Role of blood flow on artery function and exercise capacity in cystic fibrosis (CF-FLOW)

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SPECIFIC AIMS

Cystic Fibrosis (CF) is the most common fatal genetic disease in North America. The most disturbing aspect of CF is the associated premature death. Clinical manifestations of CF include not only lung dysfunction, but many systemic consequences as well.

Among the systemic consequences, **exercise intolerance has been shown to predict mortality in patients with CF independent of lung function.** Exercise capacity (VO_2 peak), an objective measurement of exercise tolerance, drops approximately 5-8% per year in patients with CF. This excessive decay in exercise capacity not only represents a 5-8 fold decline compared to healthy sedentary adults, it also leads to deterioration of lung function and more pulmonary infections. It is important to recognize that **decreases in lung function (FEV_1) do not always contribute to reductions in VO_2 peak** and that less than 2% of patients who have an FEV_1 greater than 50% predicted will have a significant drop in hemoglobin oxygen saturation (SpO_2) during maximal exercise. These data rule out the role of lung function induced hypoxemia to exercise intolerance in CF. There have been major advances in research and medical therapies to target improvements in lung function; however, non-pulmonary factors have been neglected. Based on our recent discovery that patients with CF have systemic endothelial dysfunction, it is reasonable to suspect the involvement of the blood vessels in the pathophysiology of exercise intolerance in CF - a concept that has yet to be examined. **Preventing the excessive annual reductions in exercise capacity is essential to increasing the quality of life and longevity of patients with CF**; however, a **critical barrier** to improving exercise capacity in CF is our lack of knowledge regarding the different physiological mechanisms that contribute to exercise intolerance.

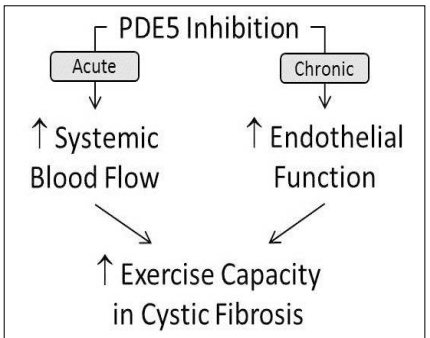

Figure 1. Working hypothesis.

In general, Phosphodiesterase type 5 (PDE5) inhibitors increase microvascular O_2 delivery and improve endothelial function. We have recently published the first evidence of systemic endothelial dysfunction in a cohort of young, healthy patients with CF. Vascular function is in part responsible for blood flow regulation, and preliminary data from our lab suggests that patients with CF also have an impairment in blood flow regulation during exercise when compared to healthy controls. Thus, the **overall goals** of this proposal are to 1) provide proof of concept for using PDE5 inhibition to improve exercise capacity in patients with CF and 2) investigate blood flow per se and endothelial dysfunction as potential mechanisms that contribute to exercise intolerance in CF. Our **central hypothesis** is that PDE5 inhibition will improve blood flow (acute treatment) and endothelial function (chronic treatment) leading to an increase in exercise capacity (**Figure 1**). We will test our central hypothesis with the following specific aims:

Aim 1. Test the hypothesis that acute PDE5 inhibition will increase systemic blood flow and contribute to an improvement in exercise capacity in patients with CF.

Exercise capacity (VO_2 peak) and indices of gas exchange (O_2 uptake kinetics, expired CO_2, VE/VO_2, VE/VCO_2), will be determined using a graded maximal exercise test on a cycle ergometer at baseline and 1 hour following the ingestion of a single dose of sildenafil (PDE5 inhibitor, 50 mg) or placebo (randomized order) in patients with CF. Secondary outcomes will include the non-invasive patented technology of the Physioflow Enduro, which will be utilized to examine differences in cardiovascular hemodynamics (heart rate, stroke volume, cardiac output, systemic vascular resistance, and ejection fraction) between patients and healthy controls during maximal cycling exercise. **This acute experiment transiently increase blood flow and test blood flow per se as a mechanism that will improve exercise capacity in CF.**

Aim 2. Test the hypothesis that chronic PDE5 inhibition will improve endothelial function and contribute to an improvement in exercise capacity in patients with CF.

