Study Protocol and Statistical Analysis Plan

Title:

The Effect of STIOLTO™ RESPIMAT® on Fatigue in Chronic Obstructive Pulmonary Disease

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PROTOCOL TITLE: A randomized, crossover, placebo controlled, double-blind phase IV trial of the effect of on STIOLTO™ RESPIMAT® on central and peripheral components of fatigue during exercise in chronic obstructive pulmonary disease
Revised: November 13, 2015

1) **Protocol Title**
A randomized, crossover, placebo controlled, double-blind phase IV trial of the effect of STIOLTO™ RESPIMAT® on central and peripheral components of fatigue during exercise in chronic obstructive pulmonary disease

2) **HSC Review History**
Not applicable

3) **Investigators**
Richard Casaburi, Ph.D., M.D. Principal Investigator
Harry B. Rossiter, Ph.D. Co-Investigator

4) **Objectives***
Patients with chronic obstructive pulmonary disease (COPD) have reduced exercise tolerance. One mechanism for this is thought to be due to dynamic hyperinflation during exercise (an increase in the end-expiratory lung volume) that contributes to the sensation of breathlessness. Whether this also contributes to inhibiting motor recruitment, and reduces the available power output (termed performance fatigue; PF), is not well understood. Preliminary data from our lab suggests that many COPD patients, unlike healthy subjects, stop exercise with a 'skeletal muscle power reserve' i.e. the ability to acutely increase muscle power output, suggesting that they are limited by mechanisms other than acute intramuscular limitations to power production. Exercise tolerance is increased by treatment with the fixed-dose combination bronchodilator, STIOLTO™ RESPIMAT®. We hypothesize that increased exercise tolerance with STIOLTO™ RESPIMAT® (reduced performance fatigue; PF) will be mediated by a combination of: 1) reduced inhibition of muscle activation (termed activation fatigue; AF) allowing patients to drive their leg muscles harder, and thus; 2) increased muscle fatigue (MF).

Primary objective: To determine the magnitude of performance fatigue (PF), activation fatigue (AF), and muscle fatigue (MF) during and after exercise in COPD patients with STIOLTO™ RESPIMAT® treatment versus placebo.

Secondary objective: To determine the association of dynamic hyperinflation with performance fatigue (PF), activation fatigue (AF), and muscle fatigue (MF) during and after constant power exercise to the limit of tolerance in COPD patients

5) **Background***
Chronic obstructive pulmonary disease (COPD) is a major worldwide cause of disability and death, with an estimated prevalence of 210 million and with more than 80 million of these being severe disease (World Health Organization). Recent data suggest that COPD is now the third leading cause of death in the USA (Miniño et al., 2010). In addition, COPD is among the top five causes of adult disability, of which

exercise intolerance consequent to dynamic hyperinflation is the main feature (Eisner et al., 2011; Casaburi & ZuWallack 2009).

Twenty to forty percent of these patients will manifest muscle abnormality, which is strongly related to mortality (Schols et al., 2005). As such, skeletal muscle function in COPD is increasingly recognized as a contributing factor to exercise limitation, impaired health-related quality of life and as a major contributor to mortality in COPD (ATS/ERS 1999; Marquis et al., 2002). Peripheral muscle dysfunction in COPD is consequent to a wide range of structural and functional changes including a dramatic loss of lower limb muscle mass (25-30%), and a switch towards more fatigable, less aerobic, muscle fibers (opposite to that seen with healthy aging) (Schols et al., 1993; Augusti et al., 2001; Engelen et al., 2002; Marquis et al., 2002; Richardson et al., 2004; Seymor et al., 2009). These detrimental skeletal muscle adaptations in COPD are accompanied by reduced performance of activities of daily living, increased sense of fatigue, and loss of autonomy. Physical inactivity itself is directly causal to chronic diseases such as obesity, hypertension, and diabetes. Therefore, ameliorating these deficits is key to the maintenance of quality of life in COPD.

The unknown causes of the decline in performance with exertion during whole-body activity - defined as performance fatigue (PF) - are a major barrier to developing effective strategies to overcome exercise limitations in COPD. PF is a highly regulated strategy conserving cellular integrity, function, and survival (McKenna & Hargreaves, 2008). Effective performance requires excitatory input to the motor cortex and motor neuron excitability, neuromuscular excitability and excitation-contraction coupling, a functioning contractile mechanism, and appropriate responses in other physiological systems to support energy supply (Bainbridge, 1919; Bigland-Richie, 1981). PF may, therefore, be conceptually divided into two components: one ‘central’ arising entirely within the central nervous system (activation fatigue, AF: inhibition of voluntary muscle activation with exertion); and another ‘peripheral’ in which fatigue occurs in the muscles themselves (muscle fatigue, MF: the reduction in power production for a given muscle activation).

In COPD patients, exertional dyspnea causes the avoidance of physical activity resulting in extreme deconditioning of the locomotor muscles; which is a major contributor to morbidity and mortality (Casaburi et al., 1999). Hence COPD patients show both increased MF (possibly from impaired gas exchange, muscle metabolism, and muscle oxygenation; Mador et al., 2000; Meyer et al., 2013), and increased AF (possibly from dynamic hyperinflation, dyspnea, cerebral deoxygenation, and/or respiratory muscle or pulmonary afferent signaling; Gagnon et al., 2012; Goodall et al., 2012). Differences in the magnitude of these effects may underlie the varied responsiveness to bronchodilation therapy in COPD (Saey et al., 2003). The degree to which these mechanisms influence independently the ‘central’ (AF) and ‘peripheral’ (MF) components of fatigue in during exercise in COPD is unknown. The answer is

crucial for effective targeting of therapeutic strategies to ameliorate exercise intolerance in COPD.

The purpose of the proposed study is to use a novel exercise testing protocol, designed and validated in our laboratories (Coelho et al., 2015), to determine the magnitude of PF, and its components (AF and MF) during and following whole body exercise in COPD. Bronchodilator treatment with the FDA-approved once-daily STIOLTO™ RESPIMAT® (tiotropium bromide and olodaterol hydrochloride) versus placebo will provide an intervention designed to acutely improve pulmonary function in COPD and reduce dynamic hyperinflation in the absence of changes to peripheral muscle metabolism and fatigue processes contributing to MF. Combined measurements of dynamic hyperinflation and muscle oxygenation dynamics will allow us to relate changes in PF, AF and MF following STIOLTO™ RESPIMAT® treatment to their putative physiological determinants. STIOLTO™ RESPIMAT® is a combination long-acting anticholinergic and beta2-adrenergic agonist that produces greater increases in forced expiratory flow in 1 second (FEV_1) compared the two drugs individually in GOLD stage 2-4 COPD patients (Ferguson et al., 2015).

This will be the first time that fatigue is measured *during* exercise in COPD with and without bronchodilator therapy. Previous studies have measured MF approximately 10 minutes after exercise cessation (Saey et al., 2003). However, preliminary data from our lab in COPD (unpublished; LA BioMed Protocol 30044-01) show that PF and MF are recovered by 90% or more after 2 minutes of recovery from exercise; meaning that the important exercise-limiting signals need to be measured *during* the exercise task itself. These preliminary data (n=13) show that MF is a minor contributor to exercise in COPD (20%), and the predominant cause of fatigue in COPD is AF (80%) (unlike in healthy young subjects). This suggestion supports the hypothesis that exercise limitation in COPD may be related to dynamic hyperinflation rather than to intramuscular limitations of muscle power production (MF).

Assessment of performance fatigue, activation fatigue and muscle fatigue
Muscle force and power production are dependent on muscle contractile velocity. Therefore assessment of fatigue (the magnitude of reduction in muscle power during contractions) requires power to be measured at a known rate of muscle shortening i.e. isometric (constant length, no shortening) or isokinetic (constant velocity) contractions (e.g. Sargeant & Dolan, 1987).

