UNIVERSITY OF CALIFORNIA, SAN DIEGO

The Role of Prior Exercise in Chronic Obstructive Pulmonary Disease and Recovery

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by

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The Thesis of Peter Weiyen Chao is approved and it is acceptable in quality and form for publication on microfilm and electronically:

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University of California, San Diego

2010

My work is dedicated to my father Ning Hsiu Chao, who will always be remembered. It is also dedicated to everyone who cannot enjoy life because of lung disease.

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LIST OF ABBREVIATIONS

Abbreviation	Where (page)	Definition
COPD	xi	Chronic Obstructive Pulmonary Disease
WHO	2	World Health Organization
FFM	2	Fat Free Mass
NHANES	3	National Heath and Nutrition Examination Survey
PFT	3	Pulmonary Function Test
FVC	4	Forced Vital Capacity
FEV_1	4	Forced Expiratory Volume in One Second
FEV_1/FVC	4	Ratio
$FEF_{25-75\%}$	4	Forced Expiratory Flow
GOLD	4	Global Initiative for Chronic Obstructive Lung Disease
AM	5	Alveolar Macrophage
DC	5	Dendritic Cells
IL-x	6	Interleukin
TGF-β	6	Transforming Growth Factor Beta
AEC	6	Alveolar Epithelial Cell
LAP	6	Latency Associated Peptide
TLR	6	Toll Like Receptor
TNF-α	6	Tumor Necrosis Factor Alpha
MMP	6	Matrix Metalloproteinase
Th-x	7	T-helper Cell
CRP	7	C-Reactive Protein
LPS	8	Lipopolysaccharide
DTPA	8	Diethylenetriaminepentacacetic acid
CNCD	10	Chronic Non-communicable Disease
ROPECAR	15	Role of Prior Exercise in COPD and Recovery
LTPAQ	16	Lifetime Physical Activity Questionnaire
MET	17	Metabolic Equivalent of Task
IRB	17	Institutional Review Board
BMI	21	Body Mass Index

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valuable insight into further research, so that fewer people will endure my father's struggle with Chronic Obstructive Pulmonary Disease.

ABSTRACT OF THE THESIS

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Chronic Obstructive Pulmonary Disease (COPD) is a debilitating airway illness with several co-morbidities, including hypertension, muscle dysfunction, clinical depression, cachexia, osteoporosis and anemia. A hallmark of the illness is breathlessness (dyspnea) due to airway obstruction, which reduces mobility and leads to a downward spiral of incapacitation. Dyspnea is a primary complaint addressed by pharmacological means, which temporarily widens airways, but cannot reverse the progression of

additional lung obstruction. Ultimately, the stress of breathelessness and comorbidities results in death, often by cardiovascular failure or cancer. Although smoking is the primary risk factor for COPD, only a small percentage of smokers develop the disease. Current research highlights new factors that enhance one's risk for COPD, including exposure to noxious air particulates (a problem in developing nations), genetic and epigenetic factors, childhood infections, and obesity. In light of such factors, we propose exercise as an additional factor for consideration. Because exercise is touted as a ubiquitous means to promote health, will long-term, consistent exercise starting from the teenage years affect the development or progression of COPD in smokers? We investigate this question using a novel retrospective activity survey comparing self-reported lifetime exercise to lung function test values in 30 smokers. To our knowledge, this is the first retrospective lifetime exercise study of individuals with at least a 10 pack-year smoking history.

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a debilitating airway disease predicted by the World Health Organization (WHO) to become the fourth global killer by 2030[1], affecting 10% of the population worldwide[2]. In contrast to mortality rates of other chronic diseases such as heart disease and stroke, which have declined between 7% and 64% in the last 30 years, COPD mortality has increased by 163%[3]. It is characterized by an inability to breathe (dyspnea) due to limited lung airflow that is not fully treatable and generally irreversible. Airflow is progressively limited by four abnormal anatomic developments (lesions): destruction of lung parenchyma (emphysema), small airway remodeling, pulmonary hypertension and chronic bronchitis[4]. Changes to small airways include fibrosis, loss of elasticity, pronounced inflammation, goblet cell metaplasia and loss of airway patency, resulting in the breathelessness (dyspnea) that characterizes COPD. Disease severity is assessed by lung function testing that measures the rate of airflow through the lungs. As airflow becomes limited over several decades, the feeling of breathelessness forces patients to diminish daily activities, leading to a downward spiral of physical deconditioning[5]. A main comorbity of COPD is skeletal muscle weakness and loss of fat free mass (FFM) due to a gradual loss of muscle throughout aging (sarcopenia) that is compounded by COPD's systemic stress[6]. Muscle wasting (particularly of type IIA/IIx fibers) reciprocally worsens respiratory function, exercise capacity, overall health status and mortality[7]. Moreover, air trapping by obstructed airways are susceptible to bacterial and viral infections that can induce an episode of severely reduced breathing capacity, termed an "acute exacerbation." Exacerbations often

require costly hospital intervention, and recovery of lung function to values prior to the episodes are rare[8].

Although the disease is predicted to become the fifth global cause of chronic disability by 2020[9], many sufferers remain undiagnosed even during late, debilitating stages[10]. Even though 10 million adults in the US are diagnosed with the disease, the National Heath and Nutrition Examination Survey (NHANES) suggests the figure could be as high as 10% of the US population, resulting in approximately 26 million sufferers[11]. Contributing to COPD's under-recognition is public perception of the disease as self-inflicted (from smoking), resulting in disproportionately low research funding for treatments or therapies[12]. In conflict with this perception, a recent long-term study (25-years) showed that less than 30% of smokers may develop the disease, with prevalence predicted to increase as people live longer[13]. The low proportion of smokers who actually develop COPD underscores its complex pathogenesis and the need for greater understanding of its molecular and genetic etiologies.

Before explaining further, the Pulmonary Function Test (PFT, also called "Spirometry") should be reviewed. Spirometry is the standard procedure that diagnoses COPD. Invented in 1846 by English surgeon John Hutchinson, spirometry found that "vital capacity" was proportional to height while inversely proportional to age[14]. It was not until 1951 that the procedure gained more credence when another surgeon, Edward A. Gaensler, included time in the measurement, producing the concept of "forced expiratory volume as a function of time[15]." Of importance is the procedure's ability to document airflow obstruction using several parameters:

1. Forced Vital Capacity (FVC) in liters
2. Forced Expiratory Volume in 1 second (FEV_1): liters/unit time
3. FVC/FEV_1 ratio: percentage
4. Forced Expiratory Flow 25-75% ($FEF_{25-75\%}$): liters/unit time

Collected data are computed as a percentage of what is considered "normal" (i.e. predicted) for the patient's age, weight and height. Airflow obstruction is diagnosed if the FEV_1/FVC ratio falls below 0.70 after the administration of an airway-dilating medication (bronchodilator)[16]. Bronchodilators are used for symptomatic relief for patients with moderate to severe COPD. A patient-preferred and commonly used β_2-adrenergic agonist is albuterol, which is short acting and relaxes pulmonary smooth muscle cells to widen airways[17]. A widely accepted standard for measuring disease severity is set by the Global Initiative for Chronic Obstructive Lung Disease (GOLD):

1. Stage 1: mild; $FEV_1/FVC < 0.70$, FEV_1 greater than or equal to 80% predicted
2. Stage 2: moderate; $FEV_1/FVC < 0.70$, FEV_1 50 to 79% predicted
3. Stage 3: severe: $FEV_1/FVC < 0.70$, FEV_1 30 to 49% predicted
4. Stage 4: very severe: $FEV_1/FVC < 0.70$, $FEV_1 < 30\%$ predicted

The term “stage” refers to categorizing the severity of a disease, and historically, COPD has been staged using the FEV_1, expressed as a percentage of predicted values for the individual's age, gender, BMI and height, among other anthropomorphic values. The FEV_1 measures how much air a person can forcibly exhale in one second, and provides a general estimate of his or her airway obstruction. In addition to dyspnea, severely

diminished FEV_1 is typically associated with several co-morbidities including cardiovascular disease, depression, osteoporosis, systemic inflammation, malnutrition, cachexia and peripheral muscle dysfunction[18].

COPD is a multi-organ system syndrome with diverse etiologies. Although smoking is the primary risk factor for developing COPD, non-smokers are also at risk, particularly in developing countries where biomass fuels are used in enclosed spaces[19]. Researchers have recently proposed several etiological mechanisms for COPD, including an oxidant-antioxidant imbalance, protease-antiprotease imbalance[20], auto-immunity, hormonal imbalances associated with obesity[21], genetic factors and early childhood infections. Most of these proposed mechanisms are associated with abnormal inflammatory processes that give rise to many of COPD's co-morbidities. Although only a small fraction of smokers develop COPD, everyone who smokes has some degree of lung inflammation[22], beginning with the neutrophil as the first responder[23]. Upon stimulation from smoke, noxious particulates or solutes, neutrophils migrate to the injury site and activate alveolar macrophages (AMs), which phagocytose offenders and release cytokines. Contents from macrophages are detected by dendritic cells (DCs) that then activate T cells, leading to the stimulating and attraction of additional leukocytes and eosinophils. Migration of these cells to the damaged area results in further cytokine and chemokine release, worsening damage and enhancing the inflammatory process.

At the center of the inflammatory process is the normally quiescent alveolar macrophage. As the primary sentinel of the lower airways against pathogens or noxious particulates, the AM suppresses leukocytes and pro-inflammatory mediators to maintain

surface area available for gas exchange via interleukin-10 (IL-10), prostaglandin and nitric oxide production (in rodents)[24]. Crucially, the AM is tonically inhibited through the $\alpha_v\beta_6$-integrin -TGF-β axis[25]. At homeostasis, AM contact with alveolar epithelial cells (AECs) triggers AECs to produce and present $\alpha_v\beta_6$-integrins on their surfaces. Latent TGF-β (produced by AMs themselves and other leukocytes) normally activates AMs to induce a inflammatory response; however, AEC $\alpha_v\beta_6$-integrins convert latent TGF-β into an active form by binding the latency associated peptide (LAP) domain of latent TGF-β. Once active, TGF-β binds to receptors on AMs and maintains them in a quiescent state. However, harmful antigens binding AM surface Toll-Like-Receptors (TLRs) induces fast AM dissociation from AECs, causing AECs to discontinue $\alpha_v\beta_6$-integrin production and halting conversion of latent TGF-β factors. Released AMs now contact accumulating latent TGF-β and initiate the inflammatory response through phagocytic programs and cytokine production (particularly IL-6 and TNF-α), recruiting leukocytes into the area. Cytokines that are of interest in COPD patients are interleukin-6 (IL-6) and Tumor Necrosis Factor-α (TNF-α), which become chronically elevated versus non-smokers and smokers with normal lung functions[26]. Once the injurious agent is cleared, a rapid slow-down system returns AMs and other leukocytes back to homeostasis in an IFN-γ and matrix metalloproteinase-9 (MMP9) dependent manner. IFN-γ secreted from activated leukocytes stimulate AM production of MMP9, which activates latent TGF-β. Buildup of activated TGF-β combined with the removal of the offending particulate/pathogen reins in active AMs to AEC contact, enabling $\alpha_v\beta_6$-integrin production and a return to homeostasis.