Following completion of Aim 1, endothelial function, exercise capacity (VO_2 peak), and the indices of gas exchange (indicated above), will be performed, only in patients with CF, at baseline and following 4 weeks of sildenafil, 20 mg thrice daily. Secondary outcomes will include measurements of arterial stiffness (pulse wave velocity), the Physioflow Enduro, and biomarkers of nitric oxide bioavailability. **This chronic experiment will test the mechanism that an increase in endothelial function will improve exercise capacity in CF.**

This proof of concept trial is the first to explore the therapeutic benefit of PDE5 inhibitors on exercise capacity in CF. This high impact proposal will also target blood flow and endothelial function as potential mechanisms that contribute to lower exercise capacity in CF. Findings from this proposal will provide the foundation to stimulate additional strategies for the treatment and prevention of exercise intolerance in CF.

A. SIGNIFICANCE

Over 20 years ago, **exercise intolerance was shown to predict mortality in patients with CF independent of lung function**[1]. Exercise intolerance is a common complaint associated with CF, and the preservation of exercise capacity is essential for survival in this population. A VO_2 peak threshold of < 32 ml/kg/min in patients with CF is predictive of a 60% chance of mortality in the subsequent 8 years following testing[2]. Exercise capacity falls approximately 5-8% per year (~2.1 ml/kg/min)[2] in CF patients, which represents a 5-8 fold decrease compared to healthy sedentary adults. Even in the presence of fairly healthy spirometric function, data from our lab demonstrates that young patients with CF have a lower exercise capacity compared to apparently healthy controls[3]. Additionally, it is important to emphasize that **decreases in FEV_1 do not always contribute to reductions in VO_2 peak**[4] and very few patients with CF (only ~1.6%) who have an FEV_1 > 50% predicted will have a significant drop in hemoglobin oxygen saturation (SpO_2) during maximal exercise. Collectively, these data suggest that it is possible to observe exercise intolerance in patients with CF even when lung function and gas exchange are preserved.

Lower exercise capacity is associated with greater mortality, steeper decline in pulmonary function, and more pulmonary infections[5]**.** There are many identified barriers associated with exercise intolerance in CF[6, 7] and the reasons for "why exercise is medicine in CF" has recently been published[8]. However, a **critical barrier** to improving exercise capacity in patients with CF is our lack of knowledge regarding the different physiological mechanisms that contribute to exercise intolerance. There have been major advances in research and medical therapies to target improvements in lung function; however, non-pulmonary factors have been neglected. Based on our recent discovery of vascular endothelial dysfunction in CF[3], it is reasonable to suspect the involvement of the blood vessels in the pathophysiology of exercise intolerance in CF - a concept that has yet to be explored. Very little work using PDE5 inhibitors in human patients with CF has been conducted and a very recent review on the topic highlights the **potential** use for PDE5 inhibitors in CF treatment[9].

There is a desperate need to understand the mechanisms which contribute to exercise intolerance in CF, and our recent findings[3] support further investigations into blood vessel dysfunction in the context of CF-mediated exercise intolerance. The **impact** of this proof of concept investigation will test PDE5 inhibitors as a potential therapy in CF and will explore blood flow and endothelial function as potential mechanisms which contribute to exercise intolerance in CF.

B. INNOVATION

It is well established that patients with CF exhibit exercise intolerance, however, the physiological mechanisms for the reduced exercise capacity in CF is unknown. **Innovation of this proposal is evident by:**

- **The novel concept that impaired blood flow and vascular dysfunction contributes to exercise intolerance in patients with CF**. **Figure 2** represents a simplistic schematic of the potential systems that could, in general, contribute to exercise intolerance. By maintaining a constant partial pressure of oxygen and knowing that lung function-mediated hypoxemia during exercise is unlikely in the proposed CF cohort[4]. Blood vessels are downstream from air and lungs and upstream from skeletal muscle (**Figure 2**); therefore, we can begin our reductionist approach with investigation into the role of the blood vessels on exercise capacity in CF. We have recently identified endothelial dysfunction at rest[3] and now have compelling evidence that patients with CF exhibit impaired blood flow regulation during exercise (preliminary data **Figure 4**).

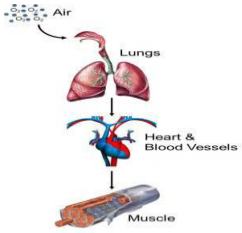

Figure 2. Factors that may contribute to exercise intolerance.