Shortening movements that produce power are highly relevant to the actions of daily living (Newham et al., 1991; Beelen & Sargeant, 1991). Standard isokinetic dynamometry, however, uses complex bulky machinery, complicating its application during whole-body activities such as treadmill walking or cycle ergometry.

Isometric assessment of muscle force, such as by potentiated twitch force measurement using magnetic stimulation (Mador et al., 2000; Saey et al., 2003),

allows changes in MF to be measured accurately and reliably. Twitch force measurements, however, can only be determined ~10 min after a whole-body exercise task (Mador et al., 2000). In healthy subjects, skeletal muscle recovers from fatigue with a half-time close to that of phosphocreatine resynthesis (about 30 s; Sargeant & Dolan, 1987; Elmer et al., 2013), whole-body exercise tolerance recovery half-time is about 4 min (Ferguson et al., 2010), and the half-time of recovery from supraspinal fatigue is about 5 min in young subjects, and prolonged in the elderly (Yoon et al., 2012). Therefore, by 10 min post-exercise, many of the varied physiological processes that integrate to cause task failure during whole-body exercise have recovered (Mador et al., 2003; McKenna & Hargreaves, 2008). This questions the validity of using twitch force measurements after exercise cessation in COPD patients, to draw conclusions on muscle function and fatigue at the point of intolerance that occurred ~10 minutes earlier.

We have provided a solution to this complexity, by instantaneously interleaving voluntary isokinetic torque measurements during standard, cadence-independent, cycle ergometry (Excalibur Sport PFM, Lode). We can establish peak isokinetic power (P_{ISO}) at any point in time before, during, or after cycle ergometry to intolerance to quantify PF (Cannon et al., 2011). Coupling this with measurement of muscle activity by surface electromyography (EMG) during interleaved maximal voluntary efforts, the dynamics of AF and MF can be determined (Ferguson et al., 2013; Coelho et al., 2015; LA BioMed Protocol 30044-01).

6) Setting of the Human Research

Patient recruitment and testing will take place at the Rehabilitation Clinical Trials Center (RCTC; consisting of 4 principal investigators, and 11 research staff, postdoctoral researchers, study coordinators). The RCTC is housed within the Chronic Disease Clinical Research Center (CDCRC). The RCTC occupies 5 new laboratory spaces in the CDCRC, which encompass an exercise-training and functional testing laboratory, an exercise physiology laboratory, a fatigue laboratory, a special pulmonary function testing facility, and a near-infrared spectroscopy laboratory (totaling 1,000 sqft). We also have 2 examination rooms that are dedicated to the RCTC, and access to an additional shared examination rooms.

7) Resources Available to Conduct the Human Research

This study is financially supported by Boehringer-Ingelheim Pharmaceuticals Inc. as part of their Investigator Initiated Studies program.

The Rehabilitation Clinical Trials Center at LA BioMed has a >15-year history in recruiting large numbers of COPD patients and healthy controls for multiple NIH and industry sponsored clinical trials and exercise studies. The RCTC commonly conducts large-scale COPD-related clinical trials, and as such there is potential access to several hundred subjects per year. Twenty-one COPD patient volunteers are

needed for this study over an approximate 1-year period, which is a small fraction of this pool. Therefore we do not anticipate any problems with patient recruitment. Several investigators and staff will contribute to the study. All have extensive experience in working with COPD patients in physiologic exercise studies. The methods used in this study were developed at LA BioMed and the PI and Co-Is are world leaders in the field, and contribute to setting the guidelines for exercise testing in patient populations. All the equipment required for the study is in standard use in our lab. The Co-I (Rossiter) will devote 10% FTE to the study, with PI (Casaburi) contributing 2% FTE. Co-I (Porszasz) will contribute 5% FTE. There will also be 4 participating physicians (Stringer, Chavoshan, Hsia, Kim). All investigators have MD and/or PhD degrees. Recruitment and regulatory compliance will be supported by contributions from study coordinators (Walker, Diaz). Physiological testing will be supported by post-doctoral scientists (Khamoui, Adami), and a clinical research associate in the RCTC lab (Cao). Staff members have been involved in a previous study using the same measurements (LA BioMed protocol 30044-01) and are well trained in their application. Our laboratory has routine staff meetings where the experimental protocols are continually reviewed and the roles of staff members clarified.

No adverse events are anticipated. However, in the event of an adverse event, our lab is equipped with a crash cart and a licensed physician will be available during all initial exercise tests. In case of emergency the lab is located in the CDCRC Building on the Harbor-UCLA Medical Center (HUMC) campus, with excellent access to the emergency facilities. HUMC is a level 1 trauma center. Four of the investigators are HUMC physicians with full and unrestricted California medical licenses.

8) Study Design

a) Recruitment Methods

Potential subjects for the study will be drawn from people known to Rehabilitation Clinical Trials Center who have previously participated in studies in our laboratory, attended clinics of Harbor-UCLA Medical Center or local patient support groups, and through a network of area pulmonologists. Subjects will be contacted by phone to determine their initial interest and invited to the study site for a complete explanation of the protocol and consent.

Subjects are reimbursed for their time and inconvenience in participating in this study. They will be informed of the following:

> "For your time and inconvenience related to your taking part in this study, you will be paid a total of $450 if you complete this study. If you do not complete the study, for any reason, you will be paid for the study visits you do complete according to the following schedule: $50 for visit 1; and $100 each for visits 2, 3, 4, and 5."

b) Inclusion and Exclusion Criteria*

Inclusion criteria

1) All patients must have a diagnosis of chronic obstructive pulmonary disease and must meet the following criteria: (a) Patients must be in a stable state of their disease with no exacerbation within the previous 4 weeks; and (b) At visit 1 spirometric must demonstrate a post-bronchodilator FEV_1 <50% of predicted normal and a post-bronchodilator FEV_1/FVC <70%.
2) At visit 1, patients will demonstrate appreciable reversibility, defined as a 12% increase in FEV_1 in response to albuterol administration.
3) Baseline dyspnea index focal score ≤ 9.
4) Male or female patients, between 45 and 90 years (inclusive) of age.
5) Patients must be current or ex-smokers with a smoking history of more than 10 pack-years
6) Patients must be able to perform technically acceptable pulmonary function tests must be able to complete multiple symptom-limited cycle ergometry tests.
7) Patients must be able to inhale medication in a competent manner from the inhalers used in the study.

Exclusion criteria

1) Patients with a significant disease other than COPD; a significant disease is defined as a disease which, in the opinion of the investigator, may (i) put the patient at risk because of participation in the study, (ii) influence the results of the study, or (iii) cause concern regarding the patient's ability to participate in the study.
2) Patients with a documented history of asthma. For patients with allergic rhinitis or atopy, medical records will be required to verify that the patient does not have asthma.
3) Patients with any of the following conditions:
   a) A history of myocardial infarction within 1 year of screening visit.
   b) Unstable or life-threatening cardiac arrhythmia.
   c) Hospitalized for heart failure within the past year.
   d) Known active tuberculosis.
   e) A malignancy for which patient has undergone resection, radiation therapy or chemotherapy within last two years (patients with treated basal cell carcinoma are allowed).
   f) A history of life-threatening pulmonary obstruction within the past two years.
   g) A history of cystic fibrosis.
   h) Clinically evident bronchiectasis.
   i) A history of significant alcohol or drug abuse within the past two years.
   j) Any contraindications for exercise testing as outlined below (see contraindications to exercise).
   k) Patients who have undergone thoracotomy with pulmonary resection.
4) Patients being treated with oral corticosteroid medication at unstable doses (i.e., less than six weeks on a stable dose) or at doses in excess of the equivalent of 10

mg of prednisone per day or 20 mg every other day.

