THESIS

Dynamic Relationship of Diffusing Capacity and Pulmonary Alveolar Vascular Recruitment during Exercise in Chronic Obstructive Pulmonary Disease

By

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For

Fulfillment of Requirements of Doctor of Philosophy

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Abstract

Background: Chronic Obstructive Pulmonary Disease (COPD) is a leading cause of global morbidity and mortality. COPD has multiple etiologies. Irreversible pulmonary-alveolar capillary damage is one of them which can be assessed by diffusing capacity of the lungs for carbon monoxide (DLCO).

Aims: The primary objective was to study the use of DLCO in predicting exercise limitation in COPD. The secondary objective was to evaluate the role of dietary nitrate precursor (beetroot juice) in improving alveolar gas exchange, pulmonary vascular function, and exercise intolerance in COPD. A third (mainly exploratory) aim was to study the expansion of pulmonary gas exchange surface area during exercise and its correlation with pulse wave velocity (PWV) as a surrogate of arterial stiffness.

Methods: 32 patients with mild to severe COPD were tested. Cycle ergometry on day 1 was performed. DLCO, noninvasive indices of gas exchange, pulmonary vascular capacitance, cardiac output (Qc), Exhaled nitric oxide (exNO), and other respiratory variables were measured before and after ergometry. Patients were randomized to 8 days of beetroot juice or placebo and on day 8 the above protocol was repeated. Effects of high nitrate juice intake on indices of arterial stiffness (Appendix D) were studied by aortic PWV and central aortic pressure (cAP) before and after exercise.

Results: Only the single breath DLCO relative to Qc and body weight were significant resting predictors of exercise intolerance. COPD patients who did expand gas exchange surface area during exercise relative to Qc had a more preserved exercise capacity. Beetroot juice showed a (non-significant) trend in improving exercise performance and pulmonary gas exchange surface area. The juice significantly lowered blood pressure, increased exNO and improved the patient overall wellbeing through objective scoring. The juice did not affect PWV before exercise in this cohort, but there was an effect of dietary nitrate on brachial systolic and pulse pressure, aortic pulse pressure and reflection magnitude determined from cAP.

Conclusion: The three sets of novel experiments showed that exercise limitation in COPD is affected by alveolar-capillary gas exchange impairment attributed to impairment of pulmonary capillary recruitment. DLCO is a good measure of pulmonary vascular health and exercise intolerance in COPD. Dietary nitrate did neither significantly improve alveolar gas exchange nor improved indices of arterial stiffness in this cohort, though positive trends were observed.

Acknowledgements

This study required a great deal of time, organization, patience, and help from others to complete. Cardiopulmonary exercise testing is an underappreciated science and the majority of clinicians are not interested in performing it because it is not only technically demanding but also intellectually challenging and difficult to interpret.

I have to commend my office staff including Angela, Becky, Vickie, and Angie for their dedication, enthusiasm, and hard work. Angie was instrumental in doing the exercise testing and her expertise was invaluable and without her talent, the project would not have come into fruition. She is one of the few respiratory staff in the whole country who could do the rarely performed intra-breath maneuver methodically and accurately.

My gratitude goes to my wife, Salome, for her organizational skills and her coordination of patient scheduling. She was extremely patient and supportive. David Sinks and Beth Anke from Care Fusion were very helpful with their teaching skills and with trouble shooting the challenging machinery and software. Beth came to my lab on several occasions and David made a special trip to Augusta to train my staff on the intricate technicality of the equipment despite his busy schedule.

I want to thank Courtney Wheatley and Alex Carlson from Mayo Clinic who helped us with procedural and logistical organizations. Courtney was very helpful in the IRB application process.

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mention my professional obligations and family responsibilities. But he kept me focused, encouraged, and optimistic about the final outcome. Dr. Johnson's passion for science, his enthusiasm for teaching, and his steadfastness for the success of my career goals is truly admirable.

I appreciate Dr. Avolio's mentorship and his supervision of the project. He has been very supportive of the whole project and walked me through this process diligently. I was very pleased to see him making a long journey to come to my research lab and showing great interest in the project.

Lastly, I want to thank Dr. Masud Behnia for helping me with the application process. His guidance and expertise were instrumental in getting me on the right research track and he was always very supportive of my academic and research ambitions.

Publications

Manuscripts (Peer reviewed Journals)

Behnia M, Wheatley CM, Avolio A, Johnson BD. Influence of Resting Lung Diffusion on exercise capacity in Patients with COPD. *BMC Pulmonary Medicine; 17 (1), 117. 2017*

Behnia M, Wheatley CM, Avolio A, Johnson BD. Alveolar-capillary reserve during exercise in patients with chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis; 12:3115-3122. 2017*

Behnia M, Wheatley CM, Avolio A, Johnson BD. Influence of dietary nitrate supplementation on gas exchange and exercise performance in patients with COPD. *Nitric Oxide; 76:53-61. 2018*

Conference Presentations

Behnia M, Wheatley CM, Avolio A, Johnson BD. Influence of lung diffusion on exercise capacity in patients with COPD. *Amer Coll Chest Phys Annual Conf, October 2016. Chest* Volume 150, Issue 4, Supplement, Page 891A

Behnia M, Avolio A, Johnson BD. Alveolar-capillary reserve during exercise in patients with chronic obstructive pulmonary disease. *Amer Coll Chest Phys Annual Conf, October 2016. Chest* Volume 150, Issue 4, Supplement, Page 851A

Behnia M, Wheatley CM, Avolio A, Johnson BD. Influence of dietary nitrate supplementation on lung function and exercise gas exchange in COPD patients. *Amer Coll Chest Phys Annual Conf, October 2017. Chest* Volume 152, Issue 4, Supplement, Page A791

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Abbreviations

AT	anaerobic threshold
ATP	adenosine triphosphate
BP	blood pressure
C_2H_2	acetylene
$CaCO_2$	arterial oxygen content
CH_4	methane
COPD	chronic obstructive pulmonary disease
CO	carbon monoxide
CO_2	carbon dioxide
COHb	carboxyhemoglobin
CPET	cardiopulmonary exercise testing
CRP	C-reactive protein
CvO_2	venous oxygen content
DLCO	diffusing capacity of lungs for carbon monoxide
ERV	end residual volume
exNO	exhaled nitric oxide
FE_{NO}	fraction of exhaled oxygen
FEV_1	forced expiratory volume in one second
$FEF_{25\%}$	forced expiratory flow at 25%
$FEF_{25-75\%}$	forced expiratory flow between 25 and 75%
FRC	forced residual capacity
FVC	forced vital capacity
GOLD	global initiative for obstructive lung disease
GxCap	pulmonary vascular capacitance

Hb	hemoglobin
IB	intra-breath
IBDLCO	intra-breath DLCO
IC	inspiratory capacity
IRB	institutional review board
IVC	inspiratory vital capacity
mMRC	modified medical research council scale
MVV	maximal voluntary ventilation
NO	nitric oxide
NOS	nitric oxide synthase
PCr	creatine phosphate
$P_{\bar{E}}CO_2$	partial pressure of exhaled mid expired CO_2
PEF	peak expiratory flow
$PetCO_2$	partial pressure of end tidal CO_2
PFT	pulmonary function testing
Q	cardiac output
Qc	pulmonary blood flow
RER	respiratory exchange ratio
SBDLCO	single breath DLCO
SGRQ	St. George respiratory questionnaire
TI	inspiratory time
TLC	total lung capacity
TNF	tumor necrosis factor
TTOT	total inspiratory cycle time
V_A	alveolar volume
V_D	dead space volume

VE	minute volume
$\dot{V}O_2$	oxygen consumption
$\dot{V}CO_2$	carbon dioxide production
V/Q	ventilation perfusion
V_T	tidal volume
VT	ventilatory threshold
WR	work rate

Chapter One

Introduction

COPD is a debilitating disease with a high economic burden, which is mainly caused by smoking, and is the fourth leading cause of morbidity and mortality in the world. Shortness of breath at rest and exercise are the major manifestations of this disease (1-3). One of the important goals of the clinicians is to determine what clinical markers or diagnostic tests are available to better understand and predict exercise limitation in patients with COPD. For years, textbooks recommended measurement of lung function such as spirometry to the extent that the majority of clinical studies published in the field focused on spirometric data and the pharmacological studies mostly emphasized the impact of interventions on spirometry in COPD.

While lung mechanics clearly play an important role, there are many other factors that contribute to exercise limitation such as heterogeneity of the disease process, lifestyle issues, such as diet and activity patterns, deconditioning, disease-related inflammatory processes, perception, as well as associated comorbidities such as cardiovascular disease (4, 5).

There are other physiological tests available that have a better predictive value than spirometry in assessment of clinical limitations and shortness of breath in COPD. One of them is diffusing capacity or DLCO, a test that has largely been underutilized by clinicians. DLCO is traditionally measured by the single breath (SB) method but it can also be measured by other less common methods, such as the intra-breath (IB) method. The intra-breath technique has been compared to other techniques such as rebreathe or open circuit and has been validated (6). One theory is that spirometry only

gives limited information regarding flow and volume; however, lung diffusion gives additional insight into gas transfer across the alveolar capillary membrane, as well as insight into pulmonary vascular health; also lung diffusion with exercise, gives insight into how the pulmonary capillary bed expands with an increasing cardiac output.

Although measurement of DLCO requires additional technical expertise, it can add a wealth of information that is simply not provided by spirometry especially when normalized to measurements of pulmonary blood flow (Qc), currently primarily a research technique. DLCO directly assesses functional surface area for gas exchange in the lungs, indirectly measures the volume of blood circulating through pulmonary vasculature and also sheds light on cardiovascular status of a COPD patient, and these cannot be assessed by spirometry alone. For example, a COPD patient with dyspnea and functional limitation may have relatively normal spirometry but significant alveolar-capillary impairment and thus the patient may be inappropriately treated (7).

With that in mind, we aimed to focus on several key questions that need further exploration in evaluation of the causes of exercise limitation in COPD population, particularly as it relates to the functional surface area of the lungs for gas exchange. Our aim includes three parts:

Part One

To quantify DLCO at rest and during exercise by using the Intra-breath (IB) method in a mild to severe COPD population. Our objective was to see if the IBDLCO method is a more sensitive measure of available gas exchange surface area than the typical SB method and if COPD subjects with the largest resting DLCO relative to pulmonary blood flow (Qc) have a more preserved exercise capacity. We preferred the IB over the SBDLCO technique because the latter is difficult to perform

requiring a 10 second breath hold at rest which is difficult for many COPD patients and more so during exercise.

Hypothesis: *DLCO will be a better predictor of exercise capacity, exertional intolerance and gas exchange abnormalities than typical airflow or lung volume measures.*

Part Two

1- To assess if the subjects who could increase their DLCO in proportion to the rise in Qc (maintenance of the relationship) would have a more preserved exercise capacity.

2- To analyze the relationship of DLCO and Qc to other noninvasive measures of respiratory gas exchange that have been associated with pulmonary vascular function. These included ventilatory efficiency, mixed expired to end tidal CO_2 ratio ($P_{\bar{E}}CO_2/PetCO_2$) as well as a more novel measure previously associated with pulmonary vascular capacitance in the heart failure population, GxCap.

Hypothesis*: A reduced ability to expand the pulmonary-capillary bed (measured as a reduced slope of the relationship between DLCO and Qc) in COPD patients with exercise will be associated with more severe disease based on quality of life (QOL) scores, but not necessarily associated with classic measures of air flow or lung volume.*

Part Three

In the last part of our study, we evaluated the role of beetroot juice as a dietary source of nitric oxide (NO), as a treatment intervention on lung diffusing capacity. Nitric oxide gas plays an important role as an inflammatory mediator especially in asthma and its measurement can be predictive of disease response to treatment and of exacerbation. However, NO is also a primary modulator of pulmonary vascular tone and a reduction of its

formation may negatively influence the ability to recruit and distend pulmonary capillaries. There are only a few studies describing the levels of NO and its importance in patients with COPD (8, 9).

1- We were also interested in determining if inorganic nitrate supplementation by use of beetroot juice, in our patients might subsequently improve exercise performance. We theorized that improving NO production in this population may improve the ability to recruit or distend pulmonary capillaries and therefore improve exercise capacity. Exhaled NO measurements were done before and after exercise testing in placebo and beetroot groups.

Hypothesis: *Supplementing the diet with high levels of nitrates and other nitric oxide precursors will improve lung surface area at rest and alveolar-capillary recruitment during exercise in patients with COPD.*

Part Four (Exploratory Aim)

To measure effects of high nitrate juice intake on arterial stiffness in COPD patients before and after exercise; we used a noninvasive device called SphygmoCor Xcel, and measured central arterial pressure waveform and pulse wave velocity to assess the stiffness.

To pursue our study aims, we recruited 32 patients with COPD. In the first part of the study, we measured NO and DLCO by different maneuvers during rest in addition to other classical lung function measurements. We then measured DLCO and other parameters during cardiopulmonary exercise testing and after its completion, and during recovery. The patients were then randomized to the beetroot juice group or placebo group for a period of 8 days. Thereafter, the exercise protocol was repeated and identical measurements during rest and exercise were taken.

Chapter 2 of the thesis is focused on a review of the literature. Its first section reviews the topic of COPD in detail which includes its definition, clinical diagnosis, subtype classification, pathology,

systemic and immunologic manifestations, and pharmacologic therapy. This is followed by in-depth review of pulmonary function testing (spirometry, lung volume, and diffusion capacity). The next section includes comprehensive review of cardiopulmonary exercise testing (CPET), its physiology and nomenclature, and pertinent clinical applications in health and disease states. The last section covers Nitrate-Nitrite-Nitric Oxide physiology, role of NO in COPD and asthma, molecular physiology of the pathway and other related topics.

Chapter 3 of the thesis deals with methodology. The first part covers IRB approval, patient selection, and informed consent. Study protocol is covered in the next part, followed by questionnaires and scales, and other study measurements including blood draw, nitric oxide measurement, and beetroot randomization. The subsequent parts systematically cover the following in technical detail: spirometry, lung volumes, diffusing capacity for carbon monoxide (IB and SB), cardiac output, CPET, and dietary nitrate.

Chapters, 4, 5, and 6, sequentially cover the three clinical studies that were performed. Study 1 examines the influence of resting lung diffusion on exercise capacity in patients with COPD. Study 2 studies alveolar-capillary reserve during exercise in patients with COPD. Study 3 evaluates influence of dietary nitrate supplementation on gas exchange and exercise performance in patients with COPD. These chapters include *summary, introduction, methods, statistical analysis, results, and discussion.*

These studies were published in peer-reviewed journals (10-12).

Chapter 7, summarizes the findings into a final *Conclusion* and enumerates the *Limitations* of project. This is followed by sections on *Future Directions*, and *List of Findings.*

The thesis comes to an end with *Appendices* and *Bibliography*.

CHAPTER TWO

Review of the Literature

A. Review of COPD

Chronic obstructive pulmonary disease or COPD is a respiratory ailment causing airflow obstruction. The disease causes shortness of breath as its major manifestation. Other symptoms include cough and sputum production in a subgroup of patients.

a) Definition

The National heart, Lung, and Blood Institute (NHLBI) and the World health Organization (WHO) started the Global Initiative for Chronic Obstructive Lung Disease (GOLD) project, which defines COPD as follows:

"COPD is a common preventable and treatable disease, is characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. Exacerbations and comorbidities contribute to the overall severity in individual patients" (1).

The major risk factor for the disease is smoking followed by indoor and outdoor pollutants, occupational hazards, and infections (2). About 80% of patients with COPD in the US are smokers but in some other countries of developing world, biomass fuel is a significant contributor. While the duration of smoking does have some correlation with the severity of disease, the exact threshold of nicotine exposure for the development of COPD is variable amongst patients. It appears that genetics and other environmental and occupational factors have some impact on the intensity of COPD. Some patients develop severe disease with fewer pack years of smoking and some hardly

develop any significant drop in their spirometry variables, with even greater than 30 pack years of smoking (13). Interestingly, 20 percent of patients who die from COPD have never smoked.

COPD is the third most common cause of death in the US, killing more than 120,000 individuals annually (3). The global burden of COPD is on the rise. In 2010 by the report of Center for Disease Control in the US, the total national cost for COPD was $30 billion in direct and $20 billion in indirect costs, totaling about $50 billion (14) . It is estimated that 16.4 million days of work are lost to COPD each year, signifying the impact of this debilitating disease. Early diagnosis and treatment of COPD is very important because it prevents further complications, reduces hospital admission and readmissions, and overall alleviates morbidity and mortality.

b) Diagnosis

The disease is universally diagnosed by pulmonary function testing (PFT) (15). One of the components of PFT is spirometry. The important values measured during spirometry are the forced expiratory volume in one second (FEV_1) and the forced vital capacity (FVC). When the post bronchodilator FEV_1/FVC on spirometry is less than 70%, typically COPD is diagnosed. Disease severity is classified based on GOLD criteria as *mild, moderate, severe, and very severe* (Fig. 2.1) (1):

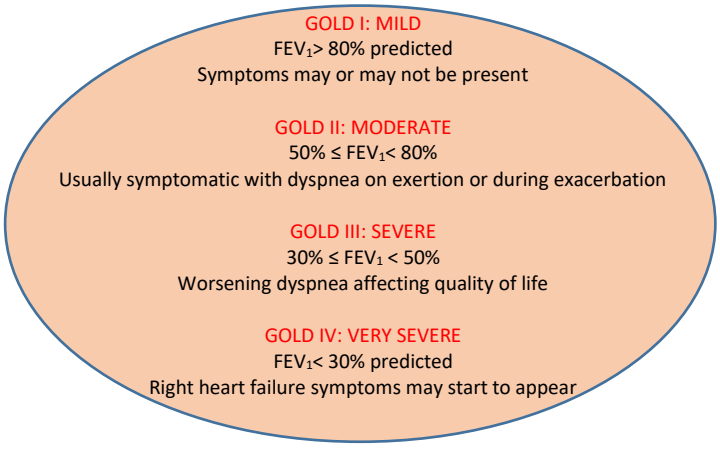


Figure 2.1. *Classification of severity of COPD based on postbronchodilator FEV_1* (1)

Using the GOLD numerical staging and number of exacerbations of COPD within the *preceding* 12 months, one can predict *future risk of exacerbation* by stratifying the disease into 4 groups (Fig. 2.2) (16):

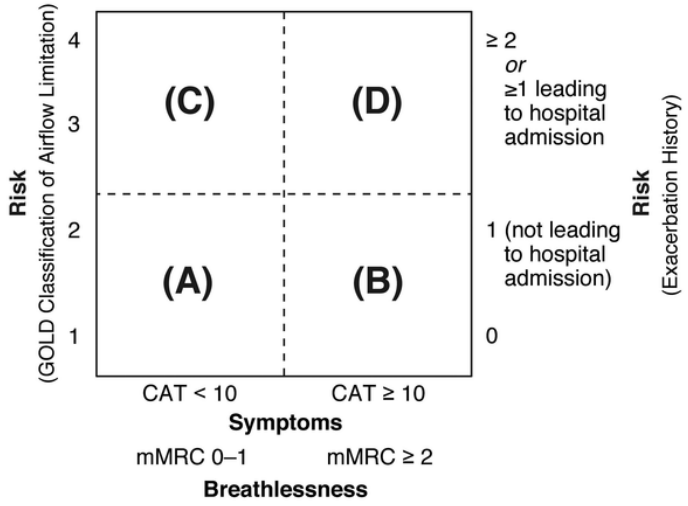
Group A: low risk, less symptoms: typically GOLD I or II (mild or moderate airflow limitation) and 0-1 exacerbation per year and no hospitalization for exacerbation, and mMRC grade 0 to 1 *(mMRC is modified Medical Research Council scale used only for assessment of breathlessness, Fig. 2.3, refer to GOLD (1))*

Group B: low risk, more symptoms: typically GOLD I or II (mild or moderate airflow limitation) and 0-1 exacerbation per year and no hospitalization for exacerbation, and mMRC grade 2 or greater

Group C: High risk, less symptoms: typically GOLD III or IV (severe or very severe airflow limitation) and/or 2 or greater exacerbations per year or 1 or greater hospitalization for exacerbation, and mMRC grade 0 to 1

Group D: High risk, more symptoms: typically GOLD III or IV (severe or very severe airflow limitation) and/or 2 or greater exacerbations per year or 1 or greater hospitalization for exacerbation, and mMRC grade 2 or greater

When assessing risk, choose the highest risk according to GOLD grade or exacerbation history. (One or more hospitalizations for COPD exacerbations should be considered high risk.)



Patient Category	Characteristics	Spirometric Classification	Exacerbations per Year	CAT	mMRC
A	Low Risk, Less Symptoms	GOLD 1–2	≤ 1	< 10	0–1
B	Low Risk, More Symptoms	GOLD 1–2	≤ 1	≥ 10	≥ 2
C	High Risk, Less Symptoms	GOLD 3–4	≥ 2	< 10	0–1
D	High Risk, More Symptoms	GOLD 3–4	≥ 2	≥ 10	≥ 2

Figure 2.2. *Schematic presentation of COPD clinical severity and classification based on severity of obstruction, mMRC, CAT, clinical assessment test score or dyspnea scale, number of exacerbations, and GOLD staging. From Global Initiative for Chronic Obstructive Lung Disease (GOLD) USA (1), reproduced with permission*

Grade 0	I only get breathless with strenuous exercise
Grade 1	I get short of breath when hurrying on a level or walking up a slight hill
Grade 2	I walk slower than people of same age on the level because of breathlessness; I have to stop for breath when walking on my own pace on the level
Grade 3	I stop for breath after walking about 100 meters or after a few minutes on the level
Grade 4	I am too breathless to leave the house or I am breathless when dressing or undressing

Figure 2.3. *Modified Medical Research Council dyspnea scale*

A limitation of using an absolute cut-off of FEV_1/FVC ratio of 0.7 in the diagnosis of COPD is that with healthy advancing age, the FEV_1/FVC decreases resulting in an overestimate of the incidence of the disease in older population. Therefore, some have advocated the fifth percentile lower limit of normal of the FEV_1/FVC ratio as a dividing line in COPD diagnosis (7).

Lung volume is another domain of lung function measurement that is not universally needed for diagnosis of COPD. However, it can be helpful to evaluate the cause of reduction in FVC such as hyperinflation, air trapping or in conditions causing a restrictive ventilatory defect.

Diffusing capacity for carbon monoxide (DLCO) is another subtype of PFT and is a robust index of the severity of emphysema (a subtype of COPD, see below) in airflow obstruction. The lower it is, the worse the severity of emphysema. DLCO and lung volume will be discussed in the subsequent chapters in detail.

Arterial blood gas measurement, chest radiography, and computed tomography are also helpful adjunctive tools in diagnosis of COPD, but are not mandatory for diagnosis. Arterial blood gas becomes useful in some clinical settings such as disease exacerbation, altered level of consciousness, and low oxygen saturation by pulse oximetry; it can also be very useful when FEV_1 reaches low levels and continuous oxygen supplementation becomes necessary.

c) Subtypes of COPD

Subtypes of COPD include emphysema, chronic bronchitis, and chronic obstructive asthma. There may be differences between the subtypes anatomically and physiologically, but their main feature is airflow obstruction. Correct diagnosis of each subtype is helpful in disease management, even though accurate diagnosis of subtypes at times can be difficult.

Chronic bronchitis: defined as a chronic productive cough for 3 months in each of two successive years in a patient whom other causes of chronic cough have been excluded (1).

Emphysema: it is a pathological term signifying abnormal and permanent enlargement of the airspaces distal to the terminal bronchioles and destruction of the airspace walls without fibrosis. Emphysema commonly occurs in subjects with moderate or severe airflow obstruction (1, 5).

Asthma: is a chronic inflammatory disorder of airways causing episodes of wheezing and breathlessness. The episodes are typically associated with airflow obstruction which can be reversible with treatment (17).

As it is shown in the Venn diagram below, there is a large degree of overlap among the subtypes of COPD (Fig. 2.4). Airway reversibility is a feature of pure asthma and is different from classical COPD where reversibility is not a feature, unless in overlap syndrome where features of both asthma and COPD are present. At times it is very difficult to differentiate between asthma with not complete airway reversibility and chronic bronchitis and emphysema with partial airflow obstruction reversibility. These asthmatics are classified as having COPD (subsets 6,7,8). Patients with emphysema or chronic bronchitis without airflow obstruction are not labelled as COPD (subsets 1,2,11).

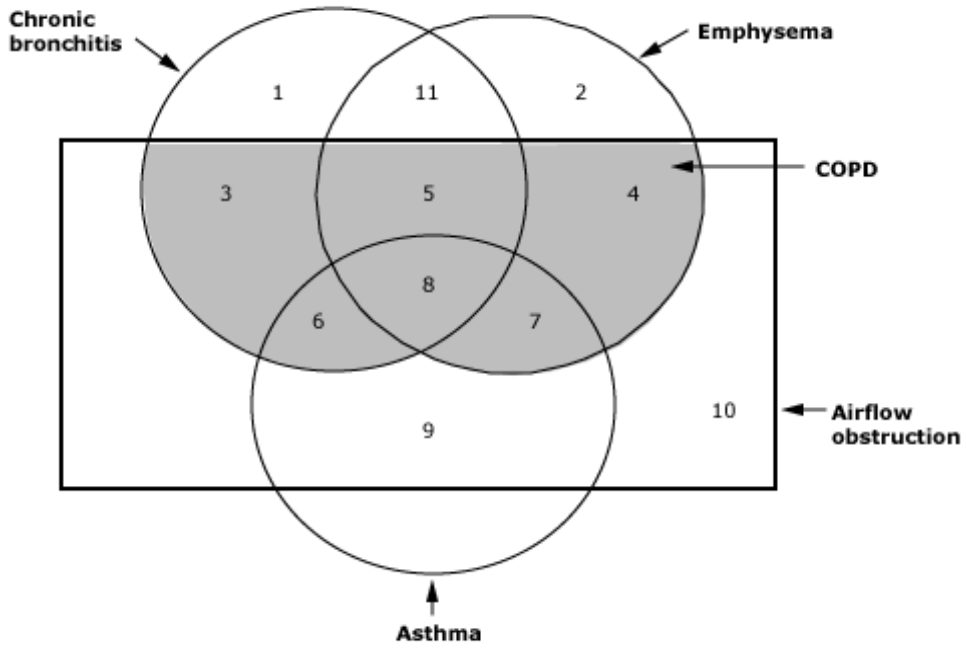


Figure 2.4*. Venn diagram depiction of subsets of obstructive airway disease. There are areas of overlap amongst different obstructive disease with some clinical phenotypes manifesting more than one disease type; i.e. chronic bronchitis, emphysema, COPD, and asthma. Shaded areas represent COPD. Airflow obstruction encompasses the majority of phenotypes. From Gibson, PG (18), reproduced with permission*

d) Pathology

The major pathology of COPD lies in the airways but lung parenchyma and vasculature can also be involved. The airways are chronically inflamed and the small airways may collapse as is seen in emphysema due to loss of tethering caused by destruction of alveolar wall (19). Emphysema destroys mainly the alveolar structures including ducts, sacs, and affected pulmonary artery capillaries (Fig. 2.5). In chronic bronchitis, mucus hypersecretion is noted while in asthma CD4+ T-lymphocytes, eosinophils, and increased Interleukin-4 and 5 are noted (20). In COPD when chronic hypoxemia prevails it causes constriction of small pulmonary arteries which in turn causes intimal hyperplasia and smooth muscle hypertrophy (21).

In COPD, inhaled irritants such as cigarette smoke stimulate fibroblast proliferation, leading to fibrosis of the small airways. Mucus hypersecretion is stimulated by cytokines such as connective tissue growth factor (Fig. 2.5) (4).

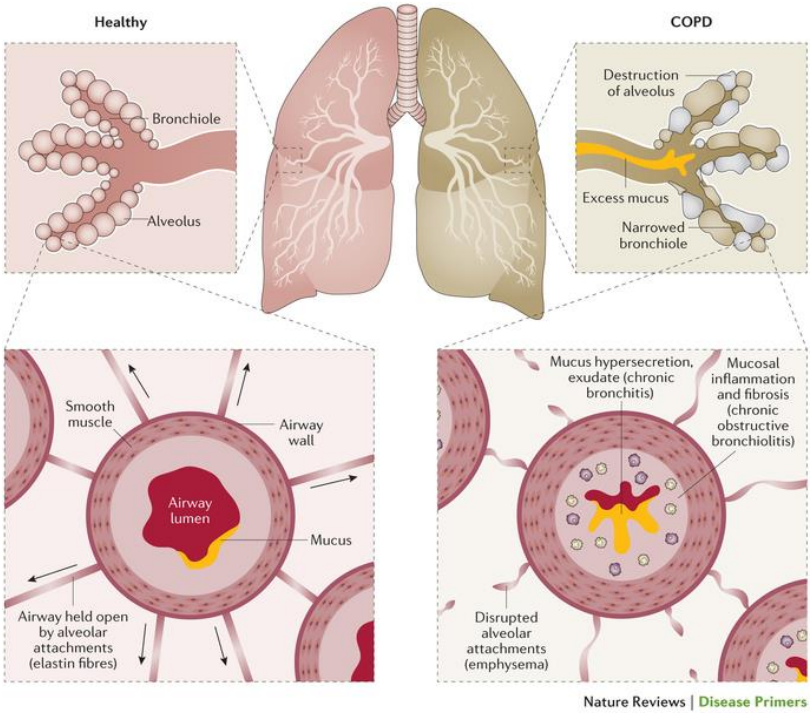


Figure 2.5. *Pathology of COPD. Destruction of lung parenchyma, collapse of airways, muscular hypertrophy, and inflammation are all features of COPD. From Barnes et al. (4), reproduced with permission*

e) COPD as a systemic disease

Circulating inflammatory mediators such as C-reactive protein (CRP) that are triggered by cigarette smoking in COPD, have an impact on other organs. Heart failure, left ventricular dysfunction, cardiomyopathy, and arterial stiffness are seen in more than 20% of patients with COPD. Other associations of systemic diseases include metabolic syndrome, hypertension, dyslipidemia, insulin resistance, and osteoporosis (22-24).

Therefore, COPD should be considered as a systemic inflammatory disease which can cause morbidity and mortality through various inflammatory pathways. For example, smoking increases levels of lipid peroxidation products and other markers, resulting in inactivation of antiproteases and increasing influx of neutrophils into lung tissues, promoting inflammation. Inflammatory cells are also increased in peripheral blood, including neutrophils and lymphocytes (25).

Furthermore, COPD patients have higher number of neutrophils in the lungs, in addition to an increase in tumor necrosis factor-alpha (TNF-α) and soluble TNF receptor. The T-cells in emphysema are predominantly T-helper cell type-1 phenotype which control the release of matrix metalloproteases. Cigarette smoke exposure induces secretion of proteolytic enzymes from cells of the innate immune system, which in turn release lung elastin fragments. Elastin is also present in arteries, arterioles and the skin; anti elastin autoimmunity in emphysema implicates an expanded role in diseases of elastin bearing organs such as skin and coronary vasculature (26).

As a systemic disease, COPD can cause cachexia and skeletal muscle abnormalities (27, 28). Decreased appendicular skeletal muscle mass is associated with low bone mineral density in men with COPD further exacerbated by frequent use of glucocorticoids. As the air flow limitation gets worse sarcopenia also intensifies (29). In COPD, atrophy of type II muscle fiber is noted (30). Some patients with COPD who have a higher energy consumption and lower fat free mass have higher levels of CRP and lipopolysaccharide-binding proteins (31). Interestingly, systemic inflammation may also blunt response to caloric supplementation (32). TNF-α can induce muscle loss by direct stimulation of protein loss, apoptosis of muscle cells, and oxidative stress-induced alteration in TNF-α (33, 34). Systemic inflammation and oxidative stress can facilitate muscle wasting synergistically (35). Loss of muscle mass causes early fatigue, exercise limitation, and shortness of breath (Fig. 2.6). Muscle loss is potentiated with use of steroids in COPD through blunting of muscle insulin-like growth factor-1 expression which in turn affects muscle protein turnover (36).

Diabetes is also seen in higher prevalence in COPD; it is hypothesized that systemic inflammation, circulating white blood cell, and lower serum albumin can all contribute to disease formation. TNF-α, IL-6, and CRP which are all cardiac risk factors, are all elevated in diabetes, too (37, 38). As for osteoporosis, prevalence of osteoporosis is significantly higher in afflicted males with COPD even in the absence of steroids. TNF-α is elevated in osteoporotic males with COPD and this cytokine

stimulates differentiation of macrophages into osteoclasts which causes bone lysis through a receptor activation member of TNF-α super family (39, 40).

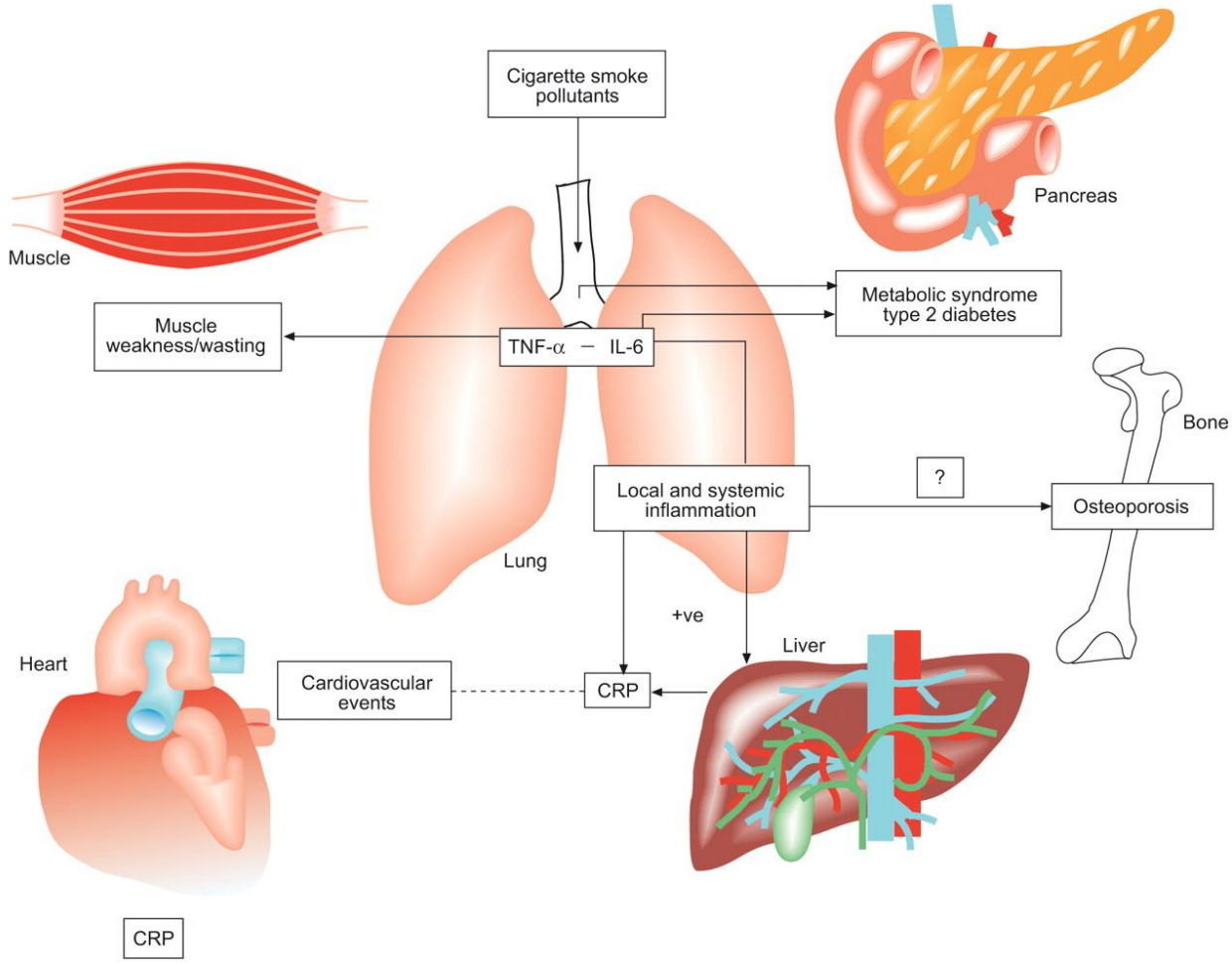


Figure 2.6. *The central role of inflammation in pathogenesis of COPD. Tumour necrosis factor (TNF)-α receptor polymorphisms are associated with pronounced severity of disease, which may be due to increased TNF-α effects. C-reactive protein (CRP) levels can also be elevated directly by TNF-α and other cytokines. Elevated CRP and fibrinogen can be very instrumental in the pathogenesis of cardiovascular illness. Reactive oxygen species released as a result of COPD may potentiate the likelihood of a patient developing cardiovascular illness, diabetes and osteoporosis. IL: interleukin; ?: unknown; +ve: positive. From Fabbri et al. (22), reproduced with permission*

f) Treatment

COPD management and treatment involve both pharmacological and nonpharmacological modalities. Smoking cessation, risk factor modification, vaccination, oxygen therapy and pulmonary rehabilitation fall under the umbrella of nonpharmacological interventions (Fig. 2.7).

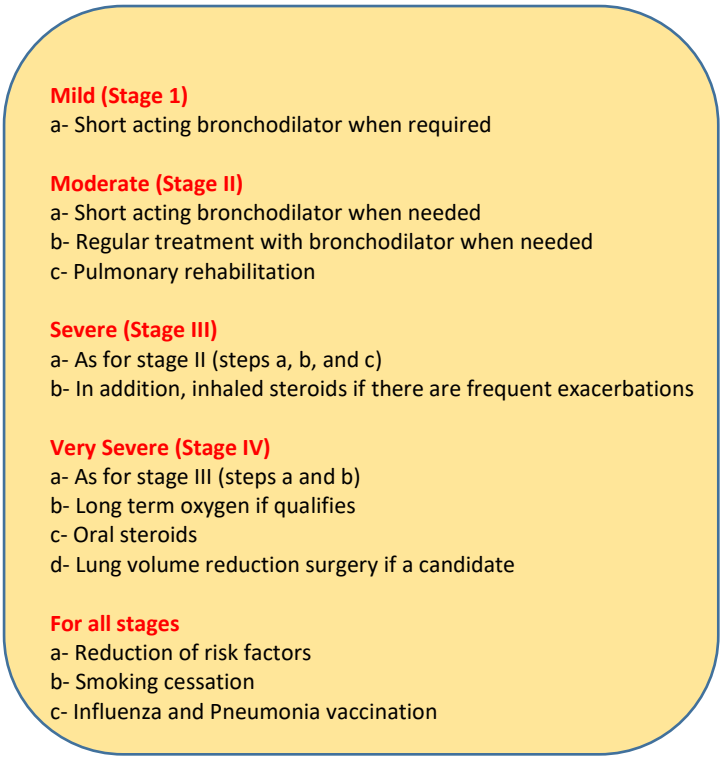


Figure 2.7. *Treatment approach to different stages of COPD*

Pharmacologic intervention encompasses several drug classes (41). The most important are inhaled *beta agonists*, coming in long acting and short acting forms, an example being albuterol. Beta agonists are bronchodilators, i.e. they dilate the smaller airways (Fig. 2.7). They improve symptoms and lung function as measured by spirometry. *Anticholinergics*, such as ipratropium or aclidinium, are another class of drugs that cause bronchodilation. They not only alleviate breathlessness, but also improve exercise capacity and improve lung mechanics. Beta agonists and anticholinergics can be used alone or in combination. But they have additive increase in FEV_1 when they are used in combination. *Inhaled glucocorticoids* are used in later stages of the disease in combination with beta agonists with the rational that COPD involves both airway and systemic inflammation and glucocorticoids suppress the inflammatory cascade.

In severe COPD with persistent symptoms despite inhaled steroids and bronchodilators, usually in GOLD stage III or IV disease, other classes of drugs can be considered. These include methylxantines (theophylline), phosphodiesterase-4 inhibitor (Roflumilast), oral glucocorticoid (prednisone), and mucoactive agents (N-acetylcysteine). Fig. 2.8 is a summary of treatment approach in COPD.

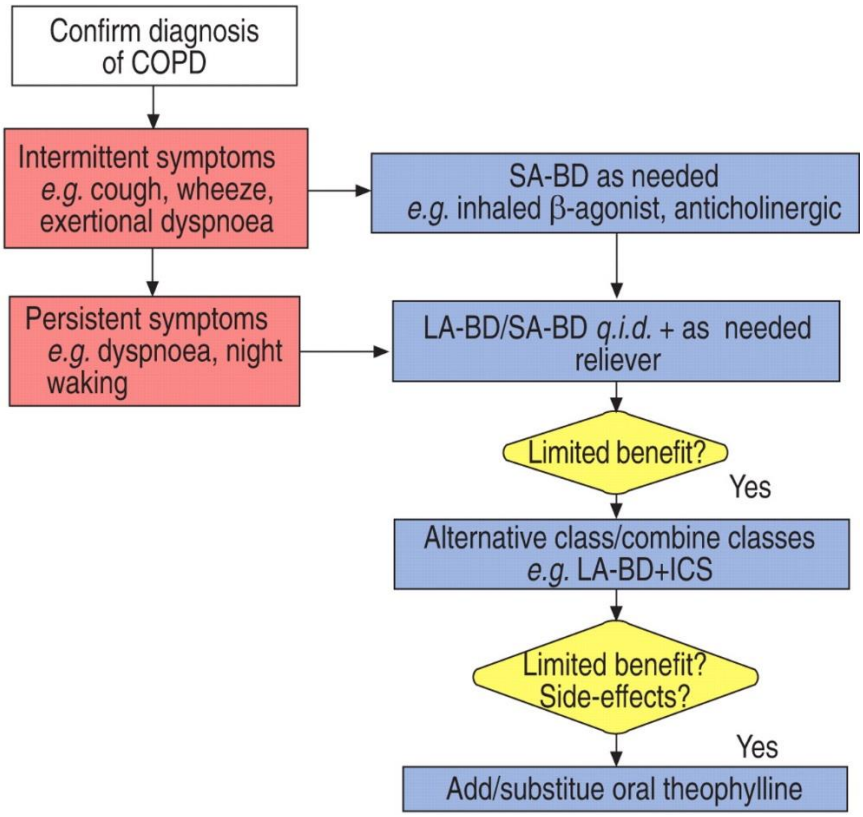


Figure 2.8. *Treatment approach to COPD. SA-BD: short-acting bronchodilator; LA-BD: long-acting bronchodilator; ICS: inhaled corticosteroid. ICS should be considered when FEV_1 is less than 50% and oral steroids and antibiotics are needed for exacerbation during the past year. From Celli et al. (7), reproduced with permission*

It should be emphasized that COPD is a systemic disease that its optimal treatment involves a multimodality approach (Fig. 2.9). While drug treatment is a very important cornerstone, pulmonary rehabilitation, smoking cessation and nutritional optimization are equally important.

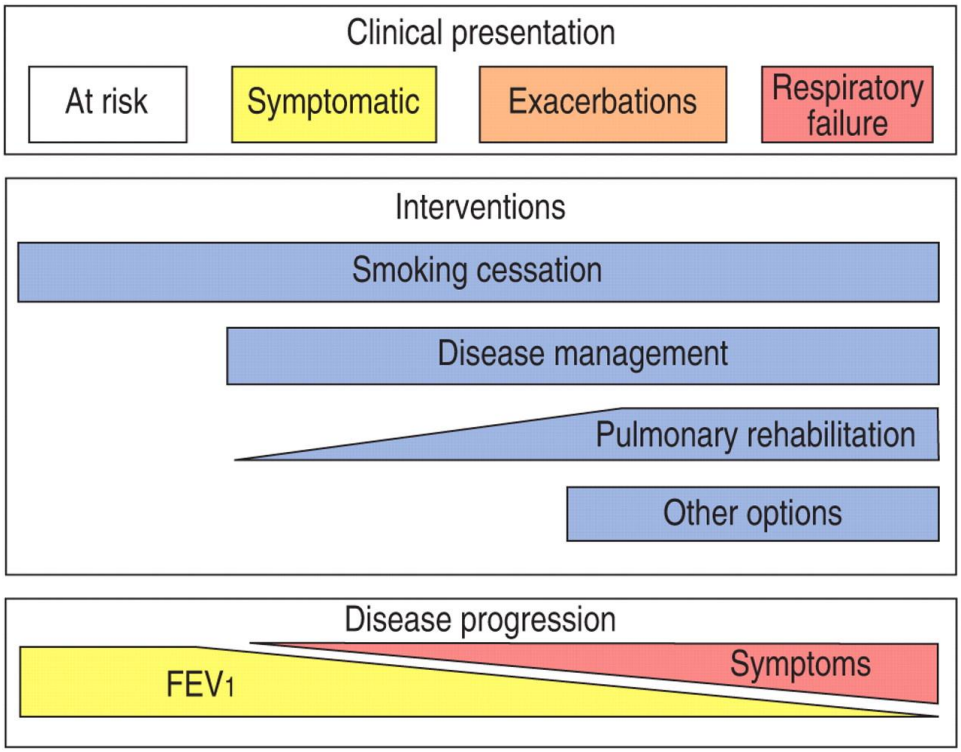


Figure 2.9. *Multimodality approach to treatment of COPD as a systemic disease. From Celli et al. (7), reproduced with permission*

B. Pulmonary Function Testing

Pulmonary function testing (PFT) is fundamental in diagnosis of COPD and other respiratory conditions. In this section the methodology of testing is reviewed.

There are 3 components to any PFT: *spirometry, lung volume, and diffusion capacity.*

a) Spirometry

This test measures the volume of exhaled air during forceful expiration at different time intervals. Spirometry includes 3 phases: 1) maximal inspiration, 2) forceful (blast) exhalation and, 3) continuous exhalation to the end. The subject's lips are sealed around a mouthpiece and normal inhalation and exhalation starts. Then at FRC (functional residual capacity) the subject takes a deep breath quickly to total lung capacity (TLC) followed by forceful exhalation until there is no more air to be expelled (residual volume) (Fig. 2.10). Several attempts (maximum of 8) are made until 3 good FEV_1 are obtained. Then the 2 largest FEV_1 readings that are within 0.150 liter of each other are accepted (42).

Forced vital capacity or FVC is the total amount of exhaled air and FEV_1 is the volume of air exhaled in one second (Figs. 2.11, 2.12). Dizziness or rarely syncope has been noted with forceful expiration. Patients are coached enthusiastically for correct performance. Start of test should be calculated correctly from time "zero" by the back extrapolation method. Computerized feedback to the technician is helpful in knowing when the starting criteria are not met. End of test is achieved by exhaling greater than 6 seconds and inability of the subject to continue further exhalation. Coughing or hesitation nulls the test. Other derived indices from spirometry include: a) $FEF_{25-75\%:}$ or mid expiratory flow in the mean forced expiratory flow between 25 and 75% of the FVC. B) PEF: in

liters/second is the peak expiratory flow from maximum forced expiration after the lungs are fully inflated. The value is obtained from flow volume curve (42).

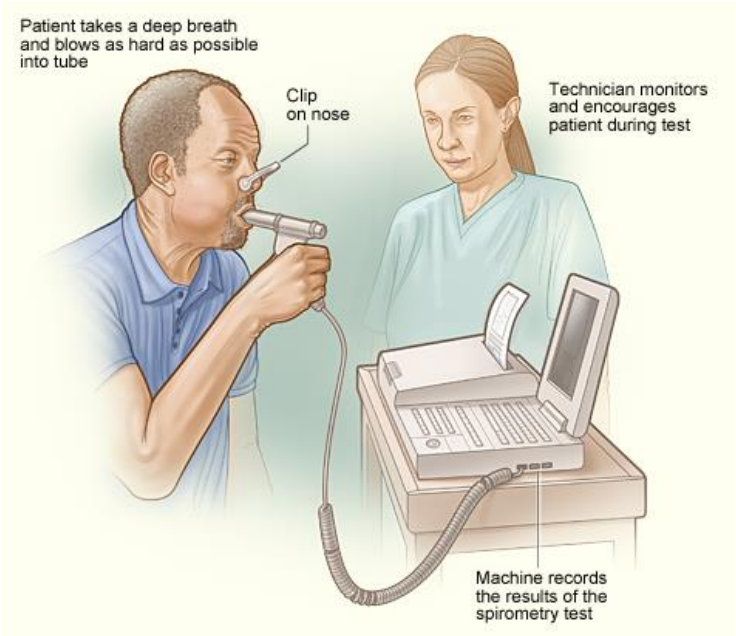


Figure 2.10. *Performing a spirometry test. Source: National Heart, Lung, and Blood Institute; National Institutes of Health; U.S. Department of Health and Human Services. (43)*

The ratio of FEV_1/FVC is a very important number used widely to differentiate between different disease patterns. As an example, in COPD this ratio is less than 0.7. Spirometry can be performed after use of a bronchodilator (albuterol) to assess reversibility of airflow obstruction. An increase in FEV_1 of at least 12 percent and 0.2 liters is required to document significant bronchodilator responsiveness (7)**.** In COPD, bronchodilator use can at times improve FEV_1 but if FEV_1 returns back to its baseline then COPD is ruled out (42).

It is recommended that at least a daily check of spirometry volume accuracy be made. System leaks should be checked daily and every quarter volume spirometers must have their calibration checked.

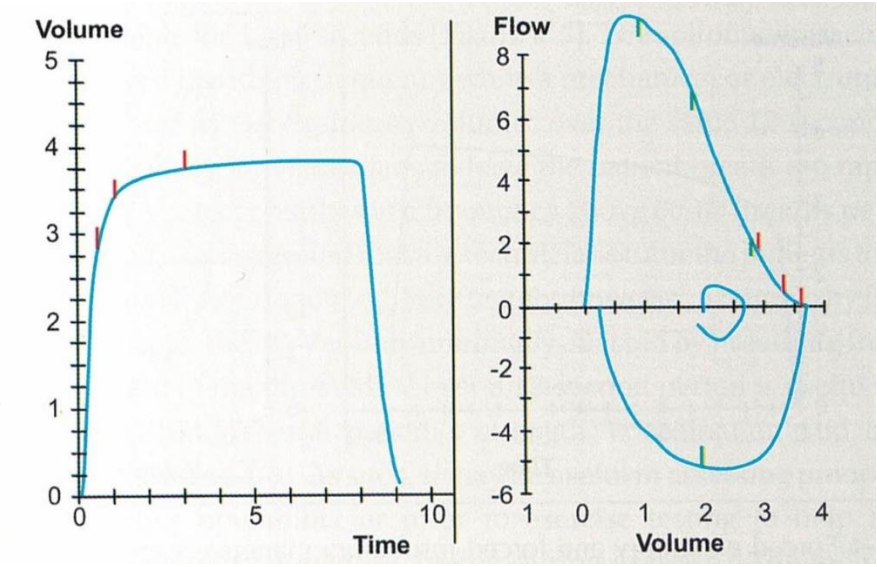


Figure 2.11. *Forced expiratory and forced inspiratory maneuvers on time-volume (left) and volume-flow (right) tracings of a man. In volume-flow, expiratory (upper) and inspiratory (lower) phases are noted. FEV_1 is 3.30 L and FVC is 3.70 L. From Hansen, JE (44), reproduced with permission*

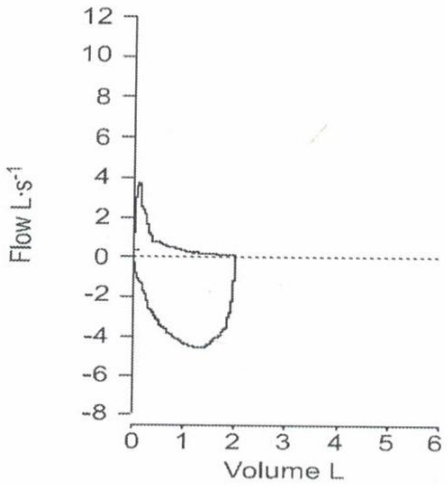


Figure 2.12. *Flow-volume loop in a patient with COPD with severe airway obstruction depicting concavity of the expiratory loop. From Miller MR, et al. (42), reproduced with permission*

b) Lung Volumes:

Lung volume measurements commonly used are vital capacity, total lung capacity, residual volume, and functional residual capacity. Lung volume nomenclature includes 4 capacities (total lung, inspiratory, functional residual, inspiratory vital) and 4 volumes (inspiratory reserve, tidal, expiratory reserve, residual). Combinations of volumes are defined as capacities (Fig. 2.13).

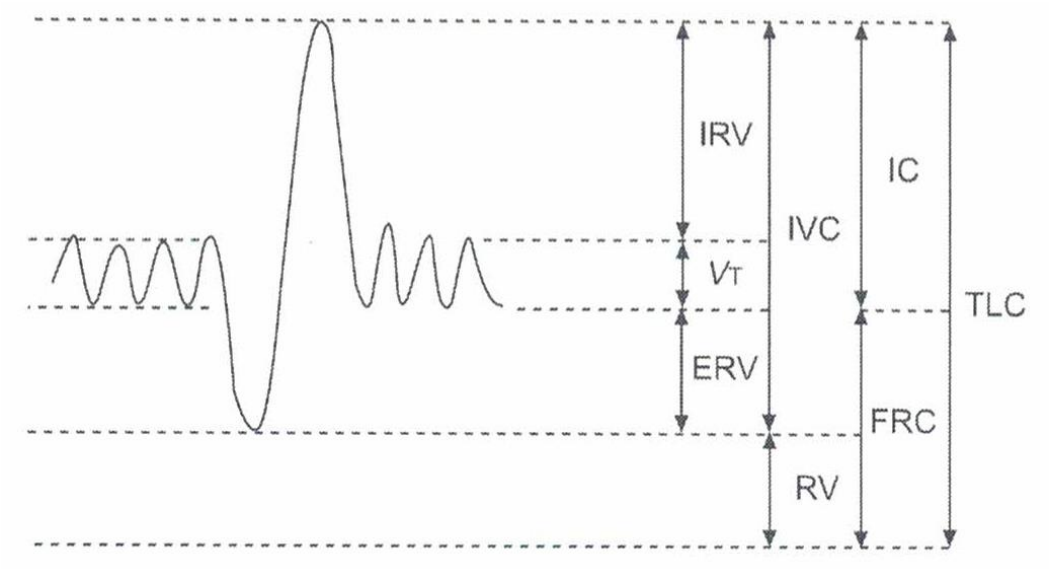


Figure 2.13. *Static lung volumes and capacities based on a volume-time spirogram of an inspiratory vital capacity (IVC). IRV: inspiratory reserve volume; VT: tidal volume (TV); ERV: expiratory reserve volume; RV: residual volume; IC: inspiratory capacity; FRC: functional residual capacity; TLC: total lung capacity. From Wanger et al. (45), reproduced with permission*

Definitions of some of the important measurements are as follows:

FRC: volume of gas present in the lungs at end expiration during tidal breathing; **ERV:** volume of gas that is maximally exhaled from end expiratory level during tidal breathing; **IC:** maximum volume of gas that can be inspired from FRC; **TLC:** sum of all the volumes of compartments or volume of the lungs after maximal inspiration.