- **This is the first study that uses PDE5 inhibitors to target improvements in exercise capacity in cystic fibrosis**. The proposed investigation not only represents an innovative proof of concept trial for the role of PDE5 inhibitors in CF, we are using PDE5 inhibition as a novel tool to test blood flow and endothelial dysfunction as two potential mechanism that contribute to exercise intolerance in CF.
- **We have employed state-of-the-art methodology**. Using the FMD test, we were the first to identify systemic endothelial dysfunction in patients with CF. We will use FMD to assess endothelial function following PDE5 inhibition. In addition, we will use the innovative assessment of monitoring cardiovascular hemodynamics during maximal exercise using the Physioflow Enduro. Physioflow is the first and only system that is fully validated for use during exercise and has never been used in patients with CF.

This proposal, through the integration of exercise physiology, vascular biology, and pharmacology, represents a major breakthrough in the approach to begin understanding the mechanisms which contribute to exercise intolerance in this deadly disease. Accordingly, this project has high clinical significance that will

help stimulate strategies for the treatment and prevention of exercise intolerance in CF that will impact clinical practice and patient care. Regardless of the outcomes, findings from this study will provide the foundation to conduct full-scale, multicenter clinical trials that will target chronic improvements in 1) exercising blood flow regulation, 2) endothelial function, and 3) exercise capacity in CF.

C. APPROACH

C1. Approach Overview. We are proposing a randomized, placebo controlled, acute experiment (aim 1) followed by an open label 4 week treatment trial (aim 2). Findings from aim 1 will isolate the transient increase in blood flow delivery as a mechanism to improve exercise capacity in CF, whereas findings from aim 2 will target an improvement in endothelial function and therefore, the improvement in exercise capacity will likely be greater in magnitude and sustainable. Nonetheless, **the current proposal will shift current research and bring new knowledge about the mechanisms associated with exercise intolerance in patients with CF**.

C2. Research Design and Methods. **Figure 3** illustrates our experimental design. On Visit 1 (preliminary day), all eligible participants (and parents of minors) will come to the Laboratory of Integrative Vascular and Exercise Physiology (LIVEP) to provide written consent/assent. Visit 1 will also be utilized to assess 1) resting oxygen saturation, 2) body composition using DXA, 3) pulmonary function test (PFT), 4) diffusion capacity of the lung for carbon monoxide (DLCO), and maximal exercise capacity on a cycle ergometer. Aim 1 will be comprised of experimental days 1 and 2. A fasting blood sample will be collected during Day 1, pre-test only. The **Pre-test** will consist of an FMD test and pulse wave velocity (PWV) assessment. One hour following sildenafil or placebo (randomized order), **Post-test** assessments of FMD, PWV and maximal exercise capacity will be determined. Aim 2 will be comprised of experimental day 3, in which a fasting blood sample, FMD, PWV, and exercise capacity will be determined 24-48 hours following the completion of 4 weeks of sildenafil treatment (20 mg thrice daily).

