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Low-Flow Domiciliary Oxygen as a Mechanism of Ongoing Oxidative Stress in COPD Patients

Jill Stulce

Low-Flow Domiciliary Oxygen as a Mechanism of Ongoing Oxidative Stress in COPD
Patients

A dissertation submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Health Related Sciences at Virginia Commonwealth University.

by

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Table of Contents

List of Tables

List of Figures

Abstract

LOW-FLOW DOMICILIARY OXYGEN AS A MECHANISM OF ONGOING OXIDATIVE STRESS IN COPD PATIENTS

Jill Stulce, Ph.D.

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Health Related Sciences at Virginia Commonwealth University.

Virginia Commonwealth University, 2015

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Healthcare costs are escalating in the U.S., with a projected 48 trillion dollars by 2021. More than ever medical researches are obligated to ensure that costly treatment modalities are safe and effective. Chronic obstructive pulmonary disease (COPD) is a costly and debilitating disease, ranked as the third leading cause of death in America. Currently, treatment for COPD consists of anti-inflammatory agents, bronchodilators, antibiotics and supplemental oxygen when hypoxemia or clinical manifestations ensue. Oxidative stress is central to the pathology of COPD. Supplemental oxygen has been substantiated as an instigator of oxidative stress; however, LFDO has not been evaluated as a mechanism of ongoing oxidative stress in individuals with COPD. Isofuran (IsoF), a biosynthetic relative of the validated oxidative stress biomarker 8-isoprostane, is preferentially synthesized during periods of increased tissue oxygen tension. This sort of

specificity allows for refinement in the assessment of supplemental oxygen as a source of oxidative stress. To address this potential this study evaluated individuals diagnosed with COPD utilizing LFDO. The study also aimed to determine if IsoF possessed clinical application in predicting the standard pulmonary function test (PFT) parameters of FEV1, FVC, FEV1/FVC and FEF25-75. The exhaled breath condensate (EBC) of 52 individuals with COPD was evaluated for the presence of IsoF. An active control group not receiving LFDO (n=26) was compared to an active treatment group receiving LFDO for a minimum of 6 hrs/day (n=26). The groups showed no statistically significant demographic differences in age, gender, height, weight, ethnicity or smoking history or in the pulmonary function test parameters of FEV1, FVC, and FEV1/FVC, with the exception of the FEF25-75 (P=0.03). The active control group generated a mean EBC IsoF level of 35.81 ± 4.91 pg/ml ($\pm$ SEM) compared to the active treatment group mean EBC IsoF level of 51.37 ± 8.27 pg/ml (P=0.057). Currently, no research has been conducted that defines baseline EBC IsoF levels in healthy or diseased lungs. No statistically significant differences in mean EBC IsoF levels were noted between the control and treatment groups; however, the results, in conjunction with the only two studies available utilizing EBC IsoF as an oxidative stress biomarker, may serve to provide benchmark information for future research regarding individuals with diseased lungs, specifically COPD.

Chapter 1- Introduction

Introduction

Americans may be living longer but not necessarily more healthfully. Some lifestyle

choices are rife with consequence. Smoking, over eating and lack of exercise are major players

in our countries overall lack of health and has translated to economic stress. Healthcare

expenditures in the U.S have been growing at an alarming rate for the past several years. Total

health expenditure in the U.S. is defined as the sum of public and private expenditure, and covers

healthcare provision (preventive and curative), family planning, nutrition activities, and

emergency aid. In the 1960's the healthcare costs constituted approximately 5.2% of the GDP.

During the 70's healthcare costs increased to 7.2% GDP, or approximately 75 billion dollars.

Currently, 17.3%, or roughly 2.5 trillion dollars, are spent annually on healthcare. A GDP of

20%, or 48 trillion dollars, is projected by 2021 (CMS Report). The United States is on track to

spend one-fifth of tax dollars exclusively on healthcare. There are many factors that contribute to

the escalating costs, such as hospitals, physicians, prescription drugs, nursing/continued care,

private health insurance administrative costs, etc.; however, this study will focus on those areas

whose cost most directly impact health care delivery, hospitals and physicians, which comprise

about 50% of the expenditures are considered the structure and process of healthcare delivery.