T leukocytes also play an important role in the inflammatory process. Naïve T cells normally stay in circulation until antigens prompt signaling from afflicted cells (such as IL-6) to attract them to the injury site. Once there, T cells ($CD4^+$ or $CD8^+$) secrete two patterns of cytokines: helper T cell 1 (Th1) [IL-2 and IFN-γ] or helper T cell 2 (Th2) [IL-4, IL-5]. The Th1 pattern potently stimulates AMs using IFN-γ, prompting secretion of inflammatory mediators such as reactive oxygen species (ROS), leukotrienes, prostaglandins and IL-12. Some studies suggest that the Th1 pattern predominates due to increased IFN-γ detection and lower IL-4[27]. However, recent evidence suggests a new phenotype termed T helper cell 17 (Th17) producing the IL-17 cytokine that is exclusive to T cells[28]. IL-17 induces AEC and fibroblast production of IL-6, and both IL-6 and IL-17 stimulate airway mucus production by airway epithelial goblet cells and submucosal glands.

Prolonged tobacco smoke (TS) exposure alters the innate and adaptive immune systems, resulting in modified lung structure and function. Normally, three levels of protection maintain lung sterility for efficient gas exchange[29]. First are physical barriers, such as a mucociliary clearance apparatus that filters incoming air of large particulates and an epithelial lining reinforced with tight cell junctions. The second and third levels are the innate and adaptive immune responses, respectively. Part of COPD's pathogenesis involves immune cells abnormally remodeling the lungs during injury repair. In stable COPD, C-reactive Protein (CRP)[30], IL-6 and TNF-α[31] remain consistently elevated, resulting in modifications to surrounding tissue. Elevated concentrations of TNF-α and IL-6 reduce type I AECs while inducing type II AEC hyperplasia. Removal of type I

AECs reduces anchoring points for AMs and $\alpha_v\beta_6$-integrins, resulting in a buildup of latent TGF-B that feeds back onto AMs, prompting additional TNF-α, IL-6 and MMP9 secretion. Although MMP9 provides negative feedback on AMs, MMP9 combined with TGF-β stimulates fibroblast hyperactivity that leads to airway fibrosis[32].

TS exposure also increases pulmonary permeability allowing accumulating inflammatory mediators to "spill over" into systemic circulation[33]. An in vitro study by Olivera et al showed that cigarette smoke transiently increases macromolecule permeability in airways[34]. In a murine model, Tamagawa et al demonstrated that following lipopolysaccharide (LPS) intratracheal injection, lung-to-blood translocation of IL-6 occurred with concomitant blood-to-lung translocation of liver-produced albumin[35]. Furthermore, a study by Wollmer measured blood-gas barrier permeability by tracking inhaled ^{99m}Tc-labeled diethylenetriaminepentacacetic acid (DTPA) clearance from the alveoli into pulmonary capillary blood, finding that subjects with the lowest lung function value (FEV_1) had the highest clearance rates. The increased permeability of the lung-to-blood barrier allowing easier inflammatory mediator translocation could contribute to the chronic systemic inflammation that accompanies COPD.

Although TS induces many immune system changes, the etiology of persistently activated T-cell populations in COPD lungs is unknown. Viruses are believed to play an important role due to the prevalence of viral infections in COPD patients. After a cleared infection, both $CD4^+$ and $CD8^+$ memory T cells remain at high concentrations for many months (in a murine model)[36]. Although the absolute number of T cells declines over 6 months, a minority of cells proliferate in the lungs to maintain long-term T cell numbers.

Lung effector memory $CD8^+$ T cells maintain a receptor expression pattern similar to those during acute activation, leading researchers to term them "persistently activated T cells[37]." However, further infection from multiple viruses (resembling infection in the real world) could alter T-helping cell immunostasis, resulting in potentially harmful T cell responses[38] due to the allocation of defenses against one particular virus that is unrelated to other invaders. Therefore, viral infection could set an inflammatory standard that is either insufficient or too extreme for the particular insult.

How Can Lifetime Exercise Affect COPD Development and/or Progression?

Many studies show exercise to have anti-inflammatory effects. Among the first cytokines to be released during exercise is IL-6, which increases up to 100-fold over baseline levels[39]. New studies show skeletal muscle to be an endocrine organ producing myokines and cytokines including IL-6 during exertion,[40]which could account for its sizable plasma concentration increase. Although IL-6 has pro-inflammatory effects, its release during moderate physical activity (without muscle damage) is accompanied by additional anti-inflammatory factors such as IL-1ra and IL-10, and is proportional to the duration, intensity and muscle mass involved[41]. Once released, IL-6 exerts inhibitory effects on TNF-α and IL-1 production[42], and induces the release of TNF-α receptors and hepatocyte-derived anti-inflammatory acute-phase-proteins[43]. In a study that injected human subjects with low-dose *Escherichia coli* endotoxin to mimic low-grade inflammation, an injection of IL-6 diminished release of TNF-α (versus a 2-3 fold increase in controls)[44].

Regular exercise has been shown to be an effective prophylactic and treatment for chronic noncommunicable diseases (CNCDs) such as cardiovascular disease, diabetes, atherosclerosis and certain cancers[45,46,47,48,49]. However, the mechanisms for its protective effects in COPD are less understood. A contributor to exercise's benefits in chronic lung disease could be skeletal muscle's newly discovered role as an endocrine organ producing anti-inflammatory factors. Because COPD often involves peripheral muscle dysfunction, muscle loss could shift the systemic inflammatory:anti-inflammatory balance[50]. A study that tracked COPD patients over the course of a year found consistently elevated inflammatory markers IL-6, TNF-α and CRP[51]. Compounding this imbalance is the presence of obesity concurrent with mild to moderate COPD[52], for adipose tissue has also been shown to be an endocrine organ releasing pro-inflammatory cytokines[53]. Because obesity (or higher fat mass) is more prevalent in mild-to-moderate COPD versus severe stages, the possibility of exercise protecting against COPD-associated lung function decline by minimizing fat buildup while spurring muscle growth should be considered.

Bacterial and viral infections in early childhood could also contribute to abnormal inflammatory responses in later adulthood, and could even account for diminished FEV_1 values. A study by Shaheen et al. found that individuals who were afflicted with pneumonia before two years of age developed lower FEV_1 values in adulthood, adjusted for age, height, smoking and asthma[54]. Other studies have linked an individual's past infections to chronic inflammation levels and increased risk of heart attack, stroke and cancer[55]. How exercise affects inflammation during infection are now being investigated. Normally, the body responds to infection by triggering the innate response via pathogen-

associated molecular patterns binding to Toll-Like-Receptors (TLR) on alveolar macrophages, myeloid and plasmacytoid dendritic cells (m/pDC)[56]. Next, activated innate immune cells release pro-inflammatory cytokines such as IL-12, TNF-α, IL-1B, IL-6, IL-1 receptor agonist (IL-1ra), IL-10 and liver-derived acute phase proteins[57]. The IL-12 cytokine bridges the innate and adaptive immunity gap by driving the differentiation of naive T-helper cells (Th0) towards the aggressive, intra-cellular pathogen-focused T-helper 1 (Th1) phenotype, characterized by IL-2 and IFN-γ secretion. IL-2 enhances CD8+ cytotoxic T cell phagocytosis of virus-infected cells, while IFN-γ has broad inflammatory effects. It should be noted that while the Th1 response is required for antiviral activity, studies show that a prolonged Th1 response is injurious to tissue via cell necrosis[58].

Many studies show that individuals who engage in regular moderate exercise experience reduced risk of respiratory infectious symptoms[596061]. It is possible that moderate exercise shifts the cellular immunity Th1 signaling pathway to the humoral Th2 pathway. T helper cells constitute either a Th1 (cellular immunity) or Th2 (humoral immunity) response pathway. An exaggerated Th1 response to influenza infection can cause long term lung damage and increased mortality[62]. A recent study using a murine model showed that moderate exercise in the early stages of H1N1 influenza virus infection via intranasal inoculation reduces the Th1 inflammatory response while increasing the Th2 humoral response[63]. Crucially, IFN-γ cytokine mRNA levels diminished on days 3 and 5 post-infection, while IL-10 (Th2 pattern) mRNA increased. IFN-γ protein levels diminished two-fold, along with other inflammatory mediators IL-

17, IL-13, leptin, SDF-1 and LIX. Meanwhile a two-fold increase was found in the Th2 cytokines IL-4, IL-12, CD30L and eotaxin. A separate study by Sim et al. with mice and influenza reached similar conclusions, finding that mice chronically exercised following influenza inoculation had lower inflammatory markers, increased appetite, less weight loss and better survival rates versus unexercised and acutely-exercised groups[64]. Although the complex nature of Th1 versus Th2 immunity renders external influences (i.e. exercise) unlikely to exert a shift in dominance from one to the other, a return to immunostasis from strongly inflammatory or strongly anti-inflammatory is possible. Given its immuno-suppressive effects, it is possible that chronic moderate exercise serves a prophylactic role against noxious particulate-induced inflammatory responses.

Overall, my hypothesis rests on two premises:

1. Chronic moderate exercise helps prevent the development of systemic inflammation by maintaining muscle mass while lowering adipose mass. The anti-inflammatory cytokines and myokines producted by skeletal muscle abates adipose tissue's pro-inflammatory mediator secretions.
2. Chronic moderate exercise reduces Th1 response dominance and lowers inflammatory cell airway infiltration, which reduces airway fibrosis and obstruction.

As dyspnea worsens with progressing COPD, patients become immobile and lose muscle mass. Long periods of physical inactivity could result in elevated levels of systemic inflammatory markers compared with physically active individuals[65]. It has

been reported that COPD patients at GOLD stage II or higher have significantly reduced physical activity versus a group of patients with normal lung functions and chronic bronchitis[66]. The recently discovered endocrine secretions of skeletal muscle diminishes concomitantly, reducing circulating anti-inflammatory cytokines and myokines that combat COPD's systemic inflammation. Meanwhile, adipose tissue increases in proportion to reduced muscle mass and contributes to systemic inflammation through pro-inflammatory cytokine release. If an individual has maintained a consistent routine of moderate exercise, then the anti-inflammatory secretions of sustained muscle mass combined with exercise's immuno-suppressive effects could counteract systemic inflammation and curtail morphological changes (airway fibrosis, emphysema) that diminish lung airflow. However, if the individual has performed strenuous exercise (job-related or other) for many years, then the inflammatory:anti-inflammatory cytokine balance is disrupted and compounded by smoking's inflammatory effects.