The gold standard in accurate measurement of lung volume (which refers to volume of gas within the lungs) is body plethysmography. It uses the concept that in a closed compartment (body box) with two compartments, the change of pressure and volume in one compartment (lung) would synchronously change the pressure and volume within the box (Boyles Law). In this method, the subject sits quietly within a sealed box with a volume of close to 600 liters and performs panting maneuvers between 60 to 90 times per minute against a closed shutter which causes the pressure and volume within the thorax to change. Simultaneously, the volume and pressure within the box

will also change. FRC can be measured by closing the shutter at the end of a quiet expiration. Then residual volume is calculated as: RV= (FRC – average ERV). TLC is calculated from (FRC + average IC).

But plethysmography method requires heavy, spacious and expensive equipment and therefore some labs use alternative techniques such as helium dilution and nitrogen washout. The caveat with the latter two is that they may underestimate lung volume in moderate to severe COPD because of lack of access of the gas to under or non-ventilated lungs (45). Helium dilution and nitrogen washout are collectively named as *gas dilution techniques*. We used nitrogen washout in our laboratory for our experiments (44).

In the *helium dilution* technique patients breathe the gas mixture at a pre concentrated level. At FRC the patient breathes helium and air until equilibrium is reached. Then FRC can be calculated. The helium technique is simple and inexpensive but a disadvantage is that it does not measure gas trapped in the lung bullae. Helium is not absorbed across the alveolar membrane.

Nitrogen washout can be utilized to calculate lung volume. Lungs contain nitrogen with an atmospheric concentration of around 80%. In the test, oxygen is delivered at 100% to lungs which slowly replaces nitrogen in the lungs causing concentration of nitrogen to fall. The exhaled gas is collected until its nitrogen content reaches 1%. Then the volume can be calculated with the following formula:

$$V_1 \text{ X } [FEN_2]_1 = V_2 \text{ X } [FEN_2]_2$$

Where V_1 is initial volume of nitrogen and $[FEN_2]_1$ is the initial exhaled nitrogen concentration before the time the breathing valve direction was changed at FRC; V_2 is the volume of collected gas and $[FEN_2]_2$ is the final nitrogen concentration. By having the values of $[FEN_2]_1$, $[FEN_2]_2$, and V_2, then V_1 which is the volume of lungs at FRC can be obtained.

Nitrogen washout takes 7 minutes to complete. Over this time period the nitrogen concentration approaches zero. When nitrogen concentration is below 1.5% the test is considered successful. It may take up to 10 minutes in severe COPD especially with bullae where N_2 is trapped. Because of movement of nitrogen from fat or surrounding air through skin into the lungs and mouth, it is not advisable to collect the gas beyond 10 minutes. Nose clip and mouthpiece must be used to prevent leakage of N_2 from the atmosphere (44).

The FRC is measured and ERV and IC can also be linked to the measurement (45). Although the advantages and disadvantages of nitrogen washout are similar to helium dilution, one additional disadvantage in severe COPD is the potential to retain carbon dioxide due to the pulmonary vasoconstriction effect from breathing 100% oxygen which exacerbates ventilation-perfusion or V/Q mismatch (Fig. 2.14).

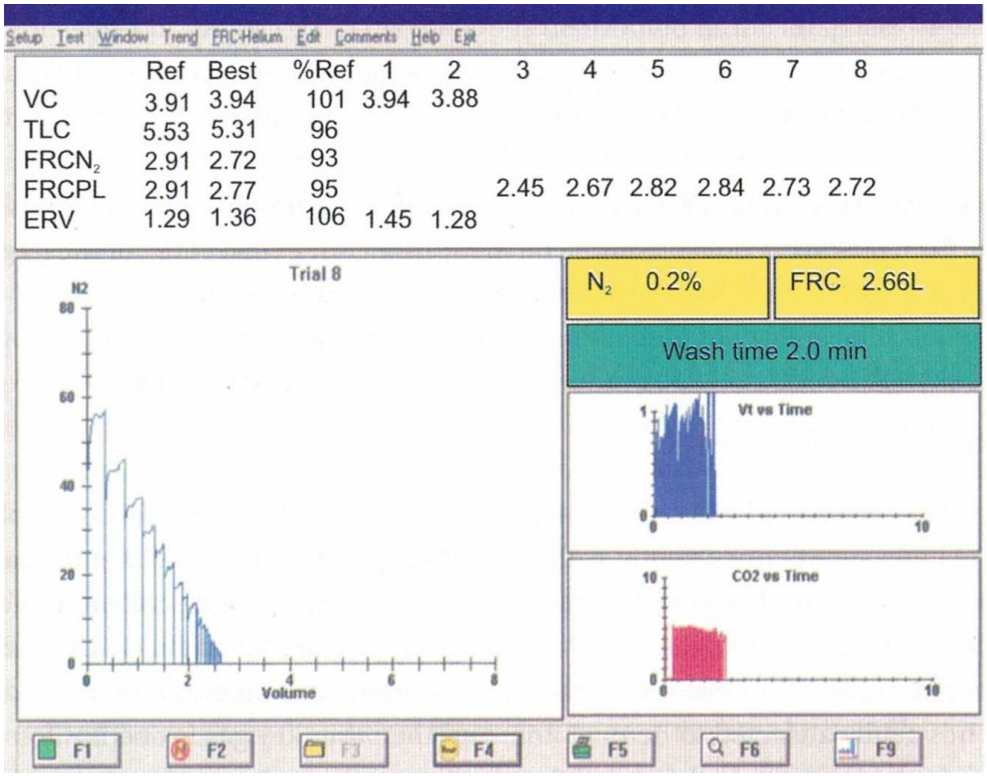


Figure 2.14. *Nitrogen washout measurement of FRC. On the right panel, there are tidal volumes (blue) and end-tidal CO_2 values (red) against time. The left panel shows N_2 concentration against washed-out volume of the lungs from which point the N_2 has been removed. When the N_2 concentration became very low, in this case 0.2% or after 2 minutes, the washout volume was stopped and the volume was recorded, which in here was 2.66mL. This is FRC. From Hansen, JE (44), reproduced with permission*

c) Diffusion Capacity

Since our research is heavily involved with measurement of diffusion capacity in COPD, we will discuss this topic in more detail. Carbon monoxide (CO) uptake from the lungs (K_{CO}) in the single breath method is measured as fall in concentration of alveolar CO per unit time divided by CO driving pressure ($P_{A,CO}$).

The rate of disappearance of CO from alveolar gas during breath-holding = K_{CO} (units: min^{-1}). K_{CO} is calculated as $\log_e [CO_0/CO_t]/BHT$ where CO_0 and CO_t are the alveolar CO concentrations at the

beginning and end of the breath-holding time (BHT). When K_{CO} is multiplied by alveolar volume (V_A) which is the volume of gas containing CO in the lung *(K_{CO} x V_A)* then "the transfer factor for the lung for CO" or "the diffusing capacity for CO(DLCO)" is obtained (46).

When CO is transferred from the environment to the lung, it goes through several physiological steps. First, it is transferred from the atmosphere to the alveoli through airways. Then it mixes and diffuses in the alveolar ducts, air sacs, and alveoli. When CO crosses the alveolar membrane, it moves from a gaseous to liquid interface. Subsequently it is mixed in the alveolar capillary plasma followed by diffusion across the red blood cell membrane. When CO gets into the red blood cell it undergoes a chain of reactions with blood hemoglobin (Fig. 2.15). The surface area of alveolar-capillary interface is so large that can approximate a tennis court, facilitating gas exchange between pulmonary circulation and the environment (46).

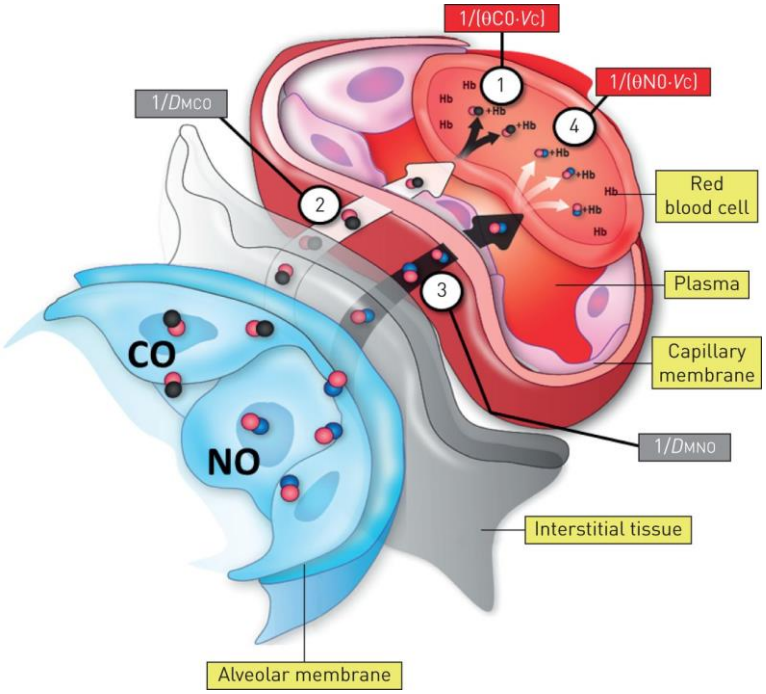


Figure 2.15. *Components of carbon monoxide or DLCO uptake. 1/DLCO represents the total resistance to CO uptake. $1/D_M$ constitutes the membrane resistance (resistance from the alveolar membrane to the red cell membrane). The diffusion and chemical combination resistance (red cell resistance) within the erythrocyte is $1/(\vartheta \cdot V_C)$. From Zavorsky et al. (47), reproduced with permission*

The CO uptake is contingent upon two transfer or conductance properties. One is diffusion properties of the alveolar-capillary membrane (D_M or membrane conductivity). The other is binding of CO and hemoglobin (Hb) which is the product of hemoglobin volume of alveolar capillary blood (V_C) by CO-Hb chemical reaction rate (θ) or (V_Cx θ) (hemoglobin conductivity) (Fig. 2.15). Therefore:

Binding of CO and Hb = CO-Hb chemical reaction X volume of Hb in alveolar capillaries (ϑ x V_C)

(*CO uptake = membrane conductivity + hemoglobin conductivity)*

Since the system is considered to be a combination of conductances in series, the equation will be solved as:

$$1/DLCO = 1/D_M + 1/ \vartheta V_C$$

Extrapulmonary reduction in lung inflation (reduced V_A) 1) Respiratory muscle weakness; 2) Thoracic deformity preventing full inflation
Diseases that decrease V_C x θ 1) Anemia; 2) Pulmonary emboli
Reduction of D_M and V_C x θ 1) Lung resection; 2) Emphysema; 3) Interstitial lung disease; 4) Pulmonary edema
Increase in V_C x θ 1) Polycythemia; 2) Left to Right shunt; 3) Asthma
Other causes of increase in V_C x θ 1) Exercise; 2) Supine position; 3) Hb binding changes

Table 2.1. *Conditions affecting diffusion capacity in the lungs. Adopted from Macintyre et al. (46), reproduced with permission*

Any factor that affects DM, θ, or V_C, affects the DLCO. These include an increase in D_M (by lung inflation), or an increase in alveolar capillary blood volume (V_C) by (exercise, polycythemia, or left to

right shunt) which all increase DLCO (48). Reduction is caused by emphysema, edema, or lung resection, affecting $1/(\vartheta \cdot V_C)$. Some conditions that decrease DLCO by reducing D_M and $\theta.V_C$ include emphysema, pulmonary edema, pulmonary vasculitis, pulmonary hypertension, and pulmonary embolism (46). Summary of the above is provided in Table 2.1.

Therefore, DLCO reflects a more comprehensive state of the lung physiology than for example spirometry, which reflects simply airflow rates through the tracheobronchial tree. DLCO reflects both anatomical features (gas volume, length of diffusion, alveolar-capillary membrane thickness and area, volume of blood in alveolar capillaries) and functional features (ventilation-perfusion matching and mismatching, membrane diffusion characteristics, hemoglobin binding properties) of the lung. A summary of alveolar-capillary gas exchange, partial pressures of oxygen, and hemoglobin dissociation curve is depicted in Fig. 2.16. It should be emphasized that as the lungs age and the subject gets older, DLCO starts to decline (Fig. 2.17).

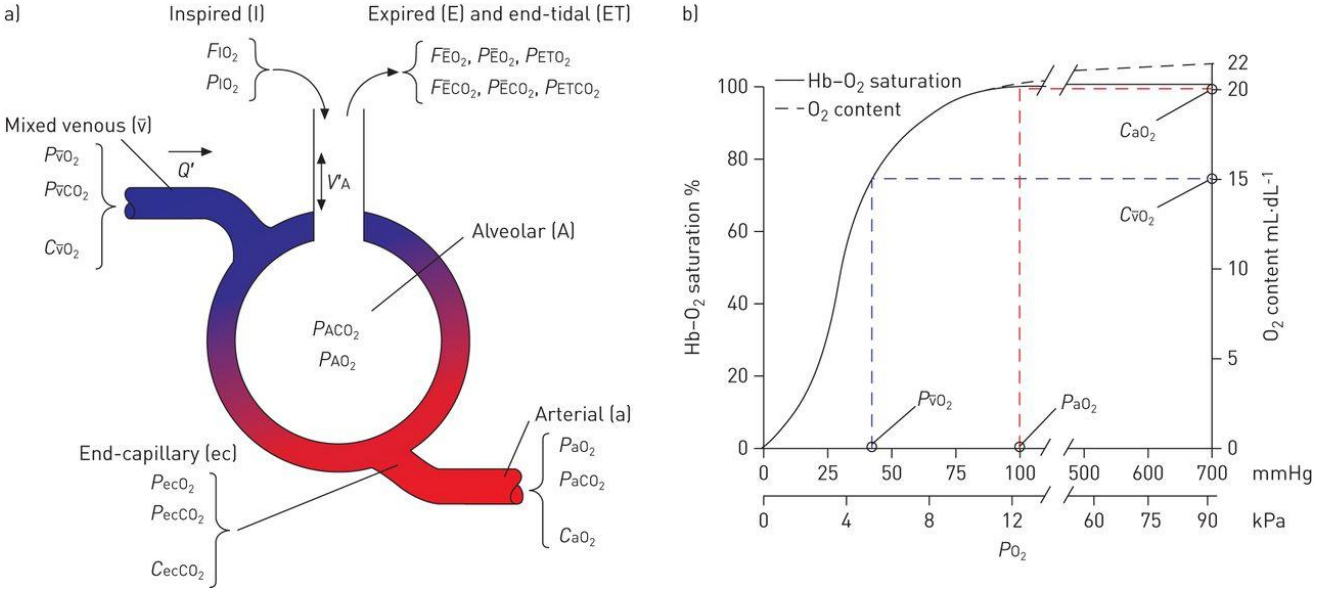


Figure 2.16. *a) Gas exchange in an alveolar-capillary unit. It should be noted that with a single-unit lung model, arterial and end-capillary values are equal. b) The haemoglobin–oxygen (Hb–O_2) dissociation curve for partial pressure of O_2 (P_{O2}), O_2 saturations and O_2 content in the venous and arterial compartments for a haemoglobin concentration of 15 g·dl^{-1}. From Petersson et al. (49), reproduced with permission*

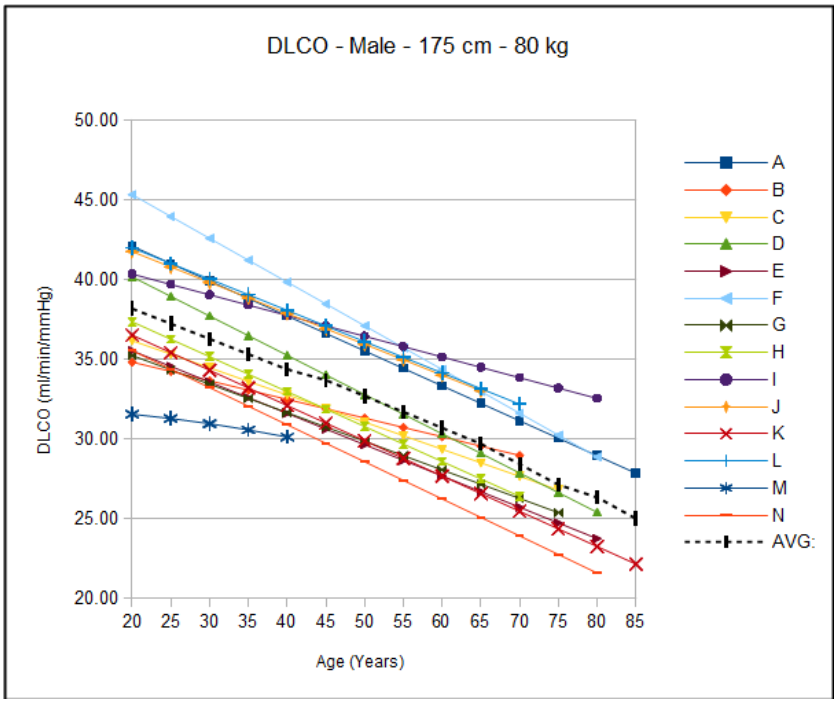


Figure 2.17. *Relationship of DLCO to natural aging. As lungs age, the diffusion capacity of the lungs decline. Personal slide of Dr. Bruce Johnson, reproduced with permission*

For calculation of DLCO in addition to CO, a tracer gas to measure V_A is also used. Helium and methane are used commonly as tracer gases. O_2 and N_2 constitute the remainder of the test gases.

Factors affecting DLCO include age, sex, height, Hb, lung volume, carboxyhemoglobin (COHb), altitude, body position, and exercise. Adjustment for Hb in the DLCO calculation is also needed:

DLCO predicted for Hb = DLCO predicted x (1.7xHb / (10.22+Hb))

P_{AO2} changes specifically by altitude also affect DLCO. COHb can be elevated in smokers and can affect DLCO by occupying Hb binding sites. Theoretically it is recommended that when COHb is elevated an adjustment factor be considered. However, for practical purposes no adjustment is required if COHb is less than 2% since the correction factor is incorporated into reference equation (46).

Measurements: DLCO is measured by several techniques which range from conventional single breath method (SBDLCO), to various rebreathe, steady state, open-circuit and intra-breath techniques (50). While the latter methods may represent in some sense more physiological quantification of functional lung surface area for gas transfer or exchange, the single breath method has been standardized with well-established predictive norms for clinical use (46).

i- Single breath (SBDLCO):

In this method, the uptake of CO from the lung by holding breath over a time period is measured. After mouthpiece and nose clip are placed, the DLCO maneuver starts with unforced exhalation to residual volume (RV). In COPD, this maneuver may take a long time; therefore, it is recommended that maneuver be limited to 6 seconds. Then at residual volume the subject takes a deep breath while connected to gas source to reach TLC (Fig. 2.18). The inspiration is rapid usually less than 4 seconds to reach 85% of the inspiratory volume (inspiratory maneuver). Then the breath-hold begins which is usually around 10 seconds and it is near atmospheric pressure by voluntarily maintaining full inspiration to minimize variability. Then the expiratory maneuver starts. This is rapid and without interruption not more than 4 seconds. During expiration, a portion of the gas related to dead space (V_D) is discarded before the true alveolar volume is collected. If V_D contaminates alveolar volume, then CO uptake is underestimated. Newer devices show concentrations of exhaled gases and avoid V_D in calculation of DLCO (46).

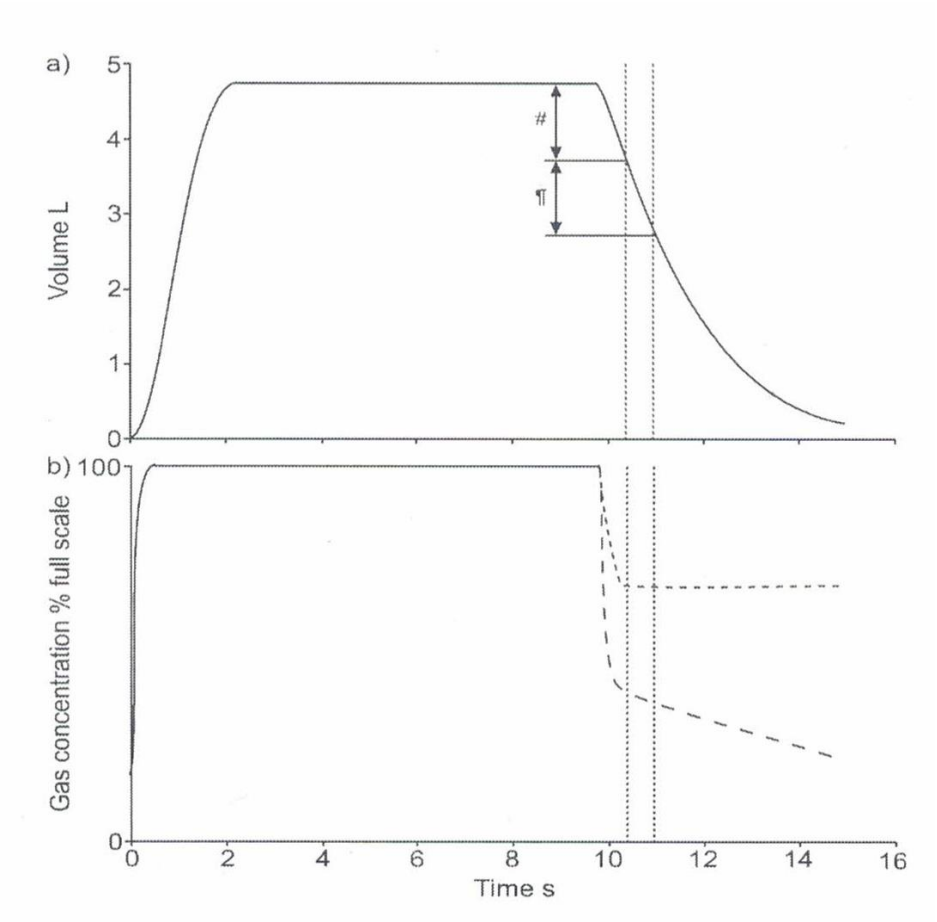


Figure 2.18. *Lung volume (a) and gas concentrations (b) during the single-breath diffusing capacity of the lung for carbon monoxide. The gas-sampling period occurs between the two dotted lines.*
- - - - tracer gas, _ _ _ _ _ carbon monoxide
#: dead space washout, ¶ sample collection. From: Macintyre et al. (46), reproduced with permission

The sample gas volume (Vs) is the volume of expired gas collected at the end of the breath hold maneuver for analysis of CO and other tracer gas concentrations . A Vs of 0.5 to 1 liter is best for analysis. Vs less than 0.5 liter can be used in patients with Vc <1 liter if there is assurance that V_D has been cleared. The key is to make sure V_D is not contributing to the collected sample otherwise. A tracer gas measures V_A as well as CO. The remainder of test gases include O_2 and N_2. The tracer gas is insoluble, is biologically inert, has gaseous diffusivity similar to CO, and is ordinarily absent in alveolar gas. Common tracer gases are helium (He) and methane (CH_4). Helium is used in derivation of most of available reference equations(46).

By measuring DLCO at several different levels of PAO_2, one can distinguish between two components of DLCO (D_M and V_C) by using the following equation:

$$1/DLCO = 1/D_M + 1/\vartheta V_C$$

At least 4 minutes should be allocated between tests to allow ample time for elimination of test gas from the lungs. This time is longer in patients with obstructive lung disease such as COPD. By using the approach of Jones and Meade, breath-hold time is the time starting from 0.3 of inspiratory time and lasting to the middle of sample collection time. Inspiratory time is the time when 90% of inspiratory volume has been inspired (51).

Determination of V_A is important in calculation of DLCO. There are methods other than single breath that are more accurate and include multiple breath and plethysmography. But because of several technical limitations, the single breath method is still the preferred one. Adjustments in Hb and PAO_2 should be made in the final calculation of DLCO. Adjustment for COHb is usually not necessary unless COHb is elevated. If COHb is less than 2 percent, then no adjustment is needed (46).

ii) Intra-breath measurement (IBDLCO):

The shortcoming of traditional methods for measurement of DLCO such as single breath is that it treats the lungs as a unified, well-mixed reservoir, generating an average DLCO which in reality pertains to a small portion of alveolar volume during which CO uptake occurs (52). But as we know blood does not distribute uniformly in all portions of the lung and there are regional differences especially in disease states. With introduction of the intra-breath method, exercise DLCO response which changes from rest because of pulmonary vasodilation and recruitment, is better detected. This method is described in detail by Huang (52, 53). In this method, a 3-gas analyzer of carbon

monoxide, acetylene, and methane (CO, C_2H_2, CH_4) measures not only DLCO during exhalation, but also simultaneously measures pulmonary capillary blood flow which can be a surrogate of cardiac output (Qc), provided that pulmonary shunting is absent (53, 54).

In this method, subjects exhale to residual volume by a single exhalation. This is followed by a rapid inhalation to total lung capacity of a mixture of CO, C_2H_2, and CH_4. Then the breath is held for 1 to 2 seconds followed by a slow exhalation at a constant rate, using a flow restrictor and an on-screen flow indicator. During exhalation gas concentrations are measured continuously. With the assistance of special software, DLCO and Qc are calculated. In this method, the DLCO is slightly lower than single-breath technique. The advantage of this technique is measurement of cardiac output noninvasively; but the shortcoming can be that absolute DLCO and Qc can misleading in presence of severe non-uniform ventilation and/or diffusion even with the use of insoluble gas such as C_2H_2 , limiting its application in patients with severe obstructive lung disease (53). Nevertheless, it is a method that can be used in research for early detection of respiratory and cardiac limitations in disease states, obtaining information that is not available from resting DLCO. This test does not involve a 10 second breath hold like traditional single breath method and is therefore easier to perform in patients with shortness of breath.

Correlation between SBDLCO and IBDLCO is variable. In a Japanese study of 88 COPD patients, no difference between IB and SB DLCO in subjects with $\%FEV_1 > 50\%$ was found. In the same study of COPD patients with FEV1 < 50%, the IBDLCO was slightly lower than the SBDLCO (55). In another study by Quantz, there was also a good correlation between the IB and SB technique in heart transplant patients (6). But the assumption that in the IB method, lungs do fill and empty homogenously and the rate of diffusion is constant over a long range of lung volumes is not

necessarily correct. IBDLCO is more increased near TLC and reduced near RV and creates some confusion when it comes to reporting (52, 56).

To obtain the best results with IBDLCO, one needs a steady expiratory flow which has been set by some to be around 0.5 L/min. This may be difficult to achieve during exercise but a flow restrictor can help (57). There is no standardization for the expiratory flow rate and different researchers have selected different rates but 0.5 L/min appears to be more reproducible.

d) Pulmonary blood flow (Qc):

This can be used as a surrogate of cardiac output and is measured by intra-breath method as described above, provided pulmonary shunt is not present. Elkayam et al. in 1984 introduced measurement of cardiac output by single breath expiratory technique (58). The absorption rate of acetylene from the alveolar gas and the change of rate of lung volume during constant expiratory flow was used to measure cardiac output (Qc) or pulmonary blood flow. The Qc was compared to cardiac output by thermodilution and the standard deviation of difference was 0.76 Lit/min with the main difference between them being 0.03 Lit/min. The study consisted of 20 patients with cardiac disease, some of whom had mild COPD. In the study there were technical difficulties in cardiac output measurement in patients with FEV_1/FVC less than 60%, but it was more accurate in higher FEV_1/FVC values. No correction for ventilation-perfusion mismatching was required (58).

In this noninvasive method, a bag in bottle system with a rolling seal spirometer was used for the measurement (58). The formula that was used for pulmonary blood flow was as follows:

$$\dot{Q}c = (\dot{V}_E \div \alpha_t) \times [(\ln F_A \div F_{A0})] \div \ln [(V_A + \alpha_t V_t) \div (V_{A0} + \alpha_t V_t)]$$

F_A: alveolar concentration of acetylene
$F_{A0} = F_I/F_IHe/F_AHe$; F_I is inspired acetylene concentration; F_IHe is inspired helium concentration
V_A: alveolar volume
V_{A0}: V_A at full inspiration and calculated from the formula $V_{A0} = (V_I - V_D)(F_IHe/F_AHe)$, where V_I is inspired volume and V_D is dead space
$\dot{V}_E$: expiratory flow rate, V_t: pulmonary parenchymal tissue volume
α_t: Bunsen coefficient for pulmonary tissue

Charloux et al. have studied this technique in detail (59). Their study published in 2010, found that DLCO could be measured with good reliability up to 73–90% of peak $\dot{V}O_2$ (oxygen consumption) during incremental exercise and 85 to 95% of peak $\dot{V}O_2$ during intermittent exercise. The coefficients of variation of DLCO and Qc measured during two successive constant-load exercise tests were 5–6% and 7–11%, respectively. The highest values of DLCO, Qc and $\dot{V}O_2$ during the incremental and intermittent tests were very similar. The main observed result was that the linear relationships between DLCO and Qc were similar regardless of the exercise type, allowing DLCO comparisons, with regards to Qc, during different exercise protocols. In the study by Elkayam et al (58) the subjects inspired gas from a bag in a bottle. The technology is now much more advanced and as we showed in our study, the Qc measurement can now be done by using the intra-breath DLCO technique with acetylene which is less cumbersome than a bag and bottle system.

Agostoni et al. used the inert gas rebreathing method for noninvasive measurement of cardiac output during exercise in a large number of healthy subjects (54). This technique has been reviewed in detail in the paper published in 2017. Agostini also studied the intra-breath method in patients with heart failure. The results compared with direct Fick and thermodilution techniques, showed very good correlation (60), suggesting this is an efficient tool particularly in heart failure patients when noninvasive measurement of cardiac output is important for prognosis and disease management.

However, Qc measurements are rarely performed in a routine clinical practice. The measurements need special software with a great deal of expertise in its performance. It is now a tool mainly used in research laboratories for noninvasive measurement of cardiac output (Fig. 2.19). But if done correctly, it can add important information in conjunction with the DLCO about the cardiovascular state of the patient as demonstrated in this thesis.

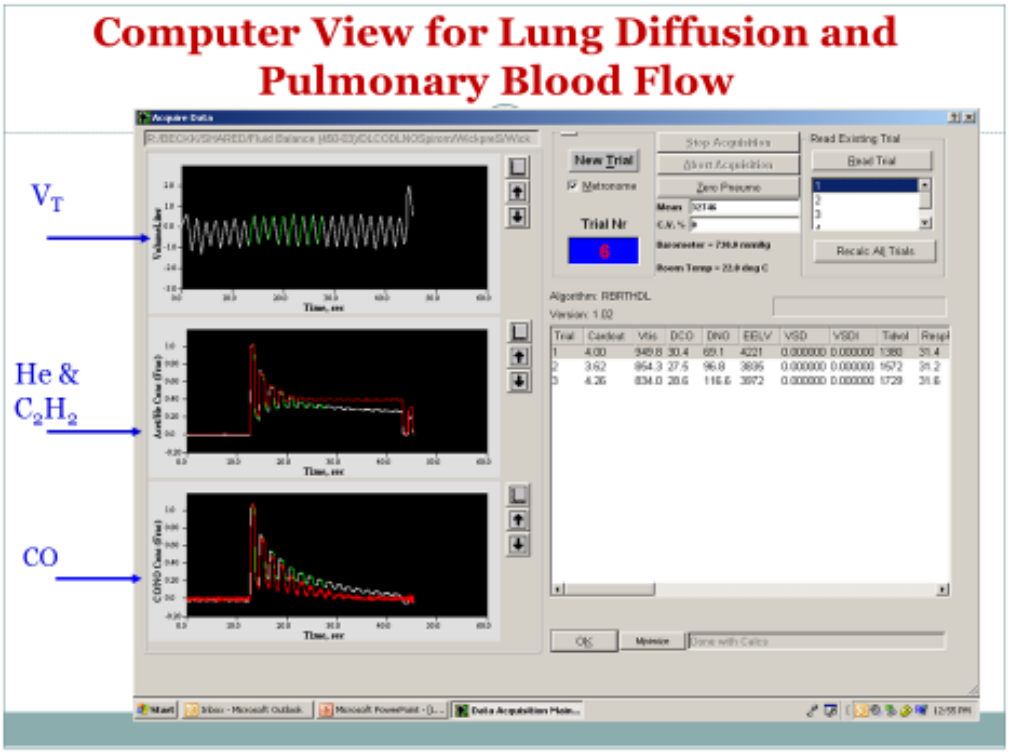


Figure 2.19. *Computer display for lung diffusion and pulmonary blood flow; personal slide of Dr. Bruce Johnson, reproduced with permission*

C. Cardiopulmonary exercise testing

CPET is a central research protocol used in this dissertation. In this test, a patient exercises on a device such as an exercise bike or treadmill with attached accessories including mask, head gear, EKG electrodes, blood pressure cuff, and pulse oximetry (Fig. 2.20). Workload is incrementally increased until symptoms of fatigue and dyspnea develop. When limiting symptoms develop, exercise is terminated and patient enters period of recovery. Continuously collected physiologic data during the test include oxygen uptake, carbon dioxide output, tidal volume, minute ventilation, oxygen saturation, and EKG data. CPET helps the clinician differentiate between causes of exercise limitation and shortness of breath: heart, lungs, vascular system, or deconditioning (61).

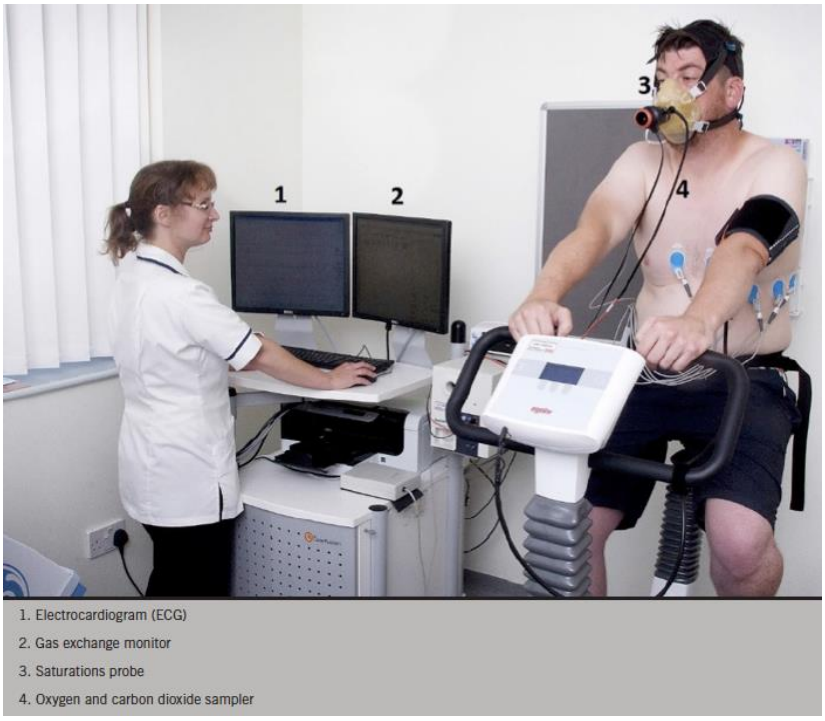


Fig 2.20. *CPET on a cycle ergometer. Facemask is strapped on the face and EKG electrodes are placed on chest. Patient breathes into the mask and data are collected continuously. Blood pressure cuff is placed. Operator records data. From Parasuraman et al. (62), reproduced with permission*

a) Biochemistry of cellular metabolism

As a preview to CPET, energy production at the cellular level will be discussed first. ATP (adenosine triphosphate) is the building block of energy for cells. The terminal phosphate moiety of ATP provides fuel for cellular metabolism. Three cellular pathways generate ATP:

1- Immediate release: Anaerobic (non-oxygen requiring) hydrolysis of creatine phosphate (PCr)

2- Short term release: Anaerobic oxidation of glycogen or glucose by pyruvic acid to produce lactic acid

3- Long term release: Aerobic (oxygen requiring) oxidation of primarily glycogen and fatty acids (Fig. 2.21)

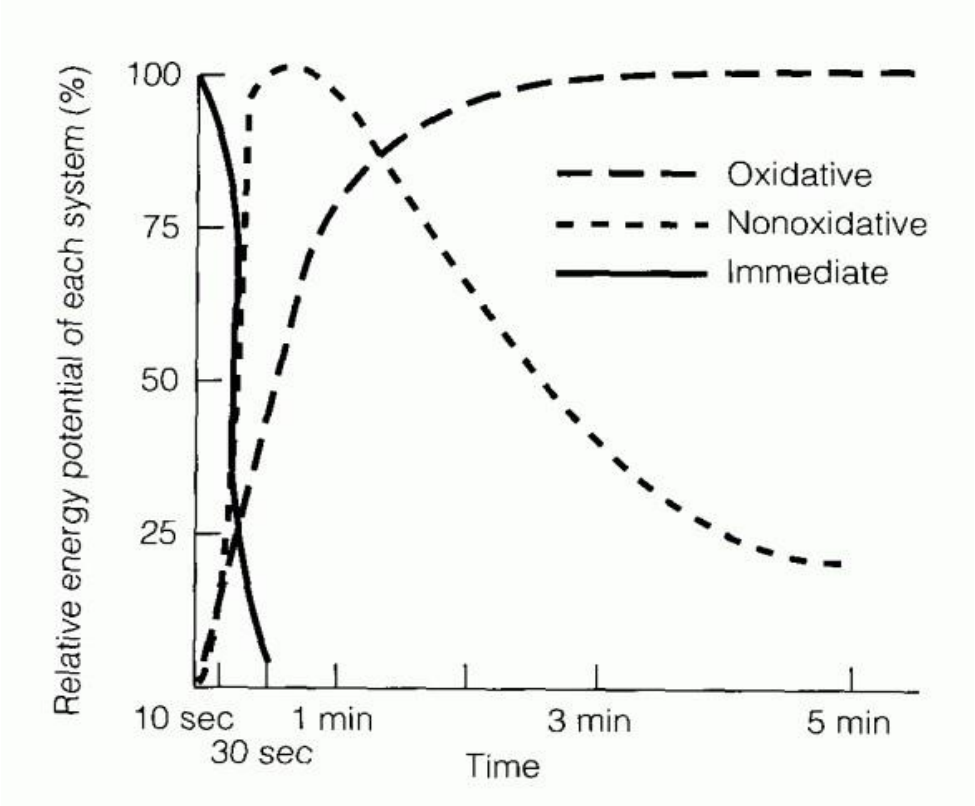


Fig 2.21. *Energy sources for muscle as a function of activity duration. The duration of each energy source is depicted. From Edington and Edgerton (63), reproduced with permission*

PCr which is stored locally in muscle is responsible for generation of high energy phosphate in early exercise when aerobic metabolism has not fully caught up with cellular demand (O_2 deficit) and during early recovery from exercise (O_2 debt). PCr is hydrolyzed to make creatine (Cr) and Pi (inorganic phosphate). The released energy from hydrolysis is utilized to regenerate ATP at the muscle myofibril during early phase of exercise (61). A patient who is less fit anaerobically, consumes PCr at a higher rate compared to a fit subject (Fig. 2.22).

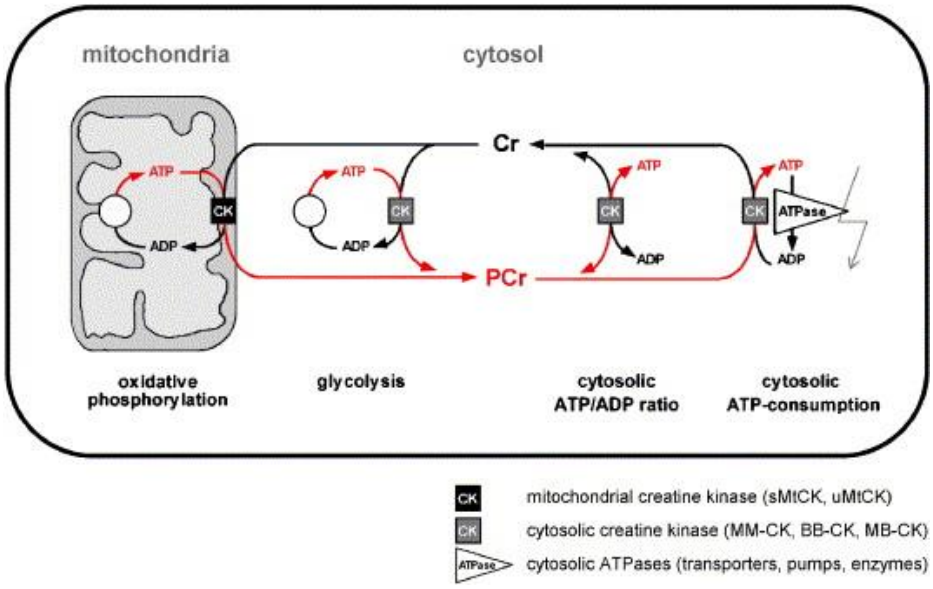


Figure 2.22. *The creatine kinase/phosphocreatine system. Isoenzymes of creatine kinase (CK) are functional in mitochondria and cytosol. Phosphocreatine (PCr) is built from creatine (Cr) by CK using ATP. PCr is then used as a temporal energy buffer to maintain ATP/ADP ratios. From Schlattner et al. (64), reproduced with permission*

Aerobic metabolism of carbohydrates (glycogen/glucose) and fatty acids are the major source of ATP. In a healthy person, 5/6 of energy is derived from aerobic metabolism of carbohydrate and 1/6 is derived from fatty acid (Figure 2.23). To sustain the cellular energy during a high demand state such as during exercise, adequate oxygen should be provided in order for aerobic generation of

carbohydrate and fatty acid to continue. In aerobic metabolism, one molecule of glucose produces 36 molecules of ATP (Fig. 2.23).

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \mathbf{36ATP}$$

When oxygen is abundant, mitochondria convert NAD^+ (nicotinamide adenine dinucleotide, oxidized) to NADH (NAD, reduced) and H^+ (Fig. 2.23). When oxygen is in poor supply, NADH and H^+ can be re-oxidized anaerobically by pyruvate through the following reaction:

$$NADH + H^+ + Pyruvate \rightarrow NAD^+ + lactate$$

NAD^+ must be formed for glycolysis to continue (anaerobic glycolysis). The amount of energy by anaerobic glycolysis of glycogen and glucose is small. Two molecules of lactate are generated in lieu of a six-carbon moiety of glycogen or glucose. Lactate is an acid and the acidic environment affects oxygen delivery. In this process the net ATP production is only 2, compared to 36 of aerobic glycolysis.

$$C_6H_{12}O_6 + 2NAD^+ + 2P_i + 2ADP \rightarrow 2 \text{ pyruvate} + 2NADH + \mathbf{2ATP} + 2H^+ + 2H_2O + heat$$

Figure 2.23 is a cartoon summary of aerobic and anaerobic metabolic pathways in generation of ATP (61).

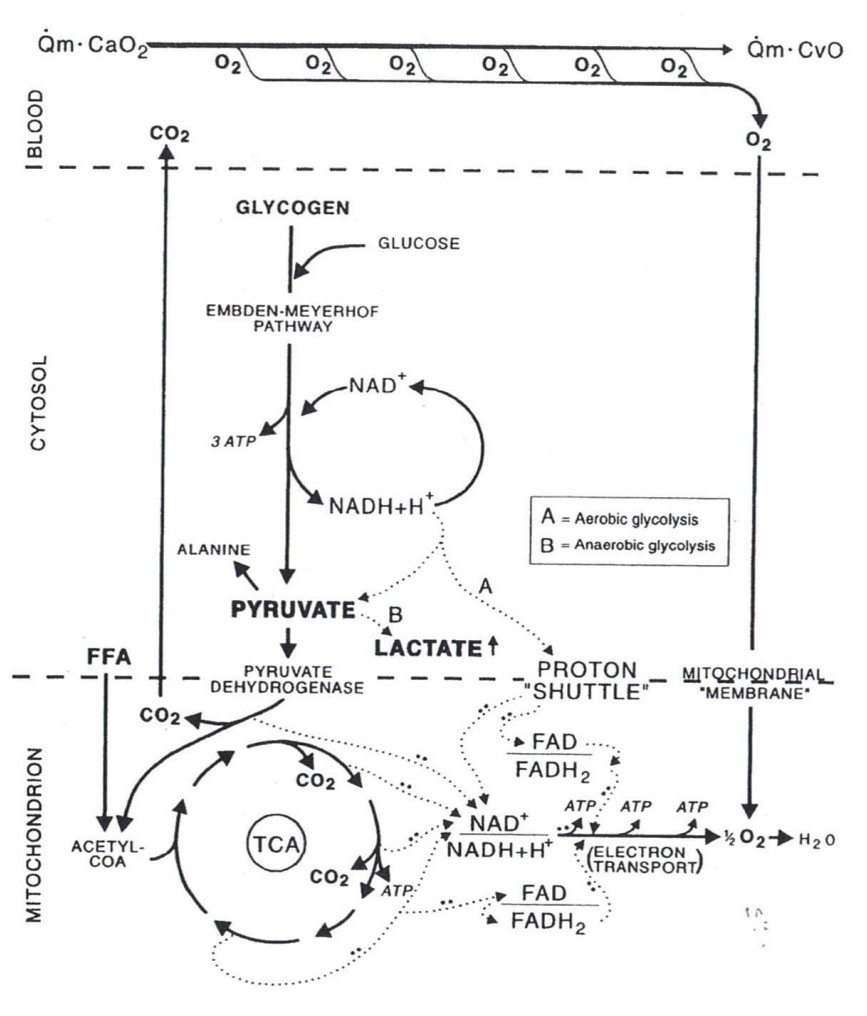


Figure 2.23. *Biochemical pathway of ATP production through aerobic and anaerobic metabolisms. Energy from carbohydrate and fatty acid catabolism are released in mitochondria, with a small amount in cell cytoplasm from conversion of glucose and glycogen to pyruvate. Energy from mitochondria and cytosol are transformed to ATP and PCr. The P moiety is released when there is a demand for energy. This must be complemented by an increase in O_2 delivery from environment to mitochondria. CO_2 as a byproduct of cellular metabolism is removed from cell by blood and carried to lungs for elimination. Acetate, made from carbohydrates, fatty acids, and amino acids in nutritionally deficient cells, reacts with oxaloacetate in mitochondria after esterification with Acetyl-CoA to form citrate in tricarboxylic acid (TCA) cycle. In TCA cycle, CO_2 is released and protons and electrons are transferred to mitochondrial electron transport chain. In this chain, ATP is regenerated from ADP and inorganic phosphate (oxidative phosphorylation). At the end of chain of transport, water is produced. Transfer of a pair of protons and electrons, results in enough energy for synthesis of 2 ATP molecules, if it begins at NAD^+, or 3 ATP molecules if it begins at FAD. From conversion of glucose to pyruvate, if NAD+ is used in cytosol, 6 ATP molecules are gained: 2 in cytosol, and 4 in mitochondria by re-oxidation of cytosolic NADH through the proton shuttle and electron transport chain. Since O_2 is recipient of protons of glycolysis pathway, it is named as aerobic metabolism. In exercise, when proton shuttle is not working at an adequate pace to re-oxidize NADH + H^+, the cytosolic NADH and H^+ increases and glycolysis will slow down. As an escape mechanism, pyruvate is converted to lactate in order to re-oxidize ($NADH+H^+$) back to NAD. Since lactate is formed without O_2, it is an aerobic process. However, the net production of ATP is only 3 compared to 36 ATP from aerobic metabolism. Therefore to generate the same amount of ATP, significantly more glucose and glycogen should be broken down. Lactate is an acid and the cellular environment becomes more acidic. In a state of high demand, both aerobic and anaerobic pathways work simultaneously to generate energy and one is not obligated to turn off while the other is working (61). Qm: pulmonary capillary blood flow, CaO_2: pulmonary arterial oxygen content, CvO: pulmonary venous oxygen content, NAD: nicotinamide adenine dinucleotide, FAD: Flavin adenine dinucleotide. From Wassermann et al. (61),* reproduced with permission

Each of the 3 pathways of ATP generation affect gas exchange ($\dot{V}O_2$ and $\dot{V}CO_2$) in a different way. When aerobic metabolism prevails, O_2 consumption and CO_2 production are in proportion to the ratio of carbohydrate to fatty acid in the substrate.

When PCr is broken to Cr and Pi, acidity is decreased because Cr is neutral. Therefore, CO_2 is consumed and its output in the airway relative to O_2 uptake diminishes, causing a mismatch between early $\dot{V}CO_2$ and $\dot{V}O_2$ early kinetics (61). In anaerobic glycolysis, lactate is buffered by HCO_3^- which in turn produces more CO_2 causing an increase of $\dot{V}CO_2$ above $\dot{V}O_2$. Therefore, each of the three pathways have a different effect on gas exchange during rest and exercise.

The excess CO_2 is transported by blood to the lungs where ventilation from rest to maximal exercise needs to increase by a factor of 10 to eliminate the excess CO_2. In the healthy state, approximately 10 ml/min of O_2 is required for every watt increase in external work rate ($\Delta\dot{V}O_2/\Delta WR=10ml/min$) which holds true for constant or incremental exercise. When the ratio is less than 10, it indicates inadequate aerobic metabolism and can be seen in several disease states (61).

In aerobic metabolism of glucose, for 6 molecules of CO_2 that is produced, 6 molecules of O_2 is consumed. Therefore, respiratory quotient ($\dot{V}CO_2$ / $\dot{V}O_2$) is 1 for carbohydrates. For lipids, the respiratory quotient is 0.71 since for production of 16 CO_2 molecules from fat metabolism, 23 molecules of O_2 is needed. At the beginning of exercise, respiratory quotient is high, indicating utilization of energy mainly from glycogen and glucose. When muscle carbohydrate is used, then energy production is more reliant on fatty acids and respiratory quotient decreases over prolonged exercise (61).

b) <u>Gas transport, Oxygen uptake and the Fick Principle:</u>

The maximal oxygen uptake ($\dot{V}O_2max$) in liters per minute is a reflection of person's maximal aerobic ability to use and transport oxygen and it signifies aerobic capacity of a person. $\dot{V}O_2max$ is the most important parameter measured during CPET and is the "gold standard" laboratory measure of cardiopulmonary fitness.

$\dot{V}O_2$ is calculated using the Fick equation as follows:

$$\dot{V}O_2 = (SV \times HR) \times (CaO_2 - CvO_2)$$

($\dot{V}O_2$ is the oxygen uptake, SV is the stroke volume, HR the heart rate, CaO_2 is the arterial oxygen content and CvO_2 is the mixed venous oxygen content (65)).

Functional aerobic impairment limits exercise by reducing subject's $\dot{V}O_2max$. Anything that affects one of the four parameters of Fick equation, affects exercise tolerance. These include a low heart rate, low stroke volume, low CaO_2 or high CvO_2. For example, if stroke volume is reduced in a patient with heart failure, $\dot{V}O_2$ is reduced.

In Fick equation, (SV x HR) is cardiac output which is Qc and (CaO_2 - CvO_2) is the difference in oxygen content of the artery and mixed venous blood. A high cardiac output increase $\dot{V}O_2$ and maximal cardiac output determines maximal oxygen uptake ($\dot{V}O_2max$). Also high CaO_2 and low CvO_2 increase $\dot{V}O_2$.

The formula for arterial-venous oxygen difference can be rearranged as follows:

CaO_2 - CvO_2 = 1.34 x hemoglobin concentration x (arterial oxygen saturation- mixed venous oxygen saturation)

Therefore, physical exercise requires an intricate balance between *ventilation, cardiac output, systemic and pulmonary circulation* to fulfill the demands of exercising muscle. The impact of each is as follows:

a- Ventilation: affected by minute ventilation which is in part regulated by the nervous system, CO_2 elimination ($\dot{V}CO_2$), physiologic dead space (VD), and oxygen in the arterial blood. Increased oxygenation is brought about by increased alveolar oxygen tension (PAO_2), decreased ventilation-perfusion mismatching, increased oxygen diffusion surface area, a lower right to left shunt, and a higher hemoglobin concentration (66).

b- Cardiac output: affected by heart rate and SV which is a function of LV filling pressure or preload and afterload (67).

c- Systemic circulation: affected by systemic blood pressure (rises in exercise) and peripheral vascular resistance (decreases in exercise), and sympathetic regulation of blood flow (less to smooth muscles and gut and more to skeletal muscles), and oxygen dissociation curve (more oxygen delivery with leftward shift) (68).

d- Pulmonary circulation: affected by pulmonary vascular resistance (drops in exercise), and pulmonary blood flow (increases in exercise) (69). The role of each step is depicted in Fig. 2.24.