When considering an approach to contain escalating costs it is necessary to understand

health trends that contribute most significantly to those costs. According to a report published by

the Agency for Healthcare Research and Quality (AHRQ) (2006), the top five diseases that

introduce the greatest economic burden to this country are mood disorders, diabetes, cancer, heart disease, lung disease and hypertension. One such disease is chronic obstructive pulmonary disease (COPD), which continues to be one of the top five leading causes of death in America. Costly treatments, increased hospital stays, loss of functionality leading to inability to work, as well as requiring in home assistance contribute to the significant expense of this disease. A deeper understanding of the pathophysiology of the disease, as well as the prescribed interventions and management, could aid in improving health states and reducing cost of these major health care categories.

A common denominator exists amongst many disease processes, oxidative stress. Oxidative stress, free radical induced damage to proteins, lipids and DNA, has been implicated as a key process in the development of heart, lung, and neural disease, cancer, diabetes, arthritis, cataracts, and, on a lesser stage, aging. Interestingly, oxidative stress is linked to those disease categories that introduce the greatest economic burden in the U.S. As such, it is imperative that researchers gain an intimate understanding of this potentially deleterious process in order to ameliorate or eliminate its health and economic impact.

Chronic obstructive pulmonary disease is one of the major pulmonary diseases that proves to be physically, spiritually and economically taxing. It is a progressive and irreversible respiratory disease primarily linked to oxidative damage caused by airway irritants, most prominently cigarette smoking. For nonsmokers, long-term exposure to second hand smoke, chronic asthma, and environmental pollutants are the most significant contributors. As the leading contributor of COPD, it is of value to understand the mechanisms of destruction induced by cigarette smoke in the airways in an effort to prevent the destruction. Cigarette smoke stimulates the generation of reactive oxygen species (ROS), a molecular form of oxygen involved in oxidative stress, and has been linked to alterations in glutathione (GSH), an intra and

2

extracellular antioxidant present in the lungs (Rahman and MacNee, 1999). Disabling the body's protective antioxidant defenses invites oxidative reactions, especially in the presence of increased ROS. Ultimately, this imbalance results in lung parenchymal damage through alterations in the lipid bilayers induced by oxidative stress.

For those who smoke, and for those who are chronically exposed to airway mutagens, it is recommended to undergo pulmonary function testing for early detection of COPD. Early detection of COPD can produce the greatest opportunity to halt the debilitating disease. Pulmonary function testing (PFT) is a simple non-invasive method of assessing lung function. Pulmonary function defects can be grouped into two main categories: obstructive and restrictive. If expiratory flow is impeded an obstructive disorder may exist; if volume is reduced a restrictive disorder may exists. Several pulmonary function tests can be performed to assist in predicting pulmonary status, such as the vital capacity (VC), forced vital capacity (FVC), peak expiratory flow rates (PEFR), forced expiratory flow rates (FEF), maximal voluntary ventilation (MVV) and forced expired volume in 1 second (FEV_1). The most frequently used parameters in predicting stages of COPD are the FEV_1 and FVC values, both of which are useful in detecting and quantifying restrictive and obstructive patterns. The FEV_1 is the amount of air forcefully expelled in the first second of exhalation. Forced vital capacity is the amount of air an individual can forcibly and maximally expel until unable to expel any more. The FEV_1/FVC represents a proportion of an individual's vital capacity expelled in the first second. When values are categorized by age, gender, race, and body height and size a meaningful interpretation can be made. PFTs are effort dependent, therefore, patient compliance and cooperation is required for accurate result valuation.