METHODS

Developing a Questionnaire—The ROPECAR Survey: A main concern was developing a survey that could quantify lifetime exercise in units applicable to our analysis. A literature search began for existing surveys, but most had limited time-frames that asked only a few years back from the current time. Also, most were designed for evaluating conditions different from COPD, such as cardiovascular diseases and diabetes. Therefore, work began on a novel survey at the expense of having unknown accuracy in terms of data reproducibility; in other words, the accuracy of the survey depended on whether the answers reported by a subject in one sitting would be similar to those reported at another sitting several days (or weeks) later. Developing a survey was a work-in-progress for nearly two months. One reason accounting for the delay was the difficulty of quantifying abstract data such as subjective, self-reported exercise—it was not readily apparent to use the final-version's units of average kcals/week. A second problem was how to sectionalize a person's lifetime in a logical manner. A third problem was how to design questions that would retain the subject's confidence in the survey, particularly when being asked to recall events from many years ago. A fourth problem was how to design questions that did not discourage subjects, especially those who were relatively sedentary.

UCSD's Institutional Review Board (IRB) approval was obtained in June 2008 for my study titled Role Of Prior Exercise in COPD And Recovery (ROPECAR); the first survey was tested in July 2008 (See Supplement 1 for the ROPECAR Survey). The ROPECAR Survey asked subjects to report lifetime exercise in 5-year blocks, starting from the age 20 until their current age. After testing with nine subjects, however, we realized that the Survey presented too much risk due its lack of proven reproducibility

and the lack of a quantifiable exercise unit. Therefore, another literature search began in September 2009 for an existing, reproducible exercise survey. With the help of our statistician Marian Renvall, we discovered and used Dr. Andrea Kriska's Lifetime Physical Activity Questionnaire (Kriska LTPAQ, see Supplement 2).

The LTPAQ was originally developed by Dr. Andrea Kriska at the University of Pittsburgh to assess a correlation between bone density in post-menopausal women and their lifetime exercise histories[67]. After obtaining permission from Dr. Kriska, the LTPAQ was adapted for use with COPD subjects. The LTPAQ was designed to be filled out by subjects with minimal oversight from a preceptor, and quantifies exercise in terms of average kcals/week per specific period of life. It consists of two components:

1. Part 1: Questions that ask subjects to self-rate their exercise histories.
2. Part 2: A table that subjects can fill in to quantify exercise in terms of kcals/week.

The first component does not quantify exercise; instead, it is a set of qualitative questions that allows subjects to grade themselves based on how active they were. These "grades" can then be compared to their self-reported kcal/week data to provide a sense of the subject's reporting consistency. For example, if subject A responded to Part 1's question of "How many average hours per week did you exercise during high school" by checking "more than 7 hours per week," but responded to Part 2's table by filling in zero hours per week for the same time period, then this subject's answers might be discarded due to inconsistency. In addition, Part 1 helps jog the subject's memory by asking them to recall long ago activities using self-rating questions. This helps subjects recall time periods more accurately for the quantitive assay in Part 2.

The table in Part 2 relied on measured Metabolic Equivalent of Task (MET) values per specific activity[68]. To obtain kcal/week, MET-hrs/week are multiplied by a subject's weight in Kg. For example, if a subject weighing 70kg played an average of 4 hours/week of basketball (which is rated at 8.0 METs), then the subject spent an average of 70kg x 4hrs/wk x 8.0 METs = 2,240kcal/wk.

To adjust the LTPAQ for our study, the survey was abridged so it could be completed within a half hour. Our experience with the prior ROPECAR survey showed that subjects lost interest after the said time, after which responses were unlikely to remain accurate. Therefore, two main components of the LTPAQ: the self-assessment and the quantitative assessment, were retained for expedited administration. Moreover, all subjects were interviewed instead of completing the survey on their own, to avoid calculation errors and unanswered questions.

Subject Recruitment: After obtaining IRB approval through the University of California, San Diego and informed consent from all interviewed subjects, a convenience sample of subjects in an existing lung study at the UCSD Hillcrest Clinical Trials Center was performed. The lung study was COPDGene™, a nationwide study of Caucasian and African-American smokers who have smoked at least 1 pack a day for 10 years (1 x 10 = 10 pack-years) to assess for genetic markers that predispose individuals to COPD. Because not all smokers (even those with >10 pack-years) develop COPD, having COPD was not a requirement. Qualified subjects were males and females over the age of 45, have had a smoking background and/or are currently smoking. See Supplement 3 for Inclusion/Exclusion Criteria. Relevant subject eligibility criteria include:

- 45 years of age or older
- 10 or more pack-years smoking history
- With or without COPD diagnosis

Subjects for COPDGene™ were recruited by paid research associates via telephone, print ads and television ads. After recruitment, subjects were assigned code numbers for privacy protection and fast identification.

Questionnaire administration: Thirty subjects currently enrolled in COPDGene™ at the UCSD Clinical Trials Center were administered a modified version of the LTPAQ after obtaining informed consent. After completing the interview, data and spirometry values were entered into an online Google™ Docs database using a formula that calculates average kcal/week expenditure for each specific time period (see Supplements 4 and 5). Specific values from spirometry were:

1. FVC (pre and post-bronchodilator values were collected for all parameters)
2. FEV_1
3. FEV_1/FVC ratio
4. PEF
5. FEF_{25-75}
6. FET
7. Pack-year smoking history
8. Body Mass Index

Spirometry: pulmonary function testing was performed following COPDGene™ protocols using the EasyOne™ spirometer and EasyWare™ software for PCs. Please see Supplement 6 for the Spirometry Protocol. Two sessions were performed, with albuterol administered in-between to assess post-bronchodilator performance.

Statistical Analysis: SYSTAT Software was used to perform Pearson coefficient analyses. Variables were considered to be of statistical significance when the p-value was 0.05 or less.

RESULTS

Thirty subjects completed full interview sessions, while three individuals did not (either did not finish spirometry or had personal time constraints). Nine subjects interviewed using the older ROPECAR survey were excluded from analysis. Seventeen were classified as Control subjects (i.e. had normal lung function), 2 were GOLD1, 6 were GOLD3, 3 were GOLD 4, and 1 was unclassified (Subject 22437J, $_{Pre}FEV_1$:60% predicted, FEV1/FVC: 0.775). All thirty subjects were overweight at the time of spirometry (17 Controls averaged 28.92 BMI, 13 GOLD averaged 27.9 BMI, see Figure 4). No statistical significance was found in pack-year smoking histories for all thirty subjects (see Figure 2). Although women were observed to have lower general Forced Vital Capacities than men, there was no difference in pack-year smoking histories between women and men. When comparing average kcal/week expenditures to disease severity defined by GOLD classifications, GOLD stages 2 and 3 had higher average kcal/week expenditures for ages Ages 14-21 and 21-34 vs. Control counterpart age ranges. However, GOLD1 Ages 34-50, was significantly lower than Control (575.16kcal vs. 1912.81kcal). GOLD3 Ages 14-21 was the only age-range in the GOLD3 category with lower kcal/week values than Control (3319.72kcal vs. 5251.58kcal), whereas Ages 21-34 and 34-50 both had substantially higher values than Control counterparts (see Figure 2).

Data adjustments were made because of confounding factors that produced unreasonably high kcal/week values for some subjects. Two subjects were removed from analysis due to either overt exaggeration (19056A reportedly performed over 35,000 kcals/week) or environmental factors (22447M worked in construction with exposure to harmful particulates). Because a male marathon runner weighing 175lb. expends ~3,500

kcal over a 26.2mi run[70], it is unlikely that most subjects expended more than 5,000 kcal/week purely on leisure-time exercise for a period of months, or even years. Therefore, a cap of 5,000kcal was placed on all subject reports due to the assumption that any calories expended per week above this value would be arbitrary. After these adjustments, the average kcal/week vs. GOLD Age Ranges was recalculated (see Figures 3 and 4). With the 5,000kcal limit, Control subjects were found to have higher average kcal/week expenditures in Age Categories 14-21 and 35-50 versus GOLD subjects. The averages between GOLD and Controls were similar for Age Category 22-34 (GOLD: 1955.68, Control: 1816.34), and leans more towards GOLD in Age Category 50+(GOLD: 282.13, Control: 137.93), although both GOLD and Control subjects performed much less exercise when older than 50 years relative to earlier Age Categories (see Figure 4).

Further analysis of GOLD subjects (n=11) found a break point in kcal/week values at approximately 1,200kcal for Ages14-21, above which values became substantially high. Below 1,200kcal/week, 6 subjects reported expending a combined average of 366.5kcal/week on leisure time exercise, while the remaining 5 subjects claimed to have expended a combined average of 7,705.43kcal/week. Of the Controls, only 3 subjects reported exercising less than 1,200kcal/week for Ages 14-21 with a combined average of 256.15kcal/week, while the remaining 14 Controls expended a combined average of 5946.92kcal/week. Therefore, an additional recalculation summary was performed with a 1,200kcal/week cap on both Controls and GOLD subjects (see Figure 5), and the subjects who expended more than value per week were termed "conscientious exercisers" (n=19). On the other hand, a cap of 700kcal/week was determined for all subjects who expended less than 1,200kcal/week because the next

closest kcal/week value below 1,200 was 632.17kcal/week (21573M). Therefore, all subjects who expended less than 700kcal/week were termed "mild exercisers" (n=9). A Chi^2 test was performed comparing the numbers of Controls and GOLDs in the Mild Group to those in the Conscientious Group (see Figure 5). A significant correlation was found: in the Mild Exercisers group, 3 subjects were Controls while 6 had lung obstruction; in the Conscientious Exercisers group, 14 were Controls while 5 had lung obstruction ($P = 0.041$).

The name "Conscientious Exerciser" is appropriate, for a strongly positive relationship was found between high kcal/week values early in life (Age 14-21) and continued activity throughout life (Pearson Correlation = 0.5882, P=0.006). The strongest trend was between Age Range 22-34 and Age Range 35-40 (Pearson = 0.9094, P=0.0000), while a slightly less substantial trend was between Age Range 14-21 to Age Range 22-34 (Pearson = 0.70, P=0.0000).