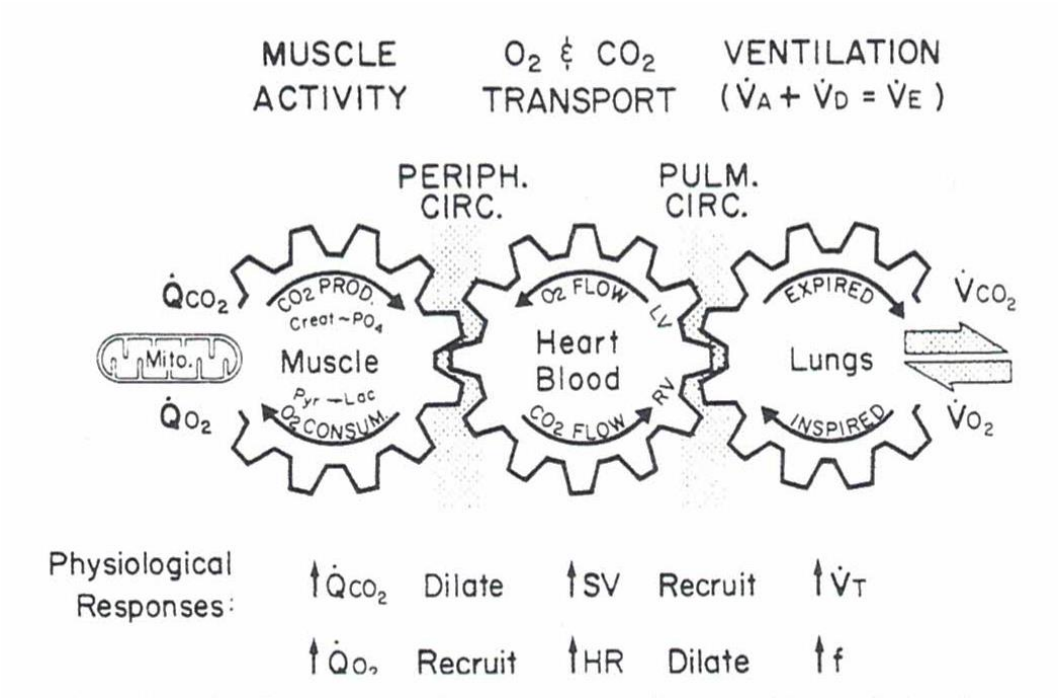


Figure 2.24. *Gas transport mechanisms involved in coupling of cellular (internal) to pulmonary (external) respiration. Gears depict interdependence of various structures of the system. Increase in utilization of oxygen by muscles (QO_2) is accomplished by: a rise in extraction of oxygen from the perfused muscle by the blood, vasodilation of systemic vascular system, increase in cardiac output and stroke volume causing elevation of cardiac output, vasodilation of pulmonary vasculature resulting in increased pulmonary blood flow, and elevation of minute ventilation. Oxygen uptake $\dot{V}O_2$ from the lungs is proportional to the pulmonary blood flow and oxygen desaturation of hemoglobin in the pulmonary vasculature. When the system is in equilibrium, $\dot{V}O2=QO_2$. Minute ventilation (VT x f) increases when CO_2 production (QCO_2) is increased. In other words: $\dot{V}CO_2=V_A x PaCO_2/PB$, where $\dot{V}CO_2$ is CO_2 production per minute, V_A is alveolar ventilation per minute, $PaCO_2$ is arterial or ideal alveolar CO_2 tension, and PB is barometric pressure. The equal size of gears does not imply equality of variables in each step. For example, a doubling of the metabolic rate does not increase the cardiac output by 2. From Wasserman et al.(61), reproduced with permission*

c) <u>Respiratory and other variable measurements</u>:

In most systems of CPET, a breath-by-breath analysis is performed. The basic parameters measured include percent O_2, percent CO_2, respiratory airflow, and heart rate which are the basis for determination of all measured and derived variables. A mask at the subject's mouth containing a non-rebreathing valve thus preventing mixing of inspired and expired gases. Oxygen and carbon dioxide tensions are measured by analyzers attached to the exercise system.

Breath-by-breath volumes of O_2 intake, CO_2 output, and expired ventilation are achieved by integration of these continuous variables over time: for O_2 (inspiration time), for CO_2 (expiration time) and VE (expired ventilation). Two sensors of O_2 and CO_2 calculate O_2 uptake and CO_2 output at any breath. Gas analyzers and flow sensors are calibrated routinely and system validation is performed regularly. EKG is monitored routinely during testing.

Gas ventilation-perfusion physiology involves 4 steps:

a- Pulmonary ventilation: air movement in and out of lungs

b- Pulmonary diffusion: O_2 and CO_2 exchange between lung and blood

c- O_2 and CO_2 transport in blood

d- Gas exchange: exchange of O_2 and CO_2 between systemic capillary blood and cells of the body

Standard measurements during a CPET include:

1- $\dot{V}O_2$max: is an important measure that defines maximal aerobic or exercise capacity.

$\dot{V}O_2$ = Heart rate x stroke volume x (arterial oxygen content – venous oxygen content).

It is measured in milliliters of O_2/kg/minute. $\dot{V}O_2$max requires maximal exercise effort and is the plateau in $\dot{V}O_2$ between the final two exercise work rates (Fig. 2.25).

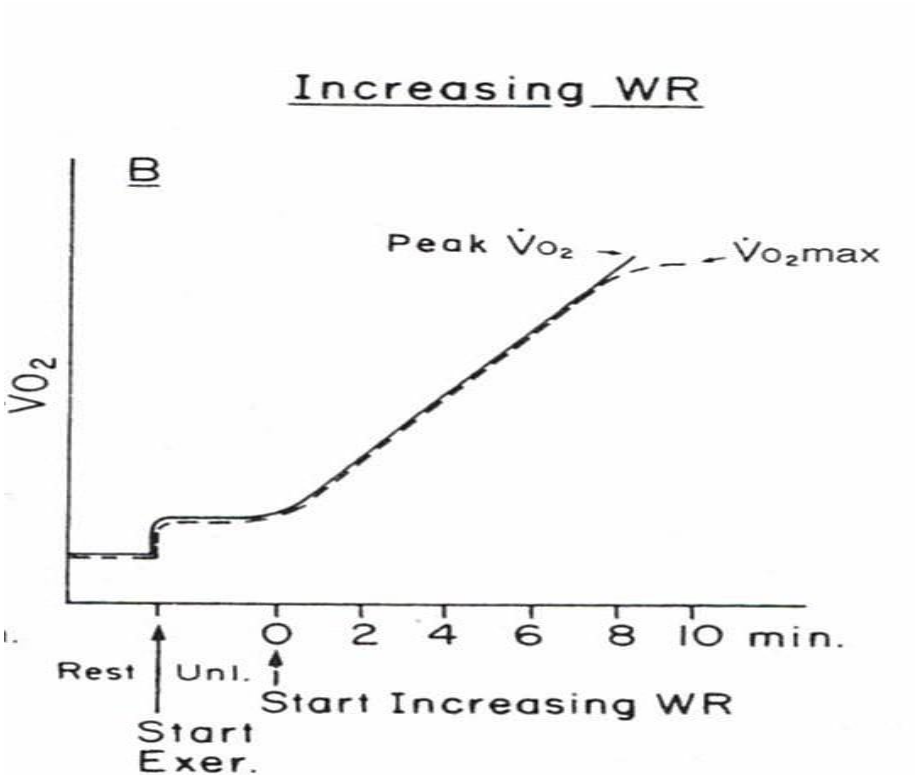


Figure 2.25. *Depiction of $\dot{V}O_2$max and differentiation between peak $\dot{V}O2$ in a maximal effort graded exercise. In $\dot{V}O_2$peak, subject has reached his maximum rate but there is still room for increase. But in $\dot{V}O_2$max subject has reached his maximum tolerable work rate. From Wasserman et al. (61), reproduced with permission*

2- Ventilatory (anaerobic) threshold (VT): level of exercise where $\dot{V}CO_2$ begins to increase relative to the increase in $\dot{V}O_2$. It signifies lactate release from muscle a byproduct of recruitment of glycolytic muscle fibers. VT occurs at 45-65% of maximal $\dot{V}O_2$ in untrained and a higher percentage in physically trained individuals (Fig. 2.26).

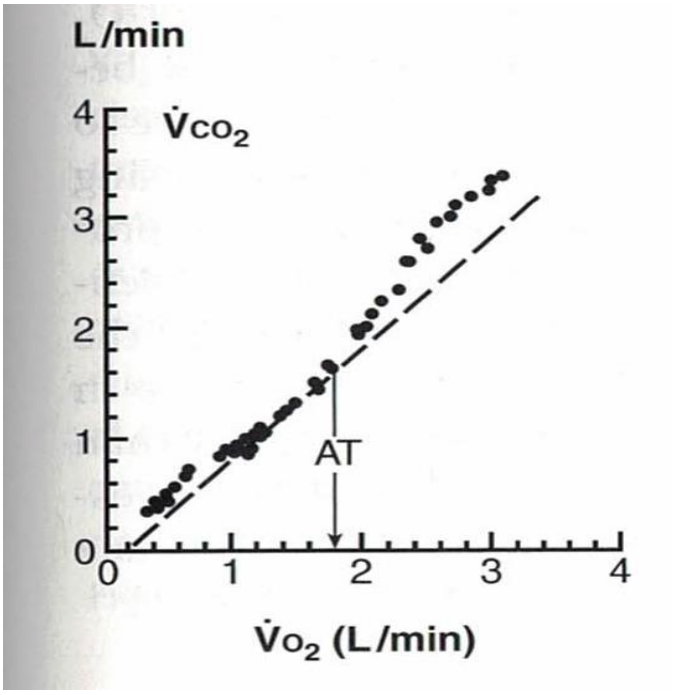


Figure 2.26. *Depiction of ventilatory or anaerobic threshold (AT) on $\dot{V}CO_2$ vs $\dot{V}O_2$ panel. The slope of the line starts to change which is marked as anaerobic threshold. From Wassermann et al. (61), reproduced with permission*

3- Peak Respiratory Exchange Ratio (RER): by definition is $\dot{V}CO_2/\dot{V}O_2$ which is obtained by expiratory gas analysis. RER is a very reliable indicator of subject effort and when >1.10 it indicates excellent effort.

4- Minute ventilation-Carbon dioxide ratio: $VE/\dot{V}CO_2$ shows ventilatory efficiency and the slope has prognostic information. Typically it requires <30 Liters of air to remove a liter of carbon dioxide produced. Thus values significantly greater than 30 indicate inefficiency or possibly a diseased state or excessive hyperventilation. An elevated ratio suggests ventilation-perfusion mismatching as seen for example in decreased cardiac output, high pulmonary arterial pressure and decreased alveolar-capillary membrane perfusion.

In addition to the above variables, other parameters such as pulse oximetry, EKG, and blood pressure are monitored during the test (Fig. 2.27).

There are other measurements that are derived during a CPET. Some of these include:

a- Oxygen uptake efficiency slope (OUES): relationship between VE (on X-axis) and $\dot{V}O_2$ on (y-axis) by using the formula: $\dot{V}O_2 = a \log_{10} VE + b$. The higher slope portends a more favorable physiological performance.

b- Partial pressure of end tidal CO_2 ($PetCO_2$): during rest and exercise, $PetCO_2$ has prognostic ramifications in heart failure and pulmonary hypertension. For example, peak exercise $PetCO_2$ is correlated with pulmonary pressure in patients with pulmonary hypertension.

c- V_D/V_T: ratio of dead space to tidal volume, is an algorithm derived variable which decreases during exercise and may drop to 0.1 at peak exercise. In some diseases, for example pulmonary hypertension, this ratio fails to decrease appropriately owing to increased dead space ventilation.

d- $\dot{V}O_2$/work rate: $\Delta\dot{V}O_2/\Delta WR$ slope can be reduced in heart failure, peripheral arterial, and lung disease.

e- O_2 pulse: is the ratio of $\dot{V}O_2$ to heart rate ($\dot{V}O_2/HR$) and provides a gauge of left ventricular stroke volume alterations during exercise. Downward displacement may indicate cardiac limitation (65).

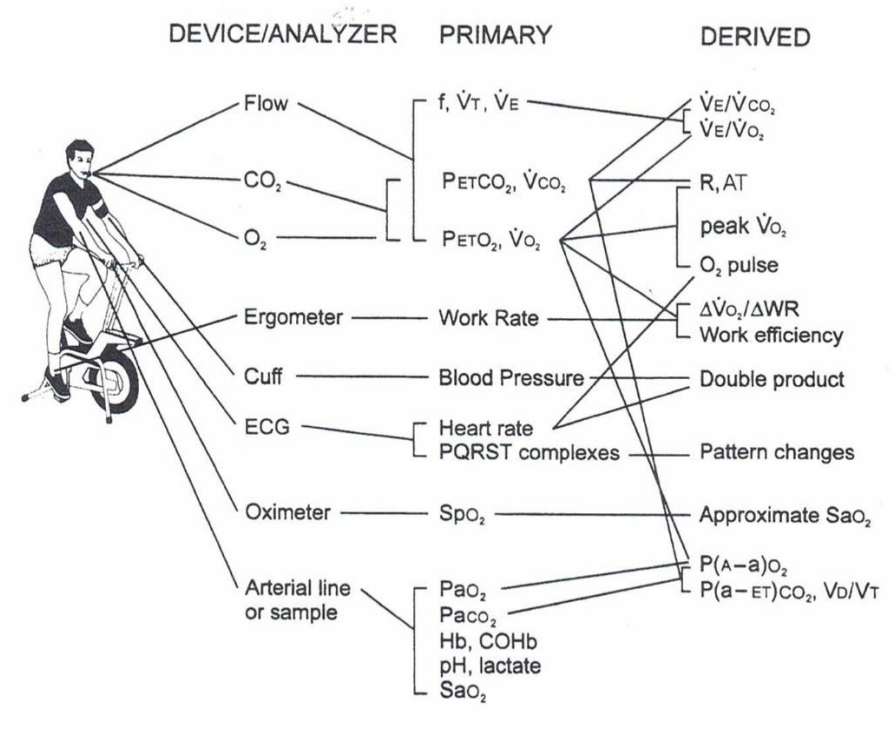


Figure 2.27. *Devices and Analyzers in measurement of variables in a traditional CPET. These analyzers measure several primary variables. The Derived variables are usually calculated from two or more primary variables. From Wasserman et al. (61), reproduced with permission*

The above measurements are plotted as 9 graphs that are traditionally labelled as "Wasserman Nine-Panel Graphical Assay" (Fig. 2.28).

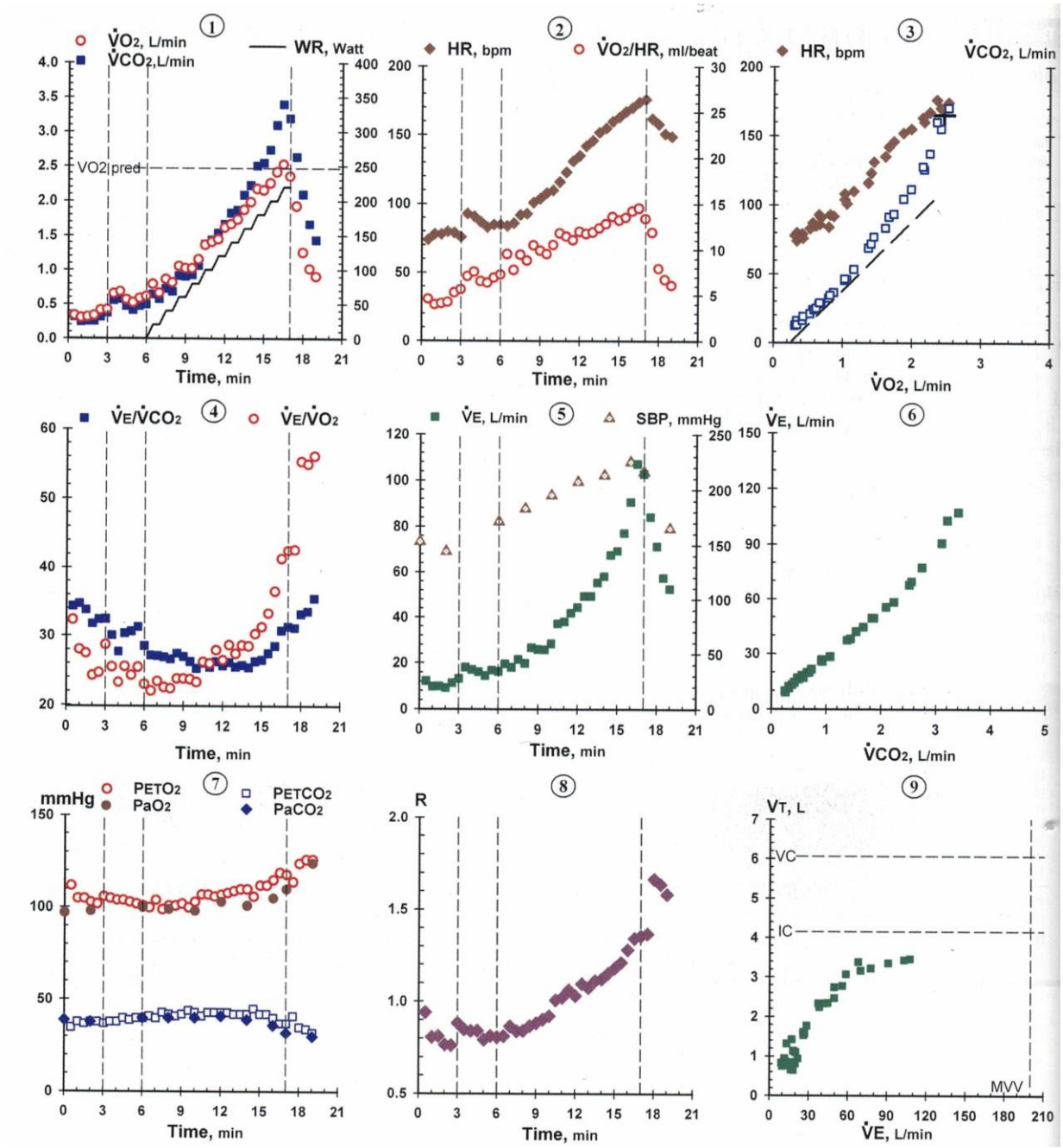


Figure 2.28. *Wasserman 9-graph panel of different variables obtained during a regular CPET in a healthy subject. Panels 1,2,4,5,7,8 all have time on the x-axis with y-axis depicting other physiologic variables. The vertical dotted lines depict unloading at 3 minutes, the change to incremental ramp exercise at 6 minutes and the end of exercise followed by recovery. Y-axis in each panel represents a different variable: O_2 and CO_2 uptake ($\dot{V}O_2$, $\dot{V}CO_2$) and Work rate in graph 1, $\dot{V}O_2/HR$ (O_2 pulse) and HR in graph 2, ventilatory equivalent for CO_2 and O_2 in graph 4 ($V_E/\dot{V}CO_2$ and $V_E/\dot{V}O_2$), minute ventilation in graphs 5 and 6, End tidal O_2 and CO_2 pressures in graph 7, and respiratory exchange ratio in graph 8. In panel 1, the increase in work rate (right y-axis) is plotted on a scale of 100 to 1 to $\dot{V}O_2$ on the left y- so that the work rate is parallel to a $\dot{V}O_2$ slope of 10 ml/min/W. In panel 3, the diagonal dashed line has a slope of 1 because both $\dot{V}O_2$ and $\dot{V}CO_2$ have identical scales and the + symbol indicates predicted values of heart rate (left y-axis) and $\dot{V}O_2$ for the subject. Arterial blood sampling is depicted in panel 7 but this is not the case in all cases of CPET. From Wasserman et. al (61), reproduced with permission*

d) CPET and COPD:

The above 9-graph panel was entertained in a normal subject. Now it will be further analyzed in a subject with COPD (Fig. 2.29).

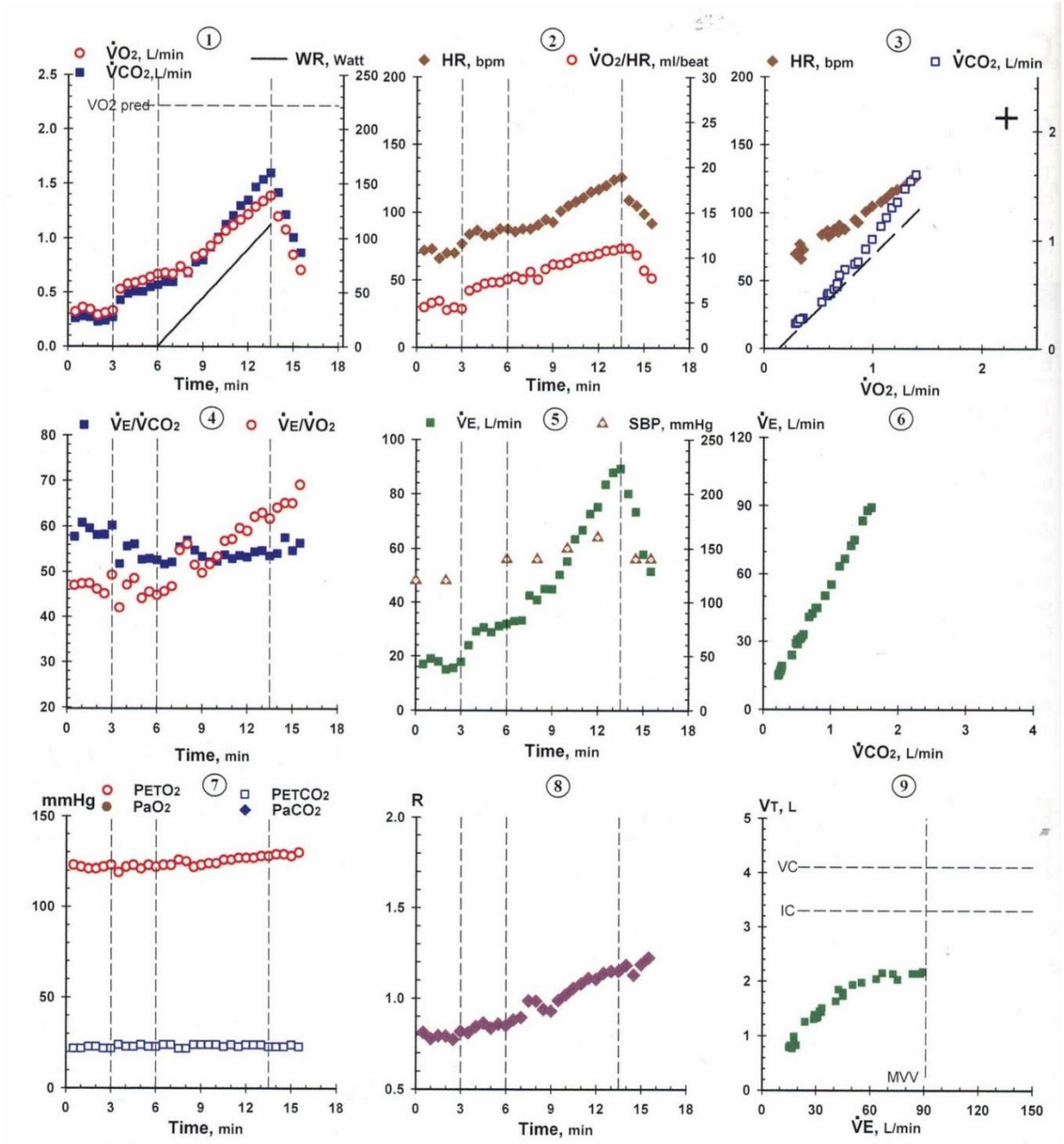


Figure 2.29. *9-panel graphical array of CPET variables in a patient with COPD. From Wasserman et al (61), reproduced with permission*

The above 9-panel array pertains to a patient with moderate COPD. When comparing graphs to the normal subject, several important differences can be noticed.

Panel 1: predicted $\dot{V}O_2$ is suppressed indicating exercise limitation.

Panel 2: shows heart rate and O_2 pulse ($\dot{V}O_2$/HR) against time. Heart rate usually increases quickly at the beginning of exercise and then increases approximately linearly with work rate to the predicted maximal heart rate (see the previous panel 2 of a normal person). But in COPD normal heart rate response is not seen because the patient prematurely stops exercise due to shortness of breath. O_2 pulse is the product of stroke volume and the arteriovenous O_2 difference. Compared to the normal person the slope of the rise is less steep.

Panel 3: this panel shows heart rate and $\dot{V}CO_2$ as functions of $\dot{V}O_2$. Heart rate elevates linearly with $\dot{V}O_2$. $\dot{V}CO_2$ usually increases as a function of $\dot{V}O_2$ with a slope of close to 1 or slightly less. When anaerobic threshold is reached, $\dot{V}CO_2$ starts to increase at a steeper slope. At times anaerobic threshold may be normal but, in some patients, may be reduced because of concomitant cardiac disease.

Panel 4: VE/$\dot{V}CO_2$ and VE/$\dot{V}O_2$ are depicted against time. When V_D/V_T, which is the physiological dead space to tidal volume ratio, and Pa_{CO2} are normal, VE/$\dot{V}O_2$ decreases and reaches a nadir at the anaerobic threshold with a value less than 28 and VE/$\dot{V}CO_2$ decreases to a nadir between anaerobic threshold and ventilator compensation point with a value less than 32. The low points of VE/$\dot{V}O_2$ and VE/$\dot{V}CO_2$ are increased in COPD, in which dead space ventilation or V_D/V_T is increased. The worse the COPD, the lower the Pa_{CO2} and the higher the VE/$\dot{V}O_2$ and VE/$\dot{V}CO_2$.

Panel 5: VE and systolic blood pressure are plotted against time. In normal subjects, V_E normally increases linearly as work level and time increase until anaerobic threshold is reached. At that point, because of lactic acidosis, the ventilation slope becomes steeper so that more CO_2 is exhaled to correct for acidosis. However, in COPD as the lungs are working hard to improve gas exchange and ventilatory reserve is very low, the ventilation cannot compensate adequately and the slope is not as steep as normal subjects.

Panel 6: in a normal subject, V_E normally increases linearly with $\dot{V}CO_2$ at a slope of 23 to 28 until lactic acidosis develops. The slope usually becomes steeper in COPD where V_D/V_T and dead space ventilation increase and Pa_{CO2} decreases, and it even becomes steeper as the disease severity exacerbates.

Panel 7: in this panel, $PetO_2$ and $PetCO_2$ are plotted against time. If an arterial line is placed, arterial O_2 and CO_2 can also be added and if not, then blood O_2 saturation by pulse oximetry can substitute them. In a normal subject, $PetO_2$ and $PetCO_2$ are close to their blood counterparts. In exercise $PetCO_2$ becomes about 4 mm Hg higher than $PaCO_2$. $PetCO_2$ increases with exercise to the anaerobic threshold when the pulmonary circulation is not affected by disease and it goes over 40 mm Hg and $PetO_2$ shows a reciprocal decrease to the AT. In COPD, $PetCO_2$ is reduced relative to $PaCO_2$ because the lung units with high ventilation-perfusion are responsible for most of the patient ventilation. If a patient has severe disease, then $PetCO_2$ can become elevated in exercise.

Panel 8: respiratory exchange ratio against time is shown in panel 8. R is $\dot{V}CO_2/\dot{V}O_2$. In a normal person at rest R is around 0.8. As exercise intensifies, then R increases because of muscle respiration and becomes steeper when bicarbonate starts buffering lactic acid. The more the lactic acid production, the steeper the curve and higher the ventilatory compensation for lactic acidosis. In COPD, the slope of R may not be as steep as in a normal subject.

Panel 9: this panel deals with tidal volume against minute ventilation. During low and moderate intensity exercise, in the normal subject tidal volume increases more than breathing frequency. Above anaerobic threshold, breathing frequency becomes more important. At peak exercise, there is still some reserve left over which is around 10 to 15 L/min which is calculate as:

Breathing reserve = maximum voluntary ventilation (L/min) – VE (L/min) at maximum exercise
(Maximum voluntary ventilation is estimated as FEV_1 X 40)

Therefore,

Breathing reserve = (FEV_1 X 40) – VE

COPD patients have a limited breathing reserve which at times can be close to 0. This is because of poor gas exchange caused by airway mechanical defect and destruction of alveoli (61).

In summary, CPET is a very important tool that when used appropriately, can provide a great deal of information about the physiological state of a subject. When analyzed, the measured and derived variables can provide information about the cause of shortness of breath, fatigue, and exercise limitation in a subject being caused by either cardiac, pulmonary, or other disease states. However, CPET can be difficult to perform and interpret and it requires training by the clinician for interpretation.

D) Nitrate-Nitrite-Nitric Oxide Physiology

a) Biochemistry:

Since our research involved beetroot which is a precursor of nitric oxide (NO), this molecule and its physiological implications in health and disease will be discussed in detail.

Nitric oxide is an important gaseous radical that has significant implications in lung physiology (Fig. 2.30). Nitric oxide can be formed in human cells from amino acid l-arginine and molecular oxygen by enzymes labelled as NOS or nitric oxide synthases in the *l-Arginine-NOS-Nitric Oxide pathway*. NOS has 3 isoforms: endothelial (eNOS), neuronal (NOS1), and inducible (iNOS or NOS2). The name does not imply unique location in a tissue but indicates the site were first discovered; each isoform may appear in a variety of cell types (70).

iNOS of airway epithelium can generate large volume of NO when upregulated by tumor necrosis factor and interferon gamma; in contrast when exposed to glucocorticoids, iNOS upregulation is suppressed (71). NO can be measured in exhaled air and is labelled as FE_{NO}. As will be discussed later, it has clinical significance in disease states. Smoking lowers the FE_{NO} by 70% (72). Although yet to be shown, measurement of FE_{NO} in COPD may be helpful in showing reversibility of airways and evaluation of glucocorticoid responsiveness (73). FE_{NO} is expressed as parts per billion (ppb) which is equivalent to nanoliters per liter.

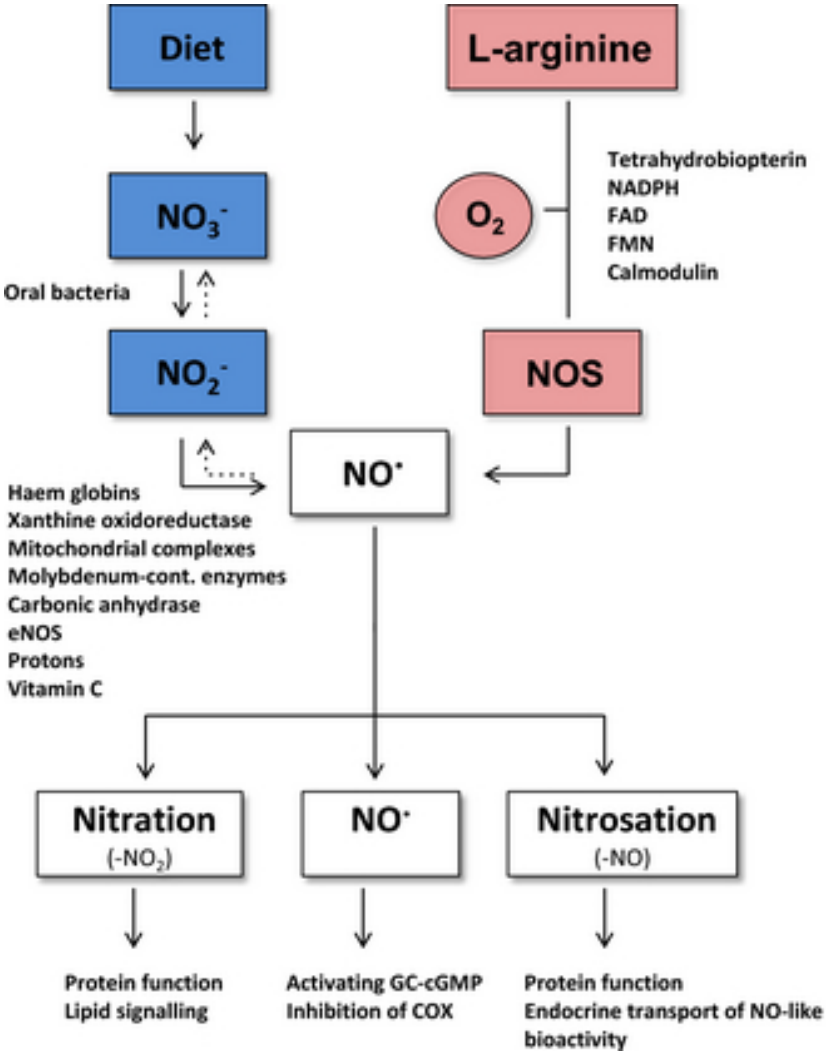


Figure 2.30. *Nitric oxide synthesis pathways, including nitric oxide synthase (NOS) and the nitrate–nitrite–NO pathway. NOS induces the oxidation of the l-arginine in the presence of O_2 and various cofactors, generating NO. In different organs, NO is oxidized to nitrite (NO_2^-) and nitrate (NO_3^-) (dashed arrows). In the other pathway, nitrate is generated from the diet; this in addition to the endogenous nitrate, can be reduced to nitrite by oral commensal bacteria. After systematic absorption of nitrite, several enzymatic and non-enzymatic pathways further reduce nitrite to NO and other reactive nitrogen intermediates. These nitrogen oxides can exert their signaling functions via nitration (–NO_2), direct NO signaling and nitrosation (–NO). FMN, Flavin mononucleotide, FAD, Flavin adenine dinucleotide, NADPH, Nicotinamide adenine dinucleotide phosphate-oxidase, COX, cytochrome C oxidase. From Hezel, MP (74), reproduced with permission*

NO synthesis by NOS is a complex process requiring numerous substrates and cofactors, l-arginine being of them. NO is involved in signaling but is rapidly oxidized which limits its signaling especially in presence of hemoglobin. NO binds rapidly to oxyhemoglobin which yields nitrate and methemoglobin. NO can also modify protein function through different mechanisms such as

activation of soluble guanylate cyclase or independent of guanylate cyclase through direct actions on DNA. In NOS pathway, NO is oxidized to nitrite and nitrate, with nitrate concentration being higher than nitrite (70).

There is another alternative for NO generation where NO_3^- (nitrate) and NO_2^- (nitrite) which are end products of NO generation, can be reduced back to NO; the *nitrate-nitrite-nitric oxide pathway* is regulated by a different pathway than the l-Arginine-NOS-Nitric Oxide pathway and is very important in tissue protection during hypoxia, regulation of blood flow, metabolism, and signaling. This pathway is upregulated by tissue hypoxia and is a backup that generates NO when the oxygen-dependent NOSs are malfunctioning. This is interesting since nitrate and nitrite which are rich in some vegetables can have potential benefits in cardiovascular physiology (75).

NO is very important in physiology. Its bioavailability is decreased in cardiovascular diseases and is augmented in hypotension and septic shock. NO is very difficult to measure in vivo; therefore, nitrate and nitrite are measured as NO markers of production. Nitrate and nitrite are increased in exercise because of circulatory shear stress which stimulates generation of NO from endothelial NOS.

b) Exercise implications of NO Pathway:

As stated above, sources of nitrates and nitrites in body include l-arginine-NOS pathway and everyday diet (Table 2.2). In dietary source, vegetables such as spinach and beetroot are high in nitrate and a serving contains higher nitrate than the entire endogenous nitrate produced by 3 isoforms in the body. Beetroot juice has a high NO_3^- content. The beetroot NO_3^- is converted to NO_2^-

by oral cavity nitrate reductase enzymes of anaerobic bacteria. The NO_2^- is then converted to NO in stomach. This process which is independent of NOS system, is dependent on the entero-salivary circulation of inorganic nitrate and is the *nitrate-nitrite-nitric oxide pathway* that was formerly described (75).

Vegetable	**Serving Size**	**Nitrate (mg)**
Potato	1 cup diced	16
Broccoli	1 cup chopped	18
Cilantro	1 cup leaves	40
Basil	1 cup leaves	44
Spinach	2 cup leaves	47
Beets	1 cup slices	188
Agurula	2 cups	192
Rhubarb	1 cup diced	343

Table 2.2. *Nitrate content of different vegetables in mg per serving size.*

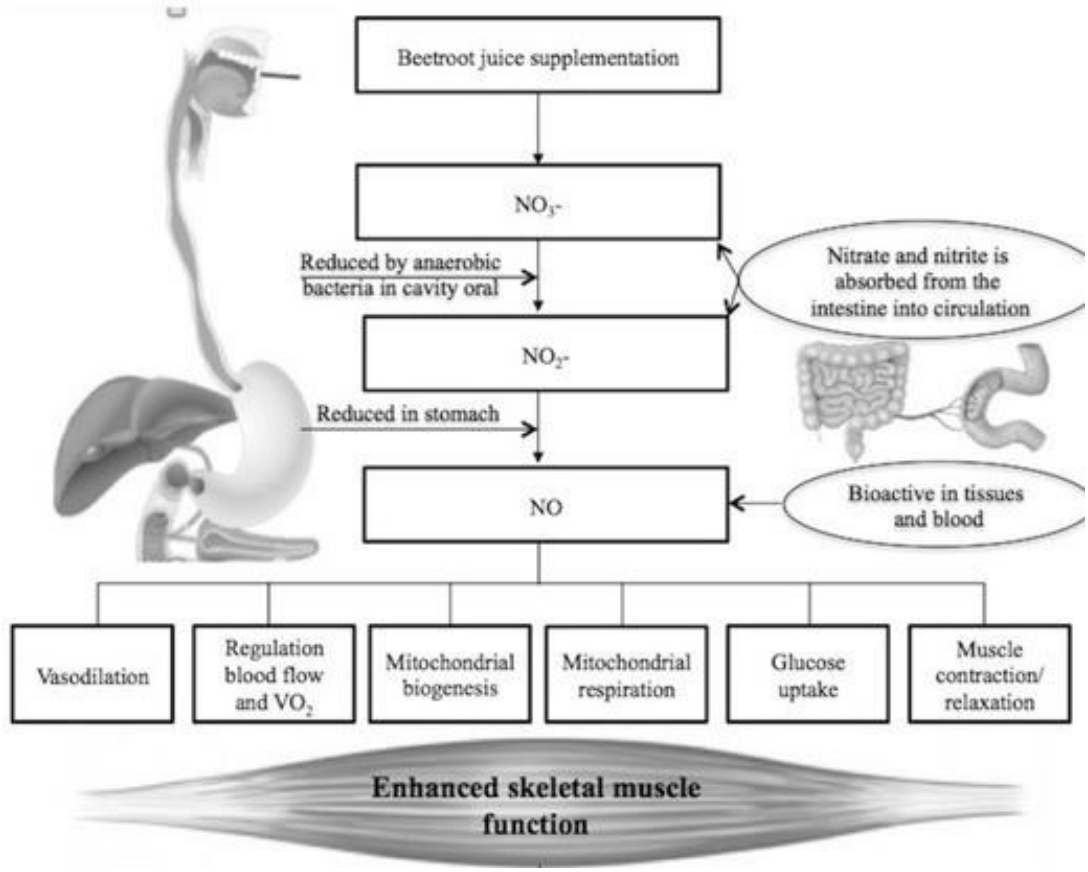


Figure 2.31. *Nitric oxide pathway of beetroot juice supplements. Nitrate of beetroot juice is reduced to nitrite and then NO. Then the remaining nitrate and nitrite are absorbed from the intestine into the circulation, converting back to NO, and becoming bioactive again with significant physiological implications. From Dominguez, et al.(76), reproduced with permission*

During hypoxic stress, such as with exercise, NO is produced in higher concentrations, causing vasodilation and greater oxygen delivery (Fig. 2.31). Furthermore, NO facilitates mitochondrial efficiency and muscle glucose metabolism and uptake, improving muscle contraction and relaxation in addition to immunomodulation (77, 78).

Studies of the role of beetroot juice in reducing oxygen cost in exercise have had mixed results. Dietary nitrate has been shown to improve performance in rowers and to improve sprint performance and cognitive function during prolonged intermittent exercise (79, 80). However, it has

not shown significant impact on O_2 kinetics and endurance in elite cyclists and has not shown improvement of repeated sprint performance (81, 82). Several confounding factors in different studies make a uniform conclusion difficult. These include duration and dose of nitrate supplementation, exercise protocol, type of sport, and exercise fitness (see below) (83). In athletes without COPD, beetroot supplementation increases the power output at each level of $\dot{V}O_2$ and also decreases the time to exhaustion at several intensities.

However, interestingly, not every study has shown a positive effect, probably due to other confounding factors such as age, diet, medications, and presence of other illnesses of the subjects, to name a few. Also, the dosing and the timing are very important. Some studies recommend dose of 6-8 mmol of NO_3^- during exercise to improve cardiovascular performance, but the efficacy in different sport types and to that effect in COPD is not fully studied. It is recommended that greater than 6 days of intake of dietary nitrate is necessary to improve the cardiorespiratory performance.

c) Nitric Oxide and COPD

This topic will be discussed in further detail in the section dealing with our nitric oxide experiments and COPD. However, it is worthwhile to discuss the topic in some length. Berry et al. studied the effects of dietary nitrate supplementation via beetroot juice on the submaximal exercise capacity of COPD patients. In their randomized, single-blind, crossover design, beetroot juice was administered at a dose of 7.58 mmol of NO_3^- (8). Relative to placebo, beetroot ingestion for 7 days, caused significant elevation of plasma NO_3 by 938% and NO_2 by 379%. Median exercise time was increased by 29 seconds and diastolic blood pressure was also reduced by around 6 mmHg. No other variables were significantly different between the two groups. In another study, Leong et al. performed a double-blind, computer-randomized placebo control crossover trial to study the effects of dietary

nitrate on exercise endurance in 19 COPD patients with stable and moderate disease (9). Patients underwent an incremental shuttle walk test to determine $\dot{V}O_2$max followed by randomization to placebo or beetroot groups. Patients performed an endurance shuttle walk test at 85% $\dot{V}O_2$max after randomization. Then they took the sample juice for 3 days and on the 4th day wash out there was a cross over between the groups. Again, blood pressure was reduced with ingestion of beetroot juice. End shuttle walk test distance and time to fatigue improved but did not reach statistical significance. In a study by Kerley et al., an increase in incremental shuttle walk distance after consumption of high nitrate juice compared to low nitrate juice was noted (25 meter compared to 14 meter). The improvement in exercise capacity was associated with statistically significant increases in serum nitrate and nitrite levels and a significant lowering of resting blood pressure (84).

These studies show that the effects of beetroot on exercise performance in COPD is not consistent and results are mixed at the best, although lowering of blood pressure probably due to vasodilatory effects was a common finding. There could be several reasons for the inconsistencies. The factors that have been suggested include deconditioning, hypoxia, hypercapnia, systemic inflammation, nutritional imbalance and medication therapy to name a few. At the physiological level, mitochondrial dysfunction and redox imbalance appear to contribute to the conflicting results that are obtained at each trial (Fig. 2.32). For example, oxidative stress shadows nitric oxide effects and is likely to be involved in the respiratory muscle dysfunction in severe COPD (85). Epigenetic modification may also play a role in muscle dysfunction in COPD. The epigenetic modifications recognized so far include DNA methylation, histone acetylation and methylation, and non-coding RNAs such as microRNAs (86). These complex, interwoven factors make prediction of dietary nitrate supplementation difficult and at times unpredictable. Therefore, there are several factors that are responsible for muscle dysfunction in COPD. In conclusion, in dietary nitrate studies, not only the dose of dietary nitrate appears to be a factor but other factors such as severity of COPD, deconditioning, muscle mass, and hypoxemia affect the end results (87).

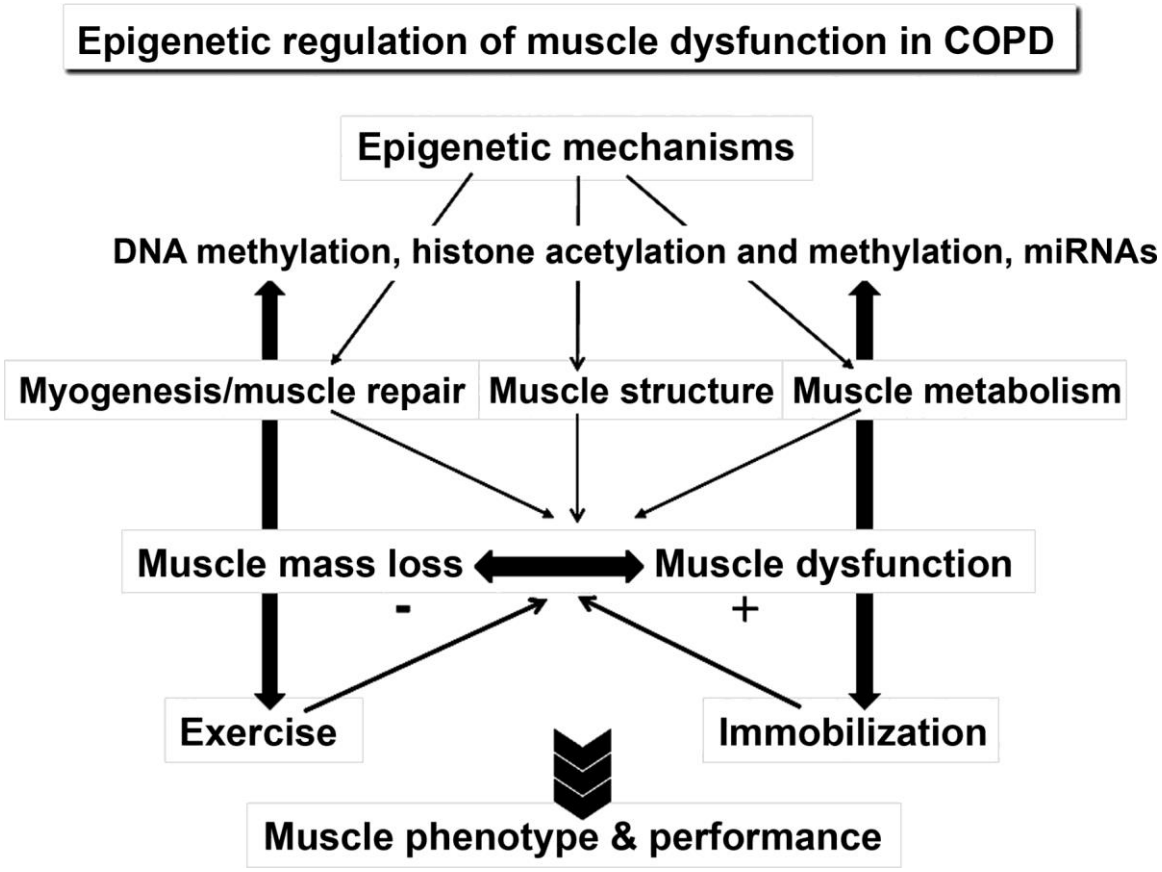


Figure 2.32. *Epigenetic regulation of muscle development and regeneration, structure, and metabolism of skeletal muscles in COPD. These mechanisms are involved in muscle mass loss and dysfunction. Environmental factors such as exercise and immobilization, which are under tight epigenetic control, also impact muscle phenotype and function in these patients; miRNA is microRNA which is a noncoding RNA. From Barreiro E, Sznajder JI (86), reproduced with permission*

Chapter 3

Methodology

The COPD study was a comprehensive clinical study requiring detailed preparation. It included various extensive organization of procedures and protocols for its successful completion. These will be further elaborated in the following sections.

a. Patient Enrollment:

1. IRB approval:

The Western Institution Review Board (WIRB) located in Pyallup, Washington, USA, approved the study design and drafted patient consent forms under the study number 1153374. Macquaire University Ethics committee, endorsed WIRB approval. The Board closely monitored the progress of the study to ensure compliance with US Federal regulations. The approval certificate is enclosed in Appendix A.

2. Patient Selection:

The patients were selected from an outpatient pulmonary clinic. All of the participants had a diagnosis of COPD based on clinical and diagnostic criteria. Inclusion criteria included a history of COPD (GOLD stage I-IV), while an inability to exercise on an exercise equipment, BMI>42, or pregnancy excluded patients from participation. Some patients had coronary artery disease. Participation was voluntary. No patient withdrew from the study after participation. All participants completed the study.

3. Informed Consent:

Before obtaining consent, the protocol was described to each patient in detail which included purpose, study design, patient recruitment, pulmonary function testing, cardiopulmonary exercise testing, and dietary beetroot supplementation. If the patient was interested in participating, then informed consent was signed. The consent form is included in the Appendix B.

b. Study Protocol:

The study design was as follows. After informed consent was obtained, St George Respiratory Questionnaire (SGRQ) was filled out (see the following section) and blood was drawn to measure hemoglobin. Then resting spirometry, lung volume and DLCO with single breath and intra-breath maneuvers were measured. The patient was instrumented with EKG leads, blood pressure cuff, and pulse oximetry, followed by placement on a semi-recumbent cycle ergometer. EKG interface was made with special wireless module. Removable breathing device with headset and circuitry was placed. Exercise was started and relative perceived exertion (RPE), dyspnea score, DLCO by intra-breath, and Q were measured every 3 minutes (see the following section). During exercise, the measurements of ($\dot{V}O_2$), ($\dot{V}CO_2$), (fb), (V_t), (VE) and derived variables (e.g., VE/$\dot{V}CO_2$) were continuously collected or calculated using a low resistance open circuit automated metabolic system (CareFusion) which will be described in detail in the next section. The test was continued until point of volitional exhaustion and was then discontinued. At 2 and 5 minutes after completion of the test, measurements of intra-breath DLCO and cardiac output in addition to exercise resting parameters were made. Then patient was randomized to either beetroot juice or placebo juice (black currant) and was given 8 days of samples, one sample bottle to be taken each day. Each patient returned in 8 days for a second study and the exercise protocol was repeated identically.

c. Questionnaires and Scales:

1. St George Respiratory Questionnaire (SGRQ):

The SGRQ is a 50-item questionnaire developed to measure health status (quality of life, QOL) in patients with obstructive lung disease. Scores are calculated for three domains: Symptoms, Activity and Impacts (Psycho-social) as well as a total score (88). Psychometric testing has demonstrated its repeatability, reliability and validity (89). Sensitivity has been demonstrated in clinical trials. A minimum change in score of 4 units has previously been established as clinically relevant. The SGRQ has been used in a range of disease groups including asthma, chronic obstructive pulmonary disease (COPD) and bronchiectasis, and in a range of settings such as randomized controlled therapy trials and population surveys (88). The SGRQ correlates significantly with other measures of disease activity such as cough, dyspnea, 6-min walk test and FEV_1 as well as other measures of general health such as the Sickness Impact Profile (SIP) score, which evaluates the impact of disease on physical and emotional functioning, and Short Form 36 (SF36) health survey which is a patient reported survey of health (90). The total score which was calculated by the Excel software sheet was included in data analysis. The complete questionnaire is enclosed in the Appendix C.

Before each test day, on the first study day and on the follow up test after juice intake, the patient completed the questionnaire and a total score was obtained.

2. RPE and Borg scales:

Rate of Perceived Exertion (RPE) is a numerical scale that classifies physical activity intensity level (Table 3.1). It was developed by Borg and has several versions. In one version, the score ranges from 1 to 10, with 1 indicating the lightest perceived intensity of activity and 10 being the maximum activity effort which is unbearable to sustain (91). The other version ranges from 6 to 20, with 6 meaning no exertion and 20 meaning maximal exertion (Table 3.2). This version is modified to estimate the heart rate by multiplying the level of exertion by 10. For example a patient at a RPE scale of 12 is estimated to have a heart rate of approximately 120 per minute (92).

The RPE score was finger pointed by the patient from a chart that was held by a nurse during the exercise. It was recorded every 2 minutes in the middle of each exercise stage.

Dyspnea Scale	Perceived Dyspnea
10	**Maximum effort:** It is impossible to continue activity any further because the patient is completely out of breath
9	**Very hard:** very difficult to sustain activity because the patient can hardly breathe and can hardly speak a few words
7-8	**Vigorous:** the patient is uncomfortable and short of breath but can speak a sentence
4-6	**Moderate:** breathing is heavy but the patient can hold a brief conversation. Semi-comfortable although becoming more challenging
2-3	**Light:** comfortable to breath and sustain a conversation and can go on for a long time
1	**Very light:** minimal amount of exertion but more than sitting on a sofa and watching TV

Table 3.1. *Rate of Perceived Exertion (RPE) scale.*

Perceived Exertion Rating	*Description of Exertion*
20	*Maximal Exertion*
19	*Extremely Difficult*
18	
17	*Very Difficult*
16	
15	*Difficult*
14	
13	*Somewhat Difficult*
12	
11	*Easy*
10	
9	*Very Easy*
8	
7	*Extremely Easy*
6	*No Exertion. Resting*

Table 3.2. *The Borg scale and its modified version of Rate of Perceived Exertion.*

d) Other Study Measurements and Beetroot Randomization:

1. Blood Draw:

Blood was drawn from antecubital vein for checking Hb (for DLCO correction) and analyzed in a hematology laboratory.

2. Blood pressure measurement, oxygen saturation, and cardiac monitoring:

Blood pressure was checked using a manual sphygmomanometer at rest and during exercise testing. One nurse was assigned for both blood pressure measurements while the patient was pedaling during the CPET and also for recording RPE and dyspnea scores during the test.

Continuous oxygen saturation was measured by Nonin 7500 Oximeter for Vmax with special probe *(Nonin Co, Plymouth, MN, USA).*

Cardiac monitoring was provided by Cardiosoft EKG from GE Marquette *(GE, Chicago, IL, USA)* with special software based 12-lead stress acquisition system. The system interfaced into the Vmax system and had automatic ST segment measurement and arrhythmia detection software.

3. Nitric Oxide Measurement:

Nitric oxide in the exhaled breath was measured using NIOX VERO *system (www.niox.com; Circassia, Oxford, UK)*. The device assesses airway inflammation in subjects as per ATS/ERS equipment recommendations. It has a short start up time and analyzes data within a minute. Measurement range for exhaled NO is 5 to 300 parts per billion (ppb) with an accuracy of 5 ppb or max 10%, and precision of <3ppb of measured value <30 ppb. Calibration is not required. To make a measurement, a filter is placed on the mouthpiece. The subject then exhales steadily for approximately 10 seconds and is guided by a visual picture on the device screen to ensure the exhalation is steady. When

maneuver is complete, the screen gives an indication and within 10 to 20 seconds, the exhaled NO value is shown on the screen (Fig. 3.1).

Figure 3.1. *NIOX system for exhaled NO measurement. From www. Niox.com*

4. Beetroot and placebo juice Randomization:

Patients were randomized to receive either beetroot or black currant juice. Beetroot was purchased from Beet It (*www.beet-it.us*) and black currant juice from Knudsen (*www.rwknudsen.com*). After randomization, the beetroot juice group of patients received 8 bottles of juice, each made of 70 ml of beetroot juice (approximately 400 mg of nitrate) plus 180 ml of water (total 250 ml). The placebo group received 8 bottles of juice, each made of 70 ml of black currant juice and 180 ml of water (total 250 ml). Black currant is devoid of nitrate. All the patients were blinded to their type of juice. Each patient had to take one bottle of juice per day for eight consecutive days, the last bottle being the day of their return for the follow up CPET study. Patients were phoned daily to remind them of taking their juice.

e. Pulmonary Function Testing and Cardiopulmonary Exercised Testing:

1. Spirometry

A spirometry was performed using the CareFusion Vmax Encore 229C Pulmonary Function *(San Diego, CA, USA).*

The system uses pneumotachograph-based pulmonary function equipment that has passed evaluation using 24-waveforms recommended by the American Thoracic Society (ATS) (42). In the first step the machine was calibrated at ambient temperature and barometric pressure. Using calibration syringe, flow volume loop was checked. Demographics were entered into the system. Then the patient was placed on a chair and connected via a mouthpiece to the breathing arm of the machine (Figs 2.20 and 3.4). A nose clip was placed and the patient was coached to take 2 to 3 breaths normally. Then a deep breath to total lung capacity was taken and blown out forcefully to residual volume, optimally for a duration of at least 6 seconds and at the end of maneuver a deep breath was taken. Spirometry was attempted at least 3 times. There should have been no coughing during the test. To meet ATS criteria, the FEV_1 and FVC should have been consistent and the largest FEV_1 and FVC should have been within 150 ml or 5% of each other. The best two of three tests were selected. Other measured parameters during the test included $FEF_{25-75\%}$, $FEF_{25\%}$, $FEF_{50\%}$, $FEF_{75\%}$, PEF, VA, and IVC. Figures 3.2, 3.3, and 3.5 include the parts used in study.