5) Patients who regularly use daytime oxygen therapy for more than one hour per day and in the investigator's opinion will be unable to abstain from the use of oxygen therapy during clinic visits.
6) Patients who desaturate to S_pO_2 <85% on screening incremental exercise testing.
7) Patients who have completed a pulmonary rehabilitation program in the six weeks prior to the screening visit or patients who are currently in a pulmonary rehabilitation program.
8) Patients who have a limitation of exercise performance as a result of factors other than fatigue or exertional dyspnea, such as arthritis in the leg, angina pectoris or claudication or morbid obesity.
9) Patients with a constant power cycle ergometry endurance time less than 4 or greater than 10 minutes after work rate adjustment procedures (described below).
10) Patients who have taken an investigational drug within one month or six half-lives (whichever is greater) prior to screening visit (Visit 1).
11) Pregnant or nursing women.
12) Women of childbearing who have the potential not to be using a highly effective method of birth control. Female patients will be considered to be of childbearing potential unless surgically sterilized by hysterectomy or bilateral tubal ligation, or post-menopausal for at least two years.
13) Patients who are currently participating in another interventional study.
14) Patients who are unable to comply with pulmonary medication restrictions prior to randomization.

All subjects will be screened for eligibility during visits 1 and 2. Eligibility will be determined by medical history, physical examination, spirometry (visit 1) and exercise testing (visit 2). Women with the potential for pregnancy will be tested using a urine-based testing kit. Eligibility will be determined according to the study inclusion and exclusion criteria

c) Local Number of Subjects

Twenty one patients are required to complete the research procedures. LA BioMed is the only study site.

Based on experience we anticipate an approximate 15% screen failure rate from spirometry and a 15% screen failure rate following the screening exercise test. Therefore we anticipate screening approximately 28 patients to complete the research in 21 subjects.

d) Study-Wide Number of Subjects*

Not applicable.

e) Study Timelines*

Each patient will be enrolled for approximately 9 weeks. This is a randomized, crossover, placebo controlled, double-blind study that includes 5 laboratory visits and three periods of 2-week washout from drug or placebo. The timeline for each patient visit is shown in Figure 1. Visit 1 will be approximately 1 hour duration. Visit 2 will be approximately 4 hours duration. Visits 3, 4, and 5 will each be approximately 2 hours duration.

Complete enrollment (n=21) is anticipated to take 12 months. We anticipate that the first-patient first-visit will be in January 2016, and completion of study visits by January 2017. Completion of primary analysis is anticipated by April 2017.

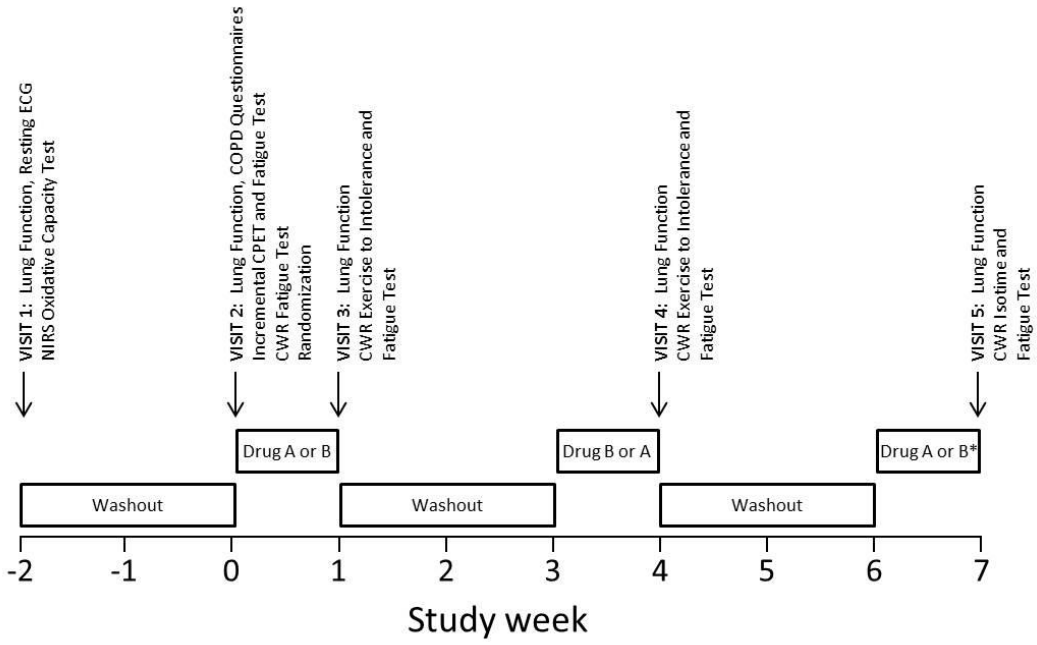

Figure 1. Study Timeline. Post-consent study timeline, including study drug administration, crossover, and washout periods. Drug A or B refers to STIOLTO™ RESPIMAT® or placebo. The administration of these drugs will be double-blinded. *The study drug A or B that resulted in a longer endurance time will be given in the week prior to Visit 5.

f) Study Endpoints*

The study endpoint is completion of Visit 5. Patient safety will be monitored throughout, and the procedures stopped if a problem arises. There are no clinical or therapeutic endpoints.

Safety endpoints include the ability to complete an exercise test safely, as judged by the investigator. Exercise tests will be stopped if clinically significant changes in the ECG occur, such as ST-segment depression, or a dangerous arrhythmia.

Adverse events (AE) and serious adverse events (SAE) are safety endpoints of the study. An AE is defined as any untoward medical occurrence, including an exacerbation of a pre-existing condition, in a patient who received a pharmaceutical product. The event does not necessarily have to have a causal relationship with this treatment. An SAE is defined as any AE which results in death, is immediately life-threatening, results in persistent or significant disability / incapacity, requires or prolongs patient hospitalization, or is to be deemed serious for any other reason if it is an important medical event when based upon appropriate medical judgment which may jeopardize the patient and may require medical or surgical intervention to prevent one of the other outcomes listed in the above definitions. Medical judgment will be used to determine the relationship between study drug or procedures and an (S)AE, considering all relevant factors, including pattern of reaction, temporal relationship, de-challenge or re-challenge, confounding factors such as concomitant medication, concomitant diseases and relevant history.

Changes in vital signs, ECG, physical examination and laboratory test results will be recorded as an (S)AE, if they are judged clinically relevant by the investigator. Vital signs, ECG, and physiological test results are recorded as part of cardiopulmonary exercise testing at each visit. These may cause exclusion of the patient from the study at any time, should they meet the exclusion criteria listed.

g) Procedures Involved in the Human Research*

All study procedures are done solely for the purposes of research.

Study design

This will be a randomized, placebo controlled, double-blind study investigating the effects of STIOLTO™ RESPIMAT® on the mechanisms of exercise intolerance in COPD patients. The study is a crossover, within-patients, design comparing the effect STIOLTO™ RESPIMAT® to placebo on dynamic hyperinflation, performance fatigue (PF), activation fatigue (AF), and muscle fatigue (MF) during and after constant power exercise to intolerance. Twenty-one patients with moderate to very severe disease will be recruited into this study.

Initially pulmonary function and cardiopulmonary exercise variables will be used to characterize recruited patients. Subsequently, constant power exercise tests (calculated from the initial incremental exercise test to bring the patient to intolerance in approx. 6 min) will be used to characterize the components of performance fatigue, ventilation, dynamic hyperinflation, skeletal muscle oxygenation, and accessory respiratory muscle activity. Patients will perform two

tests, each to the limit of tolerance, with prior STIOLTO™ RESPIMAT® bronchodilator and placebo. An independent statistician will randomize the order of these two tests, and both patients and investigators blinded to the condition. A pharmacist, who will otherwise not participate in the study, will facilitate drug administration. Unblinding information will be kept in sealed envelopes by the pharmacist in case of (S)AE.

A third constant power exercise test will be performed with the treatment that resulted in the longer endurance time. In this test the components of performance fatigue and dynamic hyperinflation will be measured at the duration at which the patient reached intolerance in the alternative treatment trial (i.e. isotime measurement). This will allow comparisons of PF, AF, and MF and their associations with ventilation, dynamic hyperinflation, skeletal muscle oxygenation, and accessory respiratory muscle activity, between STIOLTO™ RESPIMAT® and placebo at during and following exercise to intolerance and at isotime.