DISCUSSION

Without adjustments, the raw data shows no overt correlation between exercise at earlier life stages and current lung function values. While these results seem to contradict the hypothesis, some confounding factors should be noted. A primary problem is memory inaccuracy. The modifed LTPAQ was designed to be interpreted more qualitatively than quantitatively of lifetime exercise histories due to memory inaccuracies and recall bias when reporting values from long ago. Reasonable kcal/week values were obtained when subjects limited reported exercise to <10 hours week total, or limited the number of unlike activities that were performed within a given age range. On the other hand, some subjects reported exercising over 30 hours a week, which produced massive kcal/week expenditures (e.g. Subject 19056A reported exercising more than 20903.27kcal/week for ages 14-21). Because a male 180lb. marathon runner expends ~3,500 kcal over 26.2 miles[71] , subject-reported kcal/week values greater than this are likely skewed or exaggerated due to the subjective nature of recalling long ago events. Second, the ROPECAR study did not offer compensation for time and travel, therefore subjects were volunteers and could not be expected to spend extra time learning how to answer to the best of their abilities. Third, environmental factors play a considerable role in lung disease development[72] . Subject 22447M reported working in construction throughout his life with exposure to wood particulate matter and asbestos, and was hospitalized for pulmonary edema at age 45; therefore the subject's reported data might not be acceptable. Lastly, the results are limited by a very small sample size of 30 subjects (17 were GOLD-classified as Controls and only 13 were GOLD1 or more). A larger sample size of 50 subjects (GOLD subjects are necessary) will provide better results.

However, after adjusting by imposing a 5,000kcal/week cap on all subjects and excluding 2 subjects for said reasons, a correlation was found for Age Category 14-21 in Controls and GOLD subjects (see Figure 3 and 4). Controls in said age range expended on average 3159.28 kcal/week, while GOLD subjects expended on average 2128.92kcal/week, which is a difference of 1,030.32 kcal. After the adjustment, there appears to be a substantial correlation between chronic exercise when early in life (between age 14 and 21) and better lung function later in life (recall that all subjects are over age 45). When comparing kcal/week expenditures between Controls and GOLD subjects within the Age 22-34 time frame, however, the values were close with a standard deviation greater than the average (see Figure 4). The high standard deviations mean that no significant finding can be taken away from this comparison. Although Age Range 35-50 was another time frame where Controls exercised more than GOLD subjects, the standard deviations were higher than the averages for both Controls and GOLDs, therefore no significance can be derived from this finding either. The Age Range 50+ saw the greatest drop in physical activity for both groups, although the standard deviation again was higher than the average. The only conclusion from the analysis performed with a 5,000kcal/week cap is that chronic exercise at an early age (14-21) may have an effect on FEV_1 and FEV_1/FVC ratios later in life, although a larger sample size is necessary.

Next, we evaluated the perceived break point at 1,200kcal/week, above which the average kcal values became very high within the Age Range 14-21 (7,705.43kcal/week for GOLD subjects, 5946.92kcal/week for Controls). We also evaluated the break point at 700kcal/week, below which the average kcal values were 366.5kcal/week (GOLD) and 256.15kcal/week (Controls). We believe that beyond these two set points, the exercise

expenditures became either extremely high or very low; therefore, we termed everyone who performed above 1,200kcal/week "Conscientious Exercisers," and everyone below 700kcal/week "Mild Exercisers." We imposed the said limits on our values and performed a Chi^2 test comparing the numbers of Controls and GOLDs in the Mild Group to those in the Conscientious Group (see Figure 5). We found that within the Mild group, only three subjects had normal lung function while 6 had lung obstruction. On the other hand, within the Conscientious group 14 subjects had normal lung function while only 5 had obstruction ($P = 0.041$).

Upon this discovery, we evaluated whether or not the individuals who chose to be Conscientious Exercisers remained physically active throughout their lives. Indeed, we found that subjects who were very active at an early age (14-21) remained so to Age 22-34, but the likelihood that they remained similarly active from 14-21 until 35-50 was less strong. However, the strongest likelihood that active individuals remained so was between Age 22-34 until Age 35-50. In other words, if a subject started exercising heavily at Age 22-34, it was likely that he or she would remain so into the Age Range 35-50. However, no substantial correlation was found between physical activity at any Age Range and continued activity in Age 50+. These results suggest that people who become very active since an early age and smoke 10 pack-years throughout their lives will remain active until later adulthood (35-50), but will likely stop being active after age 50.

Overall, the above results suggest that the severity of exercise is less influential than the regularity of exercise performed on lung obstruction in later life. Because the average exercise values for both Controls and GOLD subjects below the 700kcal cap was so low (366.5kcal and 256.15kcal, respectively), it can be assumed that these individuals

did not regard exercise as an important part of their lifestyles. On the other hand, individuals with very high exercise expenditures since early life performed multiple sports regularly in teams, which could have established a habit of physical activity throughout their lives. Interestingly, the strongest likelihood that people will remain active once starting exercise is if they started at age 22-34, because most remained very active until age 34-50 (Pearson = 0.9094, P=0.0000).

Therefore, our data suggests that a regular regimen of lifetime exercise is correlated with better lung function values in individuals past the age of 45 who have smoked at least 10 pack-years. However, no correlation could be found between the intensity and quantity of lifetime exercise to current lung function values. Rather, it is the regularity of exercise maintained over time that shows a positive correlation with higher lung function in later life. This correlation is likely due to the immuno-suppressive effects of chronic moderate exercise on curtailing systemic inflammation. Two major mechanisms may be responsible for this.

First, chronic exercise throughout life maintains skeletal muscle mass, which in turn prevents the downward spiral that COPD-associated immobility inflicts on peripheral muscle dysfunction and cachexia. Because skeletal muscle has endocrine functions, primarily producing cytokines that shift the systemic balance from pro-inflammatory back to equilibrium, exercising to maintain muscle mass is crucial to homeostasis. Also to be considered is the effect of chronic exercise on maintaining diaphragm and breathing-associated muscle mass, as well as on the lungs itself. Chronic exercise indeed produces physiological changes throughout pulmonary vasculature and conducting airways, therefore improved lung function as a result of lifetime exercise in

lieu of smoking over 10 pack-years is not unreasonable. In addition, exercise helps prevent buildup of adipose tissue, which has been shown to be an additional endocrine organ altering the systemic cytokine balance towards a pro-inflammatory spectrum. With these considerations combined, chronic exercise should maintain healthy skeletal and breathing accessory muscle mass while lowering adipose tissue mass, enabling the body to maintain at least a neutral inflammatory state despite smoking's harmful effects.

In addition, chronic exercise could curtail lung Th1 immune pathway dominance as a response to tobacco smoke exposure by heightening the Th2 humoral pathway. Because murine studies showed that an excessive Th1 response is injurious to surrounding tissue through inflammatory-cell mediated cell necrosis, a shift from Th1 dominance to the antigen-producing humoral Th2 pathway could be beneficial to lowering TS-induced airway remodeling. However, note that specific dominance from either pathways is both an unlikely in vivo situation and injurious given the complexities of the immune system; it is a decrease away from an exaggerated response in either pathway back to neutrality that is most beneficial.

Speaking of moderation, acute exercise may be injurious to health by being either excessively immuno-suppressive, or by producing an unknown immune response when under prolonged TS exposure. Although we found that most Mild Exercisers were GOLD-classified with lung obstruction (6 vs 3 Controls), the average kcal/week expenditures of GOLDs and Controls in the Conscientious Exerciser group is interesting: 7,705.43kcal/week for GOLD subjects, 5946.92kcal/week for Controls. Subjects with lung obstruction who exercised over our perceived 1,200kcal cap were exceptionally active, while Controls over the cap were active, but not as much. Although this finding is

likely due to our small sample size, the unknown effects of acute exercise over a prolonged period should be noted.

Our results show a correlation between chronic lifetime exercise and a lower likelihood of lung obstruction in individuals over the age of 45 with a 10 pack-year smoking history. However, the study was hindered by several confounding factors, namely the difficulties of procuring an accurate assessment of lifetime exercise due to memory inaccuracies, a small sample size of only 28 subjects after exclusions and lack of study compensation for subjects. A funded study offering compensation to subjects will produce better results because subjects will be more receptive to coaching on memory recall and can spend more time during the interview process to recall more accurately. Also, a funded project can be more selective of subjects. Our study suffered from an abundance of Controls and a shortage of GOLD subjects because all subjects were convenience samples of an existing study. If subjects were paid, then a PFT could be conducted beforehand to determine lung obstruction before committing the required time for a full interview. Moreover, the LTPAQ had to be abridged so it could be administered to subjects within an acceptable time frame of a half hour. Prior experience with the older ROPECAR survey showed that volunteers became bored or uninterested after a half hour, therefore the extensive "coaching" and "memory probes" that were required by the LTPAQ were dropped for brevity to procure the most accurate assessment (via an interested study subject versus one who is in a hurry to leave).

Despite the study's shortcomings, we believe that our results have reasonable implications in COPD prophylactic measures and for pulmonary rehabilitation. Namely, exercise need not be severe, but merely moderate and consistent over time to produce the

best results. To our knowledge, the intensity of exercise required for maximal benefit in pulmonary rehabilitation programs has been a subject of debate for some time. Our results suggest not high intensity, but moderation and regularity to be beneficial. This has finding is germane, for a problem with pulmonary rehabilitation is the rate of attrition due to exercise loads and dyspnea-associated anxiety while performing rehabilitation. Our findings have useful implications for physicians who prescribe exercise regimens to adults with lung disease, for regimens that are enjoyable and not too rigorous should have higher retention rates than those with short bouts of strenuous activity. In addition, our modified LTPAQ can serve as a basis for future lifetime exercise recall questionnaires, if it is refined by a trained mathematician. A successfully reproducible survey should be useful across disciplines, ranging from cardiovascular health to diabetes to osteoporosis.

In the end, my study is an important first step towards greater epidemiological understanding of COPD pathogenesis, and the experience gathered will be invaluable to future endeavors to understand this illness.