The study of oxygen has been a topic of research for literally centuries. It is a perplexing and curious notion that an element is necessary for life but contributes to morbidity and

mortality. Oxygen delivery is a frequently prescribed therapy in both the acute and chronic care in the U.S. Low flow domiciliary oxygen (LFDO) is a prescribed therapy for those afflicted with advanced stages of COPD. Due to ethical implications a safety index for oxygen exposure has not been established. Most of the usable data regarding tolerable limits of oxygen exposure have been obtained from normal, healthy, young subjects (Klein, 1991). As a result, the effects of underlying diseases and other factors that may alter inherent oxidative protective mechanisms remain unknown. For the COPD population the benefits of LFDO therapy are well documented. Consequences of prolonged exposure to higher concentrations of oxygen has garnered much research attention; to date, only a handful of research studies have addressed the consequences of chronic exposure to low concentrations of oxygen with regard to oxidative injury. Petty, Stanford and Neff in 1971 and Steward, Hood and Block in 1975 both demonstrated histological changes in post mortem autopsies of those who had been treated with LFDO for several years. Two studies, both performed in 2004 evaluated 30-minute exposure to 28% oxygen. Phillips et al. compared healthy subjects and determined a significant ($P<0.05$) increase in BMAC, a biomarker of oxidative stress. Carpagnano et al compared healthy subjects to those with COPD and determined a significant ($P<0.05$) in 8-IsoP between groups. However, to date no studies have utilized isofuran, a biomarker synthesized only in the presence of elevated oxygen tensions, as a mechanism to determine if oxygen is the culprit for the presence of oxidative stress.

A disease process that is a direct result of unchecked oxidative stress, COPD is an ideal pathology to evaluate to learn more about the potential of LFDO as a mechanism of ongoing oxidative stress. It would be unfortunate, negligent and medically irresponsible if a prescribed therapy fostered ongoing oxidative stress in this already fragile population. Eradicating costly diseases through research and development along with the adherence to healthy lifestyles could spare the individual and nation at large significant economic, physical and emotional burden.

Purpose

Chronic obstructive pulmonary disease, a progressive and irreversible respiratory disease resulting from unchecked oxidative processes, in advanced states often requires oxygen supplementation to ameliorate distress. The purpose of this study is to identify if ongoing oxidative stress is occurring as a result of chronic exposure to low flows of domiciliary oxygen (LFDO) as evidenced by the validated biomarker, isofuran. Understanding these mechanisms may lead to a more rational basis for clinical prescription of oxygen.

The goal of this study is to gain insight into the potential of oxygen in order to safely prescribe and manage oxygen therapy. The objective of this study is to determine if LFDO as a treatment modality in COPD promotes oxidative stress. The specific aims of this study are to 1) assess for the presence of isofuran, an oxidative stress biomarker, in the exhaled breath condensate (EBC) of COPD patients on LFDO; 2) determine if a correlation exists between standard diagnostic spirometry values and exhaled breath condensate EBC biomarker (isofuran); 3) determine if a correlation exists between length of smoking history, length and concentration of oxygen exposure and EBC biomarkers (isofuran levels).

Background and Significance

The impact of oxidative stress on the health of U.S. citizens has only recently been appreciated. Oxidative stress has been linked to the top five diseases, including heart disease, lung disease, cancer, diabetes, and neural disorders, contributing to the greatest healthcare costs in terms of treatment, hospitalizations and loss of work (Druss, et al, 2001). While people may be living longer it may not necessarily be more healthfully (National Center for Health Statistics, 2010).

Chronic obstructive pulmonary disease (COPD) is a progressive disorder resulting in irreversible lung damage primarily comprised of two disorders: chronic bronchitis and

emphysema, and falls as one of the most economically burdensome. Approximately 16 million

Americans have COPD, 14 million with chronic bronchitis and 2 million with emphysema

(WHO, 2002). According to the American Lung Association June 2008 Fact Sheet, 2008 stated

that in 2006 12.1 million U.S. adults (ages 18 years and over) were estimated to have COPD but

that ~24 million U.S. adults have evidence of impaired lung function, indicating an under

diagnosis of COPD.

Smoking is considered the major contributor in developing COPD, be it direct smoking

or indirect second hand smoke exposure (American Lung Association, 2008). Associated health

care costs linked to cigarette smoking up to 2002 had been estimated at ~50 billion dollars

annually. More recent reports from the CDC show the economic costs of smoking to be at $3,393

per smoker, reaching an estimated $157 billion: $81.9 billion in productivity losses due to death

and $75.5 billion due to excess medical expenditures (CDC 2002). COPD is the fourth leading

cause of death and is projected to be the third leading cause of death by 2020 (ALA 2008). The

American Lung Association states that an estimated 800,000 adolescents will start smoking

every year. Statistics like this point to a future of continued and worsening economic and

healthcare challenges as it relates to the management of those who develop COPD.