Nasal clip

Mouthpiece

MicroGard IIC bacterial filter

Figure 3.2. *Nasal clip, mouthpiece, and bacterial filter used during PFT and CPET testing. From www.carefusion.com, reproduced with permission*

2. Lung volume and total lung capacity

Using the same Vmax system lung volume measurements were done at rest. After the machine was calibrated, mouthpiece and nose clip were placed and static lung volumes were measured using the nitrogen washout method, as described in detail in chapter 2B.

In the first phase, the patient was asked to exhale and inhale normally until FRC was achieved. Breathing should have been nice and easy for the first 4-5 breaths. Then oxygen at a concentration of 100% was delivered to the lungs when the circuit was switched over. The oxygen was dry and may have made the patient uncomfortable. The subject would breathe in and out slowly and regularly for a few minutes. Oxygen would slowly replace and "wash out" nitrogen out of the lungs and concentration of nitrogen would reduce with each breath. When nitrogen concentration was below 1% then the test was terminated. This test would generally take a few minutes. FRC should be within 10% of each other for acceptability. After FRC measurement, ERV and IC can also be linked to the measurement and to derive the total lung capacity. Maximal voluntary ventilation (MVV) is total volume of air exhaled during rapid deep breathing in one minute which is measured for 12 seconds and then multiplied by 5.

Breathing hose

Calibration Syringe

Figure 3.3. *Breathing hose and calibration syringe for PFT machine. From www.carefusion.com, reproduced with permission*

3. Diffusion capacity

a. Single breath: Classic single breath DLCO was determined following the recommendations of the ATS/ERS (46, 93). After calibration of the machine, the patient was connected to the breathing arm via MicroGard IIC bacterial filter/mouthpiece well sealed around the teeth and a nose clip was placed. Two to three normal inhalations were performed to obtain a good baseline FRC. Next, exhalation to residual volume followed by deep inspiration to 85% of vital capacity over approximately 10 seconds was performed, allowing the gas mixture to equilibrate within the lungs. The patient was then instructed to breathe out to residual volume. The gas mixture that was used was air, and helium, 0.3% CO. The first liter of expelled air was considered to be gas from anatomical dead space and was discarded with the subsequent next liter used for further calculation. Two to three trials were performed aiming for a DLCO within 3 mmHg of each other. The average between these trials were taken.

b. Intra-breath: Pulmonary Blood Flow (Qc) and diffusing capacity of the lungs for carbon monoxide (DLCO) were measured using inert and soluble gases on the CareFusion Vmax system using an intra-breath maneuver (52). For this maneuver, patients were asked to breath on a mouthpiece while wearing a nose clip. Patients were instructed to exhale to residual volume (RV) and then were switched in to an inspiratory reservoir inhaling to total lung capacity a test gas mixture containing 0.3% carbon monoxide (CO), 0.3% methane, 0.3% acetylene, 21% O_2, and balance N_2. Patients were coached to exhale slowly at a steady rate until they were near RV. From the rate of disappearance of CO and acetylene in comparison to the inert gas methane the rate of disappearance of CO and acetylene were determined. This rate of disappearance of CO provided the DLCO value. Since acetylene does not bind to hemoglobin, the rate of its disappearance is limited only by the flow of blood through the lungs, thereby providing a measure of Qc (59).

Figure 3.4. *Exercise and PFT equipment used for clinical studies. The metabolic cart and PFT system are noted on the right and on left is the Corival ergometer. Photograph taken at laboratory of M. Behnia.*

4. Cardiac output measurement:

Pulmonary blood flow was used as a surrogate estimate of cardiac output (Qc) measured by intra-breath maneuver as described above. See the section on pulmonary blood flow in chapter 2B.

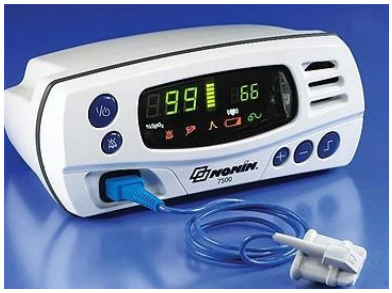
5. Cardiopulmonary exercise testing:

The system used was Vmax Encore module with mass flow sensor with Kelvin-Sensing circuitry and rapid response analyzer with special displays. Following PFT testing and cardiac output measurement at rest, patients performed cardiopulmonary exercise testing (CPET) using the Corival semi-recumbent cycle ergometer (*Lode, Netherlands*). The metabolic cart was a CareFusion Vmax Encore system. Prior to exercise, the patient was connected to a 12 lead EKG (Cardiosoft GE

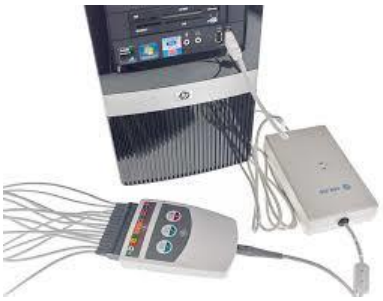
Marquette module) for continuous cardiac electrical activity monitoring. Continuous peripheral oxygen saturations were measured using the Nonin system (Fig. 3.5).

EKG electrode

CPET headset halo



Nonin Pulse oximeter



EKG Module

Flow meter assembly

Saliva trap

Figure 3.5. *Accessories used during CPET testing. For details, refer to text. From www.carefusion.com, reproduced with permission*

Each patient wore a nose clip. A CPET headset halo was placed on the head and patient was connected to a mouthpiece, saliva trap, and flow meter assembly which was then connected to the system by a breathing hose. The patient then breathed through a mouthpiece for continuous measurement of gas exchange during the exercise (Figs. 2.20 and 3.4).

The test protocol started with 20 watts for both men and women and increased by 10 watts every 2 min. During the last 30 seconds of each workload, a 12 lead EKG recording was printed; in the meantime, blood pressure (BP) was measured by a nurse, RPE score and Borg dyspnea score were rated by pointing to a board held by a nurse in front of the patient. Averages of measured heart rate and SpO_2 over this period were also determined. The goal was to obtain at least 2 to 3 work levels for each patient. Patients were encouraged to exercise to near exhaustion by achieving an RPE of 17-18 on the Borg 6-20 scale or a dyspnea score ≥7 on the 0-10 score (92). The test was terminated when the patient could not continue any further due to severe exhaustion or shortness of breath. After reaching peak exercise, an active recovery period commenced where the patient continued to pedal against light resistance and remained on the mouthpiece for one minute. Following this, the patient ceased pedaling and was given time for HR and BP to return to baseline before being dismissed.

Measured parameters: During exercise testing, the following parameters were measured directly and continuously: oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), breathing frequency (f_b), tidal volume (V_t), minute ventilation (VE).

Derived variables that were obtained included:

$VE/\dot{V}CO_2$, f_b/V_t, TI/TTOT, V_t/IC (%), $PetCO_2$ (mmHg), $PetO_2$ (mmHg), $P_{\bar{E}}CO_2$ (mmHg), O_2 pulse

These were measured continuously or calculated using a low resistance open circuit automated metabolic system.

(TI: inspiratory time, TTOT: total respiratory cycle time, IC: inspiratory capacity, O_2 pulse: $\dot{V}O_2$/heart rate, $PetCO_2$ end tidal partial pressure of carbon dioxide, $PetO_2$ end tidal partial pressure of oxygen, $P_{\bar{E}}CO_2$ expired pressure of carbon dioxide, IC: inspiratory capacity).

During CPET flow volume spirometry loops were also measured: $FEF_{25\%}$, $FEF_{50\%}$, $FEF_{75\%}$, $FEF_{25-75\%}$, IC, Vt/IC.

CHAPTER 4

Study I: Influence of Resting Lung Diffusion on Exercise Capacity in Patients with COPD

This chapter was presented as a *featured abstract* at American College of Chest Physicians Annual Meeting in 2016 in Los Angeles, CA

Published in:

Chest; volume 150 (4) Supplement; 891A, 2016 (refer to page 197)

The study was published as a manuscript in BioMed Central (Pulmonary):

Behnia M, Wheatley CM, Avolio A, Johnson BD. Influence of resting lung diffusion on exercise capacity in patients with COPD. BMC Pulm Med. 2017 Aug 25;17(1):117. doi: 10.1186/s12890-017-0454-y.

4. a. Summary

Lung diffusing capacity for carbon monoxide (DLCO) gives an overall assessment of functional lung surface area for gas exchange and can be assessed using various methods. DLCO is an important factor in exercise intolerance in patients with chronic obstructive pulmonary disease (COPD). The objective was to investigate if the intra-breath (IBDLCO) method may give a more sensitive measure of available gas exchange surface area than the more typical single breath (SBDLCO) method and if COPD patients with the largest resting DLCO relative to pulmonary blood flow (Qc) would have a more preserved exercise capacity. Informed consent was obtained. Hemoglobin measurement, spirometry, SBDLCO, IBDLCO, and Qc during IBDLCO were performed in moderate to severe COPD patients, followed by progressive cycle ergometry to exhaustion with measures of oxygen saturation (SaO_2) and expired gases. Thirty-two patients (47% female, age 66±9 yrs, BMI 30.4±6.3 kg/m^2, smoking history 35±29 pack years, 2.3±0.8 on the 0-4 GOLD classification scale) participated. The majority used multiple inhaled medications and 20% were on oral steroids. Averages were: FEV_1/FVC 58±10 % predicted, $\dot{V}O_2$peak 11.4±3.1 ml/kg/min, and IBDLCO 72% of the SBDLCO (r=0.88, SB vs IB methods). Using univariate regression, both the SB and IBDLCO (% predicted but not absolute) were predictive of $\dot{V}O_2$peak in ml/kg/min; SBDLCO/Qc (r=0.63, p<0.001) was the best predictor of $\dot{V}O_2$peak; maximal expiratory flows over the mid to lower lung volumes were the most significantly predictive spirometric measure (r=0.49, p<0.01). However, in multivariate models only BMI added additional predictive value to the SBDLCO/Qc for predicting aerobic capacity (r=0.73). Adjusting for current smoking status and gender did not significantly change the primary results. In conclusion, in patients with moderate to severe COPD, preservation of lung gas exchange surface area as assessed using the resting SBDLCO/Qc appears to be a better predictor of exercise capacity than more classic measures of lung mechanics.

4. b. Introduction

Causes contributing to exercise limitation in patients with chronic obstructive pulmonary disease (COPD) are complex (94-96). Previous studies have suggested that while lung mechanics clearly play an important role, there are many other factors that contribute to this limitation such as heterogeneity of the disease process, lifestyle issues, weight and activity patterns, deconditioning, disease related inflammatory processes, perception, as well as associated comorbidities such as cardiovascular disease (97-99). Pulmonary function measures representing the degree of obstruction and severity of hyperinflation (e.g., inspiratory capacity or IC) appear important as well as less appreciated factors such as a blunted cardiac output, either due to airway obstruction and rise in intra-thoracic pressure or from the development of pulmonary hypertension (100-102). As a result, exercise capacity as a whole has been used as a prognostic indicator in the COPD population and as such is a good assessment of the integrative factors involved in the disease (103). In addition, as stated by the GOLD initiative (Global Initiative for Chronic Obstructive Lung Disease classification for air flow obstruction), improvement in exercise tolerance is recognized as an important goal of COPD treatment.

From the lung volume reduction surgery data, it has also been found that certain patterns of disease and perhaps more severe emphysema may be associated with worse exercise tolerance (104, 105). Of the common relatively simple screening tests, a low lung diffusing capacity for carbon monoxide (DLCO) has been shown to not only suggest a more emphysematous pathophysiology but has also been a predictor of exercise capacity and in particular exercise induced oxygen desaturation (94, 106). There are different ways to quantify DLCO, from the typical SBDLCO, to various rebreathe, steady state, open-circuit and IBDLCO techniques (50). While the latter methods may represent in some sense more physiological quantification of functional lung surface area for gas transfer or

exchange, the single breath method has been standardized with well-established predictive norms for clinical use (46).

The IBDLCO is interesting in that it potentially represents a relatively simple way to quantify DLCO in patients that may struggle with longer breath hold times and there is potential for use during exercise to quantify alveolar–capillary surface area recruitment. It requires the exhalation of test gas typically near residual volume, followed by a deep inhalation and essentially instantaneous exhalation back towards RV. The expiratory sampling relies on a fast response CO analyzer and as exhalation continues towards RV, the DLCO at any point is dependent then on the exhaled lung volume and each time point would represent a DLCO that is a mix of CO uptake and mixing with other lung gases (52, 53). An advantage of this technique compared to other more discrete techniques is that the exhaled gas stream is used in its entirety to calculate DLCO.

In patients with lung disease it is likely that the intra-breath method may be more sensitive to disease pathology relative to the single breath method due to abnormalities in ventilation and perfusion and delayed time constants for ventilation with the shortened gas exchange times.

Lung diffusion is dependent on pulmonary capillary blood volume (Vc) and alveolar-capillary gas exchange surface area, usually reported as membrane diffusion capacity. While these components of DLCO can be estimated by performing DLCO at multiple oxygen concentrations or with a second gas such as nitric oxide (DLNO), a surrogate may be obtained for blood volume by examining the DLCO relative to cardiac output (Qc). Since a rise in Q tends to be the major reason for distension or recruitment of capillaries, a larger ratio would be indicative of a healthier phenotype.

Thus, in the present study the goal was to study the role of resting DLCO in predicting exercise capacity in a relative diverse group of COPD patients. More specifically, to evaluate if a higher intra-

breath to single breath DLCO ratio or a higher DLCO relative to Qc ratio would better predict exercise capacity relative to other common measures of lung mechanics; an additional goal was to see if the patients with a higher IBDLCO relative to SBDLCO or a higher SBDLCO/Qc ratio would have better preserved exercise capacity.

4. c. Methods

i- Ethics and consent

The study, ethics, and consent forms were reviewed and approved by the Western Institutional Review Board (WIRB, study number 1153374) as described in chapter 3a.

ii- Patient selection

Patients with a history of COPD that were sent for clinical pulmonary function testing and/or exercise testing were offered enrollment. This is elaborated in chapter 3a.

iii- Overview of study

After reporting to the outpatient clinic, study participants filled out the SGRQ, performed pulmonary function testing (PFTs) which included resting measures of maximal lung volumes and flow rates using classical spirometry (refer to chapter 3c and 3e). In addition, the assessment of lung diffusing capacity for carbon monoxide (DLCO) was obtained using the classical single breath technique and was also obtained using the intra-breath method which included a measure of pulmonary blood flow (Qc) (chapter 3e). A small blood sample was obtained prior to testing for assessment of hemoglobin. Patients subsequently performed cardiopulmonary exercise testing using the CareFusion Vmax Encore metabolic cart (*San Diego, CA*) with a Corival recumbent cycle ergometer (*Lode, Netherlands*), starting with 20 watts for both men and women and increasing by 10 watts every 2 min. EKG monitoring and peripheral oxygen saturation (SaO_2) for continuous monitoring

were also done. Subjects wore a nose clip and breathed on a mouthpiece for continuous measurement of gas exchange during the exercise test (chapter 3d).

During the last 30 sec of each workload, a 12 lead EKG recording was printed, blood pressure (BP) assessed, perceived dyspnea score (0-10 Borg scale) and perceived exertion (an assessment of total body effort on 6-20 scale) was rated by patients, and an average of the HR and SpO_2 over this period was determined. The goal was to obtain at least 2–3 work levels for each patient. Patients were encouraged to exercise to near exhaustion based on symptom limitation by achieving an RPE of 17-18 on the Borg 6-20 scale or a dyspnea score ≥7 on the 0-10 score (92). Upon reaching peak symptom limited exercise, patients performed active recovery where they continued to pedal with no resistance and remained on the mouthpiece for one minute. After this the patient stopped pedaling and was given time for HR and BP to return to baseline. Details are elaborated in chapter 3 sections c, d, and e.

iv- Pulmonary function and single breath DLCO

Spirometry was performed using pneumotachograph-based pulmonary function equipment that is described in chapter 3e. Classic single breath DLCO following the recommendations of the ATS/ERS (46, 93) is also detailed in the same chapter.

v- Intra-breath DLCO and pulmonary blood flow (Qc)

Pulmonary Blood Flow (Qc) and diffusing capacity of the lungs for carbon monoxide (DLCO) were measured using inert and soluble gases (52) as elaborated in chapter 3e (59, 107).

vi- St. George's respiratory questionnaire

Every patient had to fill out the St. George 50-item questionnaire before the CPET was started (88). The SGRQ is described in detail in chapter 3c (90).

vii- Gas Exchange, ventilation and lung mechanics

During exercise testing oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), breathing frequency (fb), tidal volume (V_t), minute ventilation (VE) and derived variables (e.g., $VE/\dot{V}CO_2$) were measured continuously or calculated using the low resistance open circuit automated metabolic system (CareFusion) that was described previously in chapter 3e.

viii- Statistical analysis

We were interested in the association of resting measures of DLCO measured via single breath and intra-breath methods as well as expressed relative to Qc with exercise capacity (peak $\dot{V}O_2$) in patients with moderate to severe COPD and if these measures were more highly associated to exercise capacity than more typical measures of lung mechanics. Descriptive statistics were used to describe patient characteristics and demographics while multiple regression and correlational analysis were used to determine associations between DLCO, Q, lung mechanics, QOL, disease severity and exercise capacity. Statistics were performed with a combination of EXCEL and the statistical software package JMP Statistical Discovery TM software from SAS (*www.sas.com; Cary, NC, USA).*

4d. Results

i. Patient characteristics and pulmonary function measures

Thirty-two patients completed the study. As shown in Table 4.1, on average our study cohort was older, approximately half female, above ideal body mass index and had a 35-pack year smoking history. By design, their GOLD classification ranged from 1-4 with an average classification consistent with moderate disease with an FEV_1 of 56% of age predicted and an FEV_1/FVC ratio of 59% (Table 4.2). None of the patients were on continuous home oxygen or oxygen for exercise at the time of the study. The majority of patients took a combination of inhalers that included inhaled beta-2 agonist, anticholinergic, and inhaled steroid with a minority of patients on oral steroids.

Quality of life scores based from the St George questionnaire was consistent with severity of disease as described by the GOLD classification.

Table 4.1. *Patient Characteristics of COPD patients (n=32).*

	Mean ± SD	Range
Age (years)	66 ± 9	46 - 84
% Female	47	-
Weight (Kg)	88 ± 23	36 - 155
BMI (Kg/m2)	30 ± 6	13 - 44
Smoking history (pack year)	35 ± 29	0 - 120
Current/former/never smoker (n)	6/22/4	-
GOLD Classification (1-4)	2.3 ± 0.8	1 - 4
St George Respiratory Questionnaire	44 ± 21	8 - 84
Inhaled beta agonist (%)	97	-
Inhaled anticholinergic (%)	59	-
Inhaled steroid (%)	68	-
Oral steroid (%)	20	-

GOLD. Global Initiative for Chronic Obstructive Lung Disease classification for air flow obstruction.

Table 4.2. *Pulmonary Function Variables*

	Mean ± SD	Percent Predicted (range)
FVC (L)	2.48 ± 0.69	75 ± 15
FEV_1 (L)	1.51 ± 0.58	56 ± 16
FEV_1/FVC	59 ± 11	(33 – 78)
FEF_{25-75} (L/s)	0.75 ± 0.38	26 ± 13
FEF_{75} (L/s)	0.29 ± 0.11	27 ± 13
MVV (L/m)	48 ± 19	45 ± 17

FVC: Forced Vital Capacity, FEV_1: Forced Expiratory Volume in 1 second, FEF: Forced Expiratory Flow, MVV: Maximal voluntary ventilation. All data are pre bronchodilator.

Table 4.3. *Lung Diffusing Capacity and Pulmonary Blood Flow*

	Mean ± SD	**Percent Predicted or (Range)**
Single Breath DLCO (SBDLCO, ml/min/mmHg)	13.2 ± 5.5	58 ± 23 (31 –112)
Intra-Breath DLCO (IBDLCO, ml/min/mmHg)	9.7 ± 5.9	(1.3 – 27)
IBDLCO/SBDLCO (%)	71 ± 26	(20 – 110)
Pulmonary Blood Flow (Qc, L/m, measured)	4.8 ± 0.9	(3.3 – 6.8)
Pulmonary Blood Flow–Cardiac output, (L/m, Predicted)	6.3 ± 0.4	(5.4 – 7.1)
SBDLCO/Qc ratio	2.8 ± 1.2	(1.2 – 5.7)
Hb (g/dl)	13.5 ± 1.7	(11-17)

Pulmonary Blood Flow measured with soluble gas method. Cardiac output estimated based on age, gender, BSA, from Williams, LR (108). Qc = Pulmonary Blood Flow.

ii. Resting lung diffusion measures, single breath vs intra-breath

Table 4.3 lists single breath and intra- breath DLCO measures, the measured pulmonary blood flow (Qc), and Hb values. SBDLCO averaged 13.2 ml/min/mmHg and 58% of predicted with the average IBDLCO 71% of the SB method ranging from 20 to 110% across the study population. Overall the SB and IB methods were highly correlated with an r of 0.88 (Fig. 4.1) and the IB/SBDLCO relationship was positively associated with resting IC (Fig. 4.2). The measured pulmonary blood flow (Qc) using soluble gas was 76% of the resting predicted cardiac output based on gender and body size. On average the SBDLCO was 51% of predicted in current smokers vs 56% of predicted in those that had quit or never smoked. Though the current smokers were slightly reduced relative to nonsmokers, there was no statistical difference between groups ($p<0.05$).

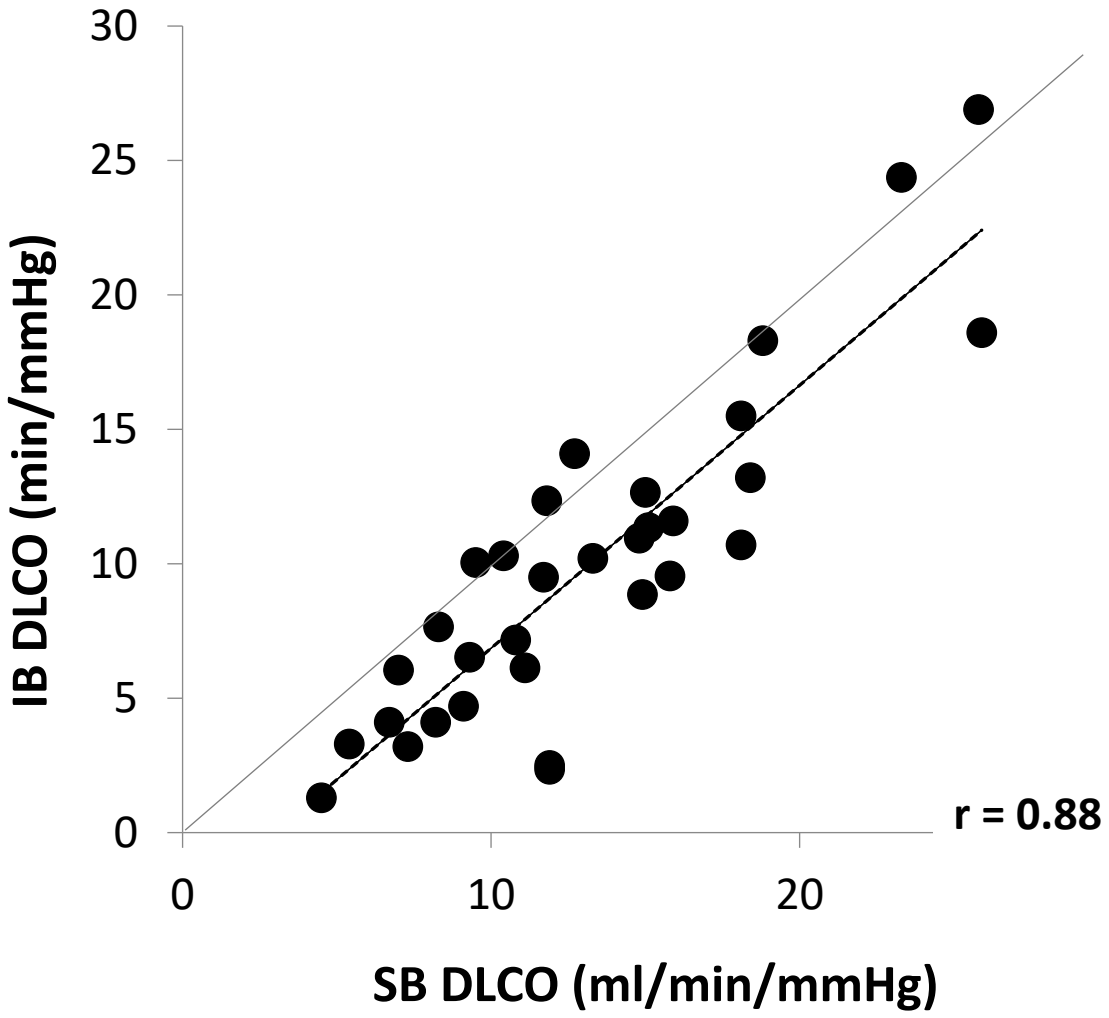


Figure 4.1. *Relationship of Single Breath DLCO to Intra-Breath DLCO (n=32). (y= 0.9780 x – 2.9202). The IBDLCO was on average lower than the SBDLCO (p<0.001) particularly in patients with values that were more significantly reduced relative to predictive values (<65% of predicted).*

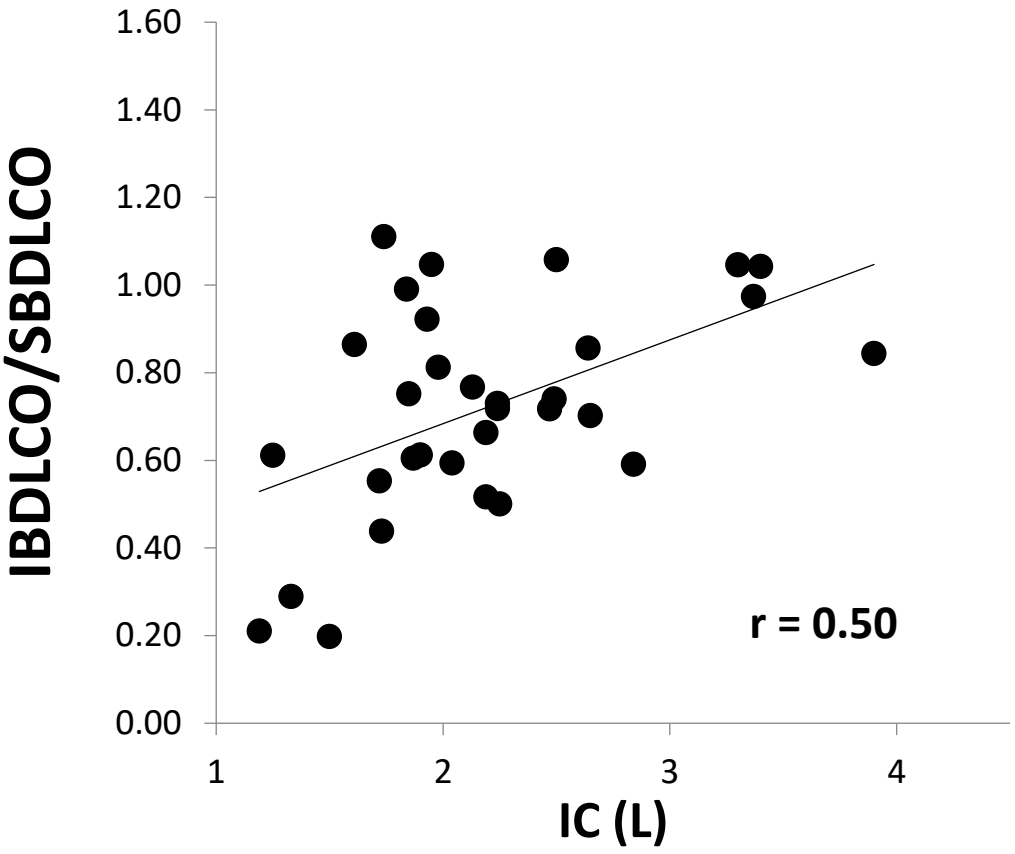


Figure 4.2. *Relationship of inspiratory capacity (IC) to IB and SB DLCO ratio in patients with COPD (n=32) (y = 0.1912 x + 0.313).*
Patients with the highest IC tended to have the highest ratio of IB to SBDLCO.

iii. Cardiopulmonary exercise responses

Cardiopulmonary exercise responses are reported in Table 4.4. On average the peak $\dot{V}O_2$ for the group was 0.98 L/min or 11.4 ml/kg/min equivalent to 50% of age and gender predicted. Average peak heart rate was 103 bpm which was 70% of their age predicted and average respiratory exchange ratio (RER) was 1.03. Inspiratory capacity consistently fell throughout exercise and at peak the tidal volume reached 65% of the IC. Oxygen pulse rose to an average of 8 ml/beat early in exercise but then plateaued thereafter and reached a max of 9.1 in peak exercise suggesting a plateauing of cardiac stroke volume. Patients complained of both general fatigue and dyspnea as major reasons for stopping the test. At peak exercise the minute ventilation averaged 73% of the maximum voluntary ventilation (MVV) with five patients exceeding their pre-test MVV.

Table 4.4. *Breathing Pattern, Lung Mechanics and Gas Exchange Responses to Exercise (n=32)*

	Rest	First work load	Peak Exercise
Heart Rate (bpm)	77 ± 10	92 ± 11	103 ± 22
RPE (6-20)	7 ± 2	11 ± 2	17 ± 2
Dyspnea (0-10)	1 ± 1	3 ± 2	7 ± 2
VE (L/min)	12.5 ± 2.7	24 ± 6	34 ± 11
Fb/VT ratio	26 ± 15	26 ± 14	30 ± 13
TI/TTOT ratio	39 ± 8	38 ± 4	39 ± 4
IC (L)	2.2 ± 0.7	1.9 ± 0.7	1.8 ± 0.7
VT/IC (%)	37 ± 13	52 ± 12	65 ± 27
$\dot{V}O_2$ ml/kg/min	3.7 ± 0.8	8.3 ± 1.4	11.4 ± 3.1
VE/$\dot{V}CO_2$ ratio	47 ± 7	37 ± 5	36 ± 5
$PetCO_2$ mmHg	35 ± 5	37 ± 4	37 ± 5
O_2Pulse	4 ± 1	8 ± 2	9 ± 3
SaO_2 (%)	96 ± 2	95 ± 3	94 ± 3

VE: Minute ventilation, fb: breathing frequency, VT: tidal volume, TI: inspiratory time, TTOT: total respiratory cycle time, IC: inspiratory capacity, $\dot{V}O_2$: oxygen consumption, $\dot{V}CO_2$: carbon dioxide production, $PetCO_2$: end tidal partial pressure of carbon dioxide, O_2pulse: $\dot{V}O_2$/heart rate, SaO_2: arterial oxygen saturation estimated from pulse oximetry.

iv. Relationship of Resting Measures of lung mechanics and lung diffusion to Exercise Capacity

Univariate correlations of resting measures of lung function, QOL, anthropometric measures and lung diffusion relative to exercise capacity (expressed in ml/kg/min as well as in L/min) are shown in Table 4.5. The values that were most significantly linked to exercise capacity based on V̇O2peak in ml/kg/min were SBDLCO relative to pulmonary blood flow (SBDLCO/Qc) (Fig. 4.3), SBDLCO (% Pred), VT/IC, (where VT is tidal volume), absolute measures of $FEF_{25-75\%}$ (Fig. 4.4), $FEF_{75\%}$ and BMI ($p<0.01$). Also associated but less significantly so ($p<0.05$ but >0.01) were the IBDLCO also relative to Qc as well as FVC and $FEF_{50\%}$. In a step wise fashion or when allowing all significant variables to compete in a multiple regression, only SBDLCO/Qc and BMI remained in a model predicting $\dot{V}O_2$peak ml/kg/min where:

[$\dot{V}O_2$ peak ml/kg/min= 2.51 (DLCO/Qc) - 0.176 (BMI) + 11.48, with an r of 0.73 and an r^2 of 0.53.]

Table 4.5. *Univariate Correlations with $\dot{V}O_2$peak expressed in L/min and ml/kg/min (n=32)*

	$\dot{V}O_2$peak ml/kg/min	p-value	$\dot{V}O_2$peak L/min	p-value
SB DLCO	0.18	0.312	0.51	0.002
SB DLCO (%Pred)	***0.49***	***0.004***	0.48	0.006
IB DLCO	0.20	0.277	0.51	0.002
IB DLCO (% Pred)	0.41	0.020	0.49	0.004
IB/SB	0.01	0.974	0.37	0.039
SB DLCO/Qc	***0.63***	***0.000***	***0.59***	***0.000***
IC (L)	0.40	0.020	***0.60***	***0.000***
VT/IC	***-0.42***	***0.017***	***-0.55***	***0.001***
FVC	0.41	0.018	0.54	0.001
FEV_1 (% Pred)	0.38	0.030	0.22	0.217
FEF_{25-75} (L/min)	***0.49***	***0.004***	0.48	0.006
FEF_{75} (L/min)	***0.47***	***0.006***	0.41	0.018
Wt (kg)	-0.30	0.092	0.55	0.001
BMI	***-0.42***	***0.017***	0.32	0.078
BSA	0.16	0.370	***0.64***	***0.000***
QOL	-0.23	0.200	-0.05	0.773
GOLD classification	-0.27	0.140	-0.20	0.275

*SB – single breath method, IB – intra-breath method. Values in columns 2 through 5 that are most significantly linked to exercise capacity based on $\dot{V}O_2$peak are in **bold** and italics. In column 1, only the variables that predict $\dot{V}O_2$peak in ml/kg/min in a multiple regression analysis, are in **bold**. All DLCO values are corrected for Hb.*

When expressing V̇O2 peak as L/min, SBDLCO/Qc, IC, VT/IC and BSA were the most predictive of exercise capacity with FVC being the strongest lung mechanics measure in a multiple regression but which lost significance when BSA was added. Thus, the best model for absolute V̇O2 was:

[$\dot{V}O_2$ peak L/min= 0.2038 (DLCO/Qc) + 0.6193 (BSA) - 0.665, with an r of 0.81 and an r^2 of 0.65.]

In rare cases of co-linearity between variables, typically during the stepwise regression, the variable that resulted in the highest R squared after selective addition or subtraction, was left in the model.

v. Influence of smoking and gender on predictors of exercise capacity

Since six of the patients in the cohort were current smokers and current smoking and the time of abstention from smoking is known to impact DLCO (109, 110), both stepwise and multivariate models accounting for smokers were performed. Under both conditions, SBDLCO/Qc and BMI still remained significant predictors ($p<0.01$) with the influence of smoking being not significant ($p=0.67$). Subgroup analysis excluding current smokers was also performed relative to $\dot{V}O_2$peak ml/kg/min, where DLCO/Q and BMI remained significant predictors ($p=0.000$ and $p=0.009$ respectively). Percent predicted SB and IBDLCO were also not significantly different between current and past smokers ($p<0.05$).

Analysis also accounted for gender in the models, including when expressing $\dot{V}O_2$peak in L/min rather than in ml/kg/min. There was no influence of gender on the relationship between SBDLCO/Qc and $\dot{V}O_2$peak. When expressing $\dot{V}O_2$peak in L/min, SBDLCO/Qc and BSA remained the most significant predictors.

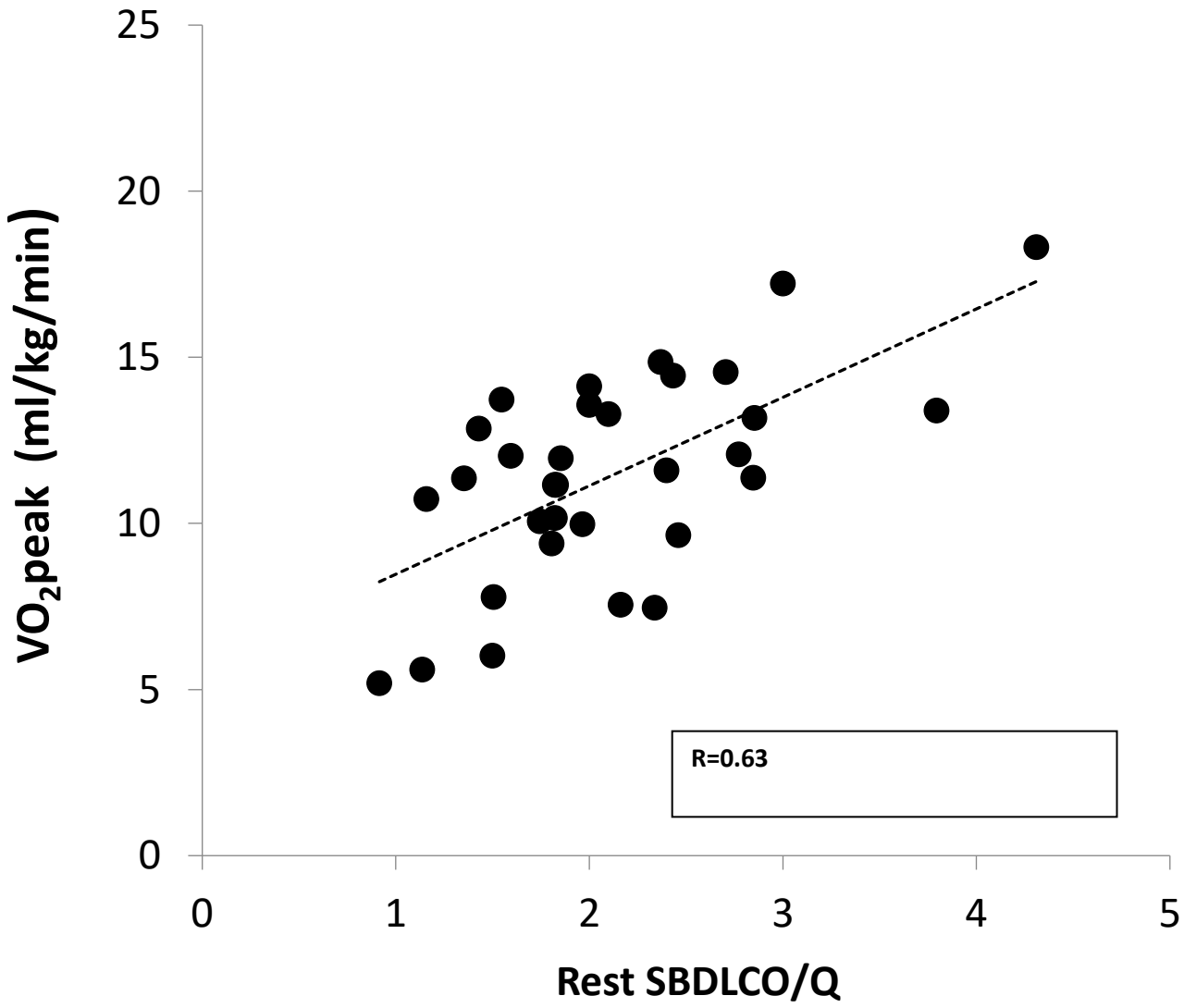


Figure 4.3. *Relationship of SBDLCO/Qc and Exercise Capacity based on $\dot{V}O_2peak$ in patients with COPD* (*y = 2.6621 x + 5.8078).*
The ratio of lung diffusing capacity to pulmonary blood flow was the best predictor of $\dot{V}O_2peak$ in this population.

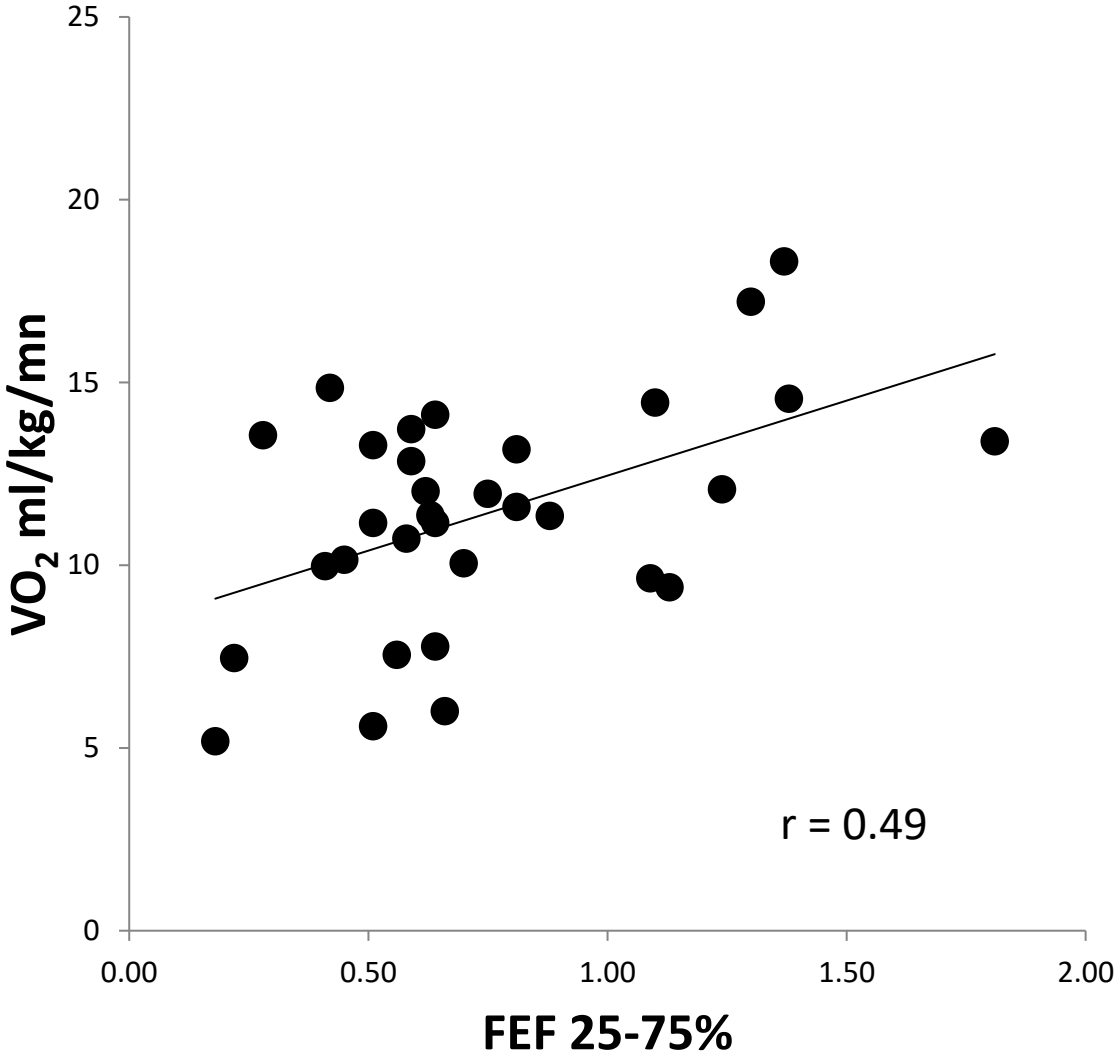


Figure 4.4. *Relationship of FEF $_{25\text{-}75\%}$ to $\dot{V}O_2$peak in patients with COPD (n=32) (y = 4.1039 x + 8.3463). $FEF_{25\text{-}75\%}$ was the best univariate lung mechanics predictor of exercise capacity but did not remain in a predictive model when DLCO and weight were added.*

4. e. Discussion

i. Primary findings

From this study it is concluded that while resting measures of hyperinflation and maximal expiratory flows, particularly over the mid to lower lung volumes were predictive of exercise capacity, lung diffusing capacity alone or expressed relative to resting pulmonary blood flow was the most predictive of exercise capacity. Furthermore, when allowed to compete in a multiple regression model, only the SBDLCO relative to Qc and measure of body weight or habitus were significant predictors and explained approximately 50-60% of the variability in exercise capacity in this population.

ii. Previous studies investigating exercise intolerance in COPD

Factors contributing to exercise intolerance in patients with COPD are complex. While a number of studies have examined predictors of exercise capacity in the COPD population, the majority of these have focused on measures of lung mechanics and while relationships are found between measures of maximal expiratory flows and volumes, measures of hyperinflation appear to be the most predictive (96, 101, 102, 111, 112). In this study we evaluated several measures of lung function, quality of life, body weight/habitus and measures of lung diffusing capacity measured differently or expressed relative to lung function and pulmonary blood flow. We found that lung mechanics, particularly flows at the mid to lower lung volumes and the inspiratory capacity relationship, or tidal volume inspiratory capacity relationship, seemed to be the most predictive. However, when allowed to compete in a model with measures of lung diffusion and measures of body weight or habitus, the measures of mechanics no longer reached significance. In particular when SBDLCO was expressed

relative to the measured Qc with BMI or when predicting $\dot{V}O_2$ in L/min, BSA together in a model the mechanics measures, were no longer contributory.

DLCO is a variable of paramount importance in pulmonary medicine. It represents a complex integration of factors including ventilation distribution, matching of ventilation to perfusion, the resistance at the alveolar-capillary membrane as well as the combination rates with hemoglobin. Since all of the above factors can be affected with more classic patterns of emphysema and COPD with ultimate destruction of the alveolar-capillary bed, preservation of DLCO is an important marker of lung health (106). For example, a major factor contributing to recruitment or distension of the pulmonary capillary bed is cardiac output or pulmonary blood flow (Qc) (113). As alveolar-capillary walls are remodeled, destroyed or even stiffened with disease, the DLCO/Qc relationship are altered. With exercise, ventilation rises and pulmonary blood flow increases resulting in elevation of DLCO. In emphysema and COPD with loss of alveolar volume, an important adaption to maintain gas exchange in the face of increased blood cell transit time is a rise in pulmonary capillary blood volume. Thus, preservation of this relationship in this population should be a discernible advantage. It was interesting that the IBDLCO, while highly correlated to the SBDLCO, was not as predictive of exercise capacity as the SB method. Our original rationale was that since the IB method was performed more quickly and at a lung volume more specific to tidal breathing, that it may be a more sensitive predictor of functional gas exchange surface area. However, during the rapid inspiratory phase of the SBDLCO method, potentially increasing pulmonary blood volume, and with the inhalation to total lung capacity, increasing alveolar volume, it is likely this gives a better overall representation of functional or possibly recruitable surface area available for use during exercise. Also, of note is the fact that the IBDLCO method appears more variable across patients and less reproducible than the SB method.

iii. Other predictors of exercise capacity in COPD

In what have become classical studies by O'Donnell and colleagues, resting IC, degree of hyperinflation with exercise, and change in IC, have all been highly predictive of exercise capacity (102). Hyperinflation is associated with expiratory flow limitation, volume constraints and less optimal respiratory muscle performance (96, 105, 111). Dynamic hyperinflation during exercise contributes to perceived respiratory discomfort. Indirect evidence of the importance of dynamic hyperinflation comes from studies that have demonstrated that alleviation of dyspnea following bronchodilator therapy and lung volume reduction surgery (LVRS) are both explained, in part, by reduced operating lung volumes (114). Additional studies have suggested that COPD patients enter into a spiral of decline associated with reduced activity, inflammation and skeletal muscle dysfunction (95, 115, 116). A high work and cost of breathing in the setting of elevated operational lung volumes and in some cases excessive expiratory muscle work and also diaphragmatic fatigue contribute to exercise intolerance (115). COPD also is associated other co-morbidities such as coronary artery disease, pulmonary vascular disease, pulmonary hypertension and right ventricular failure; all of which impair cardiac output and thus compromise oxygen delivery(116). While the reality is that these collective contributors to exercise limitation in COPD are all integrated and codependent, this study suggests that maintenance of a functional alveolar-capillary bed is an important determinant of patient's ability to exercise and likely to carry on normal daily activities.

Targeting the airways and the inflammatory pathways has been the cornerstone of therapy for COPD and emphysema which are accomplished by classes of beta-2 agonists, anticholinergics, and steroids. Using the same rational, targeting diffusing capacity, i.e. the pulmonary vasculature, by medications has been tried. However, the results have been disappointing. For example, sildenafil, a phosphodiesterase type-5 (PDE-5) inhibitor with vasodilatory properties, commonly used in treatment of pulmonary arterial hypertension (PAH), has been tried in COPD patients who did not have pulmonary hypertension. Interestingly, the drug worsened gas exchange, increased the

alveolar-arterial oxygen difference, and did not improve exercise capacity, possibly by causing ventilation-perfusion mismatch, indicating the need for more studies and new medications (26).

CHAPTER 5

Study 2: Alveolar-Capillary Reserve during Exercise in Patients with Chronic Obstructive Pulmonary Disease

This chapter was presented as a *featured abstract* at the American College of Chest Physicians Annual Meeting in 2016 in Los Angeles, CA

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DOI: 10.2147/COPD.S142523

5. a. Summary

In this chapter, measures of alveolar-capillary recruitment during exercise are quantified and their relationship to exercise capacity are explored in our cohort of COPD patients. As previously discussed, factors limiting exercise capacity in patients with chronic obstructive pulmonary disease (COPD) are complex. With evidence for accelerated pulmonary vascular aging, destruction of the alveolar–capillary bed, and hypoxic pulmonary vasoconstriction, the ability to functionally expand the alveolar-capillary surface area during exercise may become a primary limitation. 32 patients who were recruited gave informed consent (53% male, age 66±9 yrs, smoking 35±29 pkyr, GOLD 0-4 scale 2.3±0.8; mean±SD), completed the St. George QOL questionnaire, had a CBC drawn and performed spirometry, lung volume, and diffusion capacity. They also performed the IB technique for lung diffusing capacity as well as for pulmonary blood flow (IBQc). Subsequently they completed the cycle ergometry test to exhaustion with measures of oxygen saturation (SaO_2) and expired gases. The IBDLCO and IBQc were measured during the 1st workload (WL). The results showed that baseline average measures were: 44±21 for QOL (range 8-84) and 58±11 for FEV_1/FVC (range 33-78%). Peak $\dot{V}O_2$ was 11.4±3.1 ml/kg/min (49% Pred). The mean resting IBDLCO was 9.7±5.4 ml/min/mmHg and IBQc 4.7±0.9 L/min. At the 1st WL, HR increased to 92±11bpm, $\dot{V}O_2$ was 8.3±1.4 ml/kg/min and IBDLCO and IBQc increased by 46 and 43% respectively ($p<0.01$ relative to rest). The IBDLCO/Qc ratio averaged 2.0±1.1 at rest and remained essentially constant during exercise with marked variation across patients (range 0.8-4.8). Ventilatory efficiency plateaued at 37±5 during exercise. The $P_ETCO_2/PetCO_2$ ratio ranged from 0.63 to 0.67 while a noninvasive index of pulmonary capacitance, GxCap, rose 138% from rest to peak. The exercise IBDLCO/Qc ratio was related to O_2pulse ($\dot{V}O2/HR$, $r=0.58$, $p<0.01$) and patients with the highest exercise IBDLCO/Qc ratio or the greatest rise in this ratio from rest had the highest peak $\dot{V}O_2$ values ($r=0.65$ and 0.51, respectively, $p<0.05$). Of the noninvasive gas exchange measures of pulmonary vascular function, GxCap was the most closely associated with DLCO, DLCO/Qc and $\dot{V}O_2$ peak. We conclude that the COPD patients who can expand gas exchange surface area as assessed with DLCO during exercise relative to pulmonary blood flow have a more preserved exercise capacity.

5. b. Introduction

Exercise stresses our physiological reserve by increasing muscular contraction and increasing the demand for blood flow and ventilation to maintain gas exchange (117). As cardiac output rises, the typically compliant pulmonary vascular bed expands to increase lung surface area while at the same time remaining a low-pressure system for the efficient forward blood flow. In health, this expansion of the alveolar–capillary bed results in a nearly linear rise in lung diffusing capacity for carbon monoxide (DLCO) with cardiac output (Q) as exercise intensity increases (118).

In patients with COPD, as destruction of the alveolar-capillary bed occurs, areas of ventilation and perfusion mismatch create hypoxic constriction of pulmonary vessels. Furthermore, airway obstruction increases intrathoracic pressure influencing venous return to the right side of the heart (119). Remodeling of the pulmonary capillaries may also occur resulting in stiffer vessels. Thus, as the disease pathophysiology progresses, the process limits the ability of the alveolar–capillary bed to expand and potentially not only limiting the rise in gas exchange surface area, but also creating an impediment to forward blood flow or cardiac output, which could ultimately contribute to exercise intolerance in this population.

Physiology of exercise in COPD has been a topic of great interest. Previous work by Potter (119) suggested that the degree of airway obstruction during exercise may result in a blunted rise in cardiac output presumably due to the large rise in expiratory intrathoracic pressures, while Montes de Oca (120) demonstrated an association of oxygen pulse, a surrogate for stroke volume, with exercise capacity in COPD patients. Similarly Fuji (121) suggested that a higher slope of change in pulmonary arterial pressure (Ppa) relative to the change in Q was associated with a reduced exercise capacity.

In the previous chapter, we demonstrated that resting DLCO is predictive of exercise capacity in a COPD population and more significantly so when accounting for the resting pulmonary blood flow or Qc, or essentially the functional lung surface area for gas exchange relative to cardiac output (10). The DLCO is highly dependent on Q and to date most studies suggest a strong linear relationship between the two with no evidence of a DLCO plateau during heavy exercise even in highly fit individuals (122). Since a rise in cardiac output is the main determinant of pulmonary capillary recruitment, the DLCO relationship relative to Q gives a good overall estimate of the ability to expand this vascular bed.

Therefore, the focus of this study was to quantify DLCO at rest and during exercise using the intra-breath method in a moderate to severe COPD population. We hypothesized that the patients who could increase DLCO in proportion to the rise in pulmonary blood flow or Qc (maintenance of the relationship) would have a more preserved exercise capacity. We preferred the intra-breath over the single breath DLCO technique because the latter is difficult to perform with a 10 second breath hold in dyspneic COPD patients at rest and more so during exercise. The intra-breath technique has been compared to other techniques such as the rebreathe or open circuit and has been validated (50, 123, 124).