APPENDIX

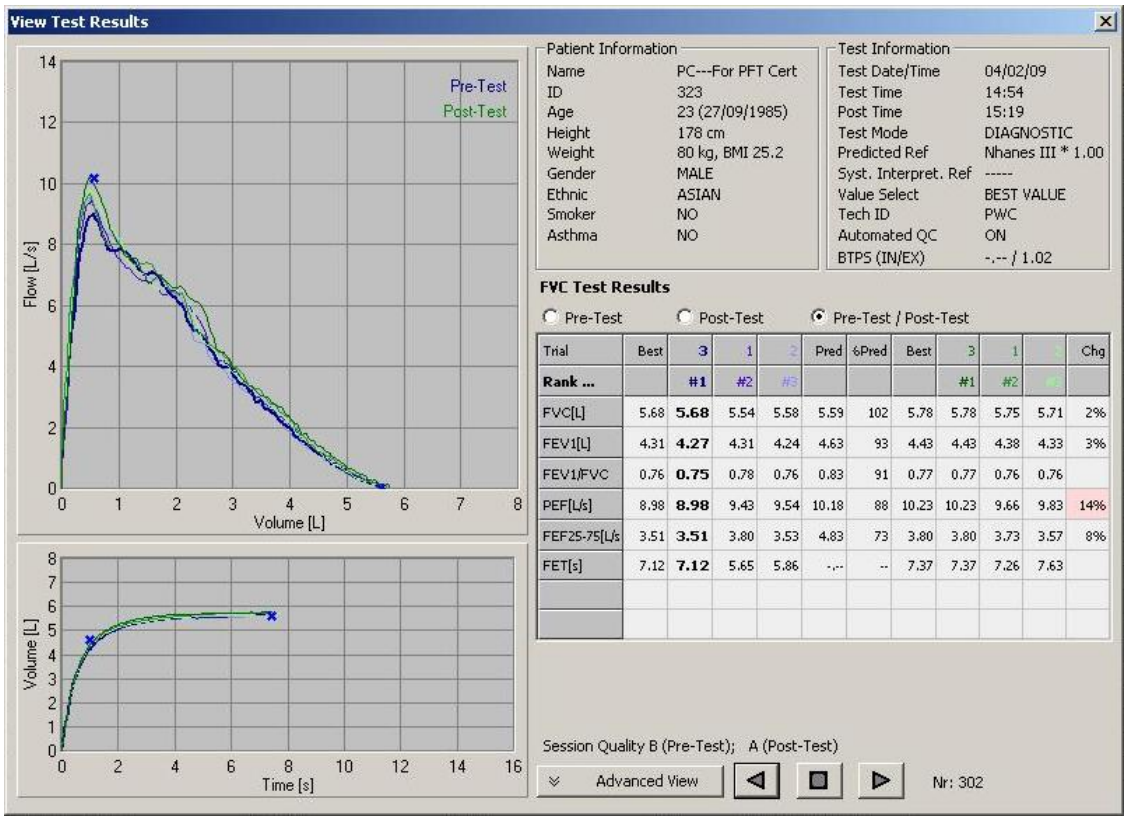

Figure 1a. A sample PFT performed with EasyWare measured using EasyOne Spirometer. The subject is a 23 year old Asian male, 178cm tall and 80kg. Because his Pre-bronchodilator administration FEV_1 is 93% of predicted and his FEV_1/FVC ratio is over 0.70 (0.91 actual), he is not classified as having lung obstruction, according to GOLD guidelines.

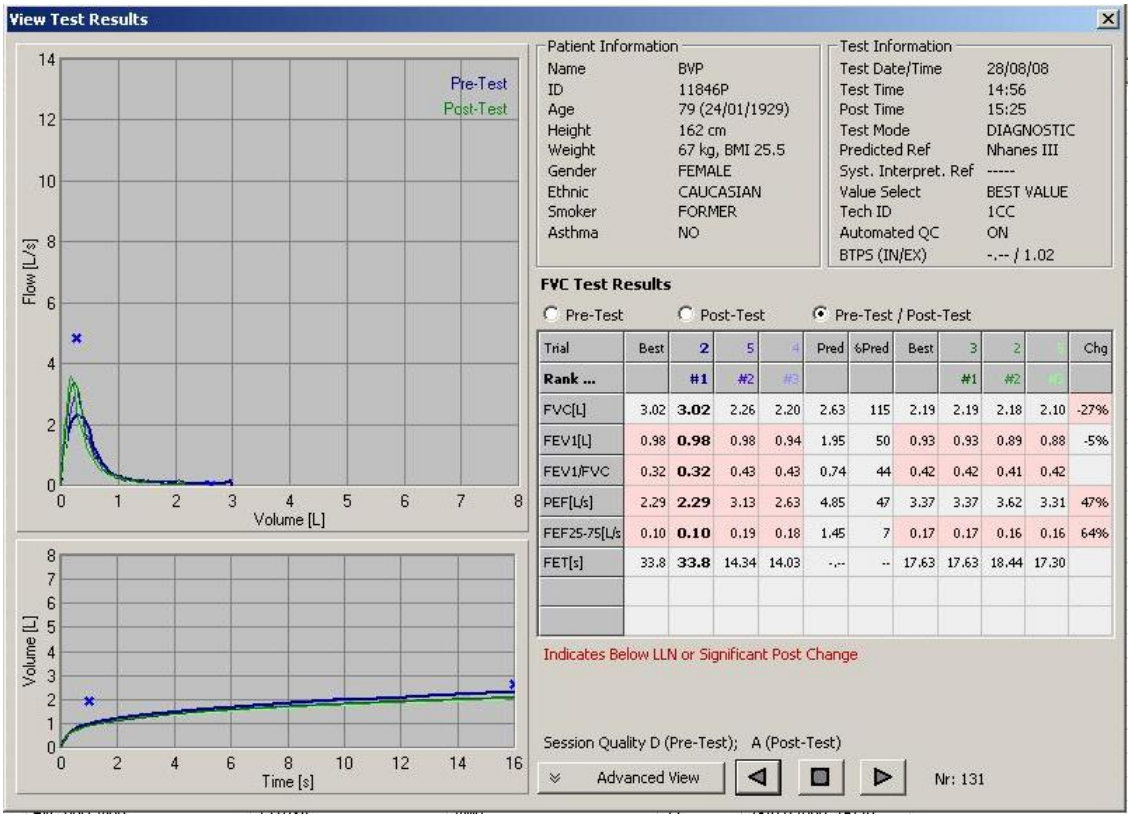

Figure 1b. A sample PFT of a 79 year-old Caucasian female subject with severely diminished lung function values. Her pre-bronchodilator FEV_1 is 50% of predicted and her FEV_1/FVC ratio is 0.44. The GOLD classification for her is borderline Stage 2-3 (moderate-severe).

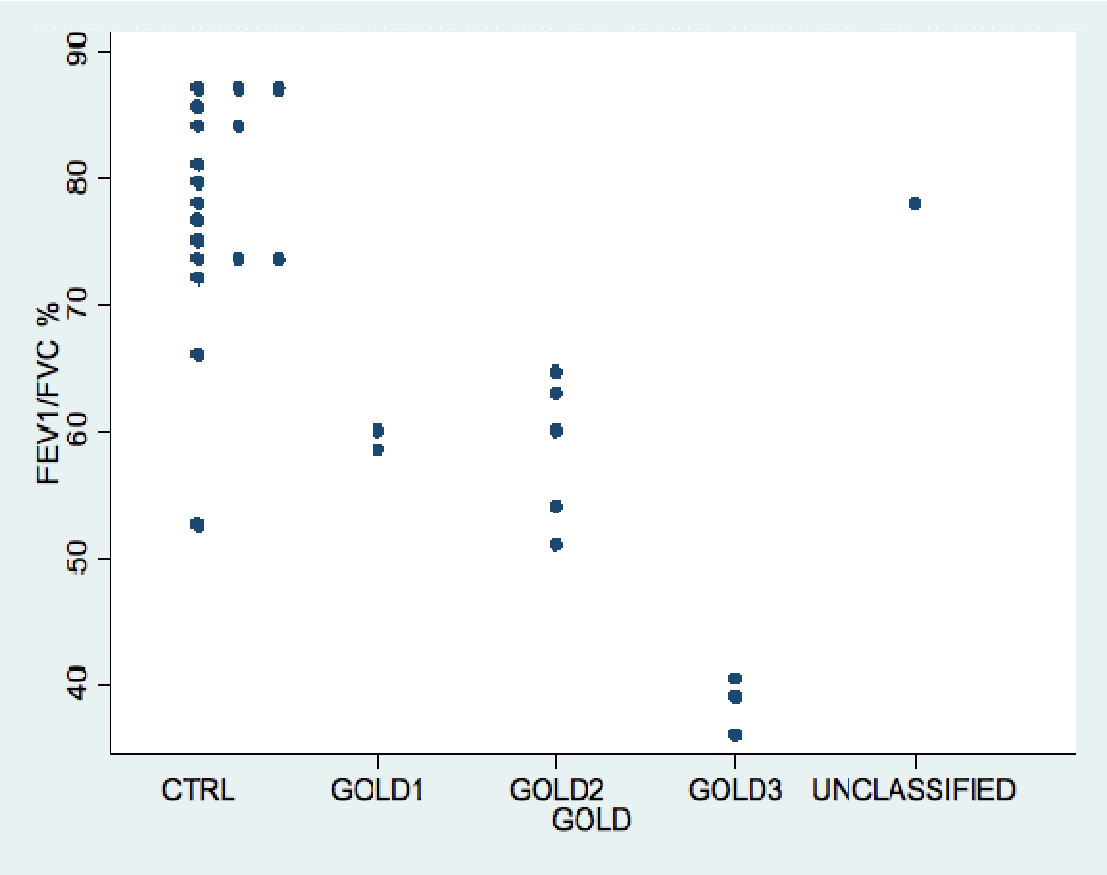

Figure 1c. A correlation between FEV_1/FVC ratios and GOLD classifications (n=30). Individuals with greater disease severity as defined by GOLD standards have lower FEV_1/FVC ratios. The unclassified subject would be considered GOLD2 due to a $_{Pre}FEV_1$ of 60%, however the subject's FEV_1/FVC ratio was too high at 0.775 (must be <0.70 to be labeled as having lung obstruction).

GOLD Severity	Pack-years	kcal 14~21	kcal 21~34	kcal34~50	kcal50+
CTRL	39.20588	5251.579	3287.72	1912.81	781.58
Std Dev	17.35841	4870.858	4526.642	2128.463	418.6319
N	17	16	15	12	3
GOLD1	50	11086.91	9196.6	575.155	1114.195
Std Dev	14.14214	.	.	582.762	1546.874
N	2	1	1	2	2
GOLD2	39.08333	7085.775	8512.307	5793.77	291.6933
Std Dev	9.779656	8262.923	13678.24	11472.63	127.9166
N	6	6	6	6	3
GOLD3	44.75	3319.72	7544.263	5831.204	.
Std Dev	13.93736	2784.839	10458.81	9761.756	.
N	4	4	3	3	0
Total	40.68966	5589.1	5288.761	3320.011	681.0263
Std Dev	15.08808	5500.397	8149.783	6719.474	720.7529
N	29	27	25	23	8

Figure 2. Summary of findings. For each disease stage group (i.e. Control or GOLD1-3), the average pack-year smoking history and average kcal/week expenditure for each Age Range is shown. Note the substantially high standard deviations for kcal/week expenditures. Blanks are due to software limitations when calculating naught values (0 for subjects who responded "sedentary").