Visit schedule

Written informed consent will be administered in the study center. Consenting subjects will discontinue long-acting bronchodilators for the entire duration of the study. All subjects will receive albuterol (either alone, or as a combination therapy; see below for details) to be taken as needed (prn) for the duration of the study. For those subjects prescribed long-acting antimuscarinic therapy at study entry, during the three washout periods of the protocol subject's therapy shall consist of combination albuterol and ipratropium (Combivent) prn. Patients taking a combination long-acting beta agonist and inhaled corticosteroid therapy (LABA+ICS) at study entry will be transitioned to ICS monotherapy for the duration of the study. The study drug (STIOLTO™ RESPIMAT® or placebo) will be administered daily during three 1-week periods during the protocol. Each time, the study drug will be taken for one week before testing is performed. The study timeline is shown in Figure 1. The study will require a total 5 visits by each patient for pulmonary function and exercise tests.

Visit 1: Baseline pulmonary function test. Visit 1 will occur after consent and will include full pulmonary function assessment including pre- and post-bronchodilator spirometry, resting ECG, and muscle NIRS test.

Visit 2: Baseline cardiopulmonary exercise tests. Visit 2 will include spirometry, an incremental exercise test, and a constant power exercise test on a cycle ergometer. This testing day will be preceded by a 14-day washout of all long acting bronchodilators, and an overnight washout of short-acting albuterol. COPD questionnaires will be completed.

Visits 3-5: Constant power exercise and fatigue tests. Visits 3-5 will each include spirometry, an exercise test (constant power exercise), and measurement of activation and muscle fatigue, dynamic hyperinflation, ventilation and gas exchange, accessory respiratory muscle activation, and leg muscle deoxygenation. The condition (active drug vs placebo) of visits 3 and 4 will be randomized and each test will be continued to the limit of tolerance. Visit 5 will be an 'isotime' test, in which the patient is stopped at the tolerable duration of the shorter test, using the condition that resulted in the longer duration. Visits 3-5 will be preceded by a 14-day washout period and 7 days using once-daily STIOLTO™ RESPIMAT® bronchodilator treatment or placebo, with the condition blinded to the patient and investigators. Subjects will take their daily dose of the study medication 90 minutes before undergoing spirometry and constant work rate testing.

Fatigue measurements (particularly AF, and MF) are technically challenging to make, and sometimes a repeat visit is required to secure robust measurements. This occurred in about 15% of visits in our previous study (LA BioMed protocol 30044-01); typically repeated measurements are needed at visit 2 where the patient is initially introduced to the requirement to make a 5-second maximal effort to measure fatigue. Should a repeated visit be necessary, the patient will be continued in the study phase i.e. taking no drug (baseline, visit 2), or taking active drug or placebo (visit 3, 4 or 5). They will be recalled within approximately 1 week for re-testing. On completion of the repeat the subject will then be advanced to the next phase of the study (crossover or isotime).

Medications

STIOLTO™ RESPIMAT®: Two actuations of STIOLTO™ RESPIMAT®, taken once daily, provides 6.2 micrograms of tiotropium bromide monohydrate and 5.5 micrograms olodaterol hydrochloride, which is equivalent to 5.0 micrograms of tiotropium and 5.0 micrograms of olodaterol.

Tiotropium bromide is a non-chiral, long-acting, inhaled anticholinergic bronchodilator initially developed for the long-term, once-daily, maintenance treatment of bronchospasm and dyspnea associated with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and emphysema. It has had FDA approval for use in COPD for over 10 years and is used in COPD more than any other bronchodilator medication.

Olodaterol hydrochloride is a long-acting beta2-adrenergic agonist bronchodilator indicated for the long-term, once-daily maintenance bronchodilator treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and/or emphysema. It has had FDA approval for use in COPD for about 2 years.

The drug product, STIOLTO™ RESPIMAT® is composed of a sterile aqueous solution of tiotropium bromide and olodaterol hydrochloride filled into a 4.5 mL plastic container crimped into an aluminum cylinder (Stiolto™ cartridge) for use with the Stiolto™ Respimat® inhaler.

The drug package insert is attached to this application.

Placebo Respimat®: The placebo Respimat® inhaler will be prepared by the manufacturer and contains no active therapeutic agent.

Albuterol: Albuterol is an FDA approved medication for pulmonary function testing. It may be used in this study as a short-acting medication during washout periods. The drug package insert is attached to this application.

Combivent: Combivent contains ipratropium bromide and albuterol. Ipratropium bromide is a marketed drug approved for use in the United States. It is a bronchodilator for maintenance treatment of bronchospasm (tightening of the airways) associated with COPD, including chronic bronchitis and emphysema. It may be used in this study as a maintenance therapy during washout periods. The drug package insert is attached to this application.

Inhaled corticosteroids (ICS): Some COPD patients take combination therapies that contain an inhaled corticosteroid and a long-acting bronchodilator in one dose. In these cases, patients will be transitioned to inhaled corticosteroid monotherapy for the duration of the study. ICS monotherapy may be used in this study as a maintenance therapy. The drug package insert is attached to this application.

Procedures
An assessment schedule for the procedures in the study is shown in Table 1.

Pulmonary function: Visit 1 will include spirometry, lung volume by body plethysmography, and single-breath carbon monoxide diffusing capacity tests. Each will be made according to ATS/ERS guidelines, at least 10 min after 200 µg of albuterol (Millar et al., 2005). Visits 2-5 will include spirometry prior to each exercise test.

Resting ECG: Visit 1 will include a resting 12 lead ECG to determine a normal electrocardiogram prior to any exercise testing.

Medical history and questionnaires: During visit 1 a medical history will be collected, including respiratory and heart history, smoking history, and medication history. Patients will also complete the St. George's Respiratory

Questionnaire (SGRQ) and the COPD Assessment Tool (CAT) to assess COPD symptoms.

Table 1. Assessment Schedule

Study phase	Consent	Screening PFT	Baseline & Randomization	Fatigue test 1	Fatigue test 2	Isotime test
Visit number	-	1	2	3	4	5
Study days	up to -17	-14	0	7	28	49
Inclusion / Exclusion criteria	X	X				
Relevant History, Physical Exam		X				
Height & Weight		X	X	X	X	X
Spirometry		X	X	X	X	X
Lung Volumes		X	X	X	X	X
DL_{CO}		X				
Resting ECG		X				
NIRS Oxidative Capacity Test		X				
CPET & ECG (Incremental)			X			
CPET & ECG (CWR)			X	X	X	X
EMG (Accessory & Leg muscles)			X	X	X	X
Isokinetic Test – Baseline Calibration			X	X	X	X
Isokinetic Test - Fatigue			X	X	X	X
Exercise Leg NIRS			X	X	X	X
Ventilation & Gas Exchange			X	X	X	X
Dynamic Hyperinflation			X	X	X	X
Comments	As required					

Non-invasive assessment of oxidative capacity by NIRS: Visit 1 will include measurement of quadriceps muscle oxygenation by near-infrared spectroscopy (NIRS; Hamamatsu Photonics KK) during a supine protocol using intermittent venous and arterial occlusion (Ryan et al., 2012). The FDA deems use of NIRS in humans as a 'non-significant risk'. The recovery dynamics of muscle O_2 consumption after brief, light muscle contractions is directly determined by mitochondrial oxidative capacity in vivo (McCully et al., 1993). We will use this for non-invasive assessment of vastus lateralis oxidative capacity by NIRS, together with an assessment of muscle vasoreactivity (Ryan et al., 2012). Briefly, the protocol consists of five key phases: 1) resting muscle oxygenation (2 minutes); 2) Ten intermittent venous occlusions (~50 mmHg cuff pressure, for 20 seconds each) to determine resting muscle blood flow; 3) Three arterial occlusions (~250 mmHg cuff pressure, for 30 seconds each) to measure resting muscle oxygen consumption; 4) a sustained arterial occlusion (maximum 5 minutes) and recovery for physiologic calibration and to characterize vasoreactivity during cuff release; 5) Ten seconds of low-intensity rhythmic knee-extension followed immediately by high-frequency intermittent arterial occlusions (~250 mmHg cuff pressure) to estimate recovery O_2 consumption dynamics for ~5 minutes; 6) a repeat of item 5 to improve signal-to-noise. The assessment takes approximately 30-35 min in total.