An additional goal of our study was to analyze the relationship of DLCO and Qc to other noninvasive measures of respiratory gas exchange that have been associated with pulmonary vascular function. These included ventilatory efficiency, mixed expired to end tidal CO_2 ratio ($P_{\bar{E}}CO_2$ /$PetCO_2$) as well as a more novel measure previously associated with pulmonary vascular capacitance in the heart failure population, GxCap, which is (O_2 pulse X $PetCO_2$) (125-127).

5. c. Methods

i- Patient inclusion

Enrollment consent, and patient inclusion and exclusion criteria were described in detail in chapter 3a. The same COPD patients that were enrolled in study I, also participated in study II.

ii- Overview of study

Study participants filled out the SGRQ before the test, performed pulmonary function testing which included resting measures of maximal lung volumes and flow rates using classical spirometry (128, 129). In addition, the assessment of lung diffusing capacity for carbon monoxide (DLCO) was obtained using the classical single breath (SB) technique and was also obtained using the intra-breath method (IB) which included a measure of pulmonary blood flow (Qc) (59). A small blood sample was obtained. Patients subsequently performed CPET on the cycle ergometer as described in chapter 3e. The test protocol started with 20 watts for both men and women and increased by 10 watts every 2 minutes. During the last 30 sec of each workload, a 12 lead EKG was recorded, blood pressure (BP) assessed, and an average of the HR and SaO_2 over this period was determined. The patient dyspnea by the Borg scale (on the 0 to 10 scale) and the total body effort by the rated perceived exertion (RPE) score (on the Borg 6 to 20 scale) were assessed. Patients were encouraged to exercise to near exhaustion by achieving an RPE of 17-18 on the Borg 6-20 scale or a dyspnea score ≥7. Upon reaching peak exercise, patients performed active recovery where they continued to pedal with no resistance and remained on the mouthpiece for one minute. After this the patients stopped pedaling and were given time for HR and BP to return to baseline. Details are outlined in chapter 3 sections c, d, and e.

iii- Pulmonary function and single breath DLCO

Spirometry was performed using pneumotachograph-based pulmonary function equipment described in chapter 3e. Classic resting single breath DLCO was determined following the recommendations of the ATS/ERS as outlined in the same chapter.

iv- Intra-breath DLCO (IBDLCO) and pulmonary Blood Flow (IBQc)

Pulmonary Blood Flow (Qc) and intra-breath DLCO were measured using inert and soluble gases with the intra-breath method which is described in detail in chapter 3e (52, 59).

v- Gas Exchange, GxCap, ventilation and lung mechanics

During exercise testing oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), breathing frequency (fb), tidal volume (V_t), minute ventilation (VE) and derived variables were measured continuously and/or calculated using the metabolic system that was described previously in chapters 3e and 4c.

In addition, measures associated with ventilation and perfusion matching or pulmonary vascular health were determined. One of the measures was partial pressure of mixed expired CO_2 ($P_{\bar{E}}CO_2$) relative to end tidal CO_2 ($PetCO_2$) or **($P_{\bar{E}}CO_2$ / $PetCO_2$)** which is associated with ventilation and perfusion matching in the lungs and is reported to be reduced in COPD patients with a negative slope during exercise in patients with pulmonary hypertension. The other gas exchange measure was GxCap which is a noninvasive estimate of pulmonary vascular capacitance calculated as oxygen pulse multiplied by $PetCO_2$, or **($\underline{O_2\ pulse}$ X $\underline{PetCO_2}$).** Oxygen pulse tracks stroke volume and $PetCO_2$ has been shown to reasonably track pulmonary arterial pressure. The last of the measures was ventilatory efficiency or **$VE/\dot{V}CO_2$** (125, 127).

vi- Statistics

We were interested in exercise limitation in the COPD population and the potential role of the alveolar–capillary bed, lung surface area for gas exchange or the ability to expand this bed as a mediating factor. Thus, our primary measures were the change in IBDLCO and the change in IBDLCO in proportion to IBQc across patients and the relationship to exercise or aerobic capacity, primarily peak $\dot{V}O_2$. So, we used multiple regression and correlational analysis to asses these relationships. In addition, general descriptive statistics were used to define our group and paired t-tests to assess changes with exercise.

5. d. Results

i. Patient characteristics and resting pulmonary function

Thirty-two patients completed all aspects of the study (Age 66 ± 9 yrs, 53% male, smoking history 35 ± 29 pack years, BMI 30±6 Kg/m^2; mean ± SD). The study cohort consisted of primarily moderate COPD patients based on the GOLD classification (2.3±0.8), but with some patients mild and others more severe (GOLD classification range 1 to 4). This degree of disease was reflected in the QOL scores (SGRQ 44±21) and medication use with the majority of patients on multiple inhalers and with 20% on oral steroids. None of the patients were on continuous oxygen or oxygen for exercise at the time of the study. Hemoglobin values were essentially within normal limits and averaged 13.5±1.7 g/dl.

The forced vital capacity (FVC) averaged 2.48±0.69L or 75% of age and gender predicted, while the forced expiratory volume in one second (FEV_1) averaged 56±16% of predicted with an FEV_1/FVC ratio of 58±11%, range 33 to 78%. The $FEF_{25-75\%}$ averaged 0.75 ± 0.38L or 26±13% of predicted. The single breath DLCO averaged 13.2 ± 5.5 ml/min/mmHg and 58±23% of age and gender predicted. Both the SGRQ and the GOLD classification somewhat associated with resting DLCO (r values ranging from 0.33 to 0.38).

ii. Cardiopulmonary exercise responses

Cardiopulmonary responses to exercise are shown in Table 5.1. Most common symptoms for stopping exercise were dyspnea, general fatigue and leg fatigue. Peak work achieved was 43 watts with a $\dot{V}O_2$peak of 11.4 ml/kg/min or 49% of age and gender predicted. Peak heart rate was 106 bpm or 70% of age predicted. Tidal volume increased early in exercise but then plateaued as ventilation was primarily rate dependent beyond the first work load. Peak minute ventilation reached 70% of the estimated breathing capacity and IC fell throughout exercise suggesting expiratory flow limitation and some hyperinflation as exercise progressed. Oxygen pulse rose to 7.8 ml per beat by the first exercise load, but then rose slowly thereafter.

Ventilatory efficiency assessed as the $VE/\dot{V}CO_2$ ratio was elevated at rest, fell with exercise but stayed elevated without a clear exercise nadir. The $P_{\bar{E}}CO_2$ /$PetCO_2$ ratio started at 0.54 at rest and stayed below a ratio of 0.70 throughout exercise. The gas exchange estimate of pulmonary capacitance, GxCap (O_2 pulse X $PetCO_2$), on average increased 138% from rest to peak exercise but plateaued or declined in approximately half (47%) of the patients over the last two workloads.

Table 5.1. *Cardiopulmonary Responses to Exercise in COPD Patients (Mean ± SD)*

n=32	Rest	First work load	70-90% of Peak	Peak
Exercise Capacity				
Work (Watts)	0	18 ± 4	33 ± 14	43 ± 19
$\dot{V}O_2$ (ml/kg/min)	3.66 ± 0.84	8.34 ± 1.40	10.00 ± 2.50	11.40 ± 3.12
Symptoms				
RPE (6-20 Borg Score)	7 ± 2	11 ± 3	15 ± 3	17 ± 2
Dyspnea (0-10 Score)	1 ± 1	3 ± 2	5 ± 2	7 ± 2
Cardiovascular				
Heart Rate (bpm)	77 ± 10	92 ± 11	100 ± 11	106 ± 12
O_2 Pulse ($\dot{V}O_2$/HR)	4.1 ± 0.9	7.8 ± 2.1	8.9 ± 2.7	9.3 ± 3.1
Blood Pressure SBP (mmHg)	118 ± 24	137 ± 17	146 ± 20	152 ± 21
DPB (mmHg)	73 ± 10	82 ± 11	86 ± 10	88 ± 11
Ventilation and Breathing Pattern				
Ventilation (L/min)	12 ± 3	24 ± 6	28 ± 9	34 ± 10
Tidal Volume (L)	0.79 ± 0.17	1.13 ± 0.37	1.15 ± 0.36	1.17 ± 0.37
Breathing Frequency (bpm)	18 ± 6	25 ± 7	28 ± 7	31 ± 5
IC (L)	2.15 ± 0.67	1.89 ± 0.71	1.78 ± 0.83	1.70 ± 0.69
VT/IC	37 ± 13	52 ± 12	61 ± 25	65 ± 27
VE/Breathing Capacity (%)	27 ± 9	51 ± 25	58 ± 21	69 ± 21
Gas Exchange – Pulmonary Vascular				
VE/$\dot{V}CO_2$ ratio	47 ± 7	37 ± 5	37 ± 5	36 ± 5
$PetCO_2$ (mmHg)	35 ± 5	37 ± 5	37 ± 5	37 ± 5
$P_{\bar{E}}CO_2$ (mmHg)	19 ± 3	23 ± 3	24 ± 3	24 ± 3
$P_{\bar{E}}CO_2$ /$PetCO_2$ ratio	0.54 ± 0.04	0.63 ± 0.05	0.66 ± 0.06	0.67 ± 0.06
GxCap	143 ± 40	289 ± 85	317 ± 102	341 ± 116

VE: Minute ventilation, fb: breathing frequency, VT: tidal volume, TI: inspiratory time, TTOT: total respiratory cycle time, IC: inspiratory capacity, $\dot{V}O_2$: oxygen consumption, $\dot{V}CO_2$: carbon dioxide production, $PetCO_2$: end tidal partial pressure of carbon dioxide, O_2 pulse: $\dot{V}O_2$/heart rate, SaO_2: arterial oxygen saturation estimated from pulse oximetry. GxCap: noninvasive estimate of pulmonary vascular capacitance (O_2 pulse X $PetCO_2$)

iii. Measures of DLCO and pulmonary blood flow

The changes in DLCO, Qc, stroke volume and DLCO relative to Qc are shown in Table 5.2. Individual changes in DLCO and DLCO/Qc from rest to the first workload are shown in Figs. 5.1 and 5.2. On average DLCO increased 45% from rest to the 1st work load. However, there was large variation in

responses with 3 patients demonstrating >10% fall in DLCO and another 4 patients demonstrating minimal change with exercise. Pulmonary blood flow increased on average 40% during the 1st work load while stroke volume increased 18%. The mean IBDLCO relative to Qc (DLCO/ Qc) stayed relatively constant with exercise increasing on average 14% from rest to the 1st work load. While the majority of patients demonstrated a rise in this ratio, 12 of the patients had no change or a fall, suggesting Qc was increasing out of proportion to lung surface area for gas exchange in these patients.

Table 5.2. *Intra-breath Lung Diffusing Capacity and Pulmonary Blood Flow at rest and during Exercise in COPD Patients (Mean ± SD)*

n = 32	**Rest**	**First Workload**	**% Change**	**Range % change**
Intra Breath DLCO (IBDLCO, ml/min/mmHg)	9.6 ± 5.9	13.3 ± 7.1	45 ± 44%	-22 to 137%
Pulmonary Blood Flow (Qc, L/m, measured)	4.8 ± 0.9	6.6 ± 1.4	40 ± 28%	0.6 to 123%
Cardiac Stroke Volume (SV ml)	62 ± 14	73 ± 20	18 ± 23	36 to 133%
IBDLCO/IBQc	2.01 ± 1.1	2.03 ± 1.0	14 ± 51%	-50 to 161%

DLCO: Lung diffusing capacity for carbon monoxide. COPD: Chronic Obstructive Pulmonary Disease, Qc: Pulmonary Blood Flow measured with soluble gas method. IB: intra-breath technique.

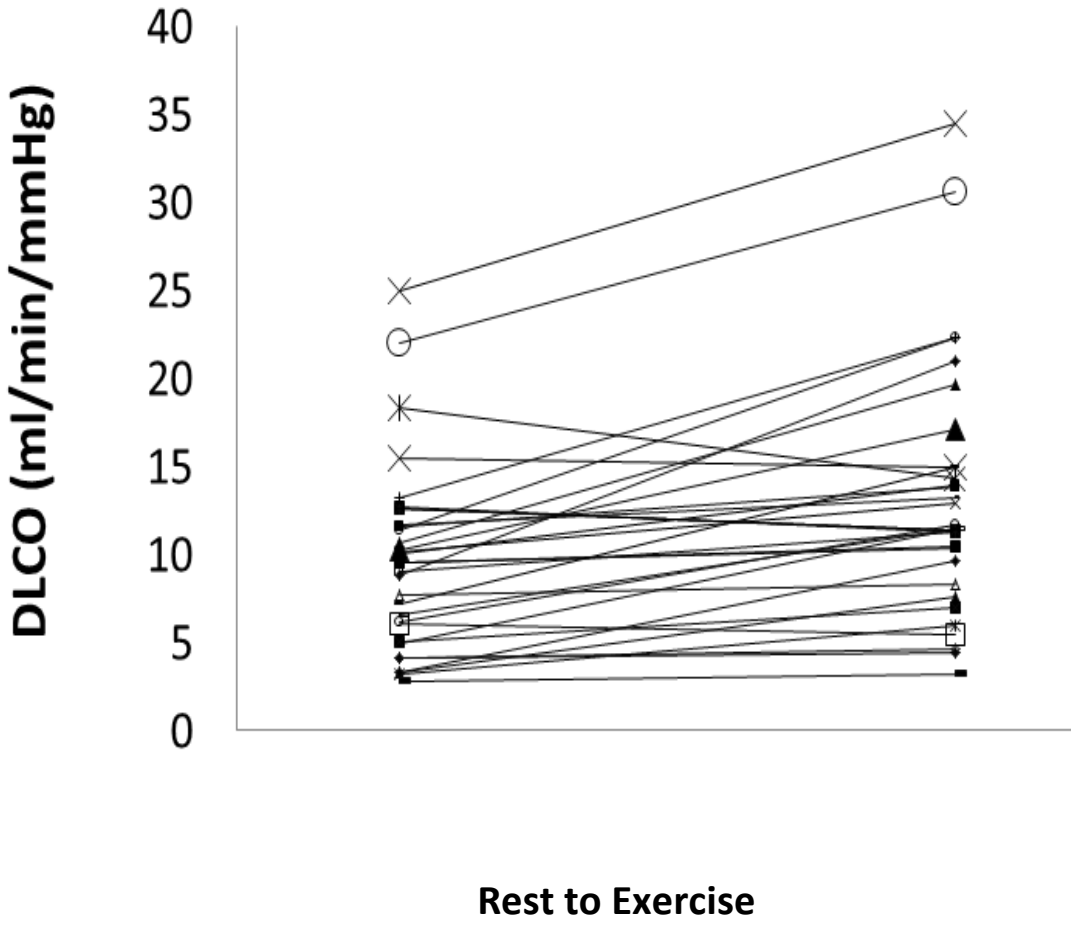

Figure 5.1. *Change in intra-breath DLCO from rest to first stage of exercise in patients with COPD*

iv. Respiratory measures associated with lung diffusion

We were interested in the relationship of other noninvasive indices associated with pulmonary vascular function and lung diffusion during exercise. Ventilatory efficiency for carbon dioxide, (V_E/VCO_2)(r=-0.44), the mixed expired to end tidal CO_2 ratio, ($P_{\bar{E}}CO_2$ /PetCO2) (r=0.40), and GxCap were all associated with the exercise IBDLCO measure ($p<0.05$). However, the GxCap was the gas exchange measure highly associated with exercise IBDLCO (Fig. 5.3) (r=0.71).

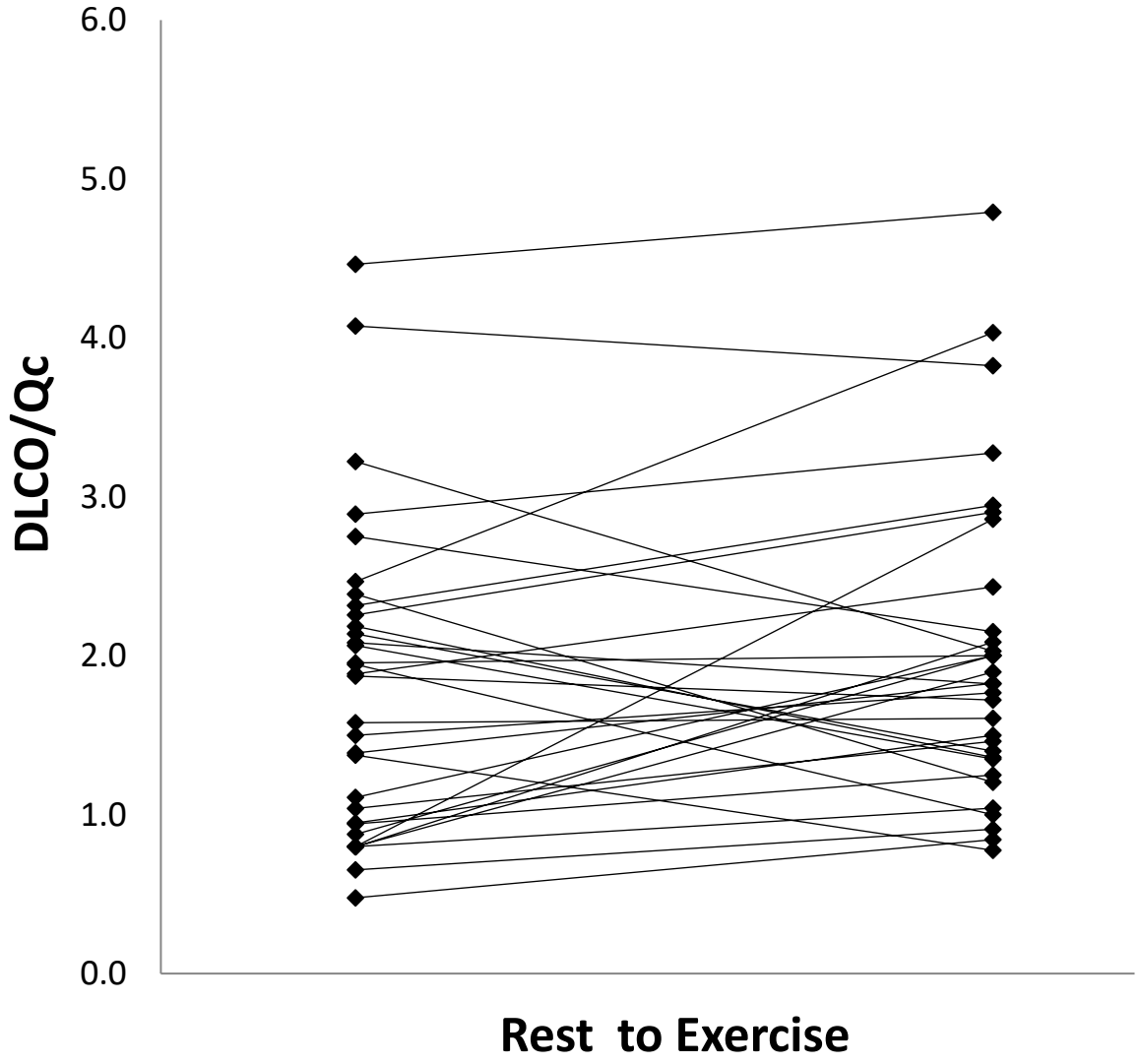

Figure 5.2. *Change in intra-breath DLCO relative to pulmonary blood flow (Qc) from rest to first exercise work load in patients with COPD.*

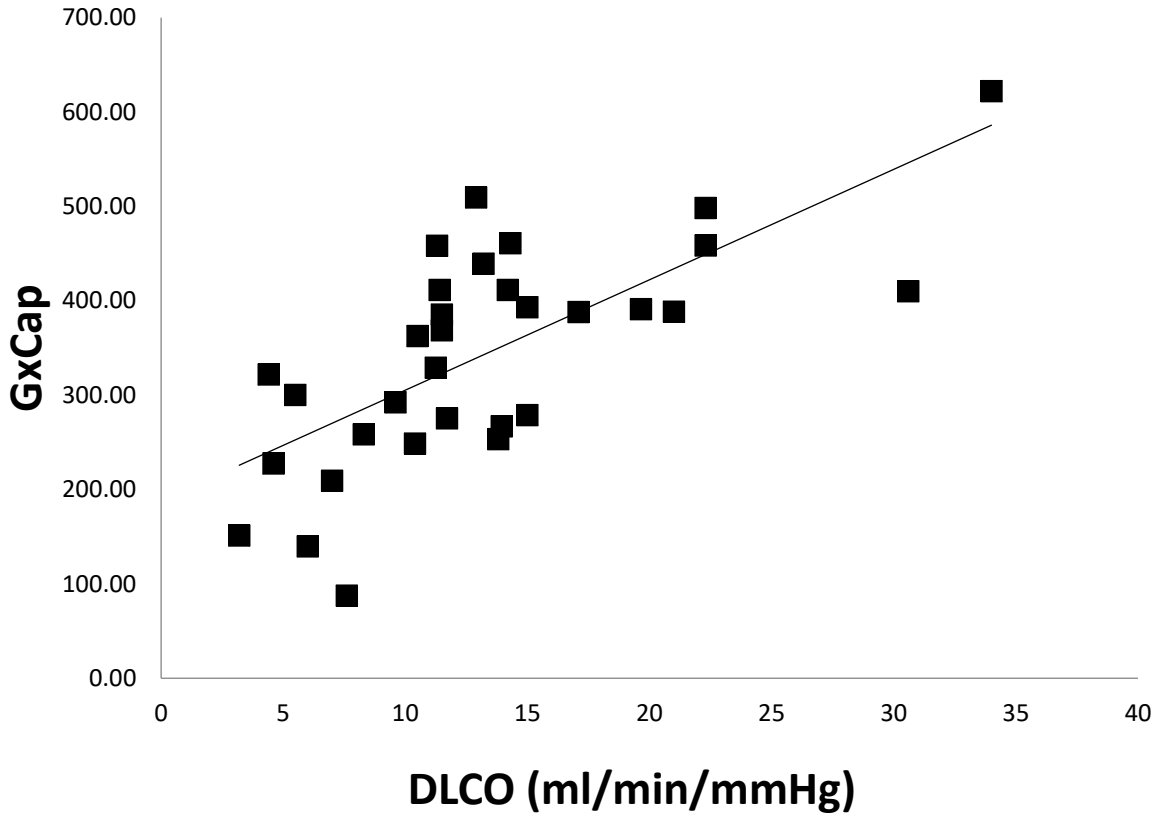

Figure 5.3. *Relationship of GxCap to DLCO in COPD patients. (y=11.70 x + 188.290)*

v. Relationship of lung diffusion, pulmonary blood flow and indices of pulmonary vascular function to exercise capacity

The exercise IBDLCO and change from rest in the IBDLCO were only marginally associated with $\dot{V}O_2$peak (r=0.43 and 0.39, p<0.05), while the relationship of exercise DLCO/Qc or the change in DLCO/Qc to $\dot{V}O_2$peak were more strongly related (r=0.63 and r=0.54, p<0.01). Fig. 5.4a shows the relationship of $\dot{V}O_2$peak and Fig. 5.4b shows the relationship of $\dot{V}CO_2$peak with DLCO/Q, respectively. The modest correlation with these measures suggests patients with the greatest recruitment in lung gas exchange surface area relative to pulmonary blood flow were the patients with the best exercise tolerance. With multiple regression including IBDLCO, the change in IBDLCO and DLCO/Qc, the DLCO/Qc from rest to exercise was the measure most significantly associated with $\dot{V}O_2$peak. DLCO/Qc was also associated with O_2pulse (r=0.63). Furthermore, exercise capacity was associated

with the $P_{\bar{E}}CO_2$ /$PetCO_2$ ratio with those with higher ratios having better exercise tolerance (r=0.57 between $P_{\bar{E}}CO_2$ /$PetCO_2$ vs $\dot{V}O_2$peak), while both the absolute measure of Gxcap during exercise as well as the change from rest to peak exercise being associated with $\dot{V}O_2$peak (r=0.53 and 0.66 – Fig. 5.5, respectively, $p<0.01$). The lung function measure most associated with DLCO or DLCO/Qc during exercise was the exercise inspiratory capacity (IC) (r=0.58 and 0.63, respectively, $p<0.01$) as shown in Fig. 5.6.

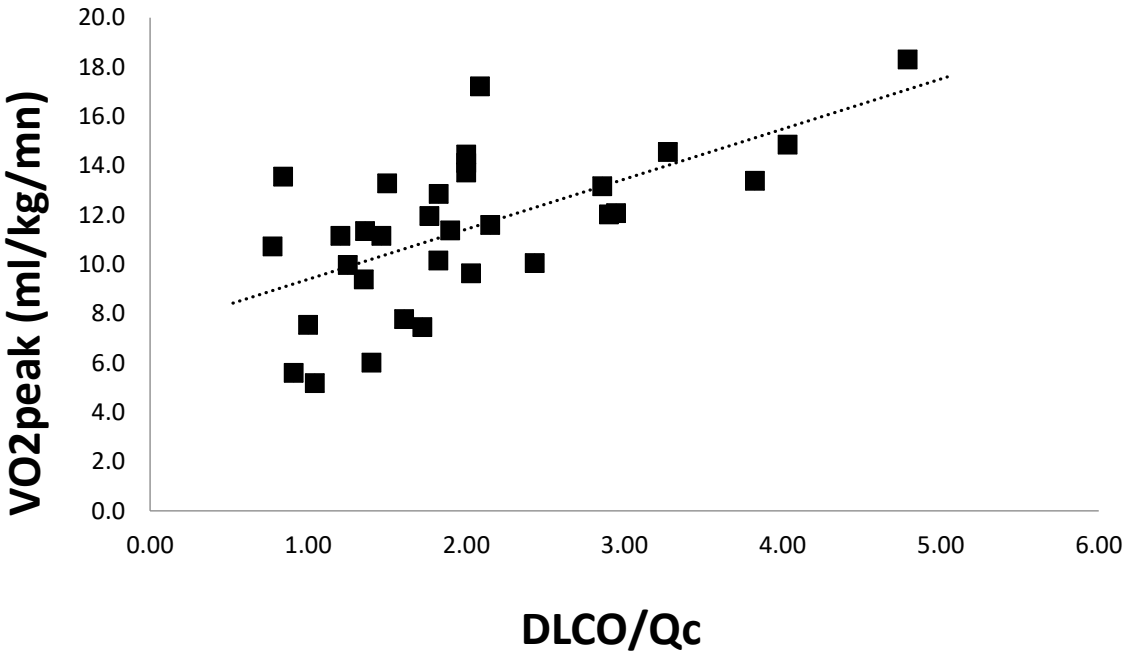

Figure 5.4a. *Relationship of $\dot{V}O_2$peak with DLCO/Q. (y= 2.0302 x + 7.3605)*

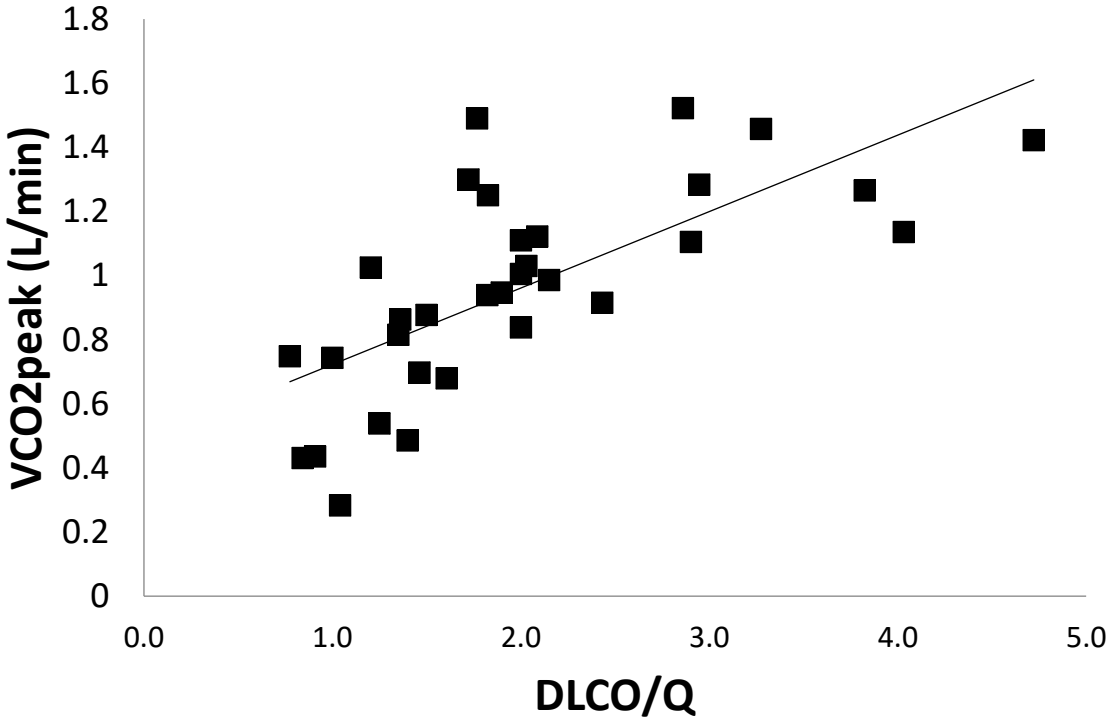

Figure 5.4b. *Relationship of $\dot{V}CO_2$peak with DLCO/Q. (y= 0.2385 x + 0.4841)*

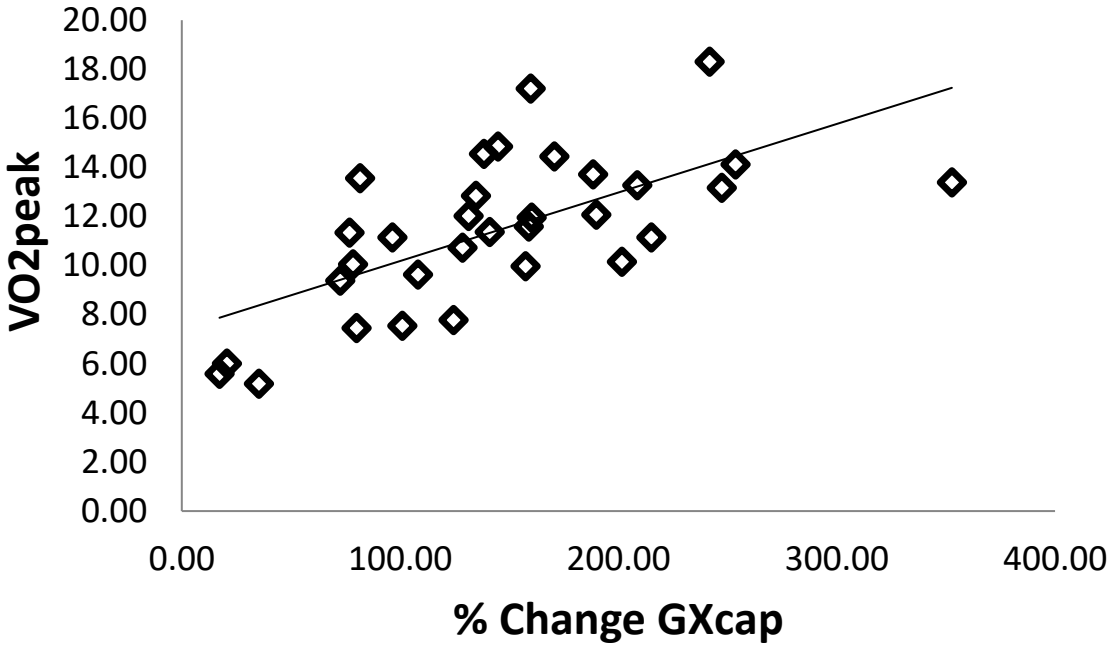

Figure 5.5. *Relationship of $\dot{V}O_2$ with the change in GxCap from rest to peak exercise (y= 0.0279 x + 7.3952)*

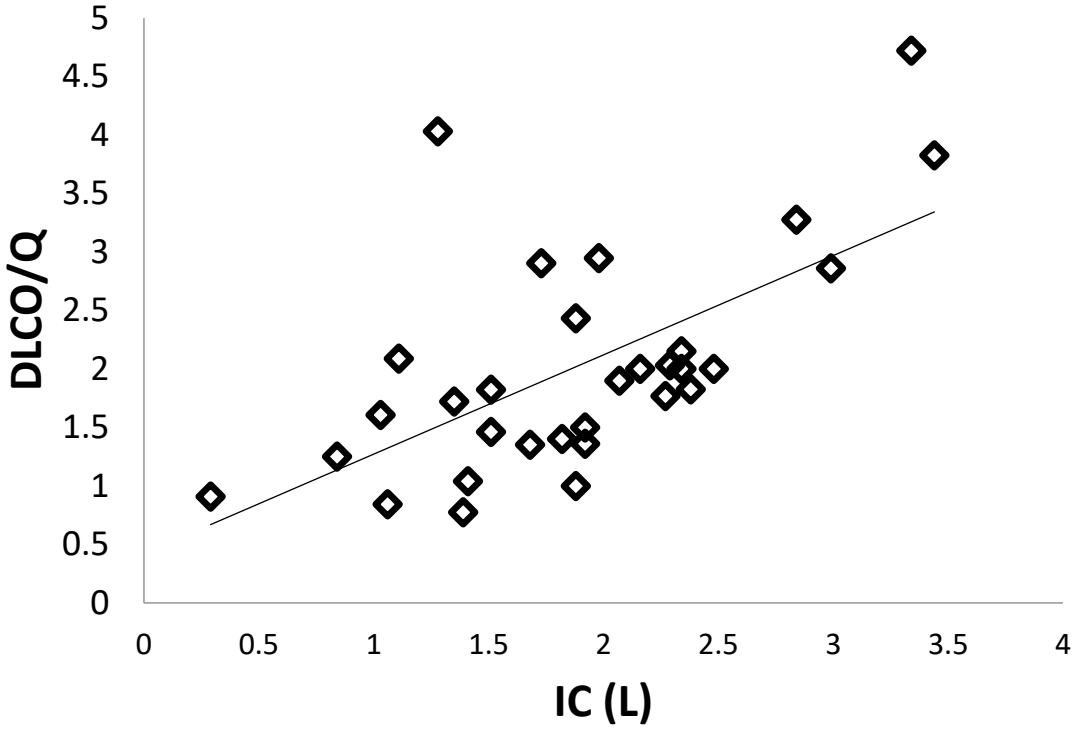

Figure 5.6. *Relationship of DLCO/Qc relative to Inspiratory Capacity* (*y = 0.8487 x + 0.4233)*

5. e. Discussion

i. Primary findings

The primary finding of this study was that COPD patients that can increase respiratory gas exchange surface area (i.e., alveolar-capillary reserve, as assessed via DLCO) relative to the rise in pulmonary blood flow (Qc) have the most preserved exercise capacity. In addition, the ability to expand the pulmonary vascular bed is associated with noninvasive indices of pulmonary capacitance as well as with inspiratory capacity assessed during exercise. While these data are correlative, this implies that a contributing factor to exercise intolerance in this population may be the inability to recruit pulmonary capillaries.

ii. Pulmonary vasculature in COPD

The majority of our patients had a history of smoking as the likely primary etiology of their airway disease. Years of exposure to inhalants causes destruction and remodeling of the alveolar-capillary bed, accelerated aging, inflammation and other chronic metabolites that circulate to cause a general vasculopathy and stiffening of vessels (130-132). There are often regions of inhomogeneous pulmonary vasoconstriction due to the ventilation and perfusion abnormalities, in total, contributing to a blunted ability to increase or expand the alveolar-capillary bed. Previous work has suggested that (121) an abnormal rise in pulmonary arterial pressure relative to the rise in cardiac output was associated with reduced exercise performance in COPD patients and presumably a resistance to forward flow through the lungs, thus a form of cardiac output limitation. However, $\dot{V}O_2$peak was not related to the degree of airflow obstruction in this study which was an interesting finding. Other studies have also suggested a strong relationship between exercise capacity and oxygen pulse, a surrogate for stroke volume (120). In addition airway obstruction itself causes an increase in intrathoracic pressure that may act as an impediment to venous return – potentially further influencing cardiac output (119). While we did not directly measure hemodynamics, there is typically a strong linear relationship between DLCO and Q in healthy individuals generally increasing

nearly proportionally with moderate to heavy exercise. Presumably this represents recruitment and distension of the pulmonary capillaries. Since DLCO is flow independent, a rise in DLCO is primarily influenced by the expansion of this bed. Thus, our measure of DLCO/Qc would primarily represent a rise in pulmonary capillary blood volume that is in contact with functional alveoli. While on average DLCO did rise with exercise and DLCO relative to Qc (DLCO/Qc) stayed relatively constant, there was a large number of patients (38%) where this ratio plateaued or fell with exercise suggesting as either pulmonary blood flow increased, or there was a non-proportional increase in gas exchange surface area (133).

iii. DLCO relative to other respiratory gas exchange measures in COPD

We also assessed several other reported respiratory gas exchange measures associated with ventilation and perfusion matching and pulmonary vascular function. Ventilatory efficiency ($\dot{V}E/\dot{V}CO_2$), the partial pressure of CO_2 relative to end tidal CO_2 ($P_{\bar{E}}CO_2$ /PetCO2) and an index of pulmonary vascular capacitance (Gxcap= O_2 pulse **X** PetCO2), are all associated with lung diffusing capacity (127). Ventilatory efficiency is commonly reported elevated in patients with pulmonary vascular disease and is associated with high dead space ventilation, hyperventilation and a rapid shallow breathing pattern (134). The $P_{\bar{E}}CO_2$ /PetCO2 ratio and the slope of change from rest to exercise has been suggested to differ between primarily pulmonary vascular disease versus more ventilation and perfusion mismatch, with lower ratios suggesting worsening disease. Furthermore, GxCap has previously been shown to correlate well with invasive measures of pulmonary vascular capacitance. It is interesting that while all these measures were associated with DLCO, the GxCap measure was the most significantly linked both to DLCO and to peak exercise performance. The premise is that oxygen pulse tends to track stroke volume changes while $PetCO_2$ tends to fall with greater pulmonary pressures in conditions such as pulmonary hypertension, thus a surrogate for pulmonary pressure. Therefore, since the DLCO is essentially dependent on the recruitment and distension of the pulmonary capillaries, it essentially should increase with greater pulmonary

vascular capacitance. While both increased with exercise, there were a significant number of patients where these rose minimally or failed to increase.

iv. Previous studies investigating exercise intolerance in COPD

A number of studies have demonstrated a clear impact of airway obstruction on exercise performance (99, 103, 135). However, as COPD progresses, it clearly becomes a systemic disease impacting many organ systems. Previous work by O'Donnell has demonstrated an association with the degree of hyperinflation and that improvement of obstruction with bronchodilators clearly enhances exercise capacity (136). Other studies have looked at the development of muscle dysfunction, muscle wasting and deconditioning in COPD patients (95, 137). It is clear that COPD is not a homogeneous disease and thus it is likely that multiple issues contribute to the loss of exercise capacity. Our study highlights the importance of the pulmonary vasculature in determining exercise tolerance in this chronic disease state.

Chapter 6

Study 3: Influence of dietary nitrate supplementation on lung function and exercise gas exchange in COPD patients

Presented as an abstract in American College of Chest Physicians Annual Meeting, October 2017, Toronto, Canada

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6. a. Summary

During exercise as pulmonary blood flow rises, pulmonary capillary blood volume increases and gas exchange surface area rises through distention and recruitment. Hypoxia and endothelial dysfunction lead to pulmonary vascular dysregulation possibly in part related to nitric oxide related pathways. In previous section of this thesis we demonstrated that pulmonary capillary recruitment is more limited in COPD patients with worse exercise tolerance. In this part of the study we attempted to determine if increasing dietary nitrate might influence lung surface for gas exchange and subsequently impact exercise performance. Our COPD cohort, filled out the SGRQ, had a baseline blood draw for Hb, performed spirometry, and had their exhaled nitric oxide (exNO) measured. Then patients performed the intra-breath (IB) technique for DLCO as well as pulmonary blood flow (Qc), followed by a progressive cycle ergometry test to exhaustion with measures of oxygen saturation (SpO_2) and expired gases along with DLCO and Qc measured during the 1st work load only. After completion, they were randomized to either take nitrate supplement (beetroot juice) or placebo (black currant juice) for 8 days. After that, they returned for a repeat of the protocol. ExNO levels rose >200% in the nitrate group ($p<0.05$) with minimal change in placebo group. The SGRQ suggested a small fall in perceived symptom limitation in the nitrate group, but no measure of resting pulmonary function differed post nitrate supplementation. With exercise, there was no influence of nitrate supplementation on $\dot{V}O_2$peak or other measures of respiratory gas exchange. There was a tendency for the exercise DLCO to increase slightly in the nitrate group with a trend towards a rise in the DLCO/Qc relationship ($p=0.06$) but not in the placebo group. The only other significant finding was a fall in the exercise blood pressure in the nitrate group, but not placebo group. Despite evidence of a rise in exhaled nitric oxide levels with nitrate supplementation, there was minimal evidence for improvement in exercise performance or pulmonary gas exchange surface area in a stable medically treated COPD population.

6. b. Introduction

COPD typically develops over many years of smoke inhalation developing into a systemic syndrome that includes inflammation, oxidative stress, insulin resistance, sympathetic activation, and hypoxemia (138, 139) . These coexisting issues have been shown to be associated with endothelial dysfunction influencing vascular health. In addition, there is some evidence that the severity of lung dysfunction may be in parallel to the severity of endothelial dysfunction (140). The pulmonary circulation may be particularly sensitive to endothelial dysfunction and in conjunction with regions of ventilation inhomogeneity, hypoxic pulmonary vasoconstriction develops which further contributes to vascular dysregulation, remodeling, destruction, and pulmonary hypertension (121, 130, 131) . Some of the dysregulation may be reversible as suggested by reported benefits from drugs such as sildenafil, which works through nitric oxide dependent pathways. Nitric oxide (NO) synthesis may also fall due to oxygen substrate limitation as a reduction in NO has been associated with the rise in pulmonary pressures at altitude.

We have previously demonstrated that in a heterogeneous population of COPD patients, lung diffusing capacity for carbon monoxide (DLCO) assessed at rest was better associated with exercise tolerance than more traditional measures of lung mechanics (e.g., FEV_1) (10). In addition, the ability to expand the pulmonary capillary bed as implied by the rise in DLCO with exercise was also preserved in patients with the greatest exercise capacity, suggesting that the pulmonary vasculature plays an important role in exercise tolerance in this population (11).

Other methods have been used to augment NO pathways and improve endothelial function. Supplements such as L-arginine or sources of inorganic nitrate (NO_3-) (e.g., beetroot Juice or sodium nitrate [NaNO3-]) have been used. Several studies have demonstrated modest benefits pertaining

to cardiovascular health, such as reducing blood pressure (BP), enhancing blood flow, and elevating the driving pressure of oxygen in the microcirculation to areas of hypoxia or exercising tissue while other studies have shown no or minimal benefit (8, 83, 141, 142).

NO however also plays an important role as an inflammatory mediator. The fraction of exhaled NO (FexNO) has been used in asthma to establish the correct diagnosis, (143, 144) predict response to therapy, (145, 146) titrate medications, (147, 148) and predict exacerbation (149-151). On the other hand, there are only a few studies describing the levels of FexNO in patients with COPD and some of the published reports are conflicting in their conclusions and unlike asthma it is not universally recommended (152-154).

Thus, the primary focus of this study was to use an inorganic nitrate supplement, beetroot juice, in a stable medically treated COPD population to determine if this might improve lung diffusing capacity and subsequently exercise performance. We hypothesized that improving nitric oxide production in this population may improve the ability to recruit or distend pulmonary capillaries and therefore improve exercise capacity.

6.c. Methods

i- Patient selection

Patients with a history of COPD who were able to complete pulmonary function testing and perform exercise testing were offered enrollment. Inclusion and exclusion criteria and regulatory oversight have been elaborated in detail in chapter 3a.

ii- Overview of study

Study participants filled out the SGRQ, performed pulmonary function testing (PFTs) which included resting measures of maximal lung volumes and flow rates using classical spirometry (45, 88, 93, 128).

DLCO measurements by SB and IB methods which included Qc were made (59). Patients subsequently performed cardiopulmonary exercise testing (CPET). The test protocol started with 20 watts for both men and women and increased by 10 watts every 2 min. EKG recording and pulse oximetry measurements were made continuously. During the last 30 sec of each workload, a 12 lead EKG was recorded, blood pressure (BP) assessed, perceived dyspnea score (0-10 scale) and perceived exertion (total body effort) was rated by patients, and an average of the HR and SaO_2 over this period was determined. Patients were encouraged to exercise to near exhaustion by achieving an RPE of 17-18 on the Borg 6-20 scale or a dyspnea score ≥7 on the 0-10 scale. Upon reaching peak exercise patients performed active recovery where they continued to pedal with no resistance and remained on the mouthpiece for one minute. After this the patient stopped pedaling and were given time for HR and BP to return to baseline. For further details please refer to chapter 3 sections a, b, c, and d. Each patient was given a sample of beetroot juice or placebo to be taken for eight days and returned on the eight day for repeat of the above protocol (Fig. 6.1).

iii- Pulmonary function and single breath DLCO

Spirometry was performed using pneumotachograph-based pulmonary function equipment that was described previously in chapter 3e (42, 93). Classic single breath DLCO measurement is described in detail in the same chapter.

iv- Intra-breath DLCO and pulmonary blood flow (Qc)

Pulmonary blood flow and IBDLCO were measured with the same technique that was elaborated in chapter 3e (59, 124). The measure of IBDLCO and IBQc was practiced several times at rest in each patient until reproducible values were obtained and performed near the end of the first workload in triplicate. If necessary, the first work load was extended before incrementing the cycle ergometer resistance in order to obtain reproducible measures.

v- St. George's respiratory questionnaire

The SGRQ is detailed in chapter 3c (128).

vi- Gas Exchange, Ventilation and Lung Mechanics

During exercise testing oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), breathing frequency (fb), tidal volume (V_t), minute ventilation (VE) and derived variables were measured continuously and/or calculated using the metabolic system that was described previously in chapters 3e and 4c.

In addition, measures associated with ventilation and perfusion matching or pulmonary vascular health were determined. These included mixed expired CO_2 ($P_{\bar{E}}CO_2$) relative to end tidal CO_2 ($PetCO_2$), GxCap, and ventilatory efficiency or VE/$\dot{V}$CO2 which were elaborated in chapter ***5.c.v*** (125, 127).

vii- Dietary supplementation

Patients were randomized to receive either beetroot or black currant juice. Randomization is detailed in chapter 3d.

viii- Exhaled nitric oxide

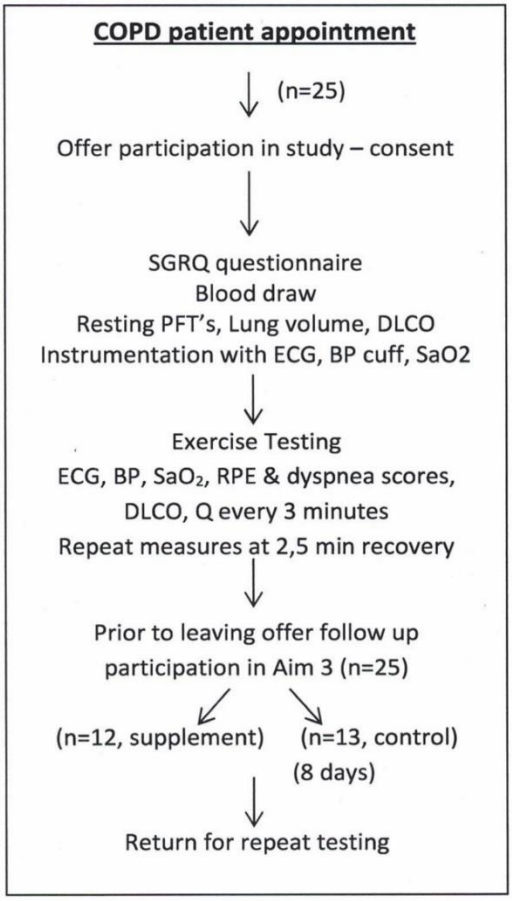
Nitric oxide in the exhaled air was measured using NIOX VERO system (www.niox.com). The device has been described in detail in chapter 3d.

ix- Statistics

We were interested in exercise limitation in the COPD population and the potential role of the alveolar–capillary bed, lung surface area for gas exchange or the ability to expand this bed as a mediating factor. Thus, our primary measures were the change in IBDLCO and the change in IBDLCO

in proportion to IBQc across patients and the relationship to exercise or aerobic capacity, primarily peak $\dot{V}O_2$. Therefore, we used multiple regression and correlational analysis to asses these relationships. In addition, general descriptive statistics were used to define our group and paired t-tests to evaluate changes with exercise. For variables found not to be normally distributed, a Wilcoxon signed rank test was used to test for differences between the groups using an α=0.05. To determine the impact of dietary supplementation with beet root juice, ANOVA was performed with post hoc testing using t-tests when the ANOVA demonstrated significance. Since this was considered a smaller, pilot study to help guide the direction for larger clinical studies and since the "influence" of the supplement on the primary target variable was not known, the number of patients recruited was small by design and no formal power calculations were performed.

Figure 1. Overview of Study Protocol.



6. d. Results

i. Patient characteristics and measures of pulmonary function

Tables 6.1, 6.2, and 6.3 give the baseline patient characteristics and standard spirometry values along with DLCO, measures of pulmonary blood flow and exhaled nitric oxide levels. The two groups (nitrate group vs placebo) were relatively well matched for age, sex, smoking history, symptoms and disease severity based on the GOLD classification and measures of lung function. In addition, they were on similar distribution of medications. Values are also reported after receiving either nitrate supplementation or placebo. The only significant changes observed were an increase in the exhaled nitric oxide levels in the nitrate group and interestingly a small drop in the SGRQ ($p<0.05$). No other significant changes were observed either between groups or post nitrate or placebo intervention in these measures.

***Table 6.1**. Patient Characteristics of COPD patients prior to receiving nitrate supplement or placebo.*

	Nitrate (n=12)	**Placebo (n=13)**
Age (years)	67 ± 8	68 ± 10
% Female	50	46
Weight (Kg)	82 ± 23	88 ± 25
BMI (Kg/m2)	28 ± 6	32 ± 6
Smoking history (pack year)	38 ± 33	36 ± 32
GOLD Classification (1-4)	2.4 ± 0.8	2.2 ± 0.6
St George Questionnaire	40 ± 19 (-17%#)	47 ± 24 (-4.2%)
Inhaled beta agonist (%)	100	100
Inhaled anticholinergic (%)	69	50
Inhaled steroid (%)	58	69
Oral steroid (%)	25	15

GOLD. Global Initiative for Chronic Obstructive Lung Disease classification for air flow obstruction.
( ) the percent change post nitrate or placebo

- *Significant difference between Nitrate group and Placebo group at baseline $p<0.05$*

 # Significant difference between pre and post nitrate or placebo $p<0.05$

Table 6.2. *Pulmonary Function Variables in COPD patients receiving nitrate supplement (n=12) or placebo (n=13)*

	Pre Nitrate	Post Nitrate	%Δ	Pre Placebo	Post Placebo	%Δ
FVC (L)	2.61 ± 0.86	2.65 ± 0.94	+1	2.36 ± 0.69	2.43 ± 0.60	+1
FEV1 (L)	1.50 ± 0.61	1.49 ± 0.69	-1	1.56 ± 0.66	1.43 ± 0.44	-3
FEV1/FVC	57 ± 10	57 ± 9	0	61 ± 11	61 ± 11	0
FEF25-75 (L/s)	0.74 ± 0.43	0.71 ± 0.42	-5	0.78 ± 0.39	0.80 ± 0.34	+15
FEF75 (L/s)	0.29 ± 0.13	0.27 ± 0.13	-5	0.29 ± 0.12	0.30 ± 0.10	+17
MVV (L/m)	50 ± 26	51 ± 33	+2	50 ± 16	50 ± 17	+1

FVC. Forced Vital Capacity, FEV_1. Forced Expiratory Volume in 1 second, FEF. Forced Expiratory Flow, MVV. Maximal voluntary ventilation. All data are pre bronchodilator.

- *Significant difference between Nitrate group and Placebo group at baseline p<0.05*

 # Significant difference between pre and post nitrate or placebo p<0.05

Table 6.3. *Resting Lung Diffusing Capacity, Pulmonary Blood Flow and Exhaled Nitric Oxide in COPD patients receiving nitrate supplement (n=12) or placebo (n=13)*

	Pre Nitrate	Post Nitrate	%Δ	Pre Placebo	Post Placebo	%Δ
Single Breath DLCO (SBDLCO, ml/min/mmHg)	11.8 ± 5.2	12.0 ± 5.8	+2	14.3 ± 5.4	14.9 ± 5.7	+4
Intra-Breath DLCO (IBDLCO, ml/min/mmHg)	9.3 ± 4.8	9.2 ± 5.2	0	11.1 ± 5.2	11.7 ± 5.8	+6
IBDLCO/SBDLCO (%)	77 ± 13	76 ± 17	+1	76 ± 14	77 ± 19	+3
Pulmonary Blood Flow (Qc, L/m, measured)	4.9 ± 0.9	4.8 ± 1.1	-4	4.8 ± 0.9	4.9 ± 0.8	+3
Pulmonary Blood Flow –Cardiac output, (L/m, Predicted)	6.3 ± 0.3			6.4 ± 0.3	`	
IBDLCO/Qc ratio	1.9 ± 1.0	1.9 ± 0.9	+2	2.3 ± 1.1	2.4 ± 1.0	+11
Exhaled Nitric Oxide (eNO, ppb)	16 ± 12	38 ± 35#	+247	25 ± 21	26 ± 22	+10
Hb (g/dl)	13.6 ± 1.7	13.6 ± 1.7	0	13.1 ± 1.2	13.2 ± 1.2	0

Pulmonary Blood Flow measured with soluble gas method. Cardiac output estimated based on age, gender, BSA, from William LR (108). Qc = Pulmonary Blood Flow. Hb = Hemoglobin, IBDLCO and SBDLCO = intra-breath and single breath lung diffusing capacity for carbon monoxide.

- *Significant difference between Nitrate group and Placebo group at baseline p<0.05*

 # Significant difference between pre and post nitrate or placebo p<0.05

ii. Exercise responses

Tables 6.4, 6.5, and 6.6 display the mean exercise data for lung diffusing capacity and pulmonary blood flow, submaximal (matched work load only) exercise ventilatory, cardiovascular and gas exchange responses and similar measures obtained at peak exercise.