Ages 14-21	Avg. Kcal/wk	Ages 22-34	Avg. Kcal/wk
GOLD:	2128.86090909091	GOLD:	1955.68272727273
Control:	3159.22058823529	Control:	1816.34294117647
Ages 35-50	Avg. Kcal/wk	Age 50+	Avg. Kcal/wk
GOLD:	651.067272727273	GOLD:	282.133636363636
Control:	1259.85705882353	Control:	137.925882352941

Figure 3. The average kcal/week values for combined GOLD1-3 subjects versus Controls are shown in each age category. The 5000kcal limit was imposed on Control and GOLD subjects who claimed to have exercised over 5,000kcal/week on leisure time physical activities.

GOLD	kcal 14~21	kcal 21~34	kcal34~50	kcal50+	age	bmi
CTRL	3159.279	1816.461	1260.151	138.7494	53.17647	28.91765
Std. Dev	1914.298	1958.602	1770.412	340.5737	6.885086	5.605046
N	17	17	17	17	17	17
GOLD1-3	2128.952	1955.865	651.2491	282.6791	57.81818	27.9
Std. Dev	2296.84	2060.377	817.353	654.575	7.84625	4.673542
N	11	11	11	11	11	11
Total	2754.508	1871.226	1020.94	195.2932	55	28.51786
Std. Dev	2094.765	1962.229	1482.073	482.2356	7.498148	5.192591
N	28	28	28	28	28	28

Figure 4. A reiteration of Fig. 3 with added Standard Deviation values and number of subjects (N) for each Age Category. Also included is the Average Age (with Std. Dev. and N) and Average BMI (with Std. Dev. and N) for Control and Combined GOLD group ("GOLD1-3"). Although the Age 14-21 and Age 34-50 Control groups averaged more kcal/week expenditure than their counterparts, the standard deviations are very high (Control: 3159.28kcal/week, 1914.29 Std. Dev.; GOLD: 2128.95kcal/week, 2296.84 Std. Dev.).

Kcal Cap	Control	GOLD	Total
700	3	6	9
	33.33	66.67	100.00
	17.65	54.55	32.14
1200	14	5	19
	73.68	26.32	100.00
	82.35	45.45	67.86
Total	17	11	28
	60.71	39.29	100.00
	100.00	100.00	100.00

Pearson chi2(1) = 4.1689 Pr = 0.041

Figure 5. A Chi^2 Chart with the 1,200 kcal/week cap in place for conscientious exercisers and a 700kcal/week cap for mild exercisers. Of GOLD subjects, 6 subjects were classified as "mild exercisers" while 5 were deemed "conscientious exercisers." Of Controls, 3 were deemed "mild" while 14 were deemed "conscientious," P = 0.041.

	Age	weight(kg)	bmi	Pkyr	Kcal:14~21	22~34
Kcal:22~34	-0.1395	-0.0924	-0.2845	0.2983	0.7016	1.0000
	0.4623	0.6274	0.1276	0.1093	0.0000	
Kcal:34~50	-0.0652	-0.1920	-0.2919	0.1765	0.5857	0.9094
	0.7323	0.3093	0.1176	0.3509	0.0007	0.0000

Figure 6. Pearson Correlation (top number) and P values (bottom number) for comparing kcal/week expenditures and Age Groups. The strongest correlation is between Age 22-34 and 34-50.

Supplement 1. ROPECAR Survey

Role of Prior Exercise in COPD and Recovery - ROPECAR

Revised 8-4-2008

Introduction

This questionnaire was designed to ask you about the average amount of activity/exercise that you have done at certain decades of your life. Overall, we would like to learn about the amount of activity that COPD patients have performed throughout their lifetimes. With the exception of sitting or sleeping, almost anything can count as an "activity" or an "exercise," depending on the intensity of actions. For example, standing and shuffling papers count as low-intensity activities while swimming or running count as moderate-intensity exercises.

We understand that it can be hard to recount the exact hours of exercise from many years ago, thus, we are only asking for a rough average that you can remember to the best of your ability. Because the questionnaire will become more complex, we designed the questions to become more complicated in a step-wise manner. Therefore, we would like to ask that you *do not skip ahead*. If you have any questions, please feel free to ask the researcher administrating this questionnaire.

Remember that there are no right or wrong answers—your unique experiences are important to the goal of this study.

ROPECAR Activity/Exercise Questionnaire

At what age did you begin smoking?
Age _____15_____. Age: 64

At what age did you quit smoking?
Age ____62_____.

From the age of **20-30** years, please estimate the hours of activity/exercise that you performed *on a weekly basis* of an "average year." This "average year" simply refers to what you assume to be a year that represents your activity/exercise level for this decade of your life. Note that if your job required physical exertion, you may include that as "exercise."

Hours per day: _____2_____.
Days per week: _____6_____.

How would you rate the intensity of your exercise during each quarter of an "average year" during this period? Please choose a number between 1 to 10, with 1 being least intense and 10 being most intense.

The following legend can be used to help you approximate your choice.

1 – Standing, shuffling papers.
3 – Walking at a medium pace, e.g. grocery shopping.
5 – Moderate aerobics; constant, elevated heart rate.
8 – Fast swimming, running, bicycling.
10 – Competition training, e.g. Tour de France

20-25
Intensity rating: _____4_____.

On average, how many packs of cigarettes did you smoke **per day**, per quarter during that age? (E.g. 1, 2½, etc.)
20-25
Packs per day: _____3/4_____.

Now, we are asking you to answer what we have asked you above *for the age of **25-30** years*. For the age of **25-30** years, please estimate—on a quarterly basis for an "average year"—the number of hours that you exercised per day, the intensity of your workouts, and the packs of cigarettes that you smoked per day.
25-30
Hours per day: _____2.5_____. Work: Insurance for schools, *extensive driving*, electronics sales, QC equipment (often flew)
Days per week: _____5_____.
Intensity rating: ____4_____.
Packs per day: ____1_____.

Please estimate again from the age of **40-50** years.
25-30
Hours per day: _____3.5_____.
Days per week: _____5_____.
Intensity rating: _____3_____.
Packs per day: _____1.2_____.

From age **30-35** years?
35-40
Hours per day: _____4_____.
Days per week: _____5_____.
Intensity rating: _____3.5_____.
Packs per day: _____1.5_____.

From age **40-45** years?
40-45
Hours per day: _____same_____.
Days per week: _____.
Intensity rating: _____.
Packs per day: _____.

From age **45-50** years?
45-50
Hours per day: _____0.5_____.
Days per week: _____7_____.
Intensity rating: _____2_____.
Packs per day: _____2_____.
Suffered depression, walking reduced significantly.

From age **50-55** years?
50-55
Hours per day: ____1_____.
Days per week: _____7_____.
Intensity rating: _____3_____.
Packs per day: _____1.5_____.

From age **55-60** years?
55-60
Hours per day: ____1.7_____.
Days per week: ____7_____.
Intensity rating: _____3_____.
Packs per day: _____1.5_____.

Thank you for taking the time to complete this questionnaire. If you have any additional comments that you would like to add, or if you would like to leave feedback regarding this survey, please write in the lines provided below:

Comments:

Supplement 2a. LTPAQ Page 1

RETROSPECTIVE PHYSICAL ACTIVITY SURVEY

Name: _____ ID no. _____ Date: _____

1. How often did you regularly participate in sports and leisure time physical activity, *excluding walking*? Please check the appropriate box.

	0-1hr/wk	2-3hrs/wk	4-7hrs/wk	>7hrs/wk
During high school and college? (14-21)?				
During years 22-34?				
During years 35-50?				
During years 50+?				

2. Were you considered more active than others your age and sex (Yes or No)?

	Yes	No
During high school and college (14-21)?		
During years 22-34?		
During years 35-50?		
During years 50+?		

3. How many miles did you normally walk each day?

	< 1 mile	1-2 miles	3-5 miles	>6 miles
Back and forth to grade school?				
Back and forth to high school and college?				
Back and forth to work during your 20s and 30s?				
During your 40s?				
During your 50s+?				

REMEMBER: 12 blocks or 20 minutes of brisk walking is equivalent to approximately 1 mile.

Supplement 2b. LTPAQ Page 2

4. Please indicate in the left-most column below all activities you have ever participated in with any regularity by placing a check in the YES column next to the activity. For each activity that you have identified participation, go across the entire row and answer for each time period: 1) the number of years you performed the activity out of the total number of years in that time period; 2) the average number of months per year you participated; 3) the average number of hours per week. If no regular participation in the activity in a particular time period, please indicate this by drawing a line through that time period.

Activity	Participate Regularly?		Age period 14-21 (8 years total). If yes, number of:			Age period 22-34 (13 years total). If yes, number of:			Age period 35-50 (16 years total). If yes, number of:			Age period 50+ If yes, number of:		
	Yes	No	Years	Mo/Year	Hrs/Wk	Years	Mo/Year	Hrs/Wk	Years	Mo/Year	Hrs/Wk	Years	Mo/Year	Hrs/Wk
Swimming														
Walking for exercise														
Running/jogging														
Bowling														
Tennis														
Golf														
Calisthenics														
Gymnastics														
Aerobic dance														
Bicycling														
Skating/ice, roller														
Hiking														
Softball/baseball														
Basketball														
Racquetball/squash														
Skiing/cross-country														
Skiing downhill														
Social dancing														
Volleyball														
Heavy outside work														
e.g. lawnmowing														
Other (describe below)														

The calculation formula for average kcal/week for a given time period is as follows:

MET of specific activity * ((years performed * months per year * 4 weeks per month * hours per week)/total years in time frame/52 weeks per year) * kilogram body weight.

Sample calculation: jogging (7.0MET) * ((1.5yrs*12mo/yr*4wks/mo*3hrs/wk)/8yrs in 14-21 age range/52wks per yr) = 3.63MET-hrs/wk * 63kg = 228.7kca/week average for Age 14-21

Supplement 3. Inclusion/Exclusion Criteria

Inclusion and exclusion criteria: (sourced from the COPDGene™ Protocol[69], pages 18-24):

COPD Subjects:

Inclusion Criteria

- Age 45 - 80 years
- Smoking history of > 10 pack-years
- Diagnosis of COPD Stages 1, 2, 3 and 4 by GOLD criteria (post-bronchodilator FEV1/FVC < 0.70)

Exclusion Criteria

- Concomitant respiratory disorder other than asthma or COPD (such as, but not limited to, diffuse bronchiectasis, cystic fibrosis, or interstitial lung disease)
- Lung surgery with removal of a lobe or more (including lung volume reduction surgery or lung transplantation)
- Lung cancer, known or suspected
- Surgical or bronchoscopic lung volume reduction
- Pregnancy or suspected pregnancy
- Uncontrolled cancer, defined as ongoing radiation therapy, ongoing chemotherapy, narcotics for pain control, or known metastatic disease
- History of radiation therapy to the chest (other than for breast cancer)
- Use of antibiotics and/or systemic steroids (new prescription or increased dose) for a COPD exacerbation or respiratory infection within the last month
- Inability to use albuterol
- First or second degree relative (parent, brother, sister, daughter, son, aunt, uncle, nephew, niece, half-sibling, grandparent, grandchild) of a subject enrolled in COPDGene™
- Subjects who indicate they are in more than one racial category
- Metal objects that may interfere with chest CT quantification including presence of a cardiac pacemaker, defibrillator, metal prosthetic heart valve, or metal shoulder prosthesis
- Subjects with affirmative answers to the following:
- chest or abdominal surgery in the past three months
- a heart attack in the last three months
- detached retina or eye surgery in the past three months
- hospitalization for any other heart problem in the past month
- Participation in the ECLIPSE study

Smokers without COPD

Inclusion Criteria

- Age 45 - 80 years
- History (current or formerly) of cigarette smoking > 10 pack-years
- Post-bronchodilator FEV1/FVC > 0.70.