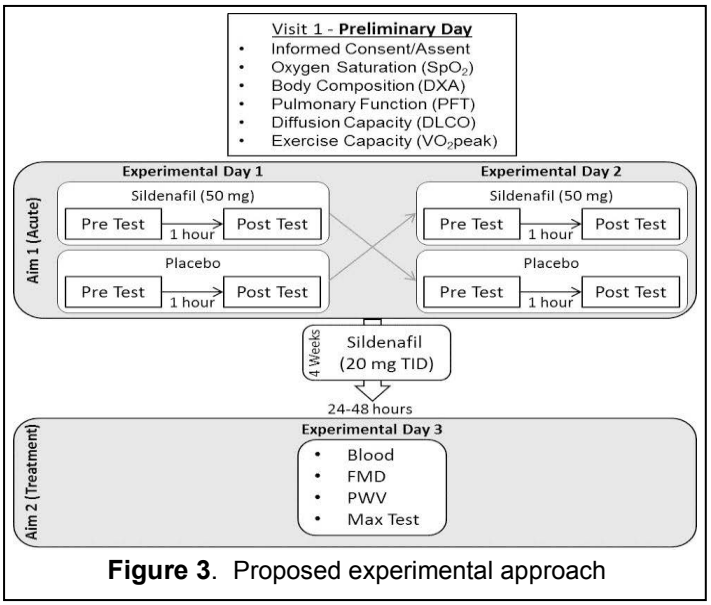

Figure 3. Proposed experimental approach

C3. Study Participants. A total of 40 patients with CF (20 children and 20 adults) and 40 demographically-matched healthy controls will be recruited for this investigation. Age in patients with CF is most often related to disease severity. Accordingly, we plan to recruit both children (9-17 years old who likely have less complications) and adults (≥18 years old who likely have more complications) with CF. Both children and adults accompanied with or without multi-organ pathophysiology (i.e. CFRD, pancreatic insufficiency, etc.) will be included. All patients will be instructed to maintain their daily routine for airway clearance and inhaled therapies. Healthy control subjects will be recruited and demographically-matched for age, sex, and BMI of the patients with CF. Healthy controls will not undergo any treatment. The treatment response to sildenafil observed in the patients will be compared to the healthy controls baseline data. Inclusion and Exclusion criteria for all subjects are presented below:

Inclusion Criteria:

- Diagnosis of CF and healthy controls
- Men and women (≥ 18 yrs. old)
- Boys and girls (9-17 yrs. old)
- FEV_1 percent predicted ≥ 50%
- Resting oxygen saturation (room air) ≥90%
- Patients with or without CF related diabetes
- Traditional CF-treatment medications
- Ability to perform reliable/reproducible PFTs
- Clinically stable for 2 weeks (no exacerbations or need for antibiotic treatment within 2 weeks of testing or major change in medical status)

Exclusion Criteria:

- Children 8 yrs. old and younger
- FEV_1 percent predicted < 50%
- Resting oxygen saturation (room air) < 90%
- Clinical diagnosis of heart disease, PAH
- Febrile illness within two weeks of visit
- Currently smoking, pregnant, or nursing
- Individuals on vaso-active medications (i.e. nitrates, beta blockers, ACE inhibitors, etc.)
- Patients with B. cepacia (only ~3% of our CF center patient population)
- Medications that interfere with Sildenafil

C4. Specific Aim 1. Test the hypothesis that acute PDE5 inhibition will increase systemic blood flow and contribute to an improvement in exercise capacity in patients with CF.

C4.1. Rationale for Aim 1. PDE5 inhibitors prolong NO-mediated vasodilation and increase microvascular O_2 delivery[10]. A single dose of Sildenafil, the most popular PDE5 inhibitor, has been shown to increase exercise capacity in Fontan patients[11], systolic heart failure[12] and congestive heart failure[13]. Our study design proposes to compare exercise capacity obtained on different days. In support, the reproducibility of repeated maximal exercise testing on a cycle ergometer in patients with CF over a 28-day period has been documented[14].

C4.2. Pilot Data for Aim 1. Data from our lab not only confirms that young patients with CF exhibit exercise intolerance[3], patients also exhibit a greater ventilatory equivalent for CO_2 compared to demographically matched healthy controls (VE/VCO_2; 33.2±0.8 vs. 30.8±0.98, respectively; p=0.022). In addition, we have generated preliminary data that suggest that patients with CF exhibit an impairment in blood flow regulation during sub-maximal exercise when compared to healthy controls (**Figure 4**). Blood flow regulation was determined by assessing brachial artery retrograde velocity at baseline and during progressive, sub-maximal cycling (lower leg) exercise at 20%, 40%, and 60% of maximal workload in patients and healthy controls. No differences in age (p=0.201) BMI (p=0.502), or FEV_1 (p=0.508) were observed between patients and healthy controls. **Impaired blood flow regulation may represent a potential mechanism** for exercise intolerance in patients with CF and acute PDE5 inhibition represents a novel method to increase blood flow and muscle oxygenation to test our stated hypothesis.

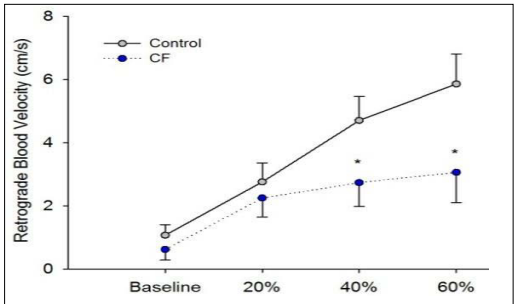

Figure 4. Brachial artery retrograde velocity at baseline and during sub-max workloads in patients with CF (n=14) and controls (n=14). Data are mean ± SEM. *Significant from control.