There was a trend for a rise in the submaximal DLCO (+14%) and expressed relative to Qc (+20%) in the nitrate group (p=0.08), with no evidence of change in the placebo group (Fig. 6.2). There were no other changes noted in cardiovascular responses or measures of pulmonary gas exchange or breathing pattern post nitrate or placebo supplementation during submaximal exercise.

Table 6.4. *Exercise Lung Diffusing Capacity, Pulmonary Blood Flow and Exhaled Nitric Oxide in COPD patients receiving nitrate supplement (n=12) or placebo (n=13)*

	Pre Nitrate	**Post Nitrate**	**%Δ**	**Pre Placebo**	**Post Placebo**	**%Δ**
Intrabreath DLCO (IBDLCO, ml/min/mmHg)	10.8 ± 7.2	12.3 ± 7.2	+14%	14.8 ± 8.3	12.9 ± 7.1	-13%
EXIBDLCO/RSTDLCO (%)	1.34 ± 0.53	1.57 ± 0.53	+17%	1.40 ± 0.69	1.28 ± 0.53	-9%
Pulmonary Blood Flow (Qc, L/m, measured)	7.0 ± 2.2	7.1 ± 2.4	+2%	6.9 ± 1.5	7.1 ± 1.12	+3%
IBDLCO/Qc ratio	1.5 ± 0.6	1.8 ± 1.2	+20%	2.2 ± 1.2	1.8 ± 1.1	-18%

Pulmonary Blood Flow measured with soluble gas method. Cardiac output estimated based on age, gender, BSA, from William, LR (108). Qc = Pulmonary Blood Flow. EXIBDLCO/RSTDLCO is the exercise intra-breath DLCO divided by the resting intra-breath DLCO.

- *Significant difference between Nitrate group and Placebo group at baseline p<0.05*

 # Significant difference between pre and post nitrate or placebo p<0.05

There was no difference in peak exercise performance post nitrate or placebo supplementation. There was also no observable impact of nitrate supplementation on other key measures of breathing pattern, respiratory gas exchange or other variables associated with pulmonary vascular function during heavier exercise. The only measure that reached statistical significance was a reduced blood pressure at peak exercise in the nitrate supplemented group, but not the placebo group (Fig. 6.3). There was a tendency for the beetroot juice group to have elevated pretreatment exercise blood pressures relative to the placebo group; however, 8 of 12 in the beetroot juice group demonstrated

a drop post treatment at peak exercise while only 2 in the placebo group demonstrated a fall in exercise blood pressure post treatment (Fig. 6.4). Differences in blood pressure between the two groups was also assessed with a Wilcoxon signed rank test showing that there was a difference in peak blood pressure response with juice vs placebo.

Table 6.5. *Cardiopulmonary Responses to Submaximal Exercise in COPD Patients receiving nitrate supplement (n=12) or placebo (n=13) (Mean ± SD)*

n=32	Pre-Nitrate	Post Nitrate	%Δ	Pre-Placebo	Post Placebo	%Δ
Exercise Capacity						
Work (Watts)	20	20 ± 4	0	20 ± 14	20 ± 19	0
$\dot{V}O_2$ (ml/kg/min)	8.1 ± 1.4	8.2 ± 1.1	+12%	8.5 ± 1.5	8.1 ± 1.3	-5%
Symptoms						
RPE (6-20 Borg Score)	11 ± 2	11 ± 3	0	12 ± 3	12 ± 2	0
Dyspnea (0-10 Score)	4 ± 2	4 ± 2	0	4 ± 2	4 ± 2	0
Cardiovascular						
Heart Rate (bpm)	91 ± 12	92 ± 9	+1%	91 ± 12	93 ± 13	+2%
O_2 Pulse ($\dot{V}O_2$/HR)	7.1 ± 1.9	6.9 ± 2.1	-2%	8.6 ± 2.3	8.1 ± 1.9#	-6%
Blood Pressure SBP (mmHg)	142 ± 15	134 ± 9#	-7%	136 ± 20	135 ± 21	-1%
DPB (mmHg)	82 ± 10	83 ± 11	+1%	81 ± 10	77 ± 12	-2%
Ventilation/Breathing Pattern						
Ventilation (L/min)	22 ± 7	23 ± 6	+3%	25 ± 7	24 ± 7	-2%
Tidal Volume (L)	1.26 ± 0.16	1.27 ± 0.56	+1%	1.13 ± 0.30	1.15 ± 0.28	+1%
Breathing Frequency (bpm)	21 ± 5	21 ± 5	0	26 ± 7	24 ± 5	-8%
IC (L)	2.01 ± 0.76	2.19 ± 1.02	-1%	1.94 ± 0.69	1.86 ± 0.54	-4%
VT/IC	56 ± 14	53 ± 14	-4%	50 ± 10	48 ± 15	-3%
VE/Breathing Capacity (%)	45 ± 13	49 ± 15	+7%	55 ± 33	52 ± 24	-4%
Gas Exchange – Pulmonary Vascular						
VE/$\dot{V}CO_2$ ratio	39 ± 5	38 ± 6	-2%	36 ± 5	35 ± 4	-2%
$PetCO_2$ (mmHg)	37 ± 6	38 ± 6	+2%	38 ± 5	39 ± 5	+2%
$P_{\bar{E}}CO_2$ (mmHg)	23 ± 3	23 ± 3	0	24 ± 3	25 ± 3	+3%
$P_{\bar{E}}CO_2$ /$PetCO_2$ ratio	0.62 ± 0.04	0.63 ± 0.06	+2%	0.64 ± 0.05	0.64 ± 0.05	0
GxCap	263 ± 82	252 ± 91	-4%	327 ± 88	305 ± 73	-5%

VE: Minute ventilation, fb: breathing frequency, VT: tidal volume, TI: inspiratory time, TTOT: total respiratory cycle time, IC: inspiratory capacity, $\dot{V}O_2$: oxygen consumption, $\dot{V}CO_2$: carbon dioxide production, $P_{\bar{E}}CO_2$: mixed expired CO_2 values, PetCO2: end tidal partial pressure of carbon dioxide, O_2pulse: $\dot{V}O_2$/heart rate, SaO_2: arterial oxygen saturation estimated from pulse oximetry. GxCap: noninvasive estimate of pulmonary vascular capacitance.

- *Significant difference between Nitrate group and Placebo group at baseline p<0.05*

 # Significant difference between pre and post nitrate or placebo p<0.05

Table 6.6. *Cardiopulmonary Responses to Maximal Exercise in COPD Patients receiving nitrate supplement (n=12) or placebo (n=13) (Mean ± SD)*

n=32	Pre Nitrate	Post Nitrate	%Δ	Pre Placebo	Post Placebo	%Δ
Exercise Capacity						
Work (Watts)	45 ± 18	48 ± 16	+6%	48 ± 21	52 ± 21	+3%
$\dot{V}O_2$ (ml/kg/min)	12.0 ± 1.6	11.7 ± 2.2	-3%	11.1 ± 3.6	10.6 ± 4.8	-4%
Symptoms						
RPE (6-20 Borg Score)	17 ± 1	17 ± 2	0	17 ± 2	17 ± 1	0
Dyspnea (0-10 Score)	7 ± 2	7 ± 1	0	7 ± 2	7 ± 2	0
Cardiovascular						
Heart Rate (bpm)	109 ± 14	109 ± 13	0	104 ± 14	106 ± 15	+1%
O_2 Pulse ($\dot{V}O_2$/HR)	8.9 ± 2.8	9.2 ± 2.9	+3%	9.8 ± 3.1	10.1 ± 3.1	+3%
Blood Pressure SBP (mmHg)	161 ± 22	150 ± 14	-7%#	145 ± 20	151 ± 21	+4%
DPB (mmHg)	92 ± 10	93 ± 11	+1%	84 ± 10	82 ± 11	-2%
Ventilation/Breathing Pattern						
Ventilation (L/min)	36 ± 13	35 ± 11	-2%	34 ± 10	36 ± 12	+4%
Tidal Volume (L)	1.24 ± 0.46	1.23 ± 0.41	-1%	1.16 ± 0.36	1.21 ± 0.33	+4%
Breathing Frequency (bpm)	30 ± 5	30 ± 6	0	31 ± 4	32 ± 6	+2%
IC (L)	1.85 ± 0.81	1.98 ± 0.72	+6%	1.85 ± 0.66	1.79 ± 0.63	-3%
VT/IC	65 ± 23	64 ± 11	-1%	64 ± 23	61 ± 22	-4%
VE/Breathing Capacity (%)	70 ± 14	68 ± 17	-2%	69 ± 26	69 ± 18	0
Gas Exchange – Pulmonary Vascular						
VE/$\dot{V}CO_2$ ratio	37 ± 4	36 ± 4	-2%	35 ± 6	34 ± 4	-2%
$PetCO_2$ (mmHg)	35 ± 5	36 ± 5	+2%	37 ± 4	38 ± 5	+2%
$P_{\bar{E}}CO_2$ (mmHg)	24 ± 3	24 ± 3	0	25 ± 4	26 ± 3	+3%
$P_{\bar{E}}CO_2$ /$PetCO_2$ ratio	0.66 ± 0.05	0.68 ± 0.06	+3%	0.69 ± 0.06	0.69 ± 0.05	0
GxCap	308 ± 94	328 ± 102	+6%	358 ± 127	380 ± 115	+6%

VE: Minute ventilation, fb: breathing frequency, VT: tidal volume, TI: inspiratory time, TTOT: total respiratory cycle time, IC: inspiratory capacity, $\dot{V}O_2$: oxygen consumption, $\dot{V}CO_2$: carbon dioxide production, $P_{\bar{E}}CO_2$: mixed expired CO_2 values, $PetCO_2$: end tidal partial pressure of carbon dioxide, O_2pulse: $\dot{V}O_2$/heart rate, SaO_2: arterial oxygen saturation estimated from pulse oximetry. GxCap: noninvasive estimate of pulmonary vascular capacitance.

- *Significant difference between Nitrate group and Placebo group at baseline p<0.05*

 # Significant difference between pre and post nitrate or placebo p<0.05

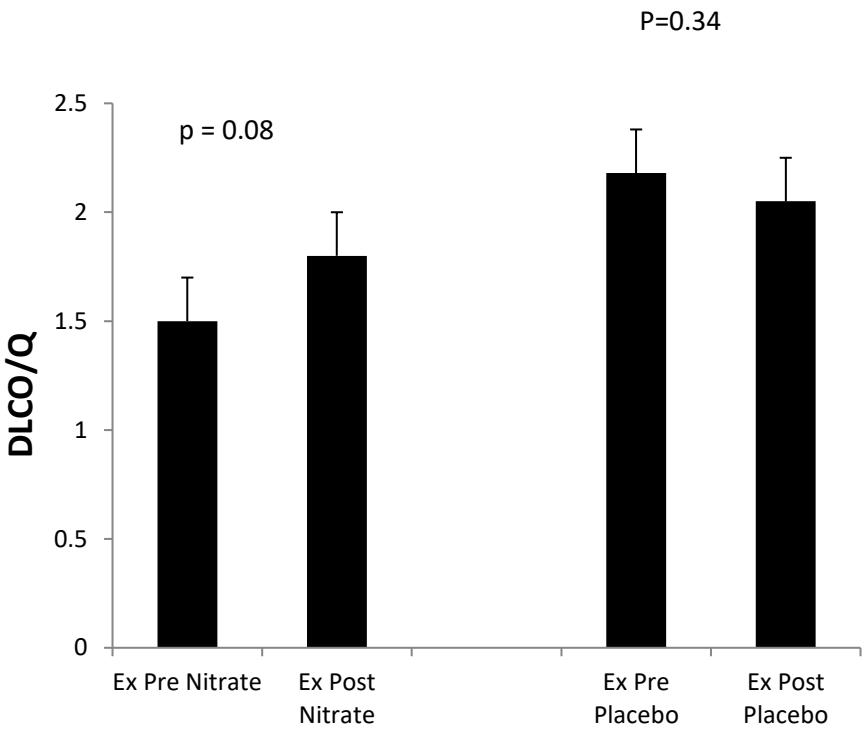


Figure 6.2. *DLCO relative to Q during submaximal exercise pre and post either nitrate supplement or placebo*

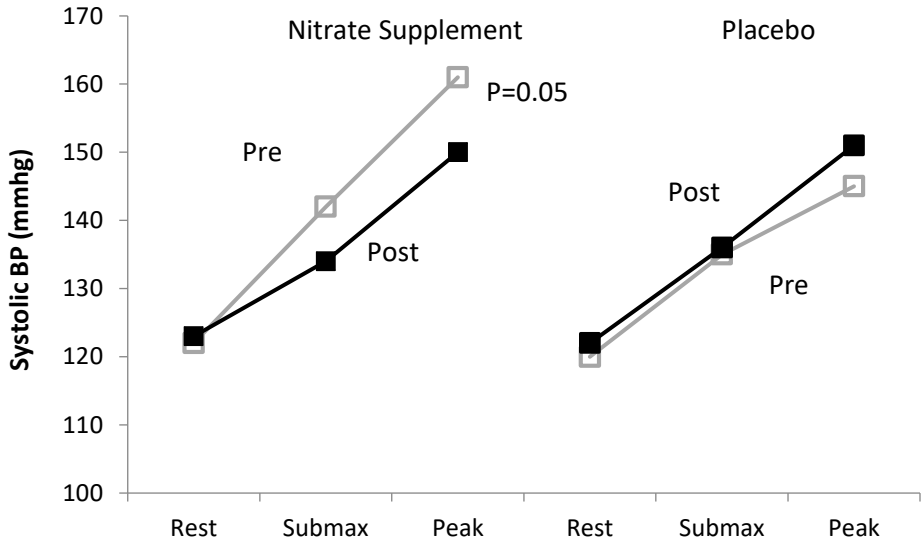


Figure 6.3. *Blood pressure responses to exercise pre and post either nitrate supplement or placebo*

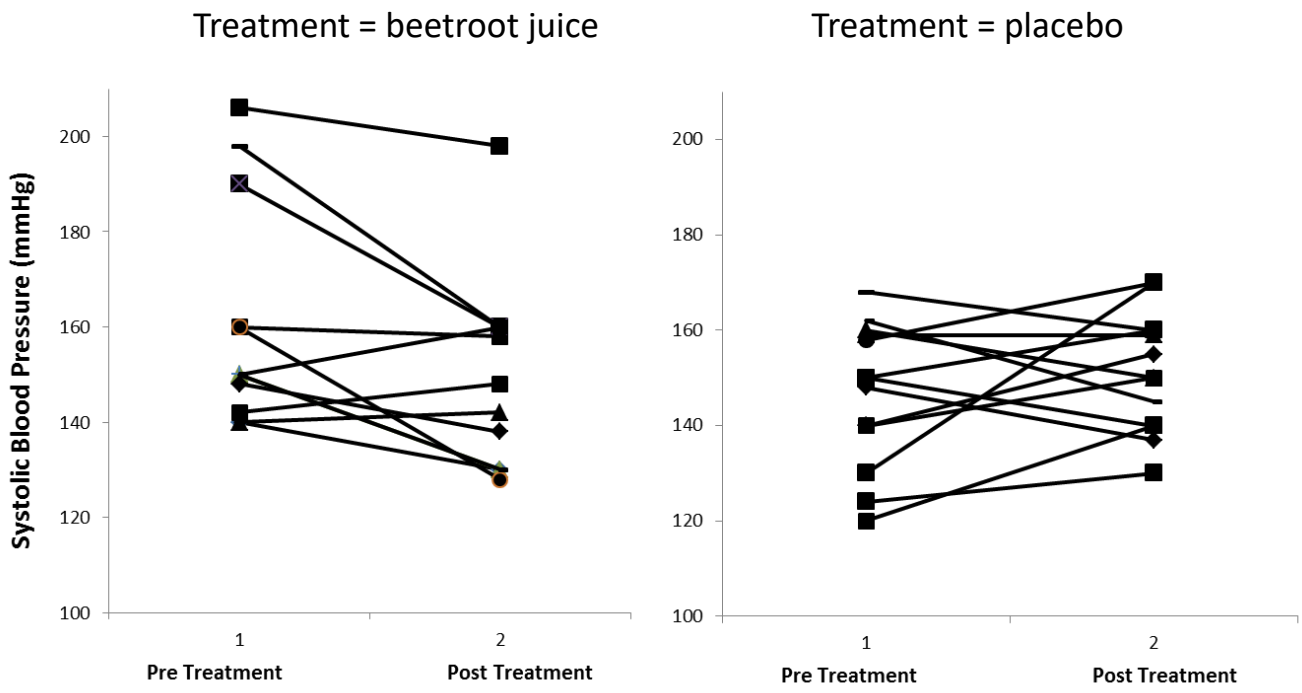


Figure 6.4. *Individual changes in peak blood pressure before and after treatment with beetroot juice or placebo. Differences in blood pressure between the two groups assessed with Wilcoxon Signed Rank Test showing that there was a difference in blood pressure between groups, α = 0.05.*

6. e. Discussion

In summary, COPD patients in the beetroot juice group demonstrated a drop in their SGRQ score, a higher exhaled nitric oxide, and a lower blood pressure at peak exercise. Chronic obstructive pulmonary disease is associated with remodeling and destruction of the pulmonary vasculature. This leads to declines in functional lung surface area for respiratory gas exchange and a reduced ability to expand it with exercise. This inability to recruit or distend pulmonary capillaries has thus been linked to exercise limitation in this population (11). Endothelial dysfunction is a part of the pathophysiology of COPD, in part related to altered nitric oxide regulation (155, 156). Therefore, we tested the hypothesis that supplementation with nitrates may improve nitric oxide formation, enhance endothelial function and improve lung surface area for respiratory gas exchange. We found that while we had evidence for increased NO formation from exhaled measurements, there was no significant impact on resting measures of airway or pulmonary vascular function. There were trends towards improved pulmonary vascular recruitment with exercise in the nitrate supplement group and evidence for reductions in systemic blood pressure, but the change in DLCO was not associated with changes in other noninvasive indices of respiratory gas exchange during exercise or an improvement in exercise capacity. Interestingly there was a trend towards a reduction in symptoms in the nitrate group relative to the placebo group, however the mechanism for this remains unclear and larger numbers would be needed to confirm this finding. Thus, while there was some evidence of a systemic influence of nitrate supplementation with inorganic supplementation, there was no definitive benefit relative in pulmonary function or exercise performance relative to placebo.

NO is a signaling molecule with multiple functions including regulation of vascular tone, mitochondrial respiration and skeletal muscle function. These factors are important in the physiological response to exercise and relative to our hypothesis for the recruitment of the pulmonary vasculature with exercise. NO is produced in two primary ways in man. The best known

is the classical L-arginine nitric oxide synthase (NOS) pathway which is oxygen dependent. The second is the entero-salivary pathway and is oxygen independent. Ultimately some nitrite is absorbed into the circulation where it acts as a storage pool for subsequent NO production. The conversion of nitrite to NO is expedited in conditions of acidosis or hypoxemia which likely occurs in regions of the pulmonary vasculature in COPD patients especially during exercise (70, 74, 75).

We previously demonstrated that resting DLCO or resting DLCO relative to pulmonary blood flow (DLCO/Qc) was predictive of exercise performance in a moderate to moderately severe COPD population (10). In addition, the ability to expand the pulmonary capillary bed during exercise was also an important factor in determining exercise capacity in this group (11). DLCO is reduced in COPD likely due to destruction of alveolar septum and the pulmonary capillary bed; but there is also remodeling, areas of poor ventilation and perfusion mismatch, with areas of hypoxic pulmonary vasoconstriction that likely contribute to the fall. A fall in NO due to less synthesis of NO due to oxygen substrate limitation or oxygen sensitivity of the NO synthase could contribute to the immediate rise in pulmonary artery pressure upon arrival at altitude as a consequence of a reduced capacity to vasodilate. Therefore, despite the remodeling, there are likely regulatory mechanisms influencing pulmonary vascular recruitment in this population that are potentially reversible.

The impact of altering NO pathways on exercise capacity in COPD patients is variable. Work by Lederer (157) did not demonstrate improvement in 6 min walk distance or peak exercise $\dot{V}O_2$ with 4 weeks of sildenafil three times daily. The authors found a reduction in quality of life and evidence for an increased alveolar to arterial oxygen difference with sildenafil. Similar findings were found by Holverda et al. (158) with acute administration of sildenafil without improvement in exercise performance in COPD patients with or without pulmonary hypertension. However, other studies have found improvements in six minute walk distance in patients with COPD taking sildenafil (159). Thus, the role of sildenafil is controversial; however, it is possible that the PDE-5 inhibitors may

override to some extent the important vaso-regulatory mechanisms that attempt to match some level of ventilation to perfusion in the lungs and it is perhaps possible that higher doses may in fact be counterproductive.

Studies have demonstrated modest benefits pertaining to cardiovascular health, such as reducing blood pressure, enhancing blood flow, and elevating the driving pressure of oxygen in the microcirculation to areas of hypoxia or exercising tissue (160, 161). However, there are limited studies in the COPD population with mixed findings.

Berry et al. studied effects of dietary nitrate supplementation via beetroot juice on the submaximal exercise capacity of COPD patients. In their randomized, single-blind, crossover design, beetroot juice was given (8). Relative to placebo, beetroot ingestion caused significant elevation of plasma NO_3 by 938% and NO_2 by 379%. Median exercise time was significantly increased and blood pressure was also significantly reduced. No other variables were significantly different between the two groups. In another study, Leong et al. performed a double-blind, computer-randomized placebo control crossover trial to study the effects of dietary nitrate on exercise endurance in stable moderate COPD patients (9). Patients underwent an incremental shuttle walk test to determine $\dot{V}O_2max$ followed by randomization to placebo or beetroot groups. Patients performed an endurance shuttle walk test at 85% $\dot{V}O_2max$ after randomization. Then they took the sample juice for 3 days and on the 4^{th} day (the wash out) there was a cross over between the groups. Again, blood pressure was reduced with ingestion of beetroot juice. End shuttle walk test distance and time to fatigue improved but did not reach statistical significance. In a study by Kerley et al., an increase in incremental shuttle walk distance after consumption of high nitrate juice (25 meters) compared to low nitrate juice (14 meters) was noted. The improvement in exercise capacity was associated with statistically significant increases in serum nitrate and nitrite levels and a significant lowering of resting blood pressure (84).

These studies show that the effects of beetroot on exercise performance in COPD is not consistent and results are mixed at best, although lowering of blood pressure probably due to vasodilatory effects was a common finding. There could be several reasons for the inconsistencies. The factors that have been suggested include deconditioning, hypoxia, hypercapnia, systemic inflammation, nutritional imbalance and medication therapy, to name a few. At the physiological level, mitochondrial dysfunction and redox imbalance seem to contribute to the conflicting results that are obtained at each trial. For example, oxidative stress shadows nitric oxide effects and is likely to be involved in the respiratory muscle dysfunction in severe COPD (85). Epigenetic modification may also play a role in muscle dysfunction in COPD. The epigenetic modifications recognized so far include DNA methylation, histone acetylation and methylation, and non-coding RNAs such as microRNAs (86). These complex, interwoven factors make prediction of dietary nitrate supplementation difficult and at times unpredictable. It seems there are several factors that are responsible for muscle dysfunction in COPD, not to include the dose of nitrate supplementation that is not clearly identified in each patient and can be variable (87).

To summarize, use of inorganic nitrate supplement for 8 days in a mild to severe COPD population increased exhaled nitric oxide levels, mildly reduced respiratory symptoms and exercise blood pressure but did not appear to significantly impact lung surface area for gas diffusion or peak exercise capacity. There are some hints of improved DLCO relative to Qc, suggesting that perhaps a mild influence on pulmonary capillary blood volume can be contributing; however, the effect is small and did not reach statistical significance.

Chapter 7

Conclusions, Limitations, Novel Aspects, Future Direction, List of Findings

Conclusions

a) In part one of the project, we aimed to better elucidate the role of gas exchange surface area as measured by DLCO in exercise limitation and shortness of breath in a COPD population. We performed static pulmonary function tests of spirometry, lung volume, and diffusion capacity in a cohort of COPD patients. Then we subjected them to cardiopulmonary exercise testing and measured various parameters of expired gas such as $\dot{V}O_2$, $\dot{V}CO_2$ and V_t. In addition, we measured diffusion capacity by the intra-breath method and pulmonary blood flow (Qc) by acetylene method. Our results showed that while resting measures of hyperinflation and maximal expiratory flows, particularly over the mid to lower lung volumes were predictive of exercise capacity, lung diffusing capacity alone or expressed relative to resting pulmonary blood flow was the most predictive of exercise capacity. Furthermore, when allowed to compete in a multiple regression model, only the SBDLCO relative to Qc and measure of body weight or habitus were significant predictors and explained approximately 50-60% of the variability in exercise capacity in this population. Hence, medical management targeting the pulmonary circulation could be very helpful and might help reduce symptoms and improve exercise tolerance in COPD patients.

b) The second aim of the project was to quantify measures of alveolar-capillary recruitment during exercise and the relationship to exercise capacity in the same cohort of COPD patients. We found that COPD patients who can expand gas exchange surface area as assessed with DLCO during exercise relative to pulmonary blood flow have a more preserved exercise capacity. We also showed that the ability to expand the pulmonary vascular bed is associated with noninvasive indices of pulmonary capacitance such as GxCap as well as with inspiratory capacity assessed during exercise. We can imply that a contributing factor to exercise intolerance in this population may be the inability to recruit pulmonary capillaries.

c) Finally, in the last part of the project we looked into the effects of beetroot juice as a source of inorganic nitrates on exercise endurance in COPD patients, influencing lung surface for gas exchange and subsequently impacting exercise performance. Patients were randomized to 2 groups of beetroot and placebo and took their juice for 8 days. Then they underwent cardiopulmonary exercise testing and the same parameters that were formerly mentioned were measured in them. We found that the COPD patients who took beetroot juice showed some evidence for improvement in exercise performance and pulmonary gas exchange surface area, although the results were not statistically significant. But there was a statistically significant difference in blood pressure drop, exhaled nitric oxide increase, and overall well-being score.

d) To summarize, exercise limitation in COPD is affected by alveolar-capillary gas exchange impairment which in turn is attributed to impairment of pulmonary circulation. SBDLCO relative to Qc and body weight are better predictors of exercise performance compared to IBDLCO and other respiratory variables in this population. Lung diffusing capacity, either alone or relative to pulmonary blood flow, is not only a good measure of pulmonary vascular health in the COPD population but also a good measure for assessing mechanisms of exercise intolerance in this population.

Limitations

There are several limitations relative to this study that needs further elaboration.

a) This was a relatively small study in a somewhat heterogeneous group of primarily moderate to moderately-severe COPD patients who performed cycle ergometry to volitional exhaustion. We essentially recruited consecutive patients with a history of COPD who were on stable medications and willing to participate. To some extent this was by design so that the study population represented a typical mixed tertiary outpatient population.

b) Lung diffusion has been shown to be predictive of exercise capacity in a number of chronic lung and heart conditions and therefore appears to be a good, more generic marker to consider for the COPD population. Larger studies would however be needed to evaluate in which specific COPD populations, it may be most predictive.

c) We only assessed DLCO during the first exercise load. While we attempted to measure this at other work levels and in some cases successfully, we only felt confident in our data at the lower work level. Patients were trained to perform the maneuver reproducibly at rest and typically performed 3 reproducible maneuvers at the lower workloads, but struggled to perform them reproducibly at the higher work intensities. Thus, we were examining their ability to recruit lung surface area with relatively modest workloads and comparing these data to their peak aerobic capacity. However, the DLCO response to exercise should be relatively linear with work load, $\dot{V}O_2$ and cardiac output and thus the ability to expand the pulmonary circulation early in exercise is likely representative of their reserve with heavier activity. The first work load also represented on average nearly 75% of peak $\dot{V}O_2$ and thus would be considered a moderately heavy load for this population.

d) We used volitional fatigue as a cessation criterion. This resulted in some patients with lower than typical RER values or other more traditional measures associated with maximal exercise, e.g., heart rate. Cycle ergometry is known to be associated with more local muscle fatigue and may underestimate true maximal exercise. However, we attempted to use similar cessation attribute for our patients and the same study staff performed all exercise testing which likely resulted in a more uniform representation of peak exercise capacity across patients. In addition, since mechanical constraint to breathing occurred, some patients were unable to hyperventilate to more typical RER values or get to a true cardiovascular limitation. Thus, we suggest that our data are representative of the typical tertiary center testing laboratory where symptom limitation is typically used as a cessation criterion.

e) We also allowed recruitment of current smokers. While patients were asked not to smoke within 24 hours of testing, we did not specifically assess carboxyhemoglobin levels and therefore could not confirm if they were smoking prior to testing. This could have influenced our DLCO measures, though previously Oglivie et al. felt that the effects of increasing COHb were sufficiently small, so that routine correction of DLCO was not necessary (162). As a result, DLCO was not until more recently clinically adjusted for increases in COHb (163). We also did not note a difference in percent predicted DLCO between smokers and non-smokers and did not find that current smoking impacted our predictive models, as shown in study I.

f) We used a soluble gas method for calculation of pulmonary blood flow or cardiac output in the absence of significant shunt. We acknowledge this method may be somewhat dependent on ventilation and perfusion matching in the lungs and therefore may also underestimate actual values.

g) We were not able to separate DLCO into its component parts, Dm and Vc (membrane diffusion capacity and pulmonary capillary blood volume); however, we did measure Qc and the ratio of

DLCO/Qc should be somewhat analogous to Vc. This ratio tended to be associated more with exercise tolerance than DLCO alone in previously published literature.

h) We did not obtain baseline and post supplement measures of pulmonary vascular pressures on our patients by right heart catheterization to better understand the relationships between pulmonary blood flow, pulmonary pressure and the rise in DLCO which would have given better insight on what was limiting the alveolar-capillary expansion. This would not have been possible because we did not have the expertise to perform heart catheterization during CPET. However, it is not likely that our patients had significant pulmonary hypertension since substantial oxygen desaturation was not seen in any patient.

i) Some of the patients were active smokers or were taking oral prednisone and these have been shown to lower FENO in COPD.

j) There is a baseline degree of deterioration in the alveolar-capillary bed in the COPD population that while predictive of the ability to increase DLCO during exercise, does not fully explain the response. Therefore, additional studies are needed to better understand factors that regulate and improve functional alveolar-capillary surface area during exercise.

k) Finally, in addition to nitrates, beetroot juice contains several potentially metabolically active compounds that could influence physiological function in patients with COPD (8). Potentially confounding compounds may be polyphenols and/or quercetin. Though we did use a juice placebo, it is possible that beetroot juice supplementation may have influenced the outcome beyond those attributed to nitrate alone.

Novel Aspects

The novelty of this project includes the following:

a) The intra-breath measurement of DLCO is not done routinely in clinical practice during CPET. It is a test that is mainly performed at research centers with strong technologic experience. The method was of novel importance especially its performance during exercise testing since single breath DLCO is difficult to do during exercise due to breath holding.

b) Measurement of Qc requires special software which is not readily available to researchers. It is innovative and requires great deal of methodological expertise by well-trained staff, accomplished by advanced research centers in the world. It is meticulously operator and patient dependent and a very minor error can make the results obsolete. But if done correctly, it is a test that can provide valuable information on cardiac output noninvasively in contrast to other techniques of invasive cardiac output measurement during exercise.

c) Our small exercise physiology laboratory, with its well-trained staff was proudly able to perform novel and technically challenging experiments that were ordinarily accomplished at advanced tertiary academic institutions.

d) The experiments elucidate a very important take home message for clinicians. The important and novel conclusion is that spirometry which has been traditionally emphasized as a very important marker of disease severity in COPD, is by itself not very reliable in predicting exercise intolerance in patients. DLCO which traditionally has not been viewed as important as spirometry in classification

of disease severity, needs to be regarded as a very important physiologic parameter in prediction of exercise capacity in this population.

e) Dynamic measurement of respiratory variables such as DLCO during exercise is more informative and a better predictor of exercise intolerance than static measurement of FEV_1 and DLCO at rest.

f) A noninvasive and novel marker such as GxCap which is a marker of pulmonary vascular capacitance and has been studied in heart failure, was for the first time studied during CPET in COPD and it can become an important marker of exercise capacity in this patient population.

g) Inorganic nitrates are proven to be very important in improving exercise function in the healthy population. We showed that they improve respiratory symptoms, lower blood pressure, and elevate exhaled nitric oxide in COPD significantly. The etiology can be multifactorial and it warrants larger clinical studies with higher doses of beetroot juice to investigate the potential benefits of this natural juice in COPD. The results could have an enormous impact on the approach to this disease and could open a novel avenue to its treatment.

Future Directions

The work presented in this thesis can be used as a working ground for future research. One area is the importance of gas exchange impairment in COPD. We propose that DLCO as a marker of gas exchange is more sensitive and predictive of exercise intolerance than spirometry in COPD. More studies need to be done to see if DLCO can be measured in a less complicated exercise regimen to better stratify COPD patients with a poor outcome. Measurement of noninvasive index of pulmonary capacitance, GxCap was the most closely associated with DLCO, DLCO/Qc and $\dot{V}O_2$peak. This could become a simple noninvasive test that can be better calibrated and be used by physicians in assessment of exercise intolerance in COPD.

Another interesting area that needs further study is arterial stiffness in COPD patients. Lung obstruction and limited ability to recruit pulmonary capillaries may limit forward flow of cardiac output and therefore cause limitation to exercise in COPD. There is emerging evidence for increased systemic vascular stiffness that may also influence cardiac output through an added afterload, beyond the pulmonary circulation. Furthermore, airway obstruction may increase central blood pressure. Vascular stiffness has been shown to be increased in COPD (164). Arterial stiffness as a manifestation of systemic disease, has been shown to cause morbidity and mortality in COPD and clinicians are nowadays encouraged to evaluate presence of arterial stiffness in their COPD patients (165). We can use non-invasive equipment to track arterial wave forms and to estimate central blood pressure and vascular stiffness at rest and during exercise in the COPD population (166) and study their correlation to DLCO and other markers of vascular recruitment such as GxCap. Some of our preliminary data on vascular stiffness is included in the appendix D and can be a harbinger of future larger studies which will need a sample size of at least 64 patients to show any statistical significance. Effects of dietary nitrate on vascular stiffness before and after exercise in COPD patients

can also be measured. All of these experiments will be novel and have not been done previously to any extent in COPD patients and can broaden our understanding of exercise limitation in this chronic disease.

Since we proved that pulmonary circulation has an important role in exercise limitation in COPD population, it will be prudent to target the circulation with specific medicines in order to reduce symptoms and improve exercise tolerance. High prevalence of COPD, a multifactorial disease, certainly makes it important enough to consider novel treatment options which are targeted toward different pathways.

Beetroot juice at higher doses could also have substantial therapeutic implications in COPD. Since this is a debilitating disease with a very high global prevalence, larger clinical trials are surely warranted to assess efficacy of inorganic nitrates in such patients.

List of Findings

We can summarize the key findings of our experiments as follows:

1. In patients with moderate to severe COPD, preservation of lung gas exchange surface area as assessed using the resting SBDLCO to Qc ratio, is a better predictor of exercise capacity than the more classic measures of lung mechanics such as spirometry.

2. COPD patients that can increase respiratory gas exchange surface area (as assessed via DLCO) relative to the rise in pulmonary blood flow (Qc) have the most preserved exercise capacity. In addition, the ability to expand the pulmonary vascular bed is associated with noninvasive indices of pulmonary capacitance (GxCap) as well as with inspiratory capacity assessed during exercise. While these data are correlative, this implies that a contributing factor to exercise intolerance in this population may be the inability to recruit pulmonary capillaries.

3. COPD patients who took the beetroot juice demonstrated a drop in their SGRQ score, a higher exhaled nitric oxide, and a lower blood pressure at peak exercise which were all statistically significant. There was a trend for improvement in exercise performance and pulmonary gas exchange surface area in our cohort but the results were not statistically significant and larger clinical trials are warranted.

Appendix A & B of this thesis have been removed as they may contain sensitive/confidential content

Appendix C

St George Respiratory Questionnaire

ST. GEORGE'S RESPIRATORY QUESTIONNAIRE
ORIGINAL ENGLISH VERSION

ST. GEORGE'S RESPIRATORY QUESTIONNAIRE (SGRQ)

This questionnaire is designed to help us learn much more about how your breathing is troubling you and how it affects your life. We are using it to find out which aspects of your illness cause you most problems, rather than what the doctors and nurses think your problems are.

Please read the instructions carefully and ask if you do not understand anything. Do not spend too long deciding about your answers.

Before completing the rest of the questionnaire:

Please tick in one box to show how you describe your current health:

Very good	Good	Fair	Poor	Very poor
☐	☐	☐	☐	☐

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continued…

St. George's Respiratory Questionnaire
PART 1

Questions about how much chest trouble you have had over the past 3 months.

Please tick (✓) *one box for each question*:

		most days a week	several days a week	a few days a month	only with chest infections	not at all
1.	Over the past 3 months, I have coughed:	☐	☐	☐	☐	☐
2.	Over the past 3 months, I have brought up phlegm (sputum):	☐	☐	☐	☐	☐
3.	Over the past 3 months, I have had shortness of breath:	☐	☐	☐	☐	☐
4.	Over the past 3 months, I have had attacks of wheezing:	☐	☐	☐	☐	☐

5. During the past 3 months how many severe or very unpleasant attacks of chest trouble have you had?

Please tick (✓) *one*:

- more than 3 attacks ☐
- 3 attacks ☐
- 2 attacks ☐
- 1 attack ☐
- no attacks ☐

6. How long did the worst attack of chest trouble last?
(Go to question 7 if you had no severe attacks)

Please tick (✓) *one*:

- a week or more ☐
- 3 or more days ☐
- 1 or 2 days ☐
- less than a day ☐

7. Over the past 3 months, in an average week, how many good days (with little chest trouble) have you had?

Please tick (✓) *one*:

- No good days ☐
- 1 or 2 good days ☐
- 3 or 4 good days ☐
- nearly every day is good ☐
- every day is good ☐

8. If you have a wheeze, is it worse in the morning?

Please tick (✓) *one:*

- No ☐
- Yes ☐

St. George's Respiratory Questionnaire
PART 2

Section 1

How would you describe your chest condition?

Please tick (✓) *one*:

- The most important problem I have ☐
- Causes me quite a lot of problems ☐
- Causes me a few problems ☐
- Causes no problem ☐

If you have ever had paid employment.

Please tick (✓) *one*:

- My chest trouble made me stop work altogether ☐
- My chest trouble interferes with my work or made me change my work ☐
- My chest trouble does not affect my work ☐

Section 2

Questions about what activities usually make you feel breathless <u>these days</u>.

Please tick (✓) in ***each box*** that applies to you ***these days***:

	True	False
Sitting or lying still	☐	☐
Getting washed or dressed	☐	☐
Walking around the home	☐	☐
Walking outside on the level	☐	☐
Walking up a flight of stairs	☐	☐
Walking up hills	☐	☐
Playing sports or games	☐	☐

continued…

St. George's Respiratory Questionnaire
PART 2

Section 3

Some more questions about your cough and breathlessness <u>these days</u>.

	Please tick (✓) in ***each box*** that applies to you ***these days***: True	False
My cough hurts	☐	☐
My cough makes me tired	☐	☐
I am breathless when I talk	☐	☐
I am breathless when I bend over	☐	☐
My cough or breathing disturbs my sleep	☐	☐
I get exhausted easily	☐	☐

Section 4

Questions about other effects that your chest trouble may have on you <u>these days</u>.

	Please tick (✓) in ***each box*** that applies to you ***these days***: True	False
My cough or breathing is embarrassing in public	☐	☐
My chest trouble is a nuisance to my family, friends or neighbours	☐	☐
I get afraid or panic when I cannot get my breath	☐	☐
I feel that I am not in control of my chest problem	☐	☐
I do not expect my chest to get any better	☐	☐
I have become frail or an invalid because of my chest	☐	☐
Exercise is not safe for me	☐	☐
Everything seems too much of an effort	☐	☐

Section 5

Questions about your medication, if you are receiving no medication go straight to section 6.

	Please tick (✓) in ***each box*** that applies to you ***these days***: True	False
My medication does not help me very much	☐	☐
I get embarrassed using my medication in public	☐	☐
I have unpleasant side effects from my medication	☐	☐
My medication interferes with my life a lot	☐	☐

continued…

St. George's Respiratory Questionnaire
PART 2

Section 6

These are questions about how your activities might be affected by your breathing.

Please tick (✓) in ***each box*** that applies to you ***because of your breathing***:

	True	False
I take a long time to get washed or dressed	☐	☐
I cannot take a bath or shower, or I take a long time	☐	☐
I walk slower than other people, or I stop for rests	☐	☐
Jobs such as housework take a long time, or I have to stop for rests	☐	☐
If I walk up one flight of stairs, I have to go slowly or stop	☐	☐
If I hurry or walk fast, I have to stop or slow down	☐	☐
My breathing makes it difficult to do things such as walk up hills, carrying things up stairs, light gardening such as weeding, dance, play bowls or play golf	☐	☐
My breathing makes it difficult to do things such as carry heavy loads, dig the garden or shovel snow, jog or walk at 5 miles per hour, play tennis or swim	☐	☐
My breathing makes it difficult to do things such as very heavy manual work, run, cycle, swim fast or play competitive sports	☐	☐

Section 7

We would like to know how your chest <u>usually</u> affects your daily life.

Please tick (✓) in ***each box*** that applies to you ***because of your chest trouble***:

	True	False
I cannot play sports or games	☐	☐
I cannot go out for entertainment or recreation	☐	☐
I cannot go out of the house to do the shopping	☐	☐
I cannot do housework	☐	☐
I cannot move far from my bed or chair	☐	☐

continued…

St. George's Respiratory Questionnaire

Here is a list of other activities that your chest trouble may prevent you doing. (You do not have to tick these, they are just to remind you of ways in which your breathlessness may affect you):

Going for walks or walking the dog

Doing things at home or in the garden

Sexual intercourse

Going out to church, pub, club or place of entertainment

Going out in bad weather or into smoky rooms

Visiting family or friends or playing with children

Please write in any other important activities that your chest trouble may stop you doing:

. . . . . .

. . . . . .

. . . . . .

. . . . . .

Now would you tick in the box (one only) which you think best describes how your chest affects you:

It does not stop me doing anything I would like to do ☐

It stops me doing one or two things I would like to do ☐

It stops me doing most of the things I would like to do ☐

It stops me doing everything I would like to do ☐

Thank you for filling in this questionnaire. Before you finish would you please check to see that you have answered all the questions.

Appendix D

Effects of Exercise on Vascular Stiffness in COPD

An ***exploratory aim*** of the dissertation was to study vascular stiffness and pulse wave velocity in our COPD cohort before and after CPET.

Introduction

Arterial stiffness is a disease with serious clinical impact. It is associated not only with atheromatous plaque burden in atherosclerosis, but also with elastin fragmentation and alterations in collagen of connective tissue of emphysematous lungs (164). Vlachopoulos et al have shown that arterial stiffness, is strongly predictive of future cardiovascular morbidity and mortality (167).

Arterial stiffness is also elevated in COPD and it has been shown that emphysema severity independent of airflow obstruction (FEV_1), age, or sex, is correlated with arterial stiffness (164). This is more manifested in the lowest quintile of lung function, as measured by FEV_1. Mid-life FEV_1 and FVC are stronger risk factors than in later life for arterial stiffness in men which was independent of smoking status and inflammatory and metabolic factors in sub-groups (168).

COPD, independent of smoking, is associated with cardiovascular morbidity and mortality. Chronic bronchitis has been shown to increase the risk of cardiovascular mortality by 50%. On average, for every 10% decrease in FEV_1, all-cause mortality is elevated by 14%, while cardiovascular mortality and nonfatal coronary events are increased by about 28% and 20%, respectively (169). In COPD, the impact of comorbidities on mortality has been studied and composite scores have been proposed. These scores help assess risk of mortality in the disease (170). The key is early identification of

patients with a high cardiovascular risk score in COPD. In patients with medium to high risk, the key is to early identify patients before major clinical events such as myocardial infarction or stroke.

Exercise reduces arterial stiffness in COPD. In a cohort of COPD patients, 4 weeks of aerobic training reduced arterial stiffness as shown by a reduction in pulse wave velocity, and it also improved walking distance, muscle endurance, systolic blood pressure, and fasting glucose (171).

Review of Primary aim

In review, in the **first** part of the thesis we showed that lung diffusing capacity (DLCO) alone or expressed relative to resting pulmonary blood flow (Qc) is the most predictive of exercise capacity in a cohort of COPD patients. In part **two** we showed that COPD patients that can increase respiratory gas exchange surface area (as assessed via DLCO) relative to the rise in pulmonary blood flow, have the most preserved exercise capacity and the ability to expand the pulmonary vascular bed is associated with noninvasive indices of pulmonary capacitance as well as with inspiratory capacity assessed during exercise. In part **three** we studied the effects of inorganic nitrates on exercise tolerance in our COPD population.

Exploratory aim

We were further interested in studying the impact of exercise on pulse wave velocity in our patients before and after exercise. We repeated our analysis in our patients and studied the effects of inorganic nitrates on pulse wave velocity before and after exercise.

Methods

Hemodynamic analysis

Central aortic pressure was measured by the SphygmoCor Xcel device (AtCor Medical, Sydney) from the brachial pulse waveform calibrated to the brachial cuff systolic and diastolic pressures. The derived central aortic pressure waveform was decomposed into forward (Pf) and backward (Pb) components using the triangulation technique, where the aortic flow is approximated by a triangular waveform with the base corresponding to ejection duration and an arbitrary height, with the peak occurring at the first inflection of the aortic pressure wave. This technique has been shown to be robust in computing indices of wave reflection (172).

Pressure augmentation and wave reflection

The augmentation of pressure during systole is quantified by the calculation of the dimensionless parameter of Augmentation Index (AIx) and the augmentation pressure (AP). AP is the difference between the peak pressure and the pressure at the first shoulder in early systole, and AIx is the ratio between AP and pulse pressure (PP).

$$AIx = AP/PP \quad (1)$$

Reflection magnitude (Ref Mag) is the ratio of the amplitude of the backward wave (Pb) and the forward wave (Pf).

$$Ref\ Mag = Pf/Pb \quad (2)$$

Pulse wave velocity

Aortic pulse wave velocity (PWV), a measure of arterial stiffness was obtained from the pulse transit time (PTT) between the carotid and femoral arteries using applanation tonometry for detection of the pulse waveform. PTT was determined from the foot-to-foot delay between the proximal (carotid) and distal (femoral) pulses. The distance for the path length (D) was obtained from the suprasternal notch to the femoral site (df) minus the distance from the suprasternal notch to the carotid site (dc) (D= df-dc). PWV was computed as

$$PWV = D/PTT \quad (3)$$

Results

Results for the hemodynamic analysis for the dietary nitrate group (N=14) and placebo (n=13) are summarized in Table 1 for pre and post exercise and pre and post beetroot juice. The data have been statistically analyzed using analysis of variance with repeated measures, with a statically significant p value of < 0.05.

The analysis was run to assess any statistical significance of (i) effect of nitrate, (ii) effect of juice intake, and (iii) effect of exercise on all the pressure and derived indices listed in Table 1. In this small cohort study using ANOVA, there was no effect of juice intake or exercise but a significant effect of nitrate on brachial systolic and pulse pressure, aortic pulse pressure and Ref Mag. The potentially intriguing finding is that nitrate subjects generally showed increased wave reflection across all subgroups which can be measured with non-invasive means.

An important observation is that the cohort may have been underpowered for detection of statistical significance in other variables. For example, taking the smallest p-value for the juice effect (PWV), a sample size of 64 would be required to detect this difference at a power of 80% and a for p value of < 0.05.

Table 1. Appendix D. *Hemodynamic data for indices of brachial and central aortic pressure for systolic (SP), diastolic (DP), mean (MP) pulse (PP), augmentation (AP) pressure; aortic augmentation index (Aix); carotid-femoral pulse wave velocity (PWV); forward (Pf) and backward (Pb) pressure amplitude; reflection magnitude (Ref Mag); heart rate (HR). p values refer to effect of disease. SD: standard deviation*

	PRE-EXERCISE								POST-EXERCISE								*p*
	PRE-JUICE				POST-JUICE				PRE-JUICE				POST-JUICE				
	Nitrate		Placebo		Nitrate		Placebo		Nitrate		Placebo		Nitrate		Placebo		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
BRACHIAL PRESSURE																	
SP (mm Hg)	141.3	14.3	147.3	25.5	137.6	21.1	138.4	17.8	153.8	22.7	152.6	17.4	149.1	16.7	150.4	16.0	0.056
DP (mm Hg)	80.9	14.2	78.5	13.1	82.6	20.0	75.6	10.4	88.4	15.5	81.4	8.7	85.4	12.8	78.7	10.7	NS
MP (mm Hg)	101.0	13.4	101.4	15.1	100.9	19.0	96.5	10.4	110.0	17.1	105.1	9.3	106.6	12.7	102.5	9.9	NS
PP (mm Hg)	60.1	10.5	68.8	21.7	55.1	15.8	62.8	17.8	65.4	15.0	71.4	17.1	62.6	13.5	71.6	17.0	0.027
AORTIC PRESSURE																	
SP (mm Hg)	127.8	12.5	131.0	24.0	125.1	19.4	123.6	15.7	138.0	20.8	134.4	15.8	132.9	13.7	131.7	14.3	NS
DP (mm Hg)	82.3	13.9	79.8	12.6	83.7	19.7	76.6	10.4	90.5	16.4	82.8	8.5	86.9	13.0	79.7	10.9	NS
MP (mm Hg)	100.8	13.6	100.0	15.0	100.6	18.9	95.1	11.1	110.3	18.8	104.2	9.9	106.1	12.3	101.5	10.9	NS
PP (mm Hg)	45.5	8.7	51.2	19.7	41.4	11.2	47.0	15.1	47.6	10.8	51.5	13.5	49.5	17.2	52.0	14.4	0.041
AP (mmHg)	13.9	6.6	12.1	8.0	11.3	6.2	11.2	5.0	14.4	7.3	11.9	5.9	11.5	6.4	12.3	7.9	NS
AIx (%)	29.6	13.5	21.8	10.7	26.8	12.7	23.8	6.4	28.8	13.0	23.2	9.5	24.9	12.0	22.2	10.2	NS
PWV (m/s)	8.2	2.3	8.8	2.6	8.9	2.0	9.2	1.4	9.1	2.4	8.9	2.2	7.8	2.9	9.3	2.0	NS
Pf (mm Hg)	31.8	7.5	34.0	7.7	28.4	8.5	32.6	8.4	35.5	10.5	38.5	6.5	34.2	7.1	36.9	12.6	NS
Pb (mm Hg)	19.4	3.7	22.4	8.4	18.3	5.5	20.2	5.5	22.5	4.8	22.2	5.1	20.6	4.5	22.4	6.7	NS
Ref Mag (%)	62.6	15.8	64.5	12.8	66.1	16.0	63.2	14.4	65.6	13.9	57.3	9.3	61.0	12.2	55.6	11.3	0.033
HR bpm	72.0	12.8	69.5	10.2	72.3	10.5	72.2	10.0	72.4	10.9	71.8	10.8	77.6	13.5	79.4	9.2	NS

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Conference Presentations

and

Journal Publications

The following abstracts this thesis have been removed as they contain published material. Please refer to the following citation for details of the articles contained in these pages.

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Behnia, M., Avolio, A., & Johnson, B. (2016). Alveolar-capillary reserve during exercise in patients with COPD. *Chest*, 150(4) Supplement, p. 851A.
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RESEARCH ARTICLE Open Access

Influence of resting lung diffusion on exercise capacity in patients with COPD

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Abstract

Background: Lung diffusing capacity for carbon monoxide (DLCO) gives an overall assessment of functional lung surface area for gas exchange and can be assessed using various methods. DLCO is an important factor in exercise intolerance in patients with chronic obstructive pulmonary disease (COPD). We investigated if the intra-breath (IBDLCO) method may give a more sensitive measure of available gas exchange surface area than the more typical single breath (SBDLCO) method and if COPD subjects with the largest resting DLCO relative to pulmonary blood flow (Qc) would have a more preserved exercise capacity.

Methods: Informed consent, hemoglobin, spirometry, SBDLCO, IBDLCO, and Qc during IBDLCO were performed in moderate to severe COPD patients, followed by progressive cycle ergometry to exhaustion with measures of oxygen saturation (SaO_2) and expired gases.

Results: Thirty two subjects (47% female, age 66 ± 9 yrs., BMI 30.4 ± 6.3 kg/m^2, smoking hx 35 ± 29 pkyrs, 2.3 ± 0.8 on the 0-4 GOLD classification scale) participated. The majority used multiple inhaled medications and 20% were on oral steroids. Averages were: FEV_1/FVC 58 ± 10%Pred, peak VO_2 11.4 ± 3.1 ml/kg/min, and IBDLCO 72% of the SBDLCO ($r = 0.88$, SB vs IB methods). Using univariate regression, both the SB and IBDLCO (% predicted but not absolute) were predictive of VO_2peak in ml/kg/min; SBDLCO/Qc ($r = 0.63$, $p < 0.001$) was the best predictor of VO_2peak; maximal expiratory flows over the mid to lower lung volumes were the most significantly predictive spirometric measure ($r = 0.49$, $p < 0.01$). However, in multivariate models only BMI added additional predictive value to the SBDLCO/Qc for predicting aerobic capacity ($r = 0.73$). Adjusting for current smoking status and gender did not significantly change the primary results.

Conclusion: In patients with moderate to severe COPD, preservation of lung gas exchange surface area as assessed using the resting SBDLCO/Qc appears to be a better predictor of exercise capacity than more classic measures of lung mechanics.

Keywords: Lung surface area, Gas exchange, Dyspnea, Gas transfer

Background

Causes contributing to exercise limitation in patients with chronic obstructive pulmonary disease (COPD) are complex [1–3]. Previous studies have suggested that while lung mechanics clearly play an important role, there are many other factors that contribute to this limitation such as heterogeneity of the disease process, lifestyle issues, such as weight and activity patterns, deconditioning, disease-related inflammatory processes, perception, as well as associated comorbidities such as cardiovascular disease [4–6]. Pulmonary function measures representing the degree of obstruction and severity of hyperinflation (e.g., inspiratory capacity or IC) appear important as well as less appreciated factors such as a blunted cardiac output, either due to airway obstruction and rise in intra-thoracic pressure or from the development of pulmonary hypertension [7–9]. As a result, exercise capacity as a whole has been used as a prognostic indicator in the COPD population and as such is a good assessment of the integrative factors involved in the disease [10]. In addition, as stated by the GOLD initiative (Global Initiative for Chronic

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Obstructive Lung Disease classification for air flow obstruction), improvement in exercise tolerance is recognized as an important goal of COPD treatment.