Exclusion Criteria

- Physician diagnosed respiratory disease other than COPD or asthma (based on subject report)
- Lung surgery with removal of a lobe or more (including lung volume reduction surgery and lung transplantation)
- Pregnancy or suspected pregnancy
- Lung cancer, known or suspected
- Uncontrolled cancer, defined as ongoing radiation therapy, ongoing chemotherapy, narcotics for pain control, or known metastatic disease
- History of radiation therapy to the chest
- Use of antibiotics (new prescription or increased dose) for a respiratory infection within the past month
- Use of systemic corticosteroids (new prescription or increased dose) for a respiratory process within the past month
- Inability to use albuterol
- First or second degree relative (parent, brother, sister, daughter, son, aunt, uncle, nephew, niece, half-sibling, grandparent, or grandchild) of a subject enrolled in COPDGene™
- Subjects who indicate they are in more than one racial category
- Metal objects that may interfere with chest CT quantification including presence of a cardiac pacemaker, defibrillator, metal prosthetic heart valve, or metal shoulder prosthesis
- Subjects with affirmative answers to the following:
- chest or abdominal surgery in the past three months
- a heart attack in the last three months
- detached retina or eye surgery in the past three months
- hospitalization for any other heart problem in the past month
- Participation in the ECLIPSE study

Supplement 4. Sample Google Docs Database

19714M	Betts	Curtis	Years	Months/Year	Hours/Week	Total MET-hrs/wk	Years	Months/Year	Hours/Week	Total MET-hrs/wk	Years	Months/Year	Hours/Week	Total MET-hrs/wk	Years	Months/Year	Hours/Week	Years in Age Period	Total MET-hrs/wk
M, African, 51yrs	CONTROL smoker	No asthma	Age 14-21				Age 22-34				Age 35-50				Age 50+				
Activity	21.5	63	Years	Months/Year	Hours/Week	Total MET-hrs/wk	Years	Months/Year	Hours/Week	Total MET-hrs/wk	Years	Months/Year	Hours/Week	Total MET-hrs/wk	Years	Months/Year	Hours/Week	Years in Age Period	Total MET-hrs/wk
Swimming	8					0				0				0					#DIV/0!
Walking for exercise	3.5		1.5	12	3	1.817307692	3	6	3	1.11834319	3	6	1.5	0.454326923076	3	3	1.5	1	3.634615384
Running/jogging	7		1.5	12	3	3.634615384	3	6	3	2.23668639	3	6	1.5	0.908653846153	3	3	1.5	1	7.269230769
Bowling						0				0				0					#DIV/0!
Tennis						0				0				0					#DIV/0!
Golf						0				0				0					#DIV/0!
Calisthenics						0				0				0					#DIV/0!
Gymnastics						0				0				0					#DIV/0!
Aerobic dance						0				0				0					#DIV/0!
Bicycling	8		1.5	12	3	4.153846153	3	6	3	2.55621301	3	6	1.5	1.038461538461	3	3	1.5	1	8.307692307
Skating/ice, rollerskating						0				0				0					#DIV/0!
Hiking	5					0	3	7	2.5	1.55325443				0					#DIV/0!
Softball/baseball						0				0				0					#DIV/0!
Basketball						0				0				0					#DIV/0!
Racquetball/squash						0				0				0					#DIV/0!
Skiing/cross-country						0				0				0					#DIV/0!
Skiing downhill						0				0				0					#DIV/0!
Social dancing	4.5					0	5	6	2	1.59763313	2	9	1	0.389423076923					#DIV/0!
Volleyball						0				0				0					#DIV/0!
Heavy outside work, e.g. lawnmowing	5.5					0				0				0					#DIV/0!
Other (describe below)						0				0				0					#DIV/0!
Total MET-hrs/wk for each time period						9.605769230				9.06213017				2.790865384615					#DIV/0!
						605.1634615				570.914201				175.8245192307					
How often did you regularly participate in sports and leisure time physical activity, excluding walking?	0-1hr/wk	2-3hrs/wk	4-7hrs/wk	Over 7hrs/wk	(Answer)	How many miles did you normally walk each day?	< 1 mile	1-2 miles	3-5 miles	>6 miles	(Answer)								
During high school and college (14-21)?				X		Back and forth to grade school?	X												
During years 22-34?		X				Back and forth to high school and college?			X										

19714M	Betts	Curtis	Years	Months/Year	Hours/Week	Total MET-hrs/wk	Years	Months/Year	Hours/Week	Total MET-hrs/wk	Years	Months/Year	Hours/Week	Total MET-hrs/wk	Years	Months/Year	Hours/Week	Years in Age Period	Total MET-hrs/wk
During years 35-50?		X				Back and forth to work during your 20s and 30s?				X									
During years 50+?		X				During your 40s?				X									
						During your 50s+?				X									
Were you considered more active than others your age and sex?	Yes	No	(Answe																
During high school and college (14-21)?		X																	
During years 22-34?		X																	
During years 35-50?		X																	
During years 50+?	X																		
Spirometry	Post-Test	Change[%]	Pretest	%Pred(Pre)	%Pred (Post)	Years Smoked	Packs/day	Pack-yrs											
FVC[L]	3.96	8	3.68	97	104.380434	35	1.5	52.5											
FEV1[L]	3.21	5	3.07	102	106.651465														
FEV1/FVC[%]	81.1		83.4	104	101.131894														
PEF[L/s]	11.33	26	8.98	110	138.786191														
FEF25-75[L/s]	3.48	-2	3.54	116	114.033898														
FET[s]	7.28		6.8																

Supplement 5. Condensed Google Docs Database

ID	Race, Gender Age	Weight (kg)	Height in meters	BMI	GOLD, Smoker, Asthma (Y/N)	Pack-Years	KCALS/WEEKAge 14-21	22-34	35-50	50+	POST FVC	FEV1	FEV1/FVC %	PEF[L/s]	FEF25-75	FET[s]	% Predicted (Pre) FVC	%FEV1	%Ratio	%PEF	%PEFL
20991Z	W, M, 53	108	1.790180933447	33.7	CTRL, N, N	25	2254.03	230.06	0	0	4.47	3.53	79	8.76	3.19	10.53	103	84	82	62	44
20171N	A, M, 61	78	1.709195224252	26.7	CTRL, N, N	22	5580	0	0	0	3.84	2.83	73.8	6.71	2.33	12.64	93	96	102	74	93
20171N	A, M, 61	78	1.709195224252	26.7	CTRL, N, N	11	4182.2	0	0	0	3.84	2.83	73.8	6.71	2.33	12.64	93	96	102	74	93
21335W	W, F, 70	58	1.598457406156	22.7	CTRL, N, N	55.5	3410.79	702.7	1204.62	1204.62	2.42	1.75	72.4	5.11	1.22	8.63	76	76	100	75	72
20931H	W, F, 47	91	1.6288220358555	34.3	CTRL, N, P (Possible)	34	1888.74	218.08	717.5	0	3.64	3.16	86.7	8.55	3.96	6.66	107	106	98	99	111
21155U	W, F, 58	115	1.680938079035	40.7	CTRL, Y, N	35	0	1142.2	2113.13	0	3.48	2.91	83.6	6.77	3.22	7.47	101	98	97	81	90
20232H	A, F, 49	78	1.539746949564	32.9	CTRL, Y, N	16.5	14165.52	4310.31	3451.13	0	2.09	1.61	77	4.37	1.37	8.82	90	74	81	73	31
21223L	W, F, 51	88	1.640466853816	32.7	CTRL, Y, N	52.5	1270.07	45.56	0	0	3.61	3.03	84.1	6.98	3.17	9.98	91	103	111	117	156
20234L	W, M, 45	90	1.758631145281	29.1	CTRL, Y, N	46.5	11948.16	3930.18	7.79	0	4.66	4.05	87	8.91	4.66	6	96	105	109	88	133
12427A	W, M, 59	93	1.790780989323	29	CTRL, Y, N	67.5	1547.78	12368.45	0	772.62	6.34	3.34	52.6	6.08	0.5	30.4	97	81	83	74	47
20739P	W, M, 46	88	1.760281667602	28.4	CTRL, Y, N	28	3490.59	1462.15	91.39	0	5.65	3.73	65.9	8.21	2.05	18.71	114	92	81	76	50
21843P	W, M, 53	91	1.881716246177	25.7	CTRL, Y, N	49.5	5372.41	620.31	496.13	367.5	6	4.47	74.5	8.92	3.49	18.53	110	103	93	94	79
21447H	W, M, 46	71	1.730832340441	23.7	CTRL, Y, N	63	16373.66	14509.21	5931.23	0	4.26	3.7	86.9	9.93	5.07	6.69	88	98	112	101	135
21338C	W, M, 46	72	1.750379897974	23.5	CTRL, Y, N	48	163.28	1635.98	2907.7	0	5.25	4.12	78.4	9.38	3.86	10.36	108	97	90	80	65
19890G	A, M, 52	69	1.739630934846	22.8	CTRL, Y, N	45	6878.77	6560.31	5604.92	0	4.31	3.14	72.8	9.7	2.16	10.27	117	102	87	112	63
19714M	A, M, 51	63	1.711792206472	21.5	CTRL, Y, N	52.5	605.16	570.91	175.83	0	3.96	3.21	81.1	11.33	3.48	7.28	97	102	104	110	116
21330M	A, F, 56	81	1.469693845669	37.5	CTRL, Y, P	15	4894.11	1009.39	252.35	0	2.06	1.75	85	6.61	2.19	4.86	109	96	87	116	48
22355H	W, M, 75	92	1.640139774388	34.2	GOLD1, N, N	60	0	0	987.23	2208	2.83	1.64	58.1	5.42	0.6	17.01	74	58	78	70	25
21332Q	W, F, 56	106	1.750309634044	34.6	GOLD1, S, N	40	11086.91	9196.6	163.08	20.39	4.9	2.97	60.5	6.97	1.23	15.46	104	80	77	73	37
22437J	W, F, 68	65	1.548716883500	27.1	GOLD2 ??, Y, Y	52	179.88	550	0	0	1.79	1.38	77.5	4.46	1.17	6.54	67	60	89	49	34
21795E	W, M, 51	83	1.779870481607	26.2	GOLD2, Y, N	49.5	9624.75	7273.55	2043.08	0	5.41	2.91	53.9	5.66	0.9	18.25	102	65	64	49	29
22354F	W, F, 53	68	1.639434974487	25.3	GOLD2, Y, N	25	572.39	627.69	700.92	439.39	2.6	1.63	62.9	3.95	0.97	9.69	82	62	75	63	33
22481M	W, M, 53	67	1.699391371780	23.2	GOLD2, Y, N	30	540.8	432.92	324.7	216.46	3.82	2.46	64.4	5.2	1.41	11.35	99	55	56	48	14
19056A	W, M, 48	73	1.860031085710	21.1	GOLD2, Y, N	48	20903.27	35924.42	29141.92	0	6.04	3.27	54.1	7.79	1.1	13.34	104	73	70	72	29
20064M	A, F, 55	59	1.709032497996	20.2	GOLD2, Y, N	42	10599.58	4622.95	2332.77	0	3.63	1.84	50.6	4.76	0.66	11.69	88	58	65	37	20
21805H	W, M, 53	95	1.800644556572	29.3	GOLD2, Y, Y	40	273.86	2192.31	219.23	219.23	4.56	2.77	60.7	8.02	0.97	17.19	70	58	83	66	23
21573M	W, F, 59	85	1.609802290541	32.8	GOLD3, N, Y	42	632.17	380.24	257.46	0	2.8	1.13	40.5	3.76	0.35	14.43	61	31	51	51	11
21844R	W, F, 63	79	1.630929998477	29.7	GOLD3, N, Y	60	1218.37	0	0	0	2.24	0.81	36	3	0.24	11.85	72	31	42	52	8
22447M	W, M, 68, had pulmonary edema	77	1.661281622971	27.9	GOLD3, Y, N	50	5430.79	19546.15	17102.88	0	3.01	1.07	35.6	4.2	0.34	17.79	52	30	57	44	16
22167G	W, M, 50	77	1.780091087510	24.3	GOLD3, Y, Y	27	5997.55	2706.4	133.27	0	3.7	1.45	39.1	5.09	0.35	16.56	55	29	53	43	7
						Heavy Gold:															