Incremental exercise test: All patients will perform a ramp-incremental cycle exercise on an electromagnetically-braked cycle ergometer (Excalibur Sport PFM, Lode NL) to the limit of tolerance after an overnight washout of short-acting bronchodilator (visit 2). Patients will breathe through a mouthpiece with nose clip in place for measurement of respired gases and volumes for breath-by-breath gas exchange and ventilatory measurements (Vmax Spectra, CareFusion USA). Tests will be administered with 3 minutes of rest and at least 3 minutes of unloaded cycling (until a steady-state is achieved), followed by an increase in power output at 5 W/min (for patients with $FEV_1 \leq 1$ L) or 10 W/min (for patients with $FEV_1 >$ 1 L) and continued to the limit of tolerance. The limit of tolerance will be determined as the point at which the patient experiences intolerable dyspnea or is unable to maintain pedaling rate above 50 rpm despite verbal encouragement. At the limit of tolerance and every 1 minute during recovery an isokinetic power test will be performed (described below). At specified times during the exercise test, inspiratory capacity (IC) measurements will be made using a maximum inhalation. These will be made at rest, every 2 min during exercise, at end-exercise, and every 1 minute during recovery

Constant work rate (CWR) exercise tests: From an unloaded pedaling baseline (~3 min), the power output will be abruptly increased to approximately 75% WRpeak (this is the peak work rate achieved during the incremental exercise test). The specific work rate chosen will be chosen to elicit intolerance in ~6 min in the placebo condition (van der Vaart et al., 2013). The patient will be instructed and encouraged to maintain the power output for as long as they can manage (except in the isotime test, as noted below). Patients will breathe through a mouthpiece with nose clip in place for measurement of respired gases and volumes for breath-by-breath gas exchange and ventilatory measurements (Vmax Spectra, CareFusion USA). A familiarization CWR test will be performed at visit 2, at least 1 hour after the incremental test. At specified times during the exercise test, inspiratory capacity (IC) measurements will be made using a maximum inhalation. These will be made at rest, every 2 min during exercise, at end-exercise, and every 1 minute during recovery

The constant power test will be repeated on at least 4 occasions. The initial constant power test will be made at least 2 hours after the incremental test and will be used to identify the ~6 min endurance time in untreated conditions (visit 2). If the endurance time is not ~6 min (in the range 4-10 minutes), then an additional visit (visit 2a) will be made to identify required power output. After this, two constant power tests will be performed to intolerance with either STIOLTO™ RESPIMAT® or placebo (visits 3 and 4). An additional, third constant power test will be performed using the treatment condition that resulted in the longest endurance time (visit 5). For this test the isokinetic measurements will be made at a time when the shorter of the two treatment tests ended,

providing isotime assessment. The condition (active drug vs placebo) of visits 3-5 will remain blinded to the participants and the experimenters until the end of the study. At the limit of tolerance and every 1 minute during recovery an isokinetic power test will be performed (described below).

Isokinetic power tests (baseline and fatigue): Patients will be familiarized with the maximal isokinetic cycling task at visit 2. Initially patients will be oriented to the cycle ergometer and optimal seat height will be determined. Following this, isokinetic ergometry tests will be performed at baseline, at the limit of tolerance, and in recovery during visits 2 (incremental exercise) and 3-5 (constant work rate tests).

At baseline subjects will cycle at a comfortable cadence with zero load for ~3 min, after which they will be asked to perform 5 pedal strokes (~4 seconds) isokinetic cycling at each of ~25%, ~50%, ~75% and 100% maximal effort. The strain of the task is minimal and only a few seconds (~30 s) recovery is necessary between each effort, but subjects will be given as long as they feel is necessary to recover. During these efforts the flywheel braking of the cycle ergometer is controlled to result in predetermined velocities equating to a constant pedal cadence of 70 rpm. Patients will complete this test twice at baseline (unfatigued condition) prior to each exercise test to ensure reproducibility. If the two repeats do not cohere to within 10%, then the procedure is repeated a third time following a short (~10 min) rest. The patient will then be given 30 minutes to recover before the incremental or CWR exercise test is performed.

During each exercise test, isokinetic fatigue tests will be made at the point of limitation (or at isotime) and during recovery. For this the cycle ergometer will be switched to isokinetic mode and five pedal strokes will be completed at maximal effort (~4 seconds) to determine PF, AF and MF. In order to track fatigue during recovery, 5-pedal-stroke maximal isokinetic efforts will also be made every minute during recovery until the fatigue has recovered to 90% of the baseline value (up to a maximum of 6 minutes; 3-4 minutes is typical).

Exercise NIRS: Tissue oxygenation during exercise will be assessed by NIRS (as described above in *Non-invasive assessment of oxidative capacity by NIRS*). NIRS probes will be attached by adhesive tape to the skin of the quadriceps (for muscle oxygenation) and the forehead (for cerebral oxygenation), and a bandage will be placed over the probe to help ensure its stability during exercise.

Measurements

Ventilation, gas exchange, heart rate, and perceived exertion during exercise: During incremental and CWR exercise, patients will breathe through a mouthpiece for breath-by-breath pulmonary gas exchange and ventilation measurement (Vmax Encore, CareFusion USA). Heart rate, arterial oxygen

saturation, and transcutaneous CO_2 partial pressure will also be measured. Patients will be asked to rate their perceived exertion ("rate your leg fatigue" and "rate your difficulty of breathing") every 2 minutes during exercise and recovery. All methods are well established in our lab. From these measurements the following variables will be determined in the incremental test: Peak oxygen uptake, lactate threshold (LT), ventilatory equivalent for CO_2 at LT as an index of ventilatory efficiency, peak ventilation (also expressed relative to maximum voluntary ventilation calculated from pulmonary function tests).

Tissue oxygenation: Tissue oxygenation dynamics [(oxygenated hemoglobin and myoglobin) / (total heme-chrompohor concentration)] will be measured in the quadriceps using spatially-resolved near-infrared spectroscopy (NIRO200, Hamamatsu; as previously described in our laboratories; Bowen et al., 2012). Kinetic modeling of deoxygenation response dynamics will be made by non-linear least squares fitting procedures to determine the association between tissue oxygenation and fatigue characteristics.

Dynamic hyperinflation (DH): DH will be evaluated from inspiratory capacity (IC) measurements at rest, every 2 min during exercise, and at end-exercise and during recovery (Somfay et al., 2001) immediately prior to the AF and MF measurement. This reproducible method allows dynamic changes in the operational lung volume to be determined.

Isokinetic power and leg muscle activity: Wireless surface electromyography (EMG) sensors (Trigno, Delsys) are fitted using adhesive patch on the quadriceps, hamstrings, and gastrocnemius. Pedal torque is measured at the crank every 2° of rotation and mean isokinetic power is determined over each complete revolution. Using these measurements a relationship between the average root mean square EMG activity (RMS EMG) and power output can be generated (Coelho et al., 2015). The baseline (unfatigued) relationship between RMS EMG and power becomes the reference value. The fatigue point is then placed in the context of the reference value (see Fig 2). Performance fatigue (PF) is the decline in power output at maximal effort between baseline and the measurement during fatigue. The activation fatigue (AF) is the power-equivalent of the reduction in RMS EMG activity between the maximal effort at baseline and during fatigue. The muscle fatigue (MF) is the difference in power output between that expected (at baseline) at the achieved RMS EMG and the measured power (see Fig 2).