C4.3. Aim 1 Experimental Design. Exercise capacity (VO_2 peak) and indices of gas exchange (O_2 uptake kinetics, expired CO_2, VE/VO_2, VE/VCO_2), will be determined using a graded maximal exercise test on a cycle ergometer (Godfrey Protocol) 1 hour following the ingestion of either a single dose of sildenafil (50 mg) or placebo (in randomized order) in patients with CF. We will allow for a washout period of at least 1 week (but no more than 2 weeks) in between treatments. Post-test values will be compared to pre-test values; however, exercise capacity will be compared to values obtained during visit 1. Secondary outcomes will include the patented technology of the Physioflow Enduro[15, 16], will be utilized to examine differences in cardiovascular hemodynamics (heart rate, stroke volume, cardiac output, systemic vascular resistance, and ejection fraction) non-invasively between case and healthy controls during maximal cycling exercise. We will also perform pre and post assessments of FMD and pulse wave velocity (PWV), non-invasive assessments of endothelial function and arterial stiffness, respectively. In addition, a fasting blood sample will be taken during pre-test of experimental day 1 to assess baseline clinical labs (lipid panel, high sensitivity C-reactive protein (hsCRP), Polymorphonuclear neutrophil (PMN), glucose, insulin, HbA1c, estradiol, progesterone, testosterone, hematocrit, and hemoglobin) and biochemical research assays (endothelial nitric oxide synthase (NOS3) expression and levels of cyclic guanosine monophosphate (cGMP) in platelets, and 8-isoprostane, nitrate/nitrite in plasma).

C4.4. Aim 1 Expected Results. Based on our published[3] and preliminary data (**Figure 4**), we predict that patients with CF will exhibit exercise intolerance compared to healthy controls. In addition, we predict that acute treatment with Sildenafil will increase systemic blood flow such that we expect that baseline brachial artery blood flow and cardiac output will be increased. Accordingly, we predict that a significant transient improvement in exercise capacity in following sildenafil will be observed, whereas placebo will elicit no change. We also predict that acute sildenafil treatment will result in more efficient ventilatory equivalents[17] and improvements in VO_2 at the anaerobic threshold (AT)[18]. Findings from this acute experiment will test the mechanism that an increase in blood flow per contributes to an improvement in exercise capacity in CF.

C4.5. Aim 1 Statistical Analyses Plan. Mixed-effects regression models (MRMs)[19] will be used to perform the standard analyses required for a two-period, two-treatment (AB/BA) cross-over design. In this analysis, MRM's will be used to compare Sildenafil vs. placebo in terms of the primary outcomes of exercise capacity and indices of gas exchange, all of which will be measured at baseline and 1 hour following ingestion of either a single dose of sildenafil or placebo. The dependent variable of interest will be the change in each outcome between baseline and post-ingestion for each treatment. The secondary outcomes (cardiac output, FMD,

PWV, etc.) will be analyzed in the same way. If necessary, adjustment will be made for the potential confounders for this aim, including age group (child vs. adult), spirometric function, diffusion limitation, presence of CFRD or pancreatic insufficiency, etc. Independent group's t-tests will be used to compare the CF patients in the sildenafil treatment phase with the healthy control participants for all outcomes measured. All analyses for Aims 1 and 2 involving MRMs will be performed using the MIXED procedure in SAS 9.3 (SAS Institute, Cary, NC, 2010) and a significance level of 0.05 will be used for all statistical tests.

C4.6. Aim 1 Power Analysis. The power calculation for Aim 1 is based on the anticipated effect size for exercise capacity, the primary outcome for this Aim. A sample size of n=20 in each sequence (AB and BA) of our cross-over design will yield 87% power to detect a difference in the mean change in VO_2 peak between sildenafil and placebo as small as that found in the cross-over study by Bocchi et al.[13] . It will also yield over 99% power to detect a similar effect size for VE/VCO_2[13], another key outcome for Aim 1.

