team (numbers provided) with any signs of infection or other concerns. To minimize the risk of scarring, the incision is made no larger than is needed to insert the biopsy needle. The skin edges are approximated with Steri-Strips at the end of the procedure.

Protections against risk
A number of procedures will be implemented to protect subjects against loss of confidentiality. Subjects will identify three methods to contact them for follow-up. Confidentiality will be protected in several ways during data management, processing, and analysis. The risk of disclosure of protected patient information will be minimized by the use of password protected databases behind the Mayo Clinic Health system.
Potential problems relate to performance of the muscle biopsy. The skeletal muscle biopsy will be performed by percutaneous technique (modified Bergstrom needle) using only lidocaine allowing for minimally invasive biopsies with low risk of bleeding and infection. This procedure has been extensively performed for more than 2 decades by Dr. Nair's group with a low risk of complications even when performing multiple biopsies sequentially in the same patient. After the biopsy, pressure will be held over the incision until hemostasis is achieved. Risks of this procedure include hematoma, infection, and pain. Hematoma likelihood is minimized by holding pressure after the biopsy to ensure hemostasis, followed by a pressure dressing. The risk of infection is minimized by using sterile surgical techniques. Pain is managed by local analgesia during the procedure and Tylenol following the procedure. Although some patients will be on chronic anticoagulation, this is routinely held for the RHC procedure and the biopsy will be performed off anticoagulation.

Safety Plan for Depressive symptoms on Questionnaires
Study team will review the questionnaires for positive answers to depressive/anxiety symptoms within one day of subject completion. The study team will contact the PI, or if not available, a Co-I, on the same day of review, to follow-up with the subject. If participants have depressive symptoms on their questionnaires, they will be discussed in person to ensure that there is no risk of suicidal ideation for the participant and will be offered more formal depression screening if requested. If there is concern for suicidal ideation, they will be withdrawn from the study and placed under immediate medical supervision.

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