From the lung volume reduction surgery data, it has also been found that certain patterns of disease and perhaps more severe emphysema may be associated with worse exercise tolerance [11, 12]. Of the common relatively simple screening tests, a low lung diffusing capacity for carbon monoxide (DLCO) has been shown to not only suggest a more emphysematous pathophysiology but has also been a predictor of exercise capacity and in particular exercise induced oxygen desaturation [1, 13]. There are different ways to quantify DLCO, from the typical single breath method (SBDLCO), to various rebreathe, steady state, open-circuit and intra-breath techniques [14]. While the latter methods may represent in some sense more physiological quantification of functional lung surface area for gas transfer or exchange, the single breath method has been standardized with well-established predictive norms for clinical use [15].

The intra-breath method (IBDLCO) is interesting in that it potentially represents a relatively simple way to quantify DLCO in patients that may struggle with longer breath hold times and there is potential for use during exercise to quantify alveolar–capillary surface area recruitment. It requires the exhalation of test gas typically near residual volume, followed by a deep inhalation and essentially instantaneous exhalation back towards residual volume (RV). The expiratory sampling relies on a fast response CO analyzer and as exhalation continues towards RV, the DLCO at any point is dependent then on the exhaled lung volume and each time point would represent a DLCO that is a mix of CO uptake and mixing with other lung gases [16, 17]. An advantage of this technique compared to other more discrete techniques is that the exhaled gas stream is used in its entirety to calculate DLCO.

In patients with lung disease it is likely that the intra-breath method may be more sensitive to disease pathology relative to the single breath method due to abnormalities in ventilation and perfusion and delayed time constants for ventilation with the shortened gas exchange times.

Lung diffusion is dependent on pulmonary capillary blood volume (Vc) and alveolar-capillary gas exchange surface area, usually reported as membrane diffusion capacity. While these components of DLCO can be estimated by performing DLCO at multiple oxygen concentrations or with a second gas such as nitric oxide (DLNO), a surrogate may be obtained for blood volume by examining the DLCO relative to cardiac output (Qc). Since a rise in Q tends to be the major reason for distension or recruitment of capillaries, a larger ratio would be indicative of a healthier phenotype.

Thus in the present study we were interested in the role of resting DLCO in predicting exercise capacity in a relative diverse group of COPD patients. More specifically we were interested if a higher intra-breath to single breath DLCO ratio or a higher DLCO relative to Qc ratio would better predict exercise capacity relative to other common measures of lung mechanics. We hypothesized that those subjects with a higher IBDLCO relative to SBDLCO or a higher SBDLCO/Qc ratio would have better preserved exercise capacity.

Methods

Ethics and consent

The study, ethics, and consent forms were reviewed and approved by the Western Institutional Review Board (WIRB, study number 1153374).

Subjects

Patients with a history of COPD that were sent for clinical pulmonary function testing and/or exercise testing were offered enrollment. Inclusion criteria included established patients with a history of COPD, on stable medications without recent exacerbation (within 3 months). Exclusion criteria included, oxygen dependence an inability to exercise and/or a BMI > 42. Both past and current smokers were allowed to participate with 7 of the participants being current smokers. Prior to participation, the study goals and requirements were reviewed with the patients. If willing to participate, patients signed informed consent.

Overview of study

After reporting to the outpatient clinic, study participants filled out the St. George's Respiratory quality of life questionnaire (SGQOL), performed pulmonary function testing (PFTs) which included resting measures of maximal lung volumes and flow rates using classical spirometry. In addition the assessment of lung diffusing capacity for carbon monoxide (DLCO) was obtained using the classical single breath (SB) technique and was also obtained using the intra-breath method (IB) which included a measure of pulmonary blood flow (Qc). A small blood sample was obtained prior to testing for assessment of hemoglobin in order to correct the measure of DLCO. Subjects subsequently performed cardiopulmonary exercise testing (CPET) using the CareFusion Vmax Encore metabolic cart (San Diego, CA) with a Corival recumbent cycle ergometer (Lode, Netherlands). The test protocol started with 20 watts for both men and women and increased by 10 watts every 2 min. Prior to exercise testing, subjects were instrumented with a 12 lead ECG, and a forehead pulse oximeter for peripheral oxygen saturation (SaO_2) for continuous monitoring. Subjects wore a nose clip and breathed on a mouthpiece for continuous measurement of gas exchange during the exercise test. During the last 30 s of each workload, a 12 lead ECG recording was printed, blood pressure (BP) assessed, perceived dyspnea score (0-10 scale) and perceived exertion (an assessment of total

body effort) was rated by subjects, and an average of the HR and SpO_2 over this period was determined. The goal was to obtain at least 2–3 work levels for each subject. Subjects were encouraged to exercise to near exhaustion based on symptom limitation by achieving an RPE of 17-18 on the Borg 6-20 scale or a dyspnea score ≥ 7 on the 0-10 score [18]. Upon reaching peak symptom limited exercise, subjects performed active recovery where they continued to pedal with no resistance and remained on the mouthpiece for 1 min. After this the subject stopped pedaling and was given time for HR and BP to return to baseline before being dismissed.

Pulmonary function and single breath DLCO

Spirometry was performed using pneumotachograph-based pulmonary function equipment that has passed evaluation using 24-wavefroms recommended by the American Thoracic Society (ATS). Classic single breath DLCO was determined using a commercial instrument that utilizes a gas chromatograph to analyze expired gas samples, following the recommendations of the ATS/ERS [15, 19].

Intrabreath lung diffusing capacity and pulmonary blood flow (qc)

Pulmonary Blood Flow (Qc) and diffusing capacity of the lungs for carbon monoxide (DLCO) were measured using inert and soluble gases on the CareFusion Vmax system using an intra-breath maneuver [17]. For this maneuver, subjects were asked to breath on a mouthpiece while wearing a nose clip. Subjects were instructed to exhale to residual volume (RV) and then were switched in to an inspiratory reservoir and took a maximal inhalation of a test gas mixture containing 0.3% carbon monoxide (CO), 0.3% methane, 0.3% acetylene, 21% O_2, and balance N_2. Subjects were coached to exhale slowly at a steady rate until they were near RV. From the rate of disappearance of CO and acetylene in comparison to the inert gas methane the rate of disappearance of CO and acetylene were determined. This rate of disappearance of CO provides the DLCO value. Since acetylene does not bind to hemoglobin the rate of its disappearance is limited only by the flow of blood through the lungs, thereby providing a measure of Q [20, 21].

QOL questionnaires. St. George's respiratory questionnaire

The SGRQ is a 50-item questionnaire developed to measure health status (quality of life, QOL) in patients with diseases of airways obstruction. Scores are calculated for three domains: Symptoms, Activity and Impacts (Psychosocial) as well as a total score. Psychometric testing has demonstrated its repeatability, reliability and validity. Sensitivity has been demonstrated in clinical trials. A minimum change in score of 4 units has previously been established as clinically relevant. The SGRQ has been used in a range of disease groups including asthma, chronic obstructive pulmonary disease (COPD) and bronchiectasis, and in a range of settings such as randomized controlled therapy trials and population surveys [22]. The SGRQ correlates significantly with other measures of disease activity such as cough, dyspnea, 6-min walk test and FEV_1 as well as other measures of general health such as the Sickness Impact Profile (SIP) score which evaluates the impact of disease on physical and emotional functioning and Short Form 36 (SF36) health survey which is a patient reported survey of health [23].

Gas exchange, ventilation and lung mechanics

During exercise testing oxygen consumption (VO_2), carbon dioxide production (VCO_2), breathing frequency (fb), tidal volume (V_t), minute ventilation (V_E) and derived variables (e.g., V_E/VCO_2) were measured continuously or calculated using a low resistance open circuit automated metabolic system (CareFusion).

Statistics

We were interested in the association of resting measures of DLCO measured via single breath or intrabreath methods as well as expressed relative to Qc with exercise capacity (peak VO_2) in patients with moderate to severe COPD and if these measures were more highly associated to exercise capacity than more typical measures of lung mechanics. Descriptive statistics were used to describe patient characteristics and demographics while multiple regression and correlational analysis were used to determine associations between DLCO, Q, lung mechanics, QOL, disease severity and exercise capacity. Statistics were performed with a combination of EXCEL and the statistical software package JMP Statistical Discovery TM software from SAS.

Results

Subject characteristics and pulmonary function measures

Thirty two subjects completed the study. As shown in Table 1, on average our study cohort was older, approximately half female, above ideal body mass index and had a 35 pack year smoking history. By design, their GOLD classification ranged from 1 to 4 with an average classification consistent with moderate disease with an FEV_1 of 56% of age predicted and an FEV_1/FVC ratio of 59% (Table 2). None of the subjects were on continuous oxygen or oxygen for exercise at the time of the study. The majority of subjects were on combination inhalation therapy that included inhaled beta-2 agonist, anticholinergic, and inhaled steroid with a minority of subjects on oral steroids. Quality of life scores based from the St George questionnaire was consistent with severity of disease as described by the GOLD classification.

Table 1 Subject characteristics (n = 32)

	Mean ± SD	Range
Age (years)	66 ± 9	46 - 84
% Female	47	-
Weight (Kg)	88 ± 23	36 - 155
BMI (Kg/m2)	30 ± 6	13 - 44
Smoking history (pack year)	35 ± 29	0 - 120
Current/former/never smoker (n)	6/22/4	-
GOLD Classification (1–4)	2.3 ± 0.8	1 - 4
St George Respiratory Questionnaire	44 ± 21	8 - 84
Inhaled beta agonist (%)	97	-
Inhaled anticholinergic (%)	59	-
Inhaled steroid (%)	68	-
Oral steroid (%)	20	-

GOLD Global Initiative for Chronic Obstructive Lung Disease classification for air flow obstruction

Resting lung diffusion measures – Single breath vs intra-breath

Table 3 lists single breath and intra breath DLCO measures, the measured pulmonary blood flow (Qc), and Hgb values. SBDLCO averaged 13.2 ml/min/mmHg and 58% of predicted with the average IBDLCO 71% of the SB method ranging from 20 to 110% across the study population. Overall the SB and IB methods were highly correlated with an r of 0.88 (Fig. 1) and the IB/SBDLCO relationship was positively associated with resting IC (Fig. 2). The measured pulmonary blood flow (Qc) using soluble gas was 76% of the resting predicted cardiac output based on gender and body size. On average the SBDLCO was 51% predicted in current smokers vs 56% predicted in those that had quit or never smoked. Though the current smokers were slightly reduced relative to nonsmokers, there was no statistical difference between groups ($p < 0.05$).

Cardiopulmonary exercise responses

Cardiopulmonary exercise responses are reported in Table 4. On average the peak VO_2 for the group was 0.98 L/min or 11.4 ml/kg/min equivalent to 50% of age and gender predicted. Average peak heart rate was 103 bpm which was 70% of their age predicted and average respiratory exchange ratio (RER) was 1.03. Inspiratory capacity consistently fell throughout exercise and at peak the tidal volume reached 65% of the IC. Oxygen pulse rose to an average of 8 ml/beat early in exercise but then plateaued thereafter and reached a max of 9.1 in peak exercise suggesting a plateauing of cardiac stroke volume. Subjects complained of both general fatigue and dyspnea as major reasons for stopping the test. At peak exercise the minute ventilation averaged 73% of the maximum voluntary ventilation (MVV) with five subjects exceeding their pre-test MVV.

Table 2 Pulmonary Function Variables

	Mean ± SD	Percent Predicted (range)
FVC (L)	2.48 ± 0.69	75 ± 15
FEV_1 (L)	1.51 ± 0.58	56 ± 16
FEV_1/FVC	59 ± 11	(33 – 78)
FEF_{25-75} (L/s)	0.75 ± 0.38	26 ± 13
FEF_{75} (L/s)	0.29 ± 0.11	27 ± 13
MVV (L/m)	48 ± 19	45 ± 17

FVC Forced Vital Capacity, *FEV_1* Forced Expiratory Volume in 1 s, *FEF* Forced Expiratory Flow, *MVV* Maximal voluntary ventilation. All data are pre bronchodilator

Relationship of resting measures of lung mechanics and lung diffusion to exercise capacity

Univariate correlations of resting measures of lung function, QOL, anthropometric measures and lung diffusion relative to exercise capacity (expressed in ml/kg/min as well as in L/min) are shown in Table 5. The values that were most significantly linked to exercise capacity based on VO_2peak in ml/kg/min were SBDLCO relative to pulmonary blood flow (SBDLCO/Qc) (Fig. 3), SBDLCO (% Pred), VT/IC, (where VT is tidal volume), absolute measures of FEF_{25-75} (Fig. 4), FEF_{75} and BMI ($p < 0.01$). Also associated but less significantly so ($p < 0.05$ but >0.01) were the IBDLCO also relative to Qc as well as FVC and FEF50%. In a step wise fashion or when allowing all significant variables to compete in a multiple regression, only SBDLCO/Qc and BMI remained in a model predicting VO_2 peak ml/kg/min where:

$$\left[VO_2peak\ ml/kg/\min = 2.51\ (DLCO/Qc)\text{-}0.176\ (BMI) + 11.48,\ with\ an\ r\ of\ 0.73\ and\ an\ R^2 of\ 0.53.\right]$$

When expressing VO_2 peak as L/min, SBDLCO/Qc, IC, VT/IC and BSA were the most predictive of exercise capacity with FVC being the strongest lung mechanics measure in a multiple regression but which lost significance when BSA was added. Thus, the best model for absolute VO_2 was:

$$\left[VO_2peak\ L/\min = 0.2038\ (DLCO/Qc) + 0.6193\ (BSA)\text{-}0.665,\ with\ an\ r\ of\ 0.81\ and\ an\ R^2 of\ 0.65.\right]$$

Influence of smoking and gender on predictors of exercise capacity

Since six of our cohort were current smokers and current smoking and the time of abstention from smoking is known to impact DLCO [24, 25], we performed both stepwise and multivariate models accounting for smokers. Under both conditions, SBDLCO/Qc and BMI

Table 3 Lung diffusing capacity and pulmonary blood flow

	Mean ± SD	Percent predicted or (Range)
Single Breath DLCO (SBDLCO, ml/min/mmHg)	13.2 ± 5.5	58 ± 23 (31 –112)
Intra Breath DLCO (IBDLCO, ml/min/mmHg)	9.7 ± 5.9	(1.3 – 27)
IBDLCO/SBDLCO (%)	71 ± 26	(20 – 110)
Pulmonary Blood Flow (Qc, L/m, measured)	4.8 ± 0.9	(3.3 – 6.8)
Pulmonary Blood Flow –Cardiac output, (L/m, Predicted)	6.3 ± 0.4	(5.4 – 7.1)
SBDLCO/Qc ratio	2.8 ± 1.2	(1.2 – 5.7)
Hgb (g/dl)	13.5 ± 1.7	(11–17)

Pulmonary Blood Flow measured with soluble gas method. Cardiac output estimated based on age, gender, BSA, from Ref (William LR). Qc = Pulmonary Blood Flow

still remained significant predictors ($p < 0.01$) with the influence of smoking being not significant ($p = 0.67$). Subgroup analysis excluding current smokers was also performed relative to VO_2 peak ml/kg/min, where DLCO/Q and BMI remained significant predictors ($p = 0.000$ and $p = 0.009$ respectively). Percent predicted SB and IBDLCO were also not significantly different between current and past smokers ($p > 0.05$).

We also accounted for gender in the models, including when expressing VO_2peak in L/min rather than in ml/kg/min. There was no influence of gender on the relationship between SBDLCO/Qc and VO_2peak. When expressing VO_2peak in L/min, SBDLCO/Qc and BSA remained the most significant predictors.

Discussion

Primary findings

From our study we conclude that while resting measures of hyperinflation and maximal expiratory flows, particularly over the mid to lower lung volumes were predictive of exercise capacity, lung diffusing capacity alone or expressed relative to resting pulmonary blood flow was the most predictive of exercise capacity. Furthermore, when allowed to compete in a multiple regression model, only the SBDLCO relative to Qc and measure of body weight or habitus were significant predictors and explained approximately 50-60% of the variability in exercise capacity in this population.

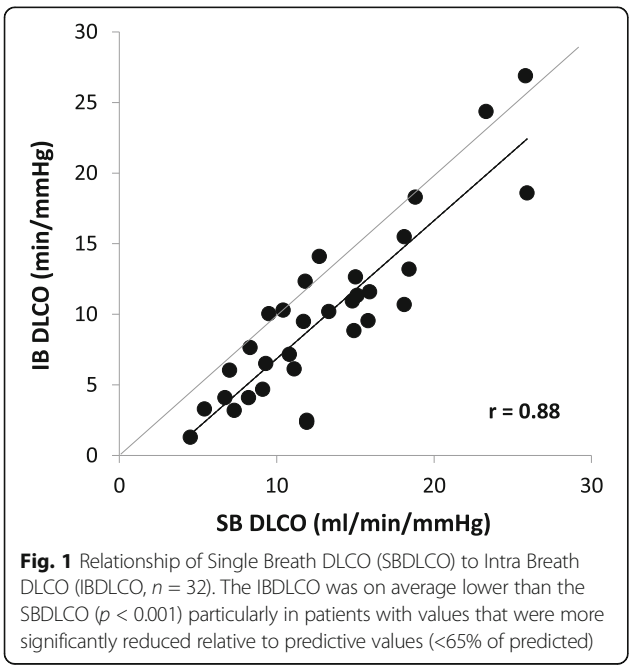

Fig. 1 Relationship of Single Breath DLCO (SBDLCO) to Intra Breath DLCO (IBDLCO, $n = 32$). The IBDLCO was on average lower than the SBDLCO ($p < 0.001$) particularly in patients with values that were more significantly reduced relative to predictive values (<65% of predicted)

Previous studies looking at exercise intolerance in COPD

Factors contributing to exercise intolerance in patients with COPD are complex. While a number of studies have examined predictors of exercise capacity in the COPD population, the majority of these have focused on measures of lung mechanics and while relationships are found between measures of maximal expiratory flows and volumes, measures of hyperinflation appear to be the most predictive [3, 8, 9, 26, 27]. In this study we evaluated several measures of lung function, quality of life, body weight/habitus and measures of lung diffusing

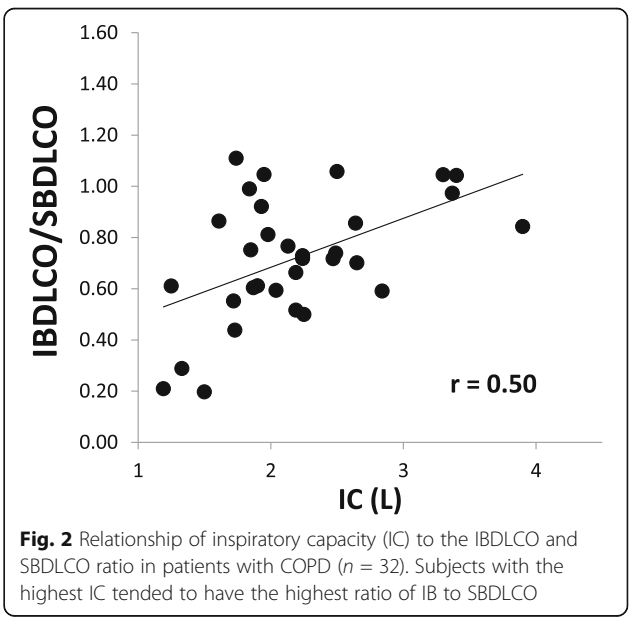

Fig. 2 Relationship of inspiratory capacity (IC) to the IBDLCO and SBDLCO ratio in patients with COPD ($n = 32$). Subjects with the highest IC tended to have the highest ratio of IB to SBDLCO

Table 4 Breathing pattern, lung mechanics and gas exchange responses to exercise (n = 32)

	Rest	First work load	Peak exercise
Heart Rate (bpm)	77 ± 10	92 ± 11	103 ± 22
RPE (6–20)	7 ± 2	11 ± 2	17 ± 2
Dyspnea (0-10)	1 ± 1	3 ± 2	7 ± 2
VE (L/min)	12.5 ± 2.7	24 ± 6	34 ± 11
Fb/VT ratio	26 ± 15	26 ± 14	30 ± 13
TI/TTOT ratio	39 ± 8	38 ± 4	39 ± 4
IC (L)	2.2 ± 0.7	1.9 ± 0.7	1.8 ± 0.7
VT/IC (%)	37 ± 13	52 ± 12	65 ± 27
VO_2 ml/kg/min	3.7 ± 0.8	8.3 ± 1.4	11.4 ± 3.1
VE/VCO_2 ratio	47 ± 7	37 ± 5	36 ± 5
$PetCO_2$ mmHg	35 ± 5	37 ± 4	37 ± 5
O_2Pulse	4 ± 1	8 ± 2	9 ± 3
SaO_2 (%)	96 ± 2	95 ± 3	94 ± 3

VE Minute ventilation, *fb* breathing frequency, *VT* tidal volume, *TI* inspiratory time, *TTOT* total respiratory cycle time, *IC* inspiratory capacity, VO_2 oxygen consumption, VCO_2 carbon dioxide production, $PetCO_2$ end tidal partial pressure of carbon dioxide, O_2pulse VO_2/heart rate, SaO_2 arterial oxygen saturation estimated from pulse oximetry

Table 5 Univariate Correlations with VO_2peak expressed in L/min and ml/kg/min

N = 32	VO_2peak ml/kg/min	p-value	VO_2peak L/min	p-value
SB DLCO	0.18	0.312	0.51	0.002
SB DLCO (%Pred)	***0.49***	***0.004***	0.48	0.006
IB DLCO	0.20	0.277	0.51	0.002
IB DLCO (% Pred)	0.41	0.020	0.49	0.004
IB/SB	0.01	0.974	0.37	0.039
SB DLCO/Qc	***0.63***	***0.000***	***0.59***	***0.000***
IC (L)	0.40	0.020	***0.60***	***0.000***
VT/IC	***−0.42***	***0.017***	***−0.55***	***0.001***
FVC	0.41	0.018	0.54	0.001
FEV_1 (% Pred)	0.38	0.030	0.22	0.217
FEF_{25-75} (L/min)	***0.49***	***0.004***	0.48	0.006
FEF_{75} (L/min)	***0.47***	***0.006***	0.41	0.018
Wt (kg)	−0.30	0.092	0.55	0.001
BMI	***−0.42***	***0.017***	0.32	0.078
BSA	0.16	0.370	***0.64***	***0.000***
QOL	−0.23	0.200	−0.05	0.773
GOLD classification	−0.27	0.140	−0.20	0.275

SB single breath method, *IB* intra-breath method. Values in columns 2 through 5 that are most significantly linked to exercise capacity based on VO_2 peak are in bold and italics. In column 1, only the variables that predict VO_2 peak in ml/kg/min in a multiple regression analysis, are in bold.

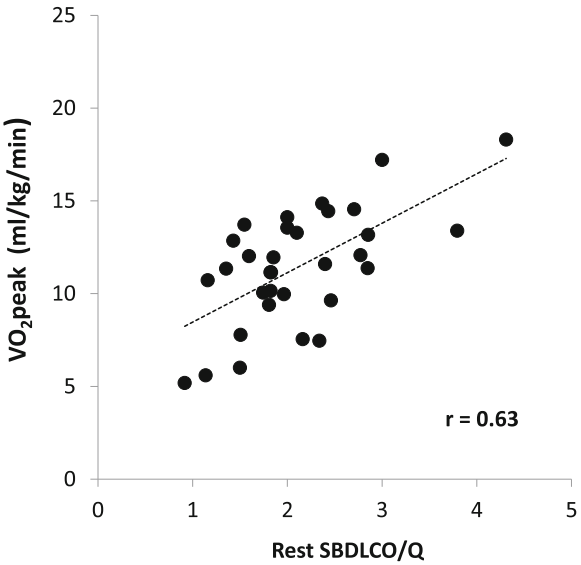

Fig. 3 Relationship of SBDLCO/Qc and Exercise Capacity based on VO_2peak in patients with COPD. The ratio of lung diffusing capacity to pulmonary blood flow was the best predictor of VO_2peak in this population

capacity measured differently or expressed relative to lung function and pulmonary blood flow. We found that lung mechanics, particularly flows at the mid to lower lung volumes and the inspiratory capacity relationship, or tidal volume inspiratory capacity relationship, seemed to be the most predictive. However when allowed to compete in a model with measures of lung diffusion and measures of body weight or habitus, the measures of

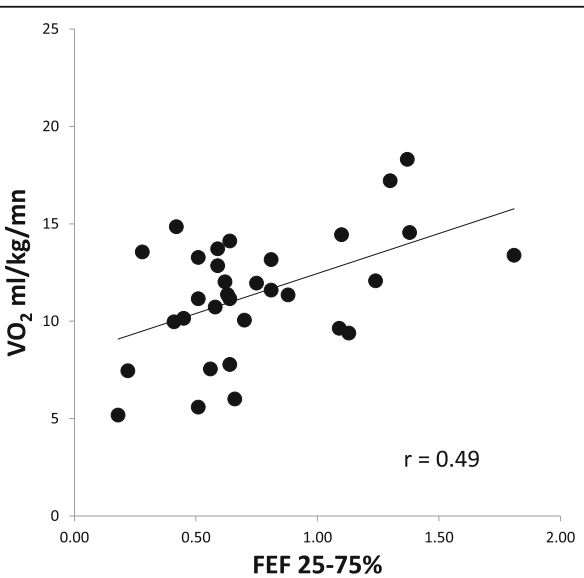

Fig. 4 Relationship of $FEF_{25-75\%}$ to VO_2peak in patients with COPD. $FEF_{25-75\%}$ was the best univariate lung mechanics predictor of exercise capacity but did not remain in a predictive model when DLCO and weight were added

mechanics no longer reached significance. In particular when SBDLCO was expressed relative to the measured Qc with BMI or when predicting VO_2 in L/min, BSA together in a model the mechanics measures, were no longer contributory.

DLCO is a variable of paramount importance in pulmonary medicine. It represents a complex integration of factors including ventilation distribution, matching of ventilation to perfusion, the resistance at the alveolar-capillary membrane as well as the combination rates with hemoglobin. Since all of the above factors can be affected with more classic patterns of emphysema and COPD with ultimate destruction of the alveolar-capillary bed, preservation of DLCO is an important marker of lung health [13]. For example, a major factor contributing to recruitment or distension of the pulmonary capillary bed is cardiac output or pulmonary blood flow (Qc) [28]. As alveolar-capillary walls are remodeled, destroyed or even stiffened with disease, the DLCO/Qc relationship would be altered. With exercise, ventilation rises and pulmonary blood flow increases resulting in elevation of DLCO. In emphysema and COPD with loss of alveolar volume, an important adaption to maintain gas exchange in the face of increased blood cell transit time is a rise in pulmonary capillary blood volume. Thus preservation of this relationship in this population should be a discernible advantage. It was interesting that the IBDLCO, while highly correlated to the SBDLCO, was not as predictive of exercise capacity as the SB method. Our original rational was that since the IB method was performed more quickly and at a lung volume more specific to tidal breathing, that it may be a more sensitive predictor of functional gas exchange surface area. However, during the rapid inspiratory phase of the SBDLCO method, potentially increasing pulmonary blood volume, and with the inhalation to total lung capacity, increasing alveolar volume, it is likely this gives a better overall representation of functional or possibly recruitable surface area available for use during exercise. Also of note was the fact that the IBDLCO method was more variable across subjects and less reproducible than the SB method.

Other predictors of exercise capacity in COPD

In what have become classical studies by O'Donnell and colleagues, resting IC, degree of hyperinflation with exercise, and change in IC, have all been highly predictive of exercise capacity [9]. Hyperinflation is associated with expiratory flow limitation, volume constraints and less optimal respiratory muscle performance [3, 12, 26]. Dynamic hyperinflation during exercise contributes to perceived respiratory discomfort. Indirect evidence of the importance of dynamic hyperinflation comes from studies that have demonstrated that alleviation of dyspnea following bronchodilator therapy and lung volume reduction surgery (LVRS) are both explained, in part, by reduced operating lung volumes [29]. Additional studies have suggested that COPD patients enter into a spiral of decline associated with reduced activity, inflammation and skeletal muscle dysfunction [2, 30, 31]. A high work and cost of breathing in the setting of elevated operational lung volumes and in some cases excessive expiratory muscle work and also diaphragmatic fatigue contribute to exercise intolerance [30]. COPD also is associated other co-morbidities such as coronary artery disease, pulmonary vascular disease, pulmonary hypertension and right ventricular failure; all of which impair cardiac output and thus compromise oxygen delivery [31]. While the reality is that these collective contributors to exercise limitation in COPD are all integrated and codependent, our work suggests that maintenance of a functional alveolar-capillary bed is an important determinant of patients ability to exercise and likely to carry on normal daily activities.

Targeting the airways and the inflammatory pathways has been the cornerstone of therapy for COPD and emphysema which are accomplished by classes of beta-2 agonists, anticholinergics, and steroids. Using the same rational, targeting diffusing capacity, i.e. the pulmonary vasculature, by medications has been tried. However, the results have been disappointing. For example, sildenafil, a phosphodiesterase type-5 (PDE-5) inhibitor with vasodilatory properties, commonly used in treatment of pulmonary arterial hypertension (PAH), has been tried in COPD patients who did not have pulmonary hypertension. Interestingly, the drug worsened gas exchange, increased the alveolar-arterial oxygen difference, and did not improve exercise capacity, possibly by causing ventilation-perfusion mismatch, indicating the need for more studies and new medications [26].

Limitations

There are several limitations relative to this study to consider. First, this was a relatively small study in a somewhat heterogeneous group of primarily moderate to moderately-severe COPD patients who performed cycle ergometry to volitional exhaustion. We essentially recruited consecutive patients with a history of COPD, on stable medications, willing to participate with minimal exclusion criteria. To some extent this was by design so that the study population represents a typical mixed tertiary outpatient population. Lung diffusion has been shown to be predictive of exercise capacity in a number of chronic lung and heart conditions and therefore appears to be a good, more generic marker to consider for the COPD population. Larger studies would however be needed to tease out in which specific COPD populations it may be most predictive. Secondly, we used volitional fatigue as a cessation criteria. This resulted in some subjects with lower than typical RER

values or other more traditional measures associated with maximal exercise, e.g., heart rate. Cycle ergometry is known to be associated with more local muscle fatigue and may underestimate true maximal exercise. However, we attempted to use similar stopping criteria for our subjects and the same study staff performed all exercise testing which likely resulted in a more uniform representation of peak exercise capacity across subjects. In addition, since mechanical constraint to breathing occurs, some subjects would be unable to hyperventilate to more typical RER values or get to a true cardiovascular limitation. Thus we feel our data are representative of the typical tertiary center testing laboratory where symptom limitation is typically used for stopping criteria.

We also allowed recruitment of current smokers. While subjects were asked not to smoke within 24 h of testing, we did not specifically assess carboxyhemoglobin levels and therefore could not confirm if they were smoking prior to testing. This could have influenced our DLCO measures, though previously Oglivie et al. felt that the effects of increasing COHb were sufficiently small, so that routine correction of DLCO was not necessary [32]. As a result, DLCO was not until more recently clinically adjusted for increases in COHb [33]. We also did not note a difference in percent predicted DLCO between smokers and non-smokers and did not find that current smoking impacted our predictive models. Finally, we used a soluble gas method for calculation of pulmonary blood flow or in the absence of significant shunt or cardiac output. We acknowledge this method may be somewhat dependent on ventilation and perfusion matching in the lungs and therefore may also underestimate actual values.

Conclusions

In conclusion, exercise limitation in COPD is affected by alveolar-capillary gas exchange impairment which in turn is attributed to impairment of pulmonary circulation. SBDLCO relative to Qc and body weight are better predictors of exercise performance compared to IBDLCO and other respiratory variables in this population. We proposed that lung diffusing capacity, either alone or relative to pulmonary blood flow, is a good measure of pulmonary vascular health in the COPD population and is also a good measure for assessing mechanisms of exercise intolerance in this population. Medical management targeting the pulmonary circulation may help reduce symptoms and improve exercise tolerance in COPD patients.

Abbreviations

BMI: Body mass index; COPD: Chronic obstructive pulmonary disease; DLCO: Diffusing capacity for carbon monoxide; IB: Intrabreath; Qc: Pulmonary blood flow; SB: Single breath; SGQOL: St George quality of life; VO_2: Oxygen consumption

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Availability of data and materials

The dataset is available for review upon request at the site of study.

Authors' contributions

MB protocol design, testing subjects, obtaining consent, drafting manuscript; CW protocol design, obtaining consent; AA protocol design; BJ protocol design, statistical analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study, ethics, and consent forms were reviewed and approved by the Western Institutional Review Board (WIRB, Pyallup, WA, study number 1153374). Each patient voluntarily participated in the study. If willing to participate, patients signed informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

Publisher's Note

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ORIGINAL RESEARCH

Alveolar–capillary reserve during exercise in patients with chronic obstructive pulmonary disease

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Background: Factors limiting exercise in patients with COPD are complex. With evidence for accelerated pulmonary vascular aging, destruction of alveolar–capillary bed, and hypoxic pulmonary vasoconstriction, the ability to functionally expand surface area during exercise may become a primary limitation.

Purpose: To quantify measures of alveolar–capillary recruitment during exercise and the relationship to exercise capacity in a cohort of COPD patients.

Methods: Thirty-two subjects gave consent (53% male, with mean ± standard deviation age 66±9 years, smoking 35±29 pack-years, and Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification of 0–4: 2.3±0.8), filled out the St George's Respiratory Questionnaire (SGRQ) to measure quality of life, had a complete blood count drawn, and underwent spirometry. The intrabreath (IB) technique for lung diffusing capacity for carbon monoxide (IBDLCO) and pulmonary blood flow (IBQc, at rest) was also performed. Subsequently, they completed a cycle ergometry test to exhaustion with measures of oxygen saturation and expired gases.

Results: Baseline average measures were 44±21 for SGRQ score and 58±11 for FEV_1/FVC. Peak oxygen consumption (VO_2) was 11.4±3.1 mL/kg/min (49% predicted). The mean resting IBDLCO was 9.7±5.4 mL/min/mmHg and IBQc was 4.7±0.9 L/min. At the first workload, heart rate (HR) increased to 92±11 bpm, VO_2 was 8.3±1.4 mL/kg/min, and IBDLCO and IBQc increased by 46% and 43%, respectively, compared to resting values ($p<0.01$). The IBDLCO/Qc ratio averaged 2.0±1.1 at rest and remained constant during exercise with marked variation across subjects (range: 0.8–4.8). Ventilatory efficiency plateaued at 37±5 during exercise, partial pressure of mix expired CO_2/partial pressure of end tidal CO_2 ratio ranged from 0.63 to 0.67, while a noninvasive index of pulmonary capacitance, O_2 pulse × $PetCO_2$ (GxCap) rose to 138%. The exercise IBDLCO/Qc ratio was related to O_2 pulse (VO_2/HR, $r=0.58$, $p<0.01$), and subjects with the highest exercise IBDLCO/Qc ratio or the greatest rise from rest had the highest peak VO_2 values ($r=0.65$ and 0.51, respectively, $p<0.05$). Of the noninvasive gas exchange measures of pulmonary vascular function, GxCap was most closely associated with DLCO, DLCO/Qc, and VO_2 peak.

Conclusion: COPD patients who can expand gas exchange surface area as assessed with DLCO during exercise relative to pulmonary blood flow have a more preserved exercise capacity.

Keywords: airflow limitation, exercise intolerance, lung gas transfer, dyspnea, COPD , diffusion capacity, cardiopulmonary exercise testing

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Introduction

Exercise stresses our physiological reserve by increasing muscular contraction and the demand for blood flow and ventilation to maintain gas exchange.[1] As cardiac output rises, the typically compliant pulmonary vascular bed expands to increase lung surface

area while at the same time retaining a low pressure system for the efficient forward blood flow. In health, this expansion of the alveolar–capillary layer results in a nearly linear rise in lung diffusing capacity for carbon monoxide (DLCO) with cardiac output (Q) as exercise intensity increases.[2]

In patients with COPD, as destruction of the alveolar–capillary bed occurs, areas of ventilation and perfusion mismatch create hypoxic constriction of pulmonary vessels. Furthermore, airway obstruction increases intrathoracic pressure influencing venous return to the right side of the heart.[3] Remodeling of the pulmonary capillaries may also occur resulting in stiffer vessels. Thus, as the disease pathophysiology progresses, the process limits the ability of the alveolar–capillary bed to expand and potentially not only limits the rise in gas exchange surface area but also creates an impediment to forward blood flow or cardiac output, which could ultimately contribute to exercise intolerance in this population.

Physiology of exercise in COPD has been a topic of great interest. Previous work by Potter[3] suggested that the degree of airway obstruction during exercise may result in a blunted rise in cardiac output presumably due to the large rise in expiratory intrathoracic pressures, while Montes de Oca[4] demonstrated an association of oxygen pulse, a surrogate for stroke volume, with exercise capacity in COPD patients. Similarly, Fuji[5] suggested that a higher slope of change in pulmonary arterial pressure relative to the change in Q was associated with a reduced exercise capacity. We and others have previously demonstrated that resting DLCO is predictive of exercise capacity in a COPD population and more significantly so when accounting for the resting pulmonary blood flow (Qc), or essentially the functional lung surface area for gas exchange relative to cardiac output.[6] The DLCO is highly dependent on Q, and to date, most studies suggest a strong linear relationship between the two with no evidence of a DLCO plateau during heavy exercise even in highly fit individuals.[7] Since a rise in cardiac output is the main determinant of pulmonary capillary recruitment, the DLCO relationship relative to Q gives a good overall estimate of the ability to expand this vascular bed.

Therefore, the focus of this study was to quantify DLCO at rest and during exercise using the intrabreath method in a moderate-to-severe COPD population (functional alveolar–capillary recruitment). We hypothesized that the subjects who could increase DLCO in proportion to the rise in Qc (maintenance of the relationship) would have a more preserved exercise capacity. We preferred the intrabreath over the single-breath DLCO (SBDLCO) technique because the latter is difficult to perform with a 10-s breath hold in dyspneic COPD patients at rest and more so during exercise. The intrabreath technique has been compared to other techniques such as the rebreathe or open circuit and has been validated.[8–10]

An additional goal of our study was to analyze the relationship of DLCO and Qc to other noninvasive measures of respiratory gas exchange that have been associated with pulmonary vascular function. These included ventilatory efficiency, partial pressure of mix expired CO_2 ($PeCO_2$)/partial pressure of end tidal CO_2 ($PetCO_2$) ratio, as well as a more novel measure previously associated with pulmonary vascular capacitance in the heart failure population, O_2 pulse × $PetCO_2$ (GxCap).[11–13]

Methods

Ethics and consent

The study, ethics, and consent forms were reviewed and approved by the Western Institutional Review Board (WIRB, study number 1153374).

Subjects

Patients with a history of COPD who were sent for clinical pulmonary function testing (PFT) and/or exercise testing were offered enrollment. Inclusion criteria was a history of moderate-to-severe COPD using the Global Initative for Chronic Obstructive Lung Disease (GOLD) criteria. Exclusion criteria included an inability to exercise, dependence on home oxygen therapy, or a body mass index (BMI) >42. Prior to participation, the study goals and requirements were reviewed with the patients. If willing to participate, subjects signed informed consent.

Overview of study

After reporting to the outpatient clinic, study participants filled out the St George's Respiratory Questionnaire (SGRQ) to measure quality of life (QOL) and performed PFTs which included resting measures of maximal lung volumes and flow rates using classical spirometry.[14,15] In addition, the assessment of DLCO was obtained using the classical SBDLCO technique and was also obtained using the intrabreath DLCO method (IBDLCO) which also included a measure of Qc.[16] A small blood sample was obtained prior to testing for assessment of hemoglobin in order to correct the measures of DLCO. Subjects subsequently performed cardiopulmonary exercise testing (CPET) using the CareFusion metabolic cart (San Diego, CA, USA) on a semi-recumbent cycle ergometer (Lode, Groningen, the Netherlands). The test protocol started with 20 W for both men and women and increased

by 10 W every 2 min. Prior to exercise testing, subjects were instrumented with a 12-lead ECG and a forehead pulse oximeter for peripheral arterial oxygen saturation (SaO_2) for continuous monitoring. Subjects wore a nose clip and breathed on a mouthpiece for measurement of gas exchange during the exercise test. During the last 30 s of each workload, a 12-lead ECG was recorded, blood pressure (BP) was assessed, and an average of the heart rate (HR) and SaO_2 over this period was determined. We assessed the patient dyspnea by the Borg scale (on the 0–10 scale) and the total body effort by the rated perceived exertion (RPE) score (on the Borg 6–20 scale). Subjects were encouraged to exercise to near exhaustion by achieving an RPE of 17–18 or a dyspnea score ≥7. Upon reaching peak exercise, subjects performed active recovery where they continued to pedal with no resistance and remained on the mouthpiece for 1 min. After this, the subjects stopped pedaling and were given time for HR and BP to return to baseline before being dismissed.

Pulmonary function and single-breath DLCO

Spirometry was performed using pneumotachograph-based pulmonary function equipment by CareFusion that had passed evaluation using 24 waveforms recommended by the American Thoracic Society (ATS). Classic resting SBDLCO was determined using CareFusion instrument following the recommendations of the ATS/ERS.[17,18]

Intrabreath lung diffusing capacity and Qc

Qc and DLCO were measured using inert and soluble gases on the CareFusion Vmax system using the intrabreath method.[16,19] For this method, subjects were asked to breathe on a mouthpiece while wearing a nose clip. Subjects were instructed to exhale to near residual volume (RV) and then were switched into an inspiratory reservoir and took a full inhalation of a test gas mixture containing 0.3% carbon monoxide (CO), 0.3% methane, 0.3% acetylene, 21% O_2, and balance N_2. Subjects were trained to exhale at a steady rate until they were back near RV. From the rate of disappearance of CO and acetylene in comparison with the inert gas methane, the rate of disappearance of CO and acetylene was determined. This rate of disappearance of CO was used to calculate IBDLCO. Since acetylene does not bind to hemoglobin, the rate of its disappearance is limited primarily by the flow of blood through the lungs, thereby providing a measure of Qc.[16,20] The measure of IBDLCO and IBQc was practiced several times at rest in each subject until reproducible values were obtained and performed near the end of the first workload in triplicate. If necessary, the first workload was extended before incrementing the cycle ergometer resistance in order to obtain reproducible measures.

QOL Questionnaires – SGRQ

The SGRQ is a 50-item questionnaire developed to measure health status (QOL) in patients with diseases causing airway obstruction. Scores are calculated for three domains: symptoms, activity, and impacts (psychosocial) as well as a total score. Psychometric testing has demonstrated its repeatability, reliability, and validity. Sensitivity has been demonstrated in clinical trials.[14] Each patient was asked to fill out the SGRQ questionnaire prior to CPET.

Gas exchange, ventilation, and lung mechanics

During exercise testing, oxygen consumption (VO_2), carbon dioxide production (VCO_2), breathing frequency (fb), tidal volume (V_t), minute ventilation (V_E), and derived variables were measured continuously and/or calculated using a low-resistance open circuit automated metabolic system (CareFusion).

In addition, measures associated with ventilation and perfusion matching or pulmonary vascular health were determined. One of the measures was $PeCO_2$ relative to $PetCO_2$ which is associated with ventilation and perfusion matching in the lungs and reported to be reduced in COPD patients having pulmonary hypertension with a negative slope during exercise. The other gas exchange measure was GxCap which is a noninvasive estimate of pulmonary vascular capacitance calculated as oxygen pulse multiplied by $PetCO_2$. Oxygen pulse tracks stroke volume and $PetCO_2$ has been shown to reasonably track pulmonary arterial pressure. The last of the measures was ventilatory efficiency or V_E/VCO_2.[11,13]

Statistics

We were interested in exercise limitation in the COPD population and the potential role of the alveolar–capillary bed, the lung surface area for gas exchange, or the ability to expand this bed as a mediating factor. Thus, our primary measures were the change in IBDLCO and the change in IBDLCO in proportion to IBQc across subjects and the relationship to exercise or aerobic capacity – primarily peak VO_2. Thus, we used multiple regression and correlational analysis to asses these relationships. In addition, general descriptive statistics were used to define our group and paired *t*-tests were used to further analyze changes with exercise.

Results

Subject characteristics and resting pulmonary function

Thirty-two subjects completed all aspects of the study (age 66±9 years, 53% male, smoking history 35±29 pack-years, BMI 30±6 kg/m^2; mean ± SD). The study cohort consisted of primarily moderate COPD patients based on the GOLD classification (2.3±0.8), but with some subjects mild and others more severe (GOLD classification range: 1–4). This degree of disease was reflected in the QOL scores (SGRQ averaged 44±21) and medication use with the majority of patients on multiple inhalers and with 20% on oral steroids. None of the subjects were on continuous oxygen at the time of the study. Hemoglobin values were essentially within normal limits and averaged 13.5±1.7 g/dL.

The forced vital capacity (FVC) averaged 2.48±0.69 L or 75% of age and gender predicted, while the forced expiratory volume in 1 s (FEV_1) averaged 56%±16% of predicted with an FEV_1/FVC ratio of 58%±11%, range 33%–78%. The $FEF_{25–75}$ averaged 0.75±0.38 L or 26%±13% of predicted. The SBDLCO averaged 13.2±5.5 mL/min/mmHg and 58%±23% of age and gender predicted. Both the SGRQ score and the GOLD classification were somewhat associated with resting DLCO (r values ranging from 0.33 to 0.38).

Cardiopulmonary exercise responses

Cardiopulmonary responses to exercise are shown in Table 1. The most common symptoms for stopping exercise were dyspnea, general fatigue, and leg fatigue. Peak work achieved was 43 W with a VO_2 peak of 11.4 mL/kg/min or 49% of age and gender predicted. Peak HR was 106 bpm or 70% of age predicted. Tidal volume increased early in exercise but then plateaued as ventilation was primarily rate dependent beyond the first workload. Peak minute ventilation reached 70% of the estimated breathing capacity and inspiratory capacity (IC) fell throughout exercise suggesting expiratory flow limitation and some hyperinflation as exercise progressed. Oxygen pulse rose to 7.8 mL per beat by the first exercise load, but then rose slowly thereafter.

Ventilatory efficiency assessed as the V_E/VCO_2 ratio was elevated at rest and fell with exercise but stayed elevated without a clear exercise nadir. The $PeCO_2/PetCO_2$ ratio started at 0.54 at rest and stayed below a ratio of 0.70 throughout exercise. The gas exchange estimate of pulmonary capacitance, GxCap, on average increased 138% from rest to peak exercise but plateaued or declined in approximately half (47%) of the subjects over the last two workloads.

Table 1 Cardiopulmonary responses to exercise in COPD patients (N=32)

	Rest	First workload	70%–90% of peak	Peak
Exercise capacity				
Work (W)	0	18±4	33±14	43±19
VO_2 (mL/kg/min)	3.66±0.84	8.34±1.40	10.00±2.50	11.40±3.12
Symptoms				
RPE (6–20 Borg Score)	7±2	11±3	15±3	17±2
Dyspnea (0–10 Score)	1±1	3±2	5±2	7±2
Cardiovascular				
Heart rate (bpm)	77±10	92±11	100±11	106±12
O_2 pulse (VO_2/HR)	4.1±0.9	7.8±2.1	8.9±2.7	9.3±3.1
SBP (mmHg)	118±24	137±17	146±20	152±21
DBP (mmHg)	73±10	82±11	86±10	88±11
Ventilation and breathing pattern				
Ventilation (L/min)	12±3	24±6	28±9	34±10
Tidal volume (L)	0.79±0.17	1.13±0.37	1.15±0.36	1.17±0.37
Breathing frequency (bpm)	18±6	25±7	28±7	31±5
IC (L)	2.15±0.67	1.89±0.71	1.78±0.83	1.70±0.69
V_t/IC	37±13	52±12	61±25	65±27
V_E/breathing capacity (%)	27±9	51±25	58±21	69±21
Gas exchange – pulmonary vascular				
V_E/VCO_2 ratio	47±7	37±5	37±5	36±5
$PetCO_2$ (mmHg)	35±5	37±5	37±5	37±5
$PeCO_2$ (mmHg)	19±3	23±3	24±3	24±3
$PeCO_2/PetCO_2$ ratio	0.54±0.04	0.63±0.05	0.66±0.06	0.67±0.06
GxCap	143±40	289±85	317±102	341±116

Notes: Data shown as mean ± standard deviation.

Abbreviations: DBP, diastolic blood pressure; fb, breathing frequency; GxCap, O_2 pulse × $PetCO_2$; HR, heart rate; IC, inspiratory capacity; O_2 pulse, VO_2/heart rate; $PeCO_2$, partial pressure of mix expired CO_2; $PetCO_2$, partial pressure of end tidal CO_2; RPE, rated perceived exertion; SaO_2, arterial oxygen saturation estimated from pulse oximetry; SBP, systolic blood pressure; TI, inspiratory time; TTOT, total respiratory cycle time; VCO_2, carbon dioxide production; V_E, minute ventilation; VO_2, oxygen consumption; V_t, tidal volume.

Measures of DLCO and pulmonary blood flow

The changes in DLCO, Qc, stroke volume, and DLCO relative to Qc are shown in Table 2. Individual changes in DLCO and DLCO/Qc from rest to the first workload are shown in Figures 1 and 2. On average, DLCO increased 45% from rest to the first workload. However, there was large variation in responses with three subjects demonstrating >10% fall in DLCO and another four subjects demonstrating minimal change with exercise. Pulmonary blood flow increased on average 40% during the first workload, while stroke volume increased 18%. The mean IBDLCO relative to Qc (DLCO/Qc) stayed relatively constant with exercise increasing on average 14% from rest to the first workload while the majority of subjects demonstrated a rise in this ratio, 12 of the subjects had no change or had a fall, suggesting Qc was

Table 2 Intrabreath lung diffusing capacity and pulmonary blood flow at rest and during exercise in COPD patients (N=32)

	Rest	First workload	% change	Range, % change
IBDLCO (mL/min/mmHg)	9.6±5.9	13.3±7.1	45%±44%	–22% to 137%
IBQc, L/min*	4.8±0.9	6.6±1.4	40%±28%	0.6% to 123%
Cardiac stroke volume (mL)	62±14	73±20	18±23	36% to 133%
IBDLCO/IBQc	2.01±1.1	2.03±1.0	14%±51%	–50% to 161%

Notes: Data shown as mean ± standard deviation. *Qc measured with soluble gas method.
Abbreviations: DLCO, lung diffusing capacity for carbon monoxide; IB, intrabreath technique; Qc, pulmonary blood flow.

increasing out of proportion to lung surface area for gas exchange in these subjects.

Respiratory measures associated with lung diffusion

We were interested in the relationship of other noninvasive indices associated with pulmonary vascular function and lung diffusion during exercise. Ventilatory efficiency for carbon dioxide, (V_E/VCO_2) (r=–0.44), the mixed expired-to-end tidal CO_2 ratio, ($PeCO_2/PetCO_2$) (r=0.40), and GxCap were all associated with the exercise IBDLCO measure ($p<0.05$). However, the GxCap was the gas exchange measure mostly associated with exercise IBDLCO (Figure 3) (r=0.71).

Relationship of lung diffusion, pulmonary blood flow, and indices of pulmonary vascular function to exercise capacity

The exercise IBDLCO and change from rest in the IBDLCO were only marginally associated with VO_2 peak (r=0.43 and 0.39, $p<0.05$), while the relationship of exercise DLCO/Qc or the change in DLCO/Qc to VO_2 peak was more strongly

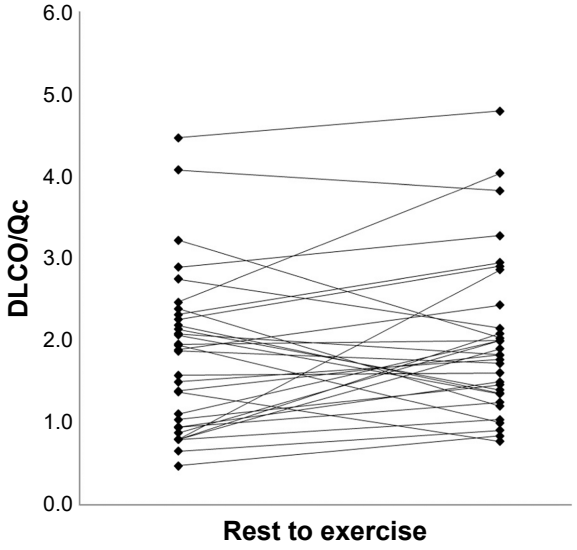

Figure 2 Change in intrabreath DLCO relative to Qc from rest to first exercise workload in patients with COPD.
Abbreviations: DLCO, diffusing capacity for carbon monoxide; Qc, pulmonary blood flow measured with soluble gas method.

related (r=0.63 and 0.54, respectively, $p<0.01$). Figure 4 shows the relationship of VO_2 peak and VCO_2 peak with DLCO/Q. The modest correlation with these measures suggests that subjects with the greatest recruitment in lung gas exchange surface area relative to pulmonary blood flow were the subjects with the best exercise tolerance. With multiple regression including IBDLCO, the change in IBDLCO and DLCO/Qc, the DLCO/Qc from rest to exercise was the measure most significantly associated with VO_2 peak. DLCO/Qc was also associated with O_2pulse (r=0.63). Furthermore, exercise capacity was associated with the $PeCO_2/PetCO_2$ ratio with those with higher ratios having better exercise tolerance (r=0.57 between $PeCO_2/PetCO_2$ vs VO_2 peak), while both the absolute measure of GxCap during exercise and the change from rest to peak exercise being associated with

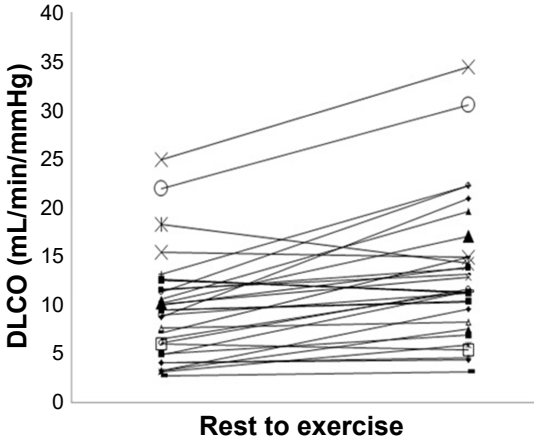

Figure 1 Change in intrabreath DLCO from rest to first stage of exercise in patients with COPD.
Abbreviation: DLCO, diffusing capacity for carbon monoxide.