Supplement 6. Spirometry Protocol

9a. Overview

Spirometry is a test that measures function of the respiratory system. It is one of the simplest, most effective tests available for the assessment of lung function. The spirometer registers the amount of air a subject breathes and the rate at which the air moves. The most common spirometric test requires that the subject take a full, deep breath and then exhale as forcefully as possible. The subject's effort is called the *forced expiratory maneuver* and most commonly only measures the amount and speed of air that is exhaled.

COPDGene® uses a spirometer (the ndd EasyOne™ Spirometer) that measures flow and volume by ultra-sound transit time. This spirometer meets American Thoracic Society spirometry standards.

Because the results of spirometry testing are used to determine the presence and severity of COPD, the measurement must be performed according to strict standards by technicians who have been properly trained and certified in how to conduct the maneuver. In addition, the equipment must be in good operating order and the calibration regularly checked. All spirometric maneuvers will be reviewed by a central reading laboratory (COPDGene® Pulmonary Function Core) to assure optimal quality of the data and to provide ongoing feedback to the pulmonary function technicians regarding the adequacy of the maneuvers.

9b. Summary of Measures

The following measurements will be obtained through spirometry testing during the COPDGene® clinic visits:

A. Forced Vital Capacity (FVC) is the total volume of air, expressed in liters, exhaled in a forced expiratory maneuver (the act of exhaling as hard and fast as possible after a maximal inspiration). The FVC is useful for detecting restrictive disorder, since lower than expected results may be a sign that the lungs cannot inflate as fully as normal. The FVC may also be reduced in people with more severe COPD and other obstructive disorders.

B. Forced Expiratory Volume at One Second (FEV_1) is the amount of air, expressed in liters, that a person breathes out during the first second of a forced expiratory maneuver. This is reduced in people with limitations such as COPD and asthma. The FEV_1 may also be reduced in patients with restrictive disorders.

C. The ratio of FEV_1 to the FVC (FEV_1/FVC) is the most sensitive and specific index of airflow limitation measured by a spirometer. It is obtained by dividing the FEV_1 by the FVC, and is expressed as a percent (i.e., 100 x FEV_1/FVC). *Note that the FEV_1/FVC ratio is the ratio of the absolute FEV_1 and absolute FVC in liters and is not usually expressed as a percent of predicted.*

D. Forced Expiratory Volume at Six Seconds (FEV_6) is the amount of air that a person breathes out during the first six seconds of a forced expiratory maneuver. Increasing interest is being shown in the FEV_6, and more particularly in the FEV_1/FEV_6 ratio, as an alternative to the FEV_1/FVC ratio. Use of the FEV_6 may be easier to obtain, particularly for patients with severe airflow limitation with long forced exhalation times.

E. The ratio of FEV_1 to the FEV_6 (FEV_1/FEV_6) is an alternative to the FEV_1/FVC ratio. A secondary objective of COPDGene® is to evaluate the utility of the FEV_1/FEV_6 ratio, particularly with respect to the assessment of COPD.

9c. Setting for Spirometry Testing

Spirometry testing ideally should be performed in a private, temperature-controlled room. All of the instruments necessary for the test should be in the room. The room should be well lit, preferably with a window, and located in a quiet area. These conditions will improve the quality and reproducibility of the results. For safety, the participant should be seated in a chair with no wheels; preferably the chair should have arms.

For some sites, it may be that testing will be done in non-clinical settings. The ndd EasyOne™ Spirometer is portable and has been shown to perform well in the field. Nonetheless, this document assumes that testing will be done in a centralized clinic facility. Sites planning to do otherwise should develop corresponding local procedures and document them in their local Manual of Procedures.

Clean mouthpieces (Spirettes™), nose-clips, and spacers should be available in the room, as should be a container to collect used Spirettes™ and used spacers. A box of facial tissue paper, paper plate or some type of container to place dentures on or in (if needed), and a trash can should be placed close to the participant. It also may be helpful for a source of drinking water to be nearby, as some subjects may get dry mouth as they are performing the maneuvers.

The ndd EasyOne™ Spirometer does not need calibration. However, a calibration check should be carried out daily to ensure that the spirometer is reading accurately. Instructions for performing the calibration check are in the ndd EasyGuide™ technical manual and appear in section 9n. The calibration syringe and adapter should always be stored next to the spirometer so that the temperature between the syringe and the spirometer are the same. This will avoid having large differences between room temperature and the spirometer temperature that could affect the results of the calibration tests. If there is the potential for a large temperature difference between the calibration syringe and the spirometer, the technician should pull and push the piston on the syringe several times to correct the problem. If spirometry is done in the field (outside of a clinical center), it is preferable to keep the spirometer and calibration syringe together overnight to avoid temperature differences at the time of calibration.

9d. Setting up the ndd EasyOne™ Spirometer

Prior to conducting the spirometry measurement, the technician should assure that the configurations of the EasyOne™ are set according to the specifications outlined under the "ndd Configuration Settings for Use in COPDGene®" below in sections 9j and 9p. Failure to have the correct settings may result in lost or deleted maneuvers, which will result in the participant being excluded from analysis in COPDGene®. Ideally, a single person should be designated as responsible for configuration of the EasyOne™ at each Clinical Center.

9e. Setting up the Computer to be Used with the Spirometer

Install the EasyWare™ software from the CD included with the ndd spirometer on all PC computers that will be used for spirometry the study. Note that a Macintosh computer can NOT be used with the spirometer. It is strongly recommended that the spirometer be connected to a PC with software installed during performance of spirometry in COPDGene™.

After installing the EasyWare™ software on your computer, follow the directions below to change the default settings:

1. Open the EasyWare™ program on your computer.
2. Open the "File" pull-down menu.
3. Select "Preferences"
4. Mark the "Screen Connector" button (***NOT*** USB Cradle or Serial Cradle)
5. Click "OK."

9f. Medication Use Prior to Testing

The subject's recent bronchodilator use needs to be recorded on the COPDGene® Spirometry Form. Some commonly used currently available bronchodilators and their classes are listed in the table in section 7d above. In COPDGene®, bronchodilators should not be withheld prior to the Study Visit. The reason for *NOT* withholding bronchodilators is to allow for all of the procedures to be completed in a single Study Visit at which time the informed consent will be signed immediately followed by all study procedures. While the assessment of bronchial hyper-responsiveness may be biased because bronchodilators are not withheld, the post-bronchodilator measurements should be unaffected, and it is the post-bronchodilator measurements that are used to define and assess the Stage of COPD.

9g. Bronchodilator Administration

In order to provide an assessment of bronchial hyper-responsiveness and to establish a diagnosis of COPD and the Stage of COPD, a "Post" bronchodilator spirometry will be performed in addition to the "Pre" bronchodilator test. In COPDGene™ study, **only albuterol** will be used as the bronchodilator. The brand names of some currently available albuterol HFA formulations are ProAir™ and Ventolin HFA™.

9h. Contraindications

The "Demographics and Physical Characteristics" and "Safety Assessment" forms should be completed prior to spirometry to assure that administration of the test or the bronchodilator does not pose a potential health risk. Since COPDGene® requires post bronchodilator assessment for COPD determination, if the participant is unwilling or unable to provide a post bronchodilator measurement, the subject will be excluded from the study. Specifically, spirometry testing should not be done if the subject has or reports any of the following:

- chest or abdominal surgery in the past three months
- a heart attack in the last three months
- detached retina or eye surgery in the past three months
- hospitalization for any other heart problem in the past month
- a resting pulse rate more than 120 beats/minute (participant should be sitting for at least 5 minutes prior to pulse rate determination)

In addition, if the participant exhibits any other co-morbidity (such as unstable angina or pneumonia) that, in the opinion of a site clinician, may affect the performance of the test or jeopardize the participant's safety, then spirometry testing should not be done. Indicate this on the Safety Assessment Form.

Note that the presence of a respiratory tract infection treated with an antibiotic in the four weeks prior to the visit is a contraindication to testing in the COPDGene® study; this is an issue of not only infection control but also for accurate diagnosis and Staging of COPD.

Ideally, sites could reschedule testing at a later date when the above situations are resolved. If participants are brought back later for spirometry testing, the site should contact the Data Coordinating Center for instructions on processing the data.

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