C4.7. Aim 1 Potential Pitfalls and Alternatives. It is possible that the state-of-the-art methodology using the Physioflow Enduro will not be sensitive to determine changes in cardiac output following acute sildenafil treatment. Accordingly, we plan to use ultrasound to confirm increases in basal brachial artery blood flow before and after treatment. In addition, we can measure femoral artery blood flow as an alternative. Patients with CF may have slight day to day variations in lung function and resultant spirometry. On each testing day we will measure spirometry and diffusion capacity and if significant changes between testing days are observed, we will statistically control for those differences where appropriate. Because we anticipate a heterogeneous sample with respect to disease severity and concomitant systemic consequences, we will document all patient characteristics and therapies, and will statistically control for potential confounders. Further, there are equivocal data that support both an improvement in FMD[20, 21] as well as no change in FMD[20, 21] following acute sildenafil treatment. Therefore, it is plausible that a single dose of sildenafil will improve systemic blood flow and transiently improve endothelial function making interpretation somewhat difficult. We plan on assessing FMD pre- and post- acute treatment; however, the acute improvement in FMD (if any) is likely 1) transient and not representative of a true improvement in vascular function, and 2) confounded by drug-mediated changes in baseline diameter and shear stress, both of which discount the "improvement" in FMD. Taken together, we are confident that changes in exercise capacity following acute sildenafil will be mediated by an increase in blood flow and microvascular O_2 delivery and not improvements FMD.

C5. Specific Aim 2. Test the hypothesis that chronic PDE5 inhibition will improve endothelial function and contribute to an improvement in exercise capacity in patients with CF.

C5.1. Rationale for Aim 2. Through production of NO, endothelial dependent vasodilation is an important function of the endothelium. The flow mediated dilation (FMD) test represents a non-invasive assessment of NO bioavailability and endothelial function[22]. Chronic PDE5 inhibition is routinely shown to improve endothelial function (in as little as 4 weeks) in patients with vascular dysfunction[23-25].

C5.2. Pilot Data for Aim 2. We have recently published in *Chest* that young, fairly healthy patients with CF exhibit endothelial dysfunction compared to healthy, demographically-matched controls[3] (**Figure 1**). Additionally, data supports improvements in endothelial function in populations who exhibit vascular dysfunction, in as little as 4 weeks following treatment with PDE5 inhibitors[23-25]. Further, we and others have reported the reproducibility of the FMD test[26-28]; however, there are no FMD reproducibility data in patients with CF. To address this knowledge gap, FMD was performed in 7 patients with CF (20±9 years, BMI = 20.9±2.9 kg/m^2, FEV_1 93±17 % predicted) during 2 sequential visits, separated by at least 14 days (mean 29±7 days). The intra-class correlation coefficients and p values for baseline diameter, FMD, and FMD/shear were 0.984; p<0.001, 0.949; p=0.001, and 0.885; p=0.009, respectively. **These data indicate that patients with CF have endothelial dysfunction, the FMD test is reproducible in CF, and PDE5 inhibitors are likely to cause an increase in FMD in patients with CF.**

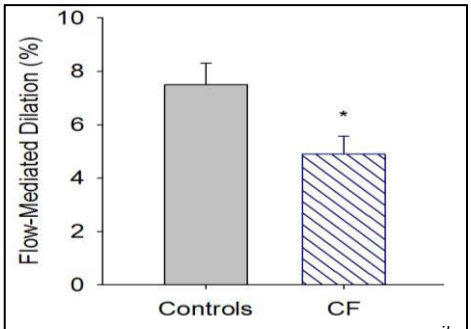

Figure X. Endothelial dysfunction in CF[3]. *Significant from controls.

C5.3. Aim 2 Experimental Design. Brachial artery FMD, exercise capacity (VO_2 peak) and indices of gas exchange (O_2 uptake kinetics, expired CO_2, VE/VO_2, VE/VCO_2) will be determined at baseline and following 4 weeks of oral sildenafil treatment (20 mg thrice daily). Secondary outcomes will include assessment of PWV

and the patented technology of the Physioflow Enduro. In addition, a fasting blood sample will be taken to determine post-treatment clinical labs and biochemical research assays to compare to baseline values.

C5.4. Aim 2 Expected Results. At baseline, we predict patients with CF to exhibit endothelial dysfunction and exercise intolerance compared to their control counterparts. In addition, we predict that FMD and exercise capacity in patients with CF will be restored to healthy control values following 4 weeks of thrice daily sildenafil treatment. Moreover, we expect to see concomitant improvements in oxygen uptake kinetics, VO_2 at the AT, and more efficient ventilatory equivalents. Findings from this aim will not only provide proof of concept for the use of PDE5 inhibitors as therapy in patients with CF, they will also elucidate endothelial dysfunction as a potential mechanism that contributes to exercise intolerance in CF.