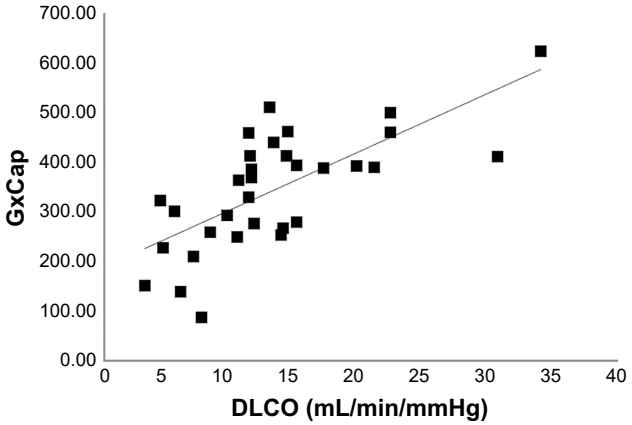

Figure 3 Relationship of GxCap to DLCO in COPD patients.
Abbreviations: DLCO, diffusing capacity for carbon monoxide; GxCap, O_2 pulse × $PetCO_2$; $PetCO_2$, partial pressure of end tidal CO_2.

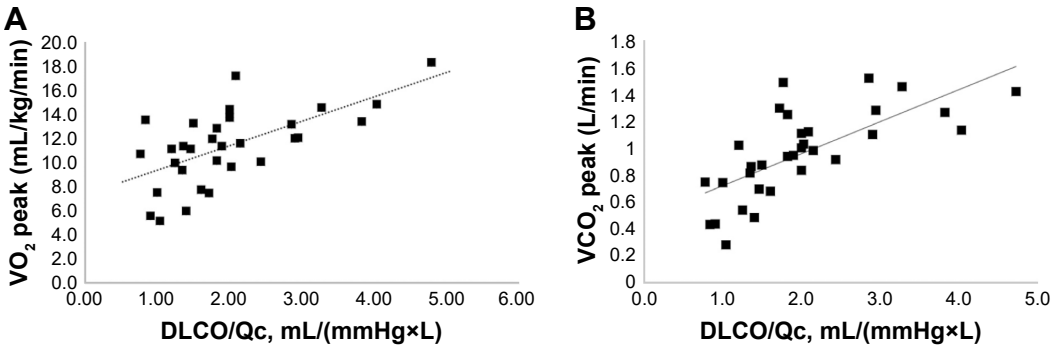

Figure 4 Relationship of VO_2 peak (**A**) and VCO_2 peak (**B**) with DLCO/Qc.
Abbreviations: DLCO, diffusing capacity for carbon monoxide; Qc, pulmonary blood flow; VCO_2, carbon dioxide production; VO_2, oxygen consumption.

VO_2 peak (r=0.53 and 0.66, respectively, $p<0.01$, Figure 5). The lung function measure most associated with DLCO or DLCO/Qc during exercise was the exercise IC (r=0.58 and 0.63, respectively, $p<0.01$, Figure 6).

Discussion

Primary findings

Our primary finding was that COPD patients who can increase respiratory gas exchange surface area (ie, alveolar–capillary reserve – as assessed by DLCO) relative to the rise in Qc have the most preserved exercise capacity. In addition, the ability to expand the pulmonary vascular bed is associated with noninvasive indices of pulmonary capacitance as well as with IC assessed during exercise. While these data are correlative, this implies that a contributing factor to exercise intolerance in this population may be the inability to recruit pulmonary capillaries.

Pulmonary vasculature in COPD

The majority of our patients had a history of smoking as the likely primary etiology of their airway disease. Years of exposure to inhalants causes destruction and remodeling of the alveolar–capillary bed, accelerated aging, inflammation, and other chronic metabolites that circulate to cause a general vasculopathy and stiffening of vessels.[21–23] There are often regions of inhomogeneous pulmonary vasoconstriction due to the ventilation and perfusion abnormalities. All of these factors contribute to a blunted ability to increase or expand the alveolar–capillary bed. Previous work has suggested that[5] an abnormal rise in pulmonary arterial pressure relative to the rise in cardiac output was associated with reduced exercise performance in COPD patients and presumably a resistance to forward flow through the lungs – thus a form of cardiac output limitation.[5] However, VO_2 peak was not related to the degree of airflow obstruction in this study which is an interesting finding. Other studies have also suggested a strong relationship between exercise capacity and oxygen pulse, a surrogate for stroke volume.[4] In addition, airway obstruction itself causes an increase in intrathoracic pressure that may act as an impediment to venous return – potentially further

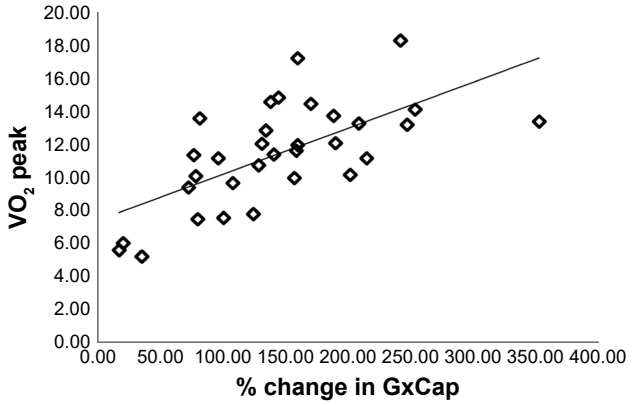

Figure 5 Relationship of VO_2 with the change in GxCap from rest to peak exercise.
Abbreviations: GxCap, O_2 pulse × $PetCO_2$; $PetCO_2$, partial pressure of end tidal CO_2; VO_2, oxygen consumption.

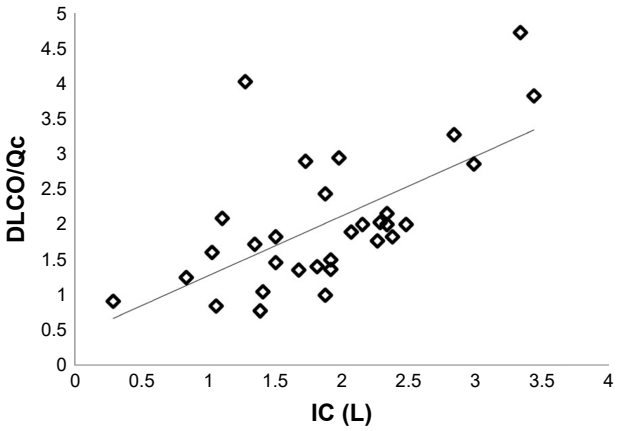

Figure 6 Relationship of DLCO/Qc relative to IC.
Note: Qc measured with soluble gas method.
Abbreviations: DLCO, diffusing capacity for carbon monoxide; IC, inspiratory capacity; Qc, pulmonary blood flow.

influencing cardiac output.[3] While we did not directly measure hemodynamics, there is typically a strong linear relationship between DLCO and Q in healthy individuals showing a steady and proportional increase with moderate-to-heavy exercise. Presumably, this represents recruitment and distension of the pulmonary capillaries. Since DLCO is flow independent, a rise in DLCO is primarily influenced by the expansion of this bed. Thus, our measure of DLCO/Qc would primarily represent a rise in pulmonary capillary blood volume that is in contact with functional alveoli. While, on average, DLCO did rise with exercise and DLCO relative to Qc (DLCO/Qc) stayed relatively constant, there were a large number of subjects (38%) where this ratio plateaued or fell with exercise, suggesting that as pulmonary blood flow increased, there was a non-proportional increase in gas exchange surface area.[24]

DLCO relative to other respiratory gas exchange measures in COPD

We also assessed several other reported respiratory gas exchange measures associated with ventilation and perfusion matching and pulmonary vascular function. Ventilatory efficiency (V_E/VCO_2), the partial pressure of CO_2 relative to end tidal CO_2 ($PeCO_2/PetCO_2$), and an index of pulmonary vascular capacitance (GxCap) are all associated with lung diffusing capacity.[13] Ventilatory efficiency is commonly reported elevated in patients with pulmonary vascular disease and is associated with high dead space ventilation, hyperventilation, and a rapid shallow breathing pattern.[25] The $PeCO_2/PetCO_2$ ratio and the slope of change from rest to exercise have been suggested to differ between primarily pulmonary vascular disease versus conditions of more ventilation and perfusion mismatch, with lower ratios suggesting worsening disease. Furthermore, GxCap has previously been shown to correlate well with invasive measures of pulmonary vascular capacitance.[13] It is interesting that while all these measures were associated with DLCO, the GxCap measure was the most significantly linked both to DLCO and to peak exercise performance. The premise is that oxygen pulse tends to track stroke volume changes, while $PetCO_2$ tends to fall with greater pulmonary vascular pressures in conditions like pulmonary hypertension – thus a surrogate for pressure. Thus, since the DLCO is essentially dependent on the recruitment and distension of the pulmonary capillaries, it essentially should increase with greater pulmonary vascular capacitance. While both increased with exercise, there were a significant number of subjects where these rose minimally or failed to increase.

Previous studies looking at exercise intolerance in COPD

A number of studies have demonstrated a clear impact of airway obstruction on exercise performance.[26–28] However, as the disease progresses, it clearly becomes a systemic disease impacting many organ systems. Previous work by O'Donnell has demonstrated an association with the degree of hyperinflation and that improvement of obstruction with bronchodilators clearly enhances exercise capacity.[29] In addition, muscle dysfunction, muscle wasting, and deconditioning also begin to develop.[30,31] It is clear that COPD is not a homogeneous disease, and thus, it is likely that multiple issues contribute to the loss of exercise capacity. Our study highlights the importance of the pulmonary vasculature in determining exercise tolerance in this chronic disease state.

Limitations and conclusion

There are a number of potential study limitations. First, the population was small, and thus, general conclusions are limited. Second, we only assessed DLCO during the first exercise load. While we attempted to measure this at other work levels and got success in some cases, we felt confident only in our data at the lower work level. Subjects were trained to perform the maneuver reproducibly at rest and typically performed three reproducible maneuvers at the lower workloads, but struggled to perform them reproducibly at the higher work intensities. Thus, we were examining their ability to recruit lung alveolar–capillary surface area with relatively modest workloads and comparing these data to their peak aerobic capacity. However, the DLCO response to exercise should be relatively linear with workload, VO_2, and cardiac output, and thus, the ability to expand the pulmonary circulation early in exercise is likely representative of their reserve with heavier activity. The first workload also represented on average nearly 75% of peak VO_2 and thus would be considered a moderately heavy load for this population. Third, we did not have direct measures of pulmonary vascular pressures to better understand the relationships between pulmonary blood flow, pulmonary pressure, and the rise in DLCO which would have given better insight into what was limiting the alveolar–capillary expansion. Finally, there is a baseline degree of deterioration in the alveolar–capillary bed in the COPD population. While DLCO increase is predictive of the ability to exercise more, it does not fully explain the exercise response. Therefore, additional studies are needed to better understand regulating factors in exercise that might improve functional alveolar–capillary surface area.

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Disclosure

The authors report no conflicts of interest in this work.

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Influence of dietary nitrate supplementation on lung function and exercise gas exchange in COPD patients

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ABSTRACT

Background: During exercise as pulmonary blood flow rises, pulmonary capillary blood volume increases and gas exchange surface area expands through distention and recruitment. We have previously demonstrated that pulmonary capillary recruitment is limited in COPD patients with poorer exercise tolerance. Hypoxia and endothelial dysfunction lead to pulmonary vascular dysregulation possibly in part related to nitric oxide related pathways.

Purpose: To determine if increasing dietary nitrate might influence lung surface area for gas exchange and subsequently impact exercise performance.

Methods: Subjects had stable, medically treated COPD (n = 25), gave informed consent, filled out the St George Respiratory Questionnaire (SGRQ), had a baseline blood draw for Hgb, performed spirometry, and had exhaled nitric oxide (exNO) measured. Then they performed the intra-breath (IB) technique for lung diffusing capacity for carbon monoxide (DLCO) as well as pulmonary blood flow (Qc). Subsequently they completed a progressive semi-recumbent cycle ergometry test to exhaustion with measures of oxygen saturation (SpO_2) and expired gases along with DLCO and Qc measured during the 1st work load only. Subjects were randomized to nitrate supplement group (beetroot juice) or placebo group (black currant juice) for 8 days and returned for repeat of the above protocol.

Results: Exhaled nitric oxide levels rose > 200% in the nitrate group ($p < 0.05$) with minimal change in placebo group. The SGRQ suggested a small fall in perceived symptom limitation in the nitrate group, but no measure of resting pulmonary function differed post nitrate supplementation. With exercise, there was no influence of nitrate supplementation on peak VO_2 or other measures of respiratory gas exchange. There was a tendency for the exercise DLCO to increase slightly in the nitrate group with a trend towards a rise in the DLCO/Qc relationship ($p = 0.08$) but not in the placebo group. The only other significant finding was a fall in the exercise blood pressure in the nitrate group, but not placebo group ($p < 0.05$).

Conclusion: Despite evidence of a rise in exhaled nitric oxide levels with nitrate supplementation, there was minimal evidence for improvement in exercise performance or pulmonary gas exchange surface area in a stable medically treated COPD population.

1. Introduction

Chronic obstructive lung disease (COPD) typically develops over many years of smoke inhalation developing into a systemic syndrome that includes inflammation, oxidative stress, insulin resistance, sympathetic activation, and hypoxemia [1,2]. These coexisting issues have been shown to be associated with endothelial dysfunction influencing vascular health. In addition, there is some evidence that the severity of lung dysfunction may be in parallel to the severity of endothelial dysfunction [3]. The pulmonary circulation may be particularly sensitive to endothelial dysfunction and in conjunction with regions of ventilation inhomogeneity, hypoxic pulmonary vasoconstriction develops which further contributes to vascular dysregulation, remodeling, destruction, and pulmonary hypertension [4–6]. Some of the dysregulation may be reversible as suggested by reported benefits from drugs such as sildenafil, which works through nitric oxide dependent pathways [7]. Nitric oxide synthesis may also fall due to oxygen substrate limitation as a reduction in NO has been associated with the rise in pulmonary pressures at altitude.

We have previously demonstrated that in a heterogeneous

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population of COPD patients, lung diffusing capacity for carbon monoxide (DLCO) assessed at rest was better associated with exercise tolerance than more traditional measures of lung mechanics (e.g., FEV_1) [8]. In addition, the ability to expand the pulmonary capillary bed as implied by the rise in DLCO with exercise was also preserved in patients with the greatest exercise capacity, suggesting that the pulmonary vasculature plays an important role in exercise tolerance in this population [9].

Other methods have been used to augment nitric oxide pathways and improve endothelial function. Supplements such as L-Arginine or sources of inorganic nitrate (NO_3^-) (e.g., beetroot juice or sodium nitrate [$NaNO3^-$]) have been used. Several studies have demonstrated modest benefits pertaining to cardiovascular health, such as reducing blood pressure (BP), enhancing blood flow, and elevating the driving pressure of oxygen in the microcirculation to areas of hypoxia or exercising tissue while other studies have shown no or minimal benefit [10–13].

Nitric oxide however also plays an important role as an inflammatory mediator. The fraction of exhaled NO (FENO) has been used in asthma to establish the correct diagnosis [14,15], predict response to therapy [16,17], titrate medications [18,19], and predict exacerbation [20–22]. On the other hand, studies describing the levels of FENO in patients with COPD are not very conclusive and measurement of nitric oxide in COPD, unlike asthma, is not universally recommended [23–25].

Thus, the primary focus of this study was to use an inorganic nitrate supplement, beetroot juice, in a stable medically treated COPD population to determine if this might improve lung diffusing capacity and subsequently exercise performance. We hypothesized that improving nitric oxide production in this population may improve the ability to recruit or distend pulmonary capillaries and therefore improve exercise capacity.

2. Methods

2.1. Subjects

The study was reviewed and approved by the Western Institutional Review Board (WIRB, study number 1153374). Patients with a history of COPD who were able to complete pulmonary function testing and perform exercise testing were offered enrollment. Inclusion criteria included a history of established mild to severe COPD, on stable medications without any recent exacerbation (within 3 months). Exclusion criteria included oxygen dependence, an inability to exercise, or a BMI > 42. Prior to participation, the study goals and requirements were reviewed with the patients. If willing to participate, they signed informed consent.

2.2. Overview of study

After reporting to the outpatient clinic, study participants filled out the St. George's Respiratory symptom questionnaire, performed pulmonary function testing (PFTs) which included resting measures of maximal lung volumes and flow rates using classical spirometry [26–29]. In addition, the assessment of lung diffusing capacity for carbon monoxide (DLCO) was obtained using the classical single breath (SB) technique and was also obtained using the intra-breath method (IB) which included a measure of pulmonary blood flow (Qc) [30]. A small blood sample was obtained prior to testing for assessment of hemoglobin in order to correct the measures of DLCO. Subjects subsequently performed cardiopulmonary exercise testing (CPET) using the CareFusion metabolic cart (San Diego, CA) on a semi-recumbent cycle ergometer (Lode, Netherlands). The test protocol started with 20 W for both men and women and increased by 10 W every 2 min. Prior to exercise testing, subjects were instrumented with a 12 lead ECG, and a forehead pulse oximeter for peripheral arterial oxygen saturation

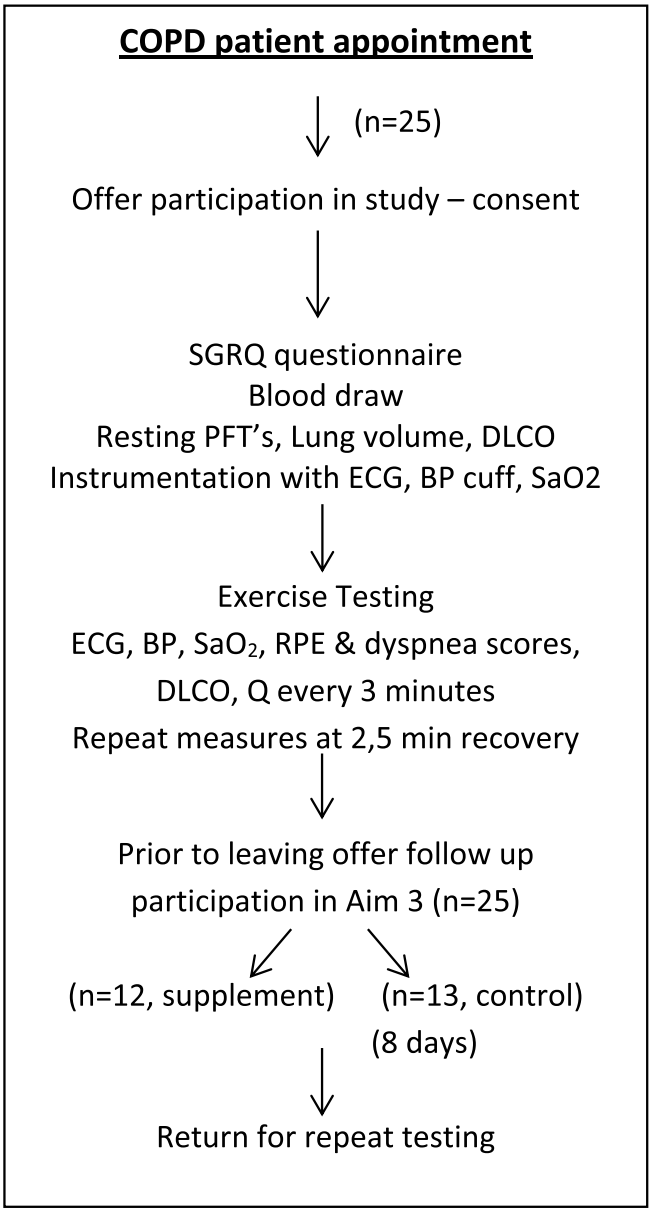

Fig. 1. Overview of study protocol.

(SaO_2) for continuous monitoring. Subjects wore a nose clip and breathed on a mouthpiece for measurement of gas exchange during the exercise test. During the last 30 s of each workload, a 12 lead ECG was recorded, blood pressure (BP) assessed, Borg's perceived dyspnea score (0–10 scale) and rate of perceived exertion (RPE) were rated by subjects, and an average of the HR and SaO_2 over this period was determined. Subjects were encouraged to exercise to near exhaustion by achieving an RPE of 17–18 on the 6–20 scale or a dyspnea score ≥7 on the 0–10 Borg scale. Upon reaching peak exercise subjects performed active recovery where they continued to pedal with no resistance and remained on the mouthpiece for 1 min. After this the subject stopped pedaling and were given time for HR and BP to return to baseline before being dismissed. The flow chart of the study is shown in Fig. 1.

2.3. Pulmonary function and single breath DLCO

Spirometry was performed using pneumotachograph-based pulmonary function equipment that had passed evaluation using 24-wavefroms recommended by the American Thoracic Society [27,31]. Classic single breath DLCO was determined using a commercial

instrument following the recommendations of the ATS/ERS.

2.4. Intra-breath lung diffusing capacity and pulmonary blood flow (Qc)

Pulmonary Blood Flow (Qc) and DLCO were measured using inert and soluble gases on the CareFusion Vmax system using an intra-breath method [30,32]. For this method, subjects were asked to breathe on a mouthpiece while wearing a nose clip. Subjects were instructed to exhale to near residual volume (RV) and then were switched into an inspiratory reservoir and took a full inhalation of a test gas mixture containing 0.3% carbon monoxide (CO), 0.3% methane, 0.3% acetylene, 21% O_2, and balance N_2. Subjects were coached to exhale at a steady rate until they were back near RV. From the rate of disappearance of CO and acetylene in comparison to the inert gas methane, the rate of disappearance of CO and acetylene were determined. This rate of disappearance of CO was used to calculate DLCO. Since acetylene does not bind to hemoglobin, the rate of its disappearance is limited primarily by the flow of blood through the lungs, thereby providing a measure of Qc. The measure of IBDLCO and IBQc was practiced several times at rest in each subject until reproducible values were obtained and performed near the end of the first workload in triplicate. If necessary the first work load was extended before incrementing the cycle ergometer resistance in order to obtain reproducible measures.

2.5. St. George's respiratory symptom questionnaire

The SGRQ is a 50-item questionnaire developed to measure health status (quality of life, QOL) in patients with diseases causing airway obstruction. Scores are calculated for three domains: Symptoms, Activity and Impacts (Psycho-social) as well as a total score. Psychometric testing has demonstrated its repeatability, reliability and validity. Sensitivity has been demonstrated in clinical trials [29].

2.6. Gas exchange, ventilation and lung mechanics

During exercise testing oxygen consumption (VO_2), carbon dioxide production (VCO_2), breathing frequency (fb), tidal volume (V_t), minute ventilation (V_E) and derived variables were measured continuously and/or calculated using the CareFusion low resistance open circuit automated metabolic system. In particular, measures associated with ventilation and perfusion matching or pulmonary vascular health were determined. These were the partial pressure of mixed expired CO_2 ($P_{et}CO_2$), end tidal CO_2 (P_eCO_2), and their relative ratio ($P_eCO_2/P_{et}CO_2$) (associated with ventilation and perfusion matching in the lungs and reported to be reduced in COPD patients with a negative slope during exercise in patients with pulmonary hypertension). Also measured were GxCap, a noninvasive estimate of pulmonary vascular capacitance calculated as oxygen pulse multiplied by $P_{et}CO_2$ (oxygen pulse tracks stroke volume and $P_{et}CO_2$ has been shown to reasonably track pulmonary arterial pressure), and ventilatory efficiency which is VE/VCO_2 [33,34].

2.7. Dietary supplementation

Patients were randomized to receive either beetroot or black currant juice. Beetroot was purchased from Beet It (www.beet-it.us) and black currant juice from Knudsen (www.rwknudsen.com). After randomization, the beetroot juice group of patients received 8 bottles of juice, each made of 70 ml of beetroot juice plus 180 ml of black currant juice (total 250 ml). The placebo group received 8 bottles of juice, each made of 70 ml of water and 180 ml of black currant juice (total 250 ml). All the patients were blinded to their type of juice. Each patient had to take one bottle of juice per day for eight consecutive days, the last bottle being the day of their return for the follow up CPET study. Patients were called every day to remind them of taking their juice.

2.8. Exhaled nitric oxide measurement

Nitric oxide in the exhaled air was measured using NIOX VERO system (www.niox.com). To make a measurement, a filter was placed on the mouthpiece. The subject then exhaled steadily for approximately 10 s and was guided by a visual monitor on the device screen to ensure the exhalation was uniform and steady. When maneuver was complete, the screen gave an indication and the final value in parts per billion was shown on the screen.

2.9. Statistics

We were interested in exercise limitation in the COPD population and the potential role of the alveolar–capillary bed, lung surface area for gas exchange or the ability to expand this bed as a mediating factor. Thus our primary measures were the change in IBDLCO and the change in IBDLCO in proportion to IBQc across subjects and the relationship to exercise or aerobic capacity; primarily peak VO_2. Thus we used multiple regression and correlational analysis to asses these relationships. In addition, general descriptive statistics were used to define our group and paired t-tests to evaluate changes with exercise. For variables found not to be normally distributed, a Wilcoxon signed rank test was used to test for differences between the groups using an $\alpha = 0.05$.

3. Results

3.1. Subject characteristics and measures of pulmonary function

Tables 1–3 give the baseline subject characteristics and standard spirometry values along with DLCO, measures of pulmonary blood flow and exhaled nitric oxide levels. The two groups (nitrate group vs placebo) were relatively well matched for age, sex, smoking history, symptoms and disease severity based on the GOLD (Global Initiative for Chronic Obstructive Lung Disease) classification and measures of lung function. In addition they were on similar distribution of medications. Values are also reported after receiving either nitrate supplementation or placebo. The only significant changes observed were an increase in the exhaled nitric oxide levels in the nitrate group and a small drop in the SGRQ ($p < 0.05$). No other significant changes were observed either between groups or post nitrate or placebo intervention in these measures.

3.2. Exercise responses

Tables 4–6 display the mean exercise data for lung diffusing capacity and pulmonary blood flow, submaximal (matched work load only) exercise ventilatory, cardiovascular and gas exchange responses and similar measures obtained at peak exercise.

Table 1
Subject Characteristics of COPD patients prior to receiving nitrate supplement or placebo.

	Nitrate (n = 12)	Placebo (n = 13)
Age (years)	67 ± 8	68 ± 10
% Female	50	46
Weight (Kg)	82 ± 23	88 ± 25
BMI (Kg/m2)	28 ± 6	32 ± 6
Smoking history (pack year)	38 ± 33	36 ± 32
GOLD Classification (1–4)	2.4 ± 0.8	2.2 ± 0.6
Inhaled beta agonist (%)	100	100
Inhaled anticholinergic (%)	69	50
Inhaled steroid (%)	58	69
Oral steroid (%)	25	15

GOLD. Global Initiative for Chronic Obstructive Lung Disease classification for air flow obstruction.
• Significant difference between Nitrate group and Placebo group at baseline $p < 0.05$.
Significant difference between pre and post nitrate or placebo $p < 0.05$.

Table 2
Pulmonary Function Variables in COPD patients receiving nitrate supplement (n = 12) or placebo (n = 13).

	Pre Nitrate	Post Nitrate	%Δ	Pre Placebo	Post Placebo	%Δ
FVC (L)	2.61 ± 0.86	2.65 ± 0.94	+1	2.36 ± 0.69	2.43 ± 0.60	+1
FEV_1 (L)	1.50 ± 0.61	1.49 ± 0.69	−1	1.56 ± 0.66	1.43 ± 0.44	−3
FEV_1/FVC	57 ± 10	57 ± 9	0	61 ± 11	61 ± 11	0
FEF_{25-75} (L/s)	0.74 ± 0.43	0.71 ± 0.42	−5	0.78 ± 0.39	0.80 ± 0.34	+15
FEF_{75} (L/s)	0.29 ± 0.13	0.27 ± 0.13	−5	0.29 ± 0.12	0.30 ± 0.10	+17
MVV (L/m)	50 ± 26	51 ± 33	+2	50 ± 16	50 ± 17	+1
St George Questionnaire	40 ± 19	34 ± 14	−17#	47 ± 24	45 ± 24	−4.2

FVC. Forced Vital Capacity, FEV_1. Forced Expiratory Volume in 1 s, FEF. Forced Expiratory Flow, MVV. Maximal voluntary ventilation. QOL. Quality of life determined from the St George Respiratory Questionnaire.
All data are pre bronchodilator.
• Significant difference between Nitrate group and Placebo group at baseline $p < 0.05$.
Significant difference between pre and post nitrate or placebo $p < 0.05$.

There was a trend for a rise in the submaximal DLCO (+14%) and expressed relative to Qc (+20%) in the nitrate group ($p = 0.08$), with no evidence of change in the placebo group (Fig. 2). There were no other changes noted in cardiovascular responses or measures of pulmonary gas exchange or breathing pattern post nitrate or placebo supplementation during submaximal exercise.

There was no difference in peak exercise performance post nitrate or placebo supplementation. There was also no observable impact of nitrate supplementation on other key measures of breathing pattern, respiratory gas exchange or other variables associated with pulmonary vascular function during heavier exercise. The only measure that reached statistical significance was a reduced blood pressure at peak exercise in the nitrate supplemented group, but not the placebo group (Fig. 3). There was a tendency for the beetroot juice group to have elevated pretreatment exercise blood pressures relative to the placebo group; however, 8 of 12 in the beetroot juice group demonstrated a drop post treatment at peak exercise while only 2 in the placebo group demonstrated a fall in exercise blood pressure post treatment (Fig. 4). Differences in blood pressure between the two groups was also assessed with a Wilcoxon Signed Rank Test showing that there was a difference in peak blood pressure response with juice vs placebo.

4. Discussion

Chronic obstructive pulmonary disease is associated with remodeling and destruction of the pulmonary vasculature. This leads to declines in functional lung surface area for respiratory gas exchange and a reduced ability to expand it with exercise. This inability to recruit or distend pulmonary capillaries has thus been linked to exercise limitation in this population [9]. Endothelial dysfunction is a part of the pathophysiology of COPD, in part related to altered nitric oxide regulation [35,36]. Therefore we tested the hypothesis that supplementation with nitrates may improve nitric oxide formation, enhance endothelial function and improve lung surface area for respiratory gas exchange. We found that while we had evidence for increased NO formation from exhaled measurements, there was no significant impact on resting measures of airway or pulmonary vascular function. There were trends towards improved pulmonary vascular recruitment with exercise in the nitrate supplement group and evidence for reductions in systemic blood pressure, but the change in DLCO was not associated with changes in other noninvasive indices of respiratory gas exchange during exercise or an improvement in exercise capacity. Interestingly there was a trend towards a reduction in symptoms in the nitrate group relative to the placebo group, however the mechanism for this remains unclear and larger numbers would be needed to confirm this finding. Thus while there was some evidence of a systemic influence of nitrate supplementation with inorganic supplementation, there was no definitive benefit relative in pulmonary function or exercise performance relative to placebo.

NO is a signaling molecule with multiple functions including regulation of vascular tone, mitochondrial respiration and skeletal muscle function. These factors are important in the physiological response to exercise and relative to our hypothesis for the recruitment of the pulmonary vasculature with exercise. NO is produced in two primary ways in man. The best known is the classical L-arginine nitric oxide synthase (NOS) pathway which is oxygen dependent. The second is the enterosalivary pathway and is oxygen independent. Ultimately some nitrite is absorbed into the circulation where it acts as a storage pool for subsequent NO production. The conversion of nitrite to NO is expedited in conditions of acidosis or hypoxemia which likely occurs in regions of the pulmonary vasculature in COPD patients, especially during exercise [37–39].

We have recently demonstrated that resting DLCO or resting DLCO relative to pulmonary blood flow (DLCO/Qc) has been predictive of exercise performance in a moderate to moderately severe COPD population [8]. In addition, our preliminary data show that the ability to

Table 3
Resting Lung Diffusing Capacity, Pulmonary Blood Flow and Exhaled Nitric Oxide in COPD patients receiving nitrate supplement (n = 12) or placebo (n = 13).

	Pre Nitrate	Post Nitrate	%Δ	Pre Placebo	Post Placebo	%Δ
Single Breath DLCO (SBDLCO, ml/min/mmHg)	11.8 ± 5.2	12.0 ± 5.8	+2	14.3 ± 5.4	14.9 ± 5.7	+4
Intra Breath DLCO (IBDLCO, ml/min/mmHg)	9.3 ± 4.8	9.2 ± 5.2	0	11.1 ± 5.2	11.7 ± 5.8	+6
IBDLCO/SBDLCO (%)	77 ± 13	76 ± 17	+1	76 ± 14	77 ± 19	+3
Pulmonary Blood Flow (Qc, L/m, measured)	4.9 ± 0.9	4.8 ± 1.1	−4	4.8 ± 0.9	4.9 ± 0.8	+3
Pulmonary Blood Flow –Cardiac output, (L/m, Predicted)	6.3 ± 0.3			6.4 ± 0.3	`	
IBDLCO/Qc ratio	1.9 ± 1.0	1.9 ± 0.9	+2	2.3 ± 1.1	2.4 ± 1.0	+11
Exhaled Nitric Oxide (eNO, ppb)	16 ± 12	38 ± 35#	+247	25 ± 21	26 ± 22	+10
Hgb (g/dl)	13.6 ± 1.7	13.6 ± 1.7	0	13.1 ± 1.2	13.2 ± 1.2	0

Pulmonary Blood Flow measured with soluble gas method. Cardiac output estimated based on age, gender, BSA, from Williams, LR(52). Qc = Pulmonary Blood Flow. Hgb = Hemoglobin, IBDLCO and SBDLCO = intra-breath and single breath lung diffusing capacity for carbon monoxide.
• Significant difference between Nitrate group and Placebo group at baseline $p < 0.05$.
Significant difference between pre and post nitrate or placebo $p < 0.05$.

Table 4
Exercise Lung Diffusing Capacity, Pulmonary Blood Flow and Exhaled Nitric Oxide in COPD patients receiving nitrate supplement (n = 12) or placebo (n = 13).

	Pre Nitrate	Post Nitrate	%Δ	Pre Placebo	Post Placebo	%Δ
Intrabreath DLCO (IBDLCO, ml/min/mmHg)	10.8 ± 7.2	12.3 ± 7.2	+14%	14.8 ± 8.3	12.9 ± 7.1	−13%
EXIBDLCO/RSTDLCO (%)	1.34 ± 0.53	1.57 ± 0.53	+17%	1.40 ± 0.69	1.28 ± 0.53	−9%
Pulmonary Blood Flow (Qc, L/m, measured)	7.0 ± 2.2	7.1 ± 2.4	+2%	6.9 ± 1.5	7.1 ± 1.12	+3%
IBDLCO/Qc ratio	1.5 ± 0.6	1.8 ± 1.2	+20%	2.2 ± 1.2	1.8 ± 1.1	−18%

Pulmonary Blood Flow measured with soluble gas method. Cardiac output estimated based on age, gender, BSA, from Williams, LR [52]. Qc = Pulmonary Blood Flow. EXIBDLCO/RSTDLCO is the exercise intra-breath DLCO divided by the resting intra-breath DLCO.
• Significant difference between Nitrate group and Placebo group at baseline $p < 0.05$.
Significant difference between pre and post nitrate or placebo $p < 0.05$.

expand the pulmonary capillary bed during exercise has also been an important factor in determining exercise capacity in this group [9]. DLCO is reduced in COPD likely due to destruction of alveolar septum and the pulmonary capillary bed; other contributing causes are remodeling, ventilation-perfusion mismatch, and hypoxic pulmonary vasoconstriction. A fall in NO due to less synthesis of NO caused by oxygen substrate limitation or oxygen sensitivity of the NO synthase, could also contribute to the immediate rise in pulmonary artery pressure upon arrival at altitude as a consequence of a reduced capacity to vasodilate [40]. Thus despite the remodeling, there are likely regulatory mechanisms influencing pulmonary vascular recruitment in this population and thus potentially reversible.

The impact of altering nitric oxide pathways on exercise capacity in COPD patients is variable. Work by Lederer [41] did not demonstrate improvement in 6 min walk distance or peak exercise VO_2 with 4 weeks of sildenafil three times daily. The authors found a reduction in quality of life and evidence for an increased alveolar to arterial oxygen difference with sildenafil. Similar findings were found by Holverda et al. [7] with acute administration of sildenafil without improvement in exercise performance in COPD patients with or without pulmonary hypertension. However, other studies have found improvements in 6 min walk distance in patients with COPD taking sildenafil [42]. Thus the impact of sildenafil is controversial. However, it is possible that the PDE-5 inhibitors such as sildenafil, may override to some extent the important vasoregulatory mechanisms that attempt to improve some level of ventilation to perfusion mismatch in the lungs and it is possible that higher doses may in fact be counterproductive [7].

4.1. Impact of dietary supplementation of inorganic nitrates

Studies have demonstrated modest benefits pertaining to cardiovascular health, such as reducing blood pressure (BP), enhancing blood flow, and elevating the driving pressure of oxygen in the microcirculation to areas of hypoxia or exercising tissue [43,44]. However, there are limited studies in the COPD population with mixed findings.

Berry et al. studied effects of dietary nitrate supplementation via beetroot juice on the submaximal exercise capacity of COPD patients. In their randomized, single-blind, crossover design, beetroot juice was

Table 5
Cardiopulmonary Responses to Submaximal Exercise in COPD Patients receiving nitrate supplement (n = 12) or placebo (n = 13) (Mean ± SD).

n = 32	Pre Nitrate	Post Nitrate	%Δ	Pre Placebo	Post Placebo	%Δ
Exercise Capacity						
Work (Watts)	20	20 ± 4	0	20 ± 14	20 ± 19	0
VO_2 (ml/kg/min)	8.1 ± 1.4	8.2 ± 1.1	+12%	8.5 ± 1.5	8.1 ± 1.3	−5%
Symptoms						
RPE (6–20 Borg Score)	11 ± 2	11 ± 3	0	12 ± 3	12 ± 2	0
Dyspnea (0–10 Score)	4 ± 2	4 ± 2	0	4 ± 2	4 ± 2	0
Cardiovascular						
Heart Rate (bpm)	91 ± 12	92 ± 9	+1%	91 ± 12	93 ± 13	+2%
O_2 Pulse (VO2/HR)	7.1 ± 1.9	6.9 ± 2.1	−2%	8.6 ± 2.3	8.1 ± 1.9#	−6%
Blood Pressure SBP (mmHg)	142 ± 15	134 ± 9#	−7%	136 ± 20	135 ± 21	−1%
DPB (mmHg)	82 ± 10	83 ± 11	+1%	81 ± 10	77 ± 12	−2%
Ventilation/Breathing Pattern						
Ventilation (L/min)	22 ± 7	23 ± 6	+3%	25 ± 7	24 ± 7	−2%
Tidal Volume (L)	1.26 ± 0.16	1.27 ± 0.56	+1%	1.13 ± 0.30	1.15 ± 0.28	+1%
Breathing Frequency (bpm)	21 ± 5	21 ± 5	0	26 ± 7	24 ± 5	−8%
IC (L)	2.01 ± 0.76	2.19 ± 1.02	−1%	1.94 ± 0.69	1.86 ± 0.54	−4%
VT/IC	56 ± 14	53 ± 14	−4%	50 ± 10	48 ± 15	−3%
VE/Breathing Capacity (%)	45 ± 13	49 ± 15	+7%	55 ± 33	52 ± 24	−4%
Gas Exchange – Pulmonary Vascular						
VE/V_{CO2} ratio	39 ± 5	38 ± 6	−2%	36 ± 5	35 ± 4	−2%
P_{etCO2} (mmHg)	37 ± 6	38 ± 6	+2%	38 ± 5	39 ± 5	+2%
PE_{CO2} (mmHg)	23 ± 3	23 ± 3	0	24 ± 3	25 ± 3	+3%
PE_{CO2}/Pet_{CO2} ratio	0.62 ± 0.04	0.63 ± 0.06	+2%	0.64 ± 0.05	0.64 ± 0.05	0
GxCap	263 ± 82	252 ± 91	−4%	327 ± 88	305 ± 73	−5%

VE: Minute ventilation, fb: breathing frequency, VT: tidal volume, TI: inspiratory time, TTOT: total respiratory cycle time, IC: inspiratory capacity, VO_2: oxygen consumption, VCO_2: carbon dioxide production, PE_{CO2}: mixed expired CO_2 values, Pet_{CO2}: end tidal partial pressure of carbon dioxide, O_2pulse: VO_2/heart rate, SaO_2: arterial oxygen saturation estimated from pulse oximetry. GxCap: noninvasive estimate of pulmonary vascular capacitance.
• Significant difference between Nitrate group and Placebo group at baseline $p < 0.05$.
Significant difference between pre and post nitrate or placebo $p < 0.05$.

Table 6
Cardiopulmonary Responses to Maximal Exercise in COPD Patients receiving nitrate supplement (n = 12) or placebo (n = 13) (Mean ± SD).

n = 32	Pre Nitrate	Post Nitrate	%Δ	Pre Placebo	Post Placebo	%Δ
Exercise Capacity						
Work (Watts)	45 ± 18	48 ± 16	+6%	48 ± 21	52 ± 21	+3%
VO_2 (ml/kg/min)	12.0 ± 1.6	11.7 ± 2.2	−3%	11.1 ± 3.6	10.6 ± 4.8	−4%
Symptoms						
RPE (6–20 Borg Score)	17 ± 1	17 ± 2	0	17 ± 2	17 ± 1	0
Dyspnea (0–10 Score)	7 ± 2	7 ± 1	0	7 ± 2	7 ± 2	0
Cardiovascular						
Heart Rate (bpm)	109 ± 14	109 ± 13	0	104 ± 14	106 ± 15	+1%
O_2 Pulse (VO2/HR)	8.9 ± 2.8	9.2 ± 2.9	+3%	9.8 ± 3.1	10.1 ± 3.1	+3%
Blood Pressure SBP (mmHg)	161 ± 22	150 ± 14	−7%#	145 ± 20	151 ± 21	+4%
DPB (mmHg)	92 ± 10	93 ± 11	+1%	84 ± 10	82 ± 11	−2%
Ventilation/Breathing Pattern						
Ventilation (L/min)	36 ± 13	35 ± 11	−2%	34 ± 10	36 ± 12	+4%
Tidal Volume (L)	1.24 ± 0.46	1.23 ± 0.41	−1%	1.16 ± 0.36	1.21 ± 0.33	+4%
Breathing Frequency (bpm)	30 ± 5	30 ± 6	0	31 ± 4	32 ± 6	+2%
IC (L)	1.85 ± 0.81	1.98 ± 0.72	+6%	1.85 ± 0.66	1.79 ± 0.63	−3%
VT/IC	65 ± 23	64 ± 11	−1%	64 ± 23	61 ± 22	−4%
VE/Breathing Capacity (%)	70 ± 14	68 ± 17	−2%	69 ± 26	69 ± 18	0
Gas Exchange – Pulmonary Vascular						
VE/VCO_2 ratio	37 ± 4	36 ± 4	−2%	35 ± 6	34 ± 4	−2%
$PetCO_2$ (mmHg)	35 ± 5	36 ± 5	+2%	37 ± 4	38 ± 5	+2%
$PECO_2$ (mmHg)	24 ± 3	24 ± 3	0	25 ± 4	26 ± 3	+3%
$PECO_2/Pet_{CO2}$ ratio	0.66 ± 0.05	0.68 ± 0.06	+3%	0.69 ± 0.06	0.69 ± 0.05	0
GxCap	308 ± 94	328 ± 102	+6%	358 ± 127	380 ± 115	+6%

VE: Minute ventilation, fb: breathing frequency, VT: tidal volume, TI: inspiratory time, TTOT: total respiratory cycle time, IC: inspiratory capacity, VO_2: oxygen consumption, VCO_2: carbon dioxide production, PE_{CO2}: mixed expired CO_2 values, Pet_{CO2}: end tidal partial pressure of carbon dioxide, O_2pulse: VO_2/heart rate, SaO_2: arterial oxygen saturation estimated from pulse oximetry. GxCap: noninvasive estimate of pulmonary vascular capacitance.
• Significant difference between Nitrate group and Placebo group at baseline $p < 0.05$.
Significant difference between pre and post nitrate or placebo $p < 0.05$.

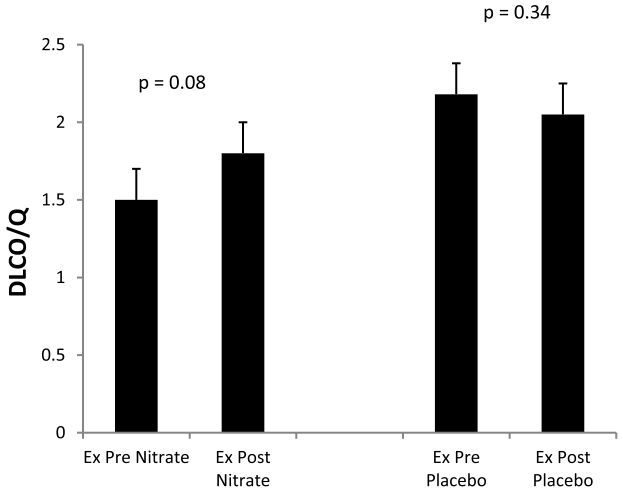

Fig. 2. DLCO relative to Q during submaximal exercise pre and post either nitrate supplement (n = 12) or placebo (n = 13) in COPD patients.

given [12]. Relative to placebo, beetroot ingestion caused significant elevation of plasma NO_3 by 938% and NO_2 by 379%. Median exercise time was significantly increased and blood pressure was also significantly reduced. No other variables were significantly different between the two groups. In another study, Leong et al. performed a double-blind, computer-randomized placebo control crossover trial to study the effects of dietary nitrate on exercise endurance in stable moderate COPD subjects [45]. Patients underwent an incremental shuttle walk test to determine VO_2max followed by randomization to placebo or beetroot groups. Subjects performed an endurance shuttle walk test at 85% VO_2max after randomization. Then they took the sample juice for 3 days and on the 4th day wash out there was a cross over between the groups. Again blood pressure was reduced with ingestion of beetroot juice. End shuttle walk test distance and time to fatigue improved but did not reach statistical significance. In a study by Kerley et al., an increase in incremental shuttle walk distance after consumption of high nitrate juice (25 m) compared to low nitrate juice (14 m) was noted. The improvement in exercise capacity was associated with statistically significant increases in serum nitrate and nitrite levels and a significant lowering of resting blood pressure [46].

These studies show that the effects of beetroot on exercise performance in COPD is not consistent and results are mixed at best, although lowering of blood pressure probably due to vasodilatory effects was a common finding. There could be several reasons for the inconsistencies. The factors that have been suggested include deconditioning, hypoxia, hypercapnia, systemic inflammation, nutritional imbalance and medication therapy to name a few. At the physiological level, mitochondrial dysfunction and redox imbalance seem to contribute to the conflicting results that are obtained at each trial. For example, oxidative stress shadows nitric oxide effects and is likely to be involved in the respiratory muscle dysfunction in severe COPD [47]. Epigenetic modification may also play a role in muscle dysfunction in COPD. The epigenetic modifications recognized so far include DNA methylation, histone acetylation and methylation, and non-coding RNAs such as microRNAs [48]. These complex, interwoven factors makes prediction of dietary nitrate supplementation difficult and at times unpredictable. It seems there are several factors that are responsible for muscle dysfunction in COPD, not to include the dose of nitrate supplementation that is not clearly identified in each patient and can be variable [49].

Thus to summarize, use of inorganic nitrate supplement for 8 days in a mild to severe COPD population increased exhaled nitric oxide levels, mildly reduced respiratory symptoms and exercise blood

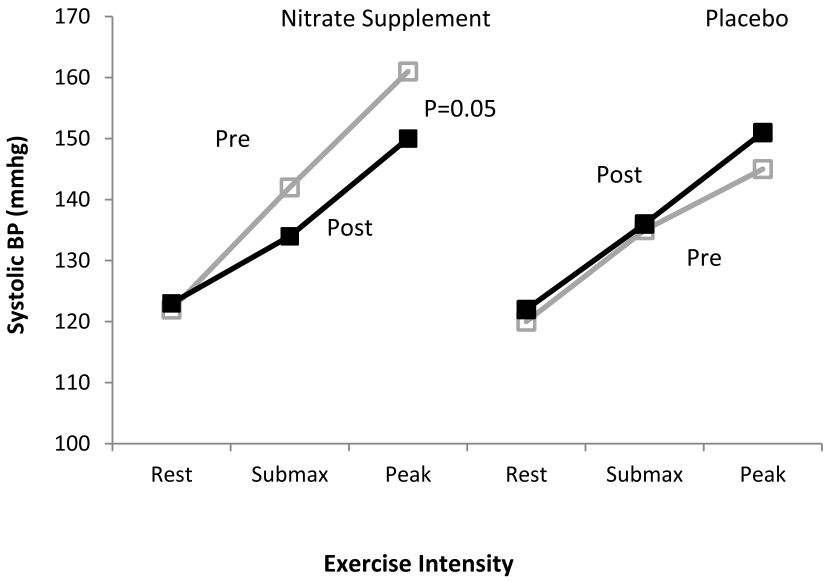

Fig. 3. Blood pressure responses to exercise pre (grey) and post (black) either nitrate supplement (n = 12) or placebo (n = 13) in COPD patients.

pressure but did not appear to significantly impact lung surface area for gas diffusion or peak exercise capacity. There are some hints of improved DLCO relative to Qc, suggesting that perhaps a mild influence on pulmonary capillary blood volume can be contributing; however, the effect is small and did not reach statistical significance.

4.2. Limitations

There are a number of limitations regarding this study. The intra-breath maneuver, while easier to perform during exercise than the single breath-hold technique, was still challenging. Thus we were only able to obtain these data at the first workload in all individuals in triplicate. We did not have additional work levels or peak values and our conclusions are thus limited to submaximal values. It is possible that recruitment or distension of this vascular bed may change with higher workloads, however previously studies have suggested a linear influence of exercise (cardiac output) on DLCO and for many of our subjects the first work level was as much as 75% of peak work. We did however have other non-invasive gas exchange measures at peak exercise that tend to correlate to DLCO, such as V_E/V_{CO2} ratio and the $P_{ECO2}/P_{et}CO_2$ ratio which were not impacted by the supplement. In addition we have previously demonstrated that the O_2 pulse multiplied by the $PetCO_2$ was associated with pulmonary capacitance in a heart failure population and this metric was also unaffected by the supplementation. Furthermore, we were not able to separate DLCO into its component parts, Dm and Vc (membrane diffusion capacity and pulmonary capillary blood volume); however, we did measure Qc and the ratio of DLCO/Qc should be somewhat analogous to Vc. In our previous studies, this ratio tended to be more highly associated with exercise tolerance than DLCO alone [9].

The study was also limited by relatively small numbers of stable COPD patients. Thus this may have led to some imbalance in the baseline measures between groups, such as pretreatment peak exercise blood pressure. However, no subjects were on oxygen therapy and none

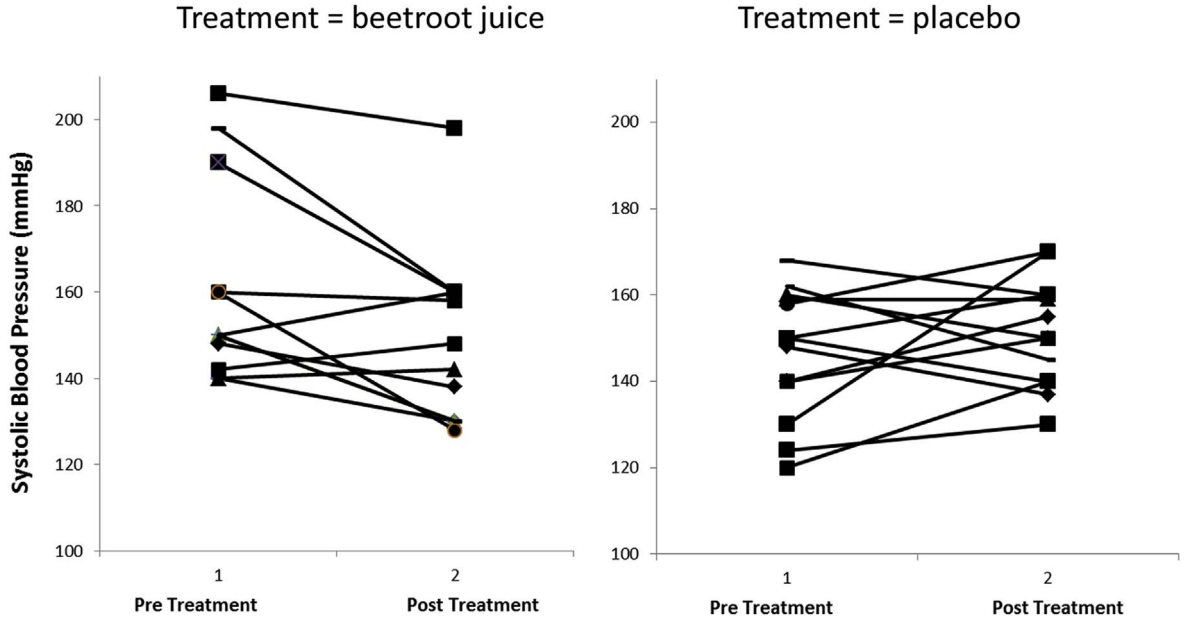

Fig. 4. Individual changes in peak blood pressure before and after treatment with beet root juice or placebo. Differences in blood pressure between the two groups assessed with Wilcoxon Signed Rank Test showing that there was a difference in blood pressure between groups, $\alpha = 0.05$.

were on vasoactive drugs at the time of the study. While we did not obtain baseline and post supplement measures of pulmonary vascular pressures on our subjects, it is not likely our subjects had significant pulmonary hypertension as we did not observe any significant oxygen desaturation with exercise. Some of the patients were active smokers or were taking oral prednisone and these have been shown to lower FENO in COPD, but these variables were similar in each study group [50,51].

Finally, as previously noted by Berry et al., in addition to nitrate beetroot juice contains several potentially metabolically active compounds that could influence physiological function in patients with COPD [12]. Potentially confounding compounds may be polyphenols and/or quercetin. Though we did use a juice placebo, it is possible that beetroot juice supplementation may have influenced the outcome beyond those attributed to nitrate alone.

Thus while there were several major limitations in this study, we view these data as thought-provoking findings in a small population of COPD patients that should form the foundation for more comprehensive and larger prospective studies that may give insight into the potential benefits of enhancing nitric oxide pathways on pulmonary vascular function.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.niox.2018.03.009.

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