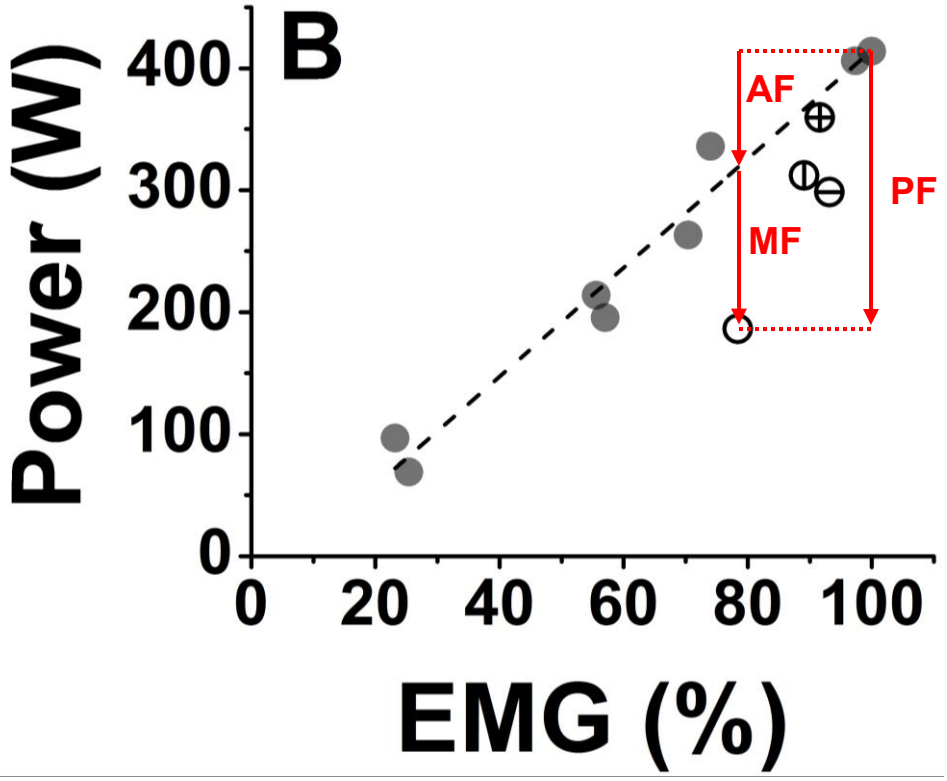

Figure 2. Relationship between isokinetic power and muscle activity (EMG). The baseline (unfatigued) relationship is shown in grey and dashed-line. At the limit of tolerance in incremental exercise fatigue is assessed from a single 5-pedal maximal effort (open circle). Recovery of fatigue is shown at 1 (horizontal bar), 2 (vertical bar), and 3(plus sign) minutes after intolerance. A graphical depiction of the method to qualify performance fatigue (PF), activation fatigue (AF), and muscle fatigue (MF) is shown in red. Note that in this example, performance fatigue at end-exercise is approximately 30% activation fatigue and 70% muscle fatigue. Recovery of muscle fatigue is essentially complete 3 minutes after end-exercise.

Accessory muscle activation: Wireless electrodes will be placed over the accessory muscle of breathing e.g. sternocleidomastoid, scalene muscles, to record electromyographic activity (Trigno, Delsys).

h) Data and Specimen Banking*

Data will be stored for at least 3 years required by the USDHSS, in password-protected PCs and secure filing cabinets in the CDCRC Building. Data will be stored indefinitely.

In order to minimize the risk of inappropriate use of medical information, no data will be labeled in a way that they can be readily identified to an individual. Participants will de-identified using a number code system and the key will be kept in a separate locked cabinet in the PI's office or records room.

Electronic documents will be stored on password-protected PCs in the CDCRC Building. Source documents will be stored in secure filing cabinets in a locked records room in the CDCRC Building. The PI and co-investigators will have access to the data.

Protected health information linking the data and samples to subject identity will be kept for 25 years after the consent is executed.

The LA BioMed training requirement (Study Volunteer Protection, HIPAA Compliance, and Good Clinical Practices) will be satisfied before any investigator handles data.

i) Data Management *

Power calculation

Formal power analyses for these measurements are difficult to compose due to the novelty of the approach. Unpublished work from our lab (LA BioMed protocol 30044-01) were able to detect an average 22% (PF = 24 Watt; p = 0.02) decrease in paired measures of isokinetic performance fatigue at isotime in 13 COPD patients with a inhaled oxygen as the intervention. This reduction in fatigue (increase in power) was due to significant reduction in activation fatigue (AF = 33 Watt reduction; p=0.03) and a non-significant increase in muscle fatigue (MF = 9 W increase; p=0.26). However, the expected difference in PF and AF following bronchodilator therapy are less than for inhaled oxygen. Saey et al., (2003) found a significant 10% difference in post-exercise quadriceps twitch force using a bronchodilator intervention vs placebo in 18 COPD patients.

Data from our laboratories, and from others where muscle fatigue was measured from change in stimulated twitch force in COPD patients, suggest that the variance within groups will be 20 times the variance explained by the main effect (that is, a σ ratio 20:1), giving an effect size for AF and MF of f=0.23. For an α of 0.05 and strong correlation between repeated measures (r=0.80; unpublished data), the actual power is 0.95 with n=21 patients (G*Power 3.1.5).

Analysis Plan

It is hypothesized that STIOLTO™ RESPIMAT® will uncover a skeletal muscle power reserve, allowing an increased MF to be elicited at the point of exercise limitation following bronchodilator therapy (Saey et al., 2003). We hypothesize that AF at the limit of tolerance will not differ between STIOLTO™ RESPIMAT® and placebo, but that AF will be lower at isotime following STIOLTO™ RESPIMAT® treatment in COPD. Differences in AF between isotime in the STIOLTO™ RESPIMAT® condition and intolerance in the placebo condition will be assessed by paired t-test. A lower AF in STIOLTO™

RESPIMAT® would suggest that the mechanism of exercise tolerance benefit of STIOLTO™ RESPIMAT® treatment is related to a reduced inhibition of muscle activation during exercise (primary objective). We also hypothesize that MF will be greater at the limit of tolerance in the STIOLTO™ RESPIMAT® condition. Differences in MF between isotime in the STIOLTO™ RESPIMAT® condition and intolerance in the placebo condition will be assessed by paired t-test. A similar MF between STIOLTO™ RESPIMAT® and placebo would be in accordance with the hypothesis. Differences in MF between isotime and intolerance within the STIOLTO™ RESPIMAT® condition will be assessed by paired t-test. A lower MF at isotime would suggest that the mechanism of exercise tolerance benefit by STIOLTO™ RESPIMAT® treatment is related to better maintenance of muscle activity resulting in increased muscle fatigue during exercise (primary objective). The rate of recovery of PF, AF, and MF will be assessed by repeated measures ANOVA among the baseline (pre-exercise, unfatigued) condition and fatigue measurements at intolerance and every minute during recovery from exercise (up to 6 minutes). It is hypothesized that PF will recover to baseline within 3 minutes following exercise intolerance.