C5.5. Aim 2 Statistical Analyses Plan. Mixed-effects regression models[19] will be used to perform a within-subject repeated measures analysis of variance (ANOVA) in terms of mean change from baseline to 4 weeks for each of the primary outcomes associated with this aim (FMD, exercise capacity, and indices of gas exchange). One of the advantages of the MRM methodology is that one is able to incorporate subject-specific parameters into the within-subject analyses. In all MRM analyses, adjustment will be made for any significant covariates (age group, changes in spirometry or diffusion capacity, presence of CFRD or pancreatic insufficiency, etc.). The same analysis will be used for each of the secondary outcomes (PWV, Physioflow Enduro measurements, NOS3, cGMP, 8-isoprostane, nitrate/nitrite, etc.).

C5.6. Aim 2 Power Analysis. The power calculation for Aim 2 is based on the anticipated effect size for 1) FMD[23] and 2) exercise capacity[12] following 4 weeks of PDE5 inhibition. A sample size of n=40 will yield 97% power to detect a difference in the mean change in VO_2 peak between baseline and 4 weeks as small as that found by Lewis et al.[12]. A sample size of n=40 will also yield >99% power for detecting a mean change in FMD between baseline and 4 weeks as small as that found by Rosano et al.[23].

C5.7. Aim 2 Potential Pitfalls and Alternatives. PDE5 inhibitors are generally less effective in the presence of down regulated NOS3 and very little is known about NOS3 expression in patients with CF. Therefore the efficacy of sildenafil in CF may be patient/genotype specific depending on NO bioavailability. Accordingly, we plan on assess NOS3 expression and cGMP levels in circulating platelets at baseline and following treatment which will complement the proposed use of FMD and provide further insight in the unlikely event that sildenafil does not improve either FMD or exercise capacity. It is important to note that insignificant findings for FMD or exercise capacity are just as important and it is plausible that a change in exercise capacity will be observed with no change in endothelial function (and vice versa). These findings will lend support for future investigations into the skeletal muscle as a barrier to improve exercise capacity in CF. It is important to mention that the FDA has recently issued a statement warning against the use of sildenafil to treat pulmonary arterial hypertension in children due to the increased risk of death[29]. However, this elevated risk was only seen in the high dose treatment group (up to 80 mg TID), and only following >2 years of treatment**. No increased risk of death was observed using the dose we are proposing**. Further, we are proposing the use of sildenafil to look at a mechanism, and our trial will 1) use a smaller dose, 2) only last 4 weeks, and 3) will exclude patients with PAH. Accordingly, we fell that our proposed use of sildenafil is safe.

C6. Subject Recruitment. The PI has developed a strong collaboration with Dr. McKie, the Georgia Regents University (GRU) CF center director and both Dr. McKie and Dr. Forseen (adult CF center director) are very enthusiastic about the current proposal (See letters from Drs. McKie and Foreseen). The GRU CF center currently cares for approximately 95 pediatric and 85 adult patients with CF, the majority of whom would qualify for the study. In fact, over the past 2 summers (5 months total) we have successfully recruited/ tested 42 patients with CF. Thus, we are confident we can recruit enough patients and achieve our targeted goals.

The main source of control subject recruitment will be the subjects that are either enrolled or previously enrolled in other studies at the Georgia Prevention Center (GPC). Last year, the GPC saw approximately 12,000 subject visits, which included approximately 50% children and 50% adults. In addition, we will list the study on the GRU website under “Studies in Progress,” place classified ads in local newspapers, and utilize free online sources which attracts participants from the GRU community and Augusta as a whole. We anticipate through all the aforementioned recruitment strategies we will be able to meet our recruitment goals.

C7. Subject Safety. The overall proposal is associated with minimal risk and great benefit. A complete description of our Data Safety Monitoring Plan (DSMP) is located in the Protection of Human Subjects section.

C8. Role of the Study Physician. Dr. McKie will play an integral role in the successful completion of this project. Not only has she been instrumental in the collection and interpretation of our preliminary data, she brings clinical expertise and experience in CF patient care and research. Dr. McKie will help screen patients with CF prior to enrollment and verify they meet the inclusion/exclusion criteria. In addition, Dr. McKie will continue to assist in subject recruitment, data analysis and interpretation, data dissemination, and provide medical oversight for the entire proposed investigation.