To investigate the physiological mechanisms underlying fatigue, we will determine the associations among physiological measurements and PF, AF or MF. Firstly we hypothesize that the dynamics of AF will be associated with those of dynamic hyperinflation. By reducing dynamic hyperinflation using STIOLTO™ RESPIMAT® we hypothesize that dyspneagenesis will be reduced (e.g. reducing respiratory muscle work during exercise by reducing end-expiratory lung volume, and/or from reducing pulmonary mechanoreceptor activity during hyperinflation). We will measure dynamic hyperinflation with inspiratory capacity measurements during and following exercise and determine the association between dynamic hyperinflation and AF (secondary objective). Secondly, we hypothesize that MF at the limit of tolerance will be associated with increased muscle deoxygenation – the greater the MF the lower the muscle oxygenation consistent with a limitation to oxidative metabolism (secondary objective). These secondary objectives will be assessed using linear regression to determine the proportion of AF and MF that can be "explained" by e.g. dynamic hyperinflation, or leg muscle deoxygenation at isotime, end-exercise and during recovery.

j) Confidentiality

Data will be de-identified and stored on password-protected PCs and in locked cabinets. The PI and co-investigators will have access to the data. Data will be stored for at least three years, with the aim of indefinite storage. Data will be stored locally.

k) Provisions to Monitor the Data to Ensure the Safety of Subjects*

The data will be handled as source documents. These will include all data with patient demographics, history, questionnaires, pulmonary function, muscle

oxidative capacity and exercise testing results. The documents will be filed in the subject's source document binder. This binder will also contain the properly executed consent form, the HIPAA (PHI) form as well as demographic data. The patient identifiers will be kept in this folder, but under a separate section from the study source data. This section will be marked as 'confidential' and the binders will be kept in a locked cabinet in a locked room. Access to the patient identifiers will be permitted only for the study coordinator and the person(s) with direct contact with the patient.

The PI will be responsible for reporting any adverse events to the local IRB utilizing the adverse event reporting policy established by the local IRB. All serious adverse events and non-serious adverse events which are relevant for a reported serious adverse event and Adverse Events of Special Interest (AESI) shall be reported to Boehringer Ingelheim Pharmaceuticals, Inc. Data monitoring will made on a per-procedure basis by the PI or co-investigators.

Per its policy, periodic data monitoring will be undertaken by a member of the Compliance Office at Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center.

l) Withdrawal of Subjects*

Participants will be free to withdraw at any time. Participants may choose to stop study treatment or leave the study at any time without it affecting their future care. Subjects are asked to discuss this decision with the study doctor. Subjects will be asked to return to the clinic for a follow-up visit and to return any unused study medication, but do not have to do this.

Participants are told that the study doctor may decide to withdraw them from this study at any time even without their consent. Participants will be withdrawn for any of the following reasons:

- Consent is withdrawn
- The subject is unwilling or unable to follow the rules of the protocol
- The subject becomes pregnant
- The subject experiences a medical emergency that makes it necessary to stop taking study treatment or that requires treatment assignment to be revealed to the study doctor and/or staff
- At the decision of the PI of one of the participating physicians

9) Risks to Subjects*

Identification of risk and protections to minimize risk

Our primary concern is the safety of the participant during exercise testing. Participants will perform high-intensity exercise that may lead to physical discomforts (e.g. fatigue, delayed onset muscle soreness), but especially dyspnea in this patient population. The risks associated with participating in this study may

include, although unlikely, muscle cramps, muscle strain and/or joint injury, delayed muscle soreness, dyspnea, syncope, and fatigue. They may feel delayed muscle soreness (24-48 hr) after exercise. There is a risk of a cardiac event, such as a cardiac ischemia, cardiac arrest, dangerous arrhythmia, or acute cardiac collapse. This risk is approximately 1-2 occurrences in 10,000 tests of 'high risk' subjects with cardiovascular disease (American College of Sports Medicine). Similarly, an assessment of risk during high-intensity interval exercise testing and training of patients with coronary heart disease revealed approximately 1 event in over 23,000 hr of exercise, such as arrhythmia or myocardial infarction (Rognmo et al., 2012).

A person trained in CPR will be present during testing and a defibrillator is located within the laboratory in case of a cardiac event. A licensed physician will be available within the CDCRC for all study visits and will be in the room during each subject's initial incremental test. Participants will be coached to identify to the experimenters any unexpected symptoms and the exercise stopped immediately to mitigate risk during exercise testing. All tests are done with a 12-lead ECG and the test is stopped immediately should arrhythmic or ischemic patterns develop. All tests are done with pulse oximetry and the exercise is stopped should oxygen saturation fall below 85%.

All medications have the potential to cause side effects. The drugs involved in this study may involve risks that are already known, as well as risks that are currently unknown. Serious allergic reactions that can be life threatening may occur with any medication. However, we will take precautions to reduce these risks. Patients will be instructed to immediately report any allergic reactions to the PI, such as:

- a rash
- having a hard time breathing
- wheezing
- sudden dizziness, especially when standing
- swelling around the mouth, throat, or eyes
- fast pulse
- sweating

Placebo Respimat®
There are no known risks associated with using the placebo Respimat®.

STIOLTO™ RESPIMAT®
Combination treatment with tiotropium plus olodaterol (STIOLTO™ RESPIMAT®) has been studied in 6 completed clinical trials. The following side effects occurred in more than 3% of subjects:

- nasopharyngitis
- cough
- back pain

Other side effects that occurred in less than 3% of patients are:

- dehydration
- dizziness, insomnia
- glaucoma, intraocular pressure increased, vision blurred
- atrial fibrillation, palpitations, supraventricular tachycardia, tachycardia, hypertension
- epistaxis, pharyngitis, dysphonia, bronchospasm, laryngitis, sinusitis
- dry mouth, constipation, oropharyngeal candidiasis, dysphagia, gastroesophageal reflux disease, gingivitis, glossitis, stomatitis, intestinal obstruction including ileus paralytic
- rash, pruritus, angioneurotic edema, urticaria, skin infection, and skin ulcer, dry skin, hypersensitivity (including immediate reactions)
- arthralgia, joint swelling
- urinary retention, dysuria, and urinary tract infection

Use of short acting bronchodilators
The study is a crossover design. Prior to beginning each new condition a 2 week washout from the study drug or placebo is required. This means that the COPD patients in the study will not be able to take long-acting anticholinergic or long-acting beta-agonist bronchodilators for several 2 week periods during the study. During this time they will be provided with a short-acting brochodilator replacement medication (albuterol, or in some cases, Combivent) and be given instructions how to use it. In addition, any patients on combination therapies that include a long-acting bronchodilator will be required to discontinue its use prior to the study. This may include combination inhaled corticosteroid medications. These patients will be transitioned to a short-acting bronchodilator (albuterol, or in some cases, Combivent), and an inhaled corticosteroid monotherapy as appropriate.

Albuterol
Four puffs of albuterol will be given at visit 1 during the lung function tests, and will be provided as a short-acting bronchodilator medication. Albuterol is an FDA approved bronchodilator with mild side effects. Reported risks include:

- Very common (greater than 10%): Pharyngitis (sore throat).
- Common (3% - 7%): Headache, rapid heart rate, muscle and joint pain, dizziness, stuffy and runny nose, and unpleasant awareness of palpitations (strong heart beat).
- Uncommon (less than 3%): Chest pain, infection, diarrhea, inflammation of the tongue, accidental injury, anxiety, shortness of breath, ear disorder, ear pain and urinary tract infection.

Combivent
Combivent is an FDA approved short-acting bronchodilator containing albuterol and ipratropium bromide.

- Common side effects (greater than 2%) associated with Combivent include: Cough, shortness of breath, headache, bronchitis, sore throat, respiratory infection
- Uncommon side effects (less than 2%) include: High blood pressure, dizziness, tremor, muscle spasms or muscle pain, diarrhea, nausea, dry mouth, constipation, vomiting, weakness, flu-like illness, chest discomfort, eye pain, low blood potassium, palpitations (strong heat beat), fast heartbeat, skin itching or rash, pain in nose or throat, wheezing

Patients taking an inhaled corticosteroid in a combined therapy will transition to an FDA approved monotherapy at the beginning of the study. Risks of corticosteroids include:

- Common side effects (greater than 3%) associated with inhaled corticosteroids include: upper respiratory infections, throat irritation, upper respiratory inflammation, sinus infection, hoarseness, mouth or throat infection, cough, bronchitis, headache
- Uncommon side effects (less than 3%) include: runny nose or post-nasal drip, nasal sinus disorders, laryngitis, diarrhea, viral gastrointestinal infections, dyspeptic symptoms, gastrointestinal discomfort and pain, dry mouth, joint pain, muscle pain, muscle stiffness/tightness/rigidity, dizziness, migraines, fever, viral infections, pain, chest symptoms, viral skin infections, muscle injuries, soft tissue injuries, urinary infections

Pulmonary function tests represent minimal risk and are done routinely. In the past ten years there has been no adverse event precipitated by pulmonary function tests in our laboratory. Some people find the breathing tests tiring and occasionally patients experience a sense of dizziness, headache or shortness of breath that usually resolves quickly (common, mild seriousness). A few may experience a feeling of claustrophobia while in the body-plethysmograph. This usually passes as soon as the door is opened (uncommon, mild seriousness). The carbon monoxide content of the gas mixture used to measure lung diffusion capacity is low (0.3%) and the duplicate tests are done five minutes apart; therefore this test does not increase significantly carboxy-hemoglobin concentration.

Risks of adhesive tape allergy or discomfort from EMG and NIRS probes will be minimized using pinned-bandages in participants with adhesive allergies or those expressing adverse discomfort. The attachment and removal of the NIRS probe and EMG electrodes with adhesive may present a minimal, though brief, discomfort. Mild discomfort is common with the occlusion of extremity blood flow with the inflation of the blood pressure cuff during the resting NIRS measurement of oxidative capacity.

The risks associated with reading and filling out questionnaires are some psychological discomfort and anxiety.

Only personal information essential to the research will be recorded, including age, anthropometric data such as height and weight, and physiologic data gathered during the experiments. These variables are important to define the patients enrolled for future publications. Personal health information is protected by conducting all data collection, storage, or transfer according to HIPAA Privacy regulations. Identifiable data will be securely stored and access is limited to the principal investigator and authorized assistants. Data will be coded as early in the research as possible and the code linking the data is stored in a locked cabinet. None of the data are considered to be sensitive or place the subject at legal risk. Personally identifiable data will not be disclosed to anyone other than the research team without the written consent of the subjects or their legal representative.

10) Potential Benefits to Subjects*

There are no direct benefits to the subjects from their participation in the study.

11) Provisions to Protect the Privacy Interests of Subjects

The collection of data will be within the confines of the local research laboratories in the CDCRC Building within areas that insure subject privacy away from other ongoing activities and personnel. Only investigators and the necessary support staff will interact with the subjects during historical and experimental data collection.

The investigators and the support personnel involved are highly experienced with appropriate interaction with research subjects. This expertise and the physical means of providing privacy (see above) are the main methods in place in order to maximize the subjects' sense of ease during the study-related activities.

The patients' historical and experimental data will be secured. Only the investigators and the necessary support personnel with a need to know will have access to the files.

Subjects are informed in the consent form:

"We will do our best to make sure that the personal information in your medical record is kept private. However, we cannot guarantee total privacy. We may have to give out your personal information if required by law. If information from this study is published or presented at scientific meetings, we will not use your name or other personal information that can identify you.

Organizations that may look at and/or copy your medical records for research, quality assurance, and data analysis include:

- The maker of the study medication, Boehringer-Ingelheim Pharmaceuticals Inc., and its representatives
- The Office of Human Research Protection and other government agencies, like the Food and Drug Administration (FDA), involved in keeping research safe for people

- The Institutional Review Board at the Los Angeles Biomedical Research Institute or its staff

The ways your study doctor will use your study-related health information and the people who may receive it are identified in a separate form entitled "Authorization For Release of Protected Health Information (PHI)." "

12) Compensation for Research-Related Injury

Participants are informed:

"If you are injured because of your taking part in this research study medical care will be available. This care will not necessarily be free of charge. If you are injured as a direct result of taking the drugs used in this study or as a result of a procedure that would not have been performed on you if you were not in the study, you will be provided with appropriate medical care including treatment and hospitalization if necessary. The care will not necessarily be free of charge. Financial compensation for any injury from this research is not available. The study sponsor Los Angeles Biomedical Research Institute will not pay for the normal progress of your disease, or any injury or complication due to the medical condition you already have. Financial compensation for such things as lost wages, disability or discomfort due to an injury is not available."

13) Economic Burden to Subjects

Participants are informed:

"You do not have to pay for study drugs, study visits, supplemental medications or tests that are part of the study.

We will conduct all tests and procedures required for this study at no cost to you. You (and/or your health care payer) will continue to be responsible for the costs of your regular medical care and any medications you take for any condition that is not part of this study.

Boehringer-Ingelheim Pharmaceuticals Inc. will supply the study medication at no charge while you take part in this study. The costs of the cost of getting the medications ready and giving it to you are also provided by the sponsor, Los Angeles Biomedical Research Institute. Even though it probably won't happen, it is possible that the sponsor may not be able to continue to provide the medications for some reason. If this would happen, the study may have to close. Your study doctor will talk with you about this, if it happens.

For your time and inconvenience related to your taking part in this study, you will be paid a total of $450 if you complete this study. If you do not complete the study, for any reason, you will be paid for the study visits you do complete according to the following schedule: $50 for visit 1; and $100 each for visits 2, 3, 4, and 5. If you are asked to repeat a visit, you will also be paid at the same rate that visit."

14) Consent Process

The consent will be obtained at the CDCRC Building before any study-related procedures are performed. Subjects will be told that they have as much time as they need to consider consenting and will be given the opportunity to discuss the study with friends, family and their health care provider. At each visit subjects will be reminded that participation in the study is voluntary and their questions, if any, will be answered. We will follow the SOP: Informed Consent Process for Research (HRP-090).

Non-English Speaking Subjects

Since our area has a significant Spanish-speaking population, this ethno-linguistic population will also be considered for recruitment. An IRB-approved Spanish Language Consent form will be officially produced if needed. Several of the study associates are natively fluent in Spanish and will assist with communication during the actual experiments.

Waiver or Alteration of the Consent Process (consent will not be obtained, required information will not be disclosed, or the research involves deception)

Not Applicable.

Subjects who are not yet adults (infants, children, teenagers)

Subjects who are not yet adults are excluded from this research.

Cognitively Impaired Adults

Subjects who are cognitively impaired are excluded from this research.

Adults Unable to Consent

Adults who are unable to consent are excluded from this research.

15) Process to Document Consent in Writing

We will follow the standard operating procedure (Written Documentation of Consent; HRP-091) for written documentation of consent.

16) Vulnerable Populations

Vulnerable populations such as, pregnant women, adults unable to give consent, individuals who are not yet adults and prisoners are excluded from this research.

17) Drugs or Devices

The study drug, STIOLTO™ RESPIMAT® and matching placebo, will be prepared by the manufacturer who is experienced in placebo controlled trials. Medication will be packaged with a coded label and shipped to each study site. The LA BioMed Research Pharmacy will prepare blinded study medication, utilizing randomization

codes supplied by an independent statistician who is otherwise not involved in the study. Blinded drug supply for each patient will be kept in temperature and humidity controlled cabinets in the Research Pharmacy.

The study drug and matching placebo will be provided by Boehringer-Ingelheim Pharmaceuticals Inc. Supplemental medication (albuterol, Combivent, and ICS) will be obtained commercially by the research team. These drugs will be stored in a locked cabinet in the Drug Supply Room in the CDCRC and supplied to study participants as needed in unblinded containers.

18) Multi-Site Human Research*

Not applicable.

19) Community-Based Participatory Research

Not applicable.

20) Sharing of Results with Subjects

The results of this study will not be shared directly with the participants. As part of the consent process it will be explained to the participants that they can contact the Principal Investigator should they wish to find out about the results of the study, or for any other information about the study.

The results of the pulmonary function tests and cardiopulmonary exercise tests may be shared with the patient on request. Patients are likely to have performed this test previously, and the results of this test may provide information on the progression of their disease. Patients will be provided with a copy of their incremental exercise test results on request. Information on pulmonary function or clinical exercise testing will be provided by the PI or participating physician. Other results will be explained on request by the PI or his staff, but these are experimental procedures and have no specific diagnostic or clinical benefit to the individual patient.

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