C9. Study Timetable. **Table 1** illustrates a conservative number of subject visits per month throughout the project period. One preliminary day is required for all subjects. For patients, the completion of both aims will require a total of 4 visits (1 preliminary day and 3 experimental days; 40 patients x 4 visits = 160 visits), whereas healthy controls will only require 2 subject visits (1 preliminary day and 1 experimental day; 40 healthy controls x 2 visits = 80 visits). Completion of both aims would require a total of 240 subject visits.

Table 1. Proposed number of subject visits by month

	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec	Total
2013									Study Initiation		12	12	24
2014	12	12	12	12	12	16	16	16	12	12	12	12	156
2015	12	12	12	12	12	Analysis/Finalization							240

The proposed timeline is based on the 1) number of patients/healthy controls who can be recruited and studied each month, and 2) the number of visits needed for study completion. In our experience, recruitment of children is more effective when school is out; therefore, we will plan to test more subjects during the summer months (June, July, and August). Research assays will be batched and likely performed during the second year. Abstracts will be presented and manuscripts will be prepared when applicable. We have allowed for a buffer period during the last 3 months allowing for subject visit overflow and finalization of the study.

C10. Collaboration among the Investigative Team. Our group has been working diligently over the past 2 ½ years to uncover the recently discovered vascular phenotype at rest[3] and during exercise (**Figure 4**). Together, we have published our FMD findings in *Chest*[3] and presented our research at the 2011[30] and 2012[31] NACFC conference, and the 2012 American College of Sports Medicine[32] and 2012 Experimental Biology[33] national meetings. We are committed to research aimed to improve the quality of life and longevity in patients with CF.

C11. Consultant Arrangements. For the current proposal, we have arranged to include Dr. Clinton Webb, PhD as a consultant. Dr. Webb holds the Kupperman Chair in Cardiovascular disease and is the Department chair of Physiology at GRU. Dr. Webb is internationally recognized as an expert on PDE5 inhibition and vascular function[34-36] and has provided invaluable suggestions to strengthen our experimental approach. In addition, Dr Webb has agreed to assist interpretation throughout our study (See letter of support from Dr. Webb).

C12. Specific Techniques

Maximal Exercise Capacity Test. Subjects will perform the maximal exercise tests on an electronically braked cycle ergometer using the Godfrey protocol. Expired gasses will be collected using a Parvo Medics True One metabolic cart for determination of exercise capacity (VO_2 peak), CO_2 retention, O_2 uptake kinetics, ventilatory equivalents, and AT. In addition, blood pressures (Suntech Tango) will be assessed during each stage of exercise, SpO_2 (Nonin) will also be monitored throughout the exercise tests, and we will use the Physioflow Enduro to monitor cardiovascular hemodynamics during the maximal exercise tests in all subjects.

Pulmonary Function Testing. Pulmonary function testing will be repeated several times (according to ATS standards[37]) to ensure that lung function is not changing over time. The National Health and Nutrition Examination Survey (NHANES) III spirometric reference standards will be used to determine the % predicted data set. The diffusing capacity of carbon monoxide (DLCO) test will be used to determine diffusion limitations. Briefly, subjects will exhale to RV and then take a deep breath which will include a mixture of gas (10% He, 21% O_2, 0.3% CO, and balance N_2) and will be held for 10 seconds. Concentrations of CO will be analyzed. All values will be presented as % predicted and normalized for TLC and hemoglobin.

Brachial Artery FMD. The brachial artery FMD test will be performed according to the recent tutorial on the ultrasonic assessment of FMD[22] and shear rate will be calculated as the stimulus of the vasodilatory response.

Arterial Stiffness. SphygmoCor Pulse Wave Analysis System (SphygmoCor, PWV Medical, Sydney, Australia), which uses applanation tonometry, will be used for accurate, non-invasive assessment of arterial stiffness. Aortic augmentation index will also be determined and is an index of arterial stiffness.

Physioflow Enduro. The Physioflow Enduro device uses the patented technology of signal Morphology-based impedance cardiography. This non-invasive, wireless, device produces continuous, sensitive, and reproducible assessments of cardiovascular hemodynamics (i.e. HR, SV, CO, SVR, and EF) that has been validated against the invasive thermodilution Swan-Ganz catheter technique.

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