While smoking is implicated as the initial inducer of oxidative stress in COPD pathology,

continued oxidative stress may be occurring as a result of treatment modalities. Superimposing

further damage on an individual with COPD through chronic exposure to greater than room air

concentrations of oxygen via low flow domiciliary oxygen therapy (LFDO) is neither in the best

interest of the COPD patient nor the community at large. It is well understood that oxygen

therapy for those suffering with advanced stages of COPD benefit from LFDO, however, it needs

to be appreciated if this necessary treatment is a contributing factor to ongoing oxidative stress.

To identify whether or not this phenomenon is occurring as a result can assist in guiding

researchers to a better understanding and development of COPD health management modalities, as well as a better understanding of the untoward effects of chronic exposure of LFDO and the potentially destructive effects of oxygen free radicals.

Oxidative stress occurs as a result of an imbalance between reactive oxygen species (ROS) and the system's ability to neutralize these highly reactive intermediates. This imbalance of a normal redox state has deleterious effects on the surrounding protein, lipid and DNA structures as a result of peroxide and free radical production and activity. Oxidative stress has been linked to aging, autism, Alzheimer's, cancer, COPD and cardiac disease to name a few (Halliwell, 2007; Valko et al, 2007; Singh et al, 1995; Ramond et al, 2011; Dean et al, 2011). It should be understood, however, that ROS are integral in health states as well, such as activation of the immune system; it is the imbalance of ROS and endogenous antioxidants that result in pathology (Segal, 2005). Although physiologic changes attributable to oxygen toxicity, such as decreases in vital capacity, pulmonary compliance, and diffusing capacity, together with increases in arteriovenous shunting and ration of dead space to tidal volume, early detection of toxicity requires more sensitive, specific, and preferably easy, tests (Klein, 1991).

In the past decade significant research has been conducted to aid in better understanding the inflammatory cascades occurring within the body in response to various stressors, such as oxidative stress (Montuschi, 2007; Barnes, 2006). As a result 8-iso-PGF2α, in addition to many other nonenzymatically generated prostaglandins, have been identified as a biomarker consistent with oxidative stress occurring within the lungs (Montuschi, 2007; Barnes, 2006).

More recently, research has demonstrated that isofurans, biosynthetic relatives of the isoprostanes, are formed under conditions where elevated oxygen tensions exist, allowing for research to expand in the area of hyperoxia. Both biomarkers can be evaluated in exhaled breath, making them an ideal to study pathologies occurring within the lungs.

Breath analysis is not new to medicine. Hippocrates would assess breath odor to diagnose disease states; however, not until the 1970's was it demonstrated by Linus Pauling that over 250 substances were identifiable in exhaled breath via gas chromoatgraphy mass spectrometry (GC-MS) (Dweik and Amann, 2008). More efficient devices have been developed to collect and store exhaled breath for biomarker analysis. Researchers suggest using these biomarkers to diagnose and prognose, as well as manage disease states (Montuschi, 2007).

The identification of a biomarker sensitive to endogenous changes to oxygen tension within the body provides an opportunity for researchers to better understand oxidative processes. Currently there is no information available in the literature that defines basal levels of isofuran in the exhaled breath of individuals diagnosed with advanced COPD with or without domiciliary oxygen therapy. This study aims to address this gap in foundational knowledge. It also aims to gain insight into whether or not chronic exposure to oxygen therapy serves as a mechanism of ongoing oxidative stress within the lungs of individuals with advanced COPD.

Conceptual Framework

"The well-being of the soul can be obtained only after that of the body has been secured," insight provided by Maimonides, a revered 12th century Jewish author, physician and philosopher, in his three volume series entitled *The Guide for the Perplexed*, demonstrating an early appreciation that quality of life is directly linked to physical health (Epstein and Sherwood, 1996). Current appreciation of the effect physical health has on quality of life, through outcomes research, has expanded to become a multidisciplinary effort involving researchers, healthcare providers, epidemiologists, economists, sociologists, statisticians and ethicists (Epstein and Sherwood, 1996).

The idea that quality healthcare can be achieved based on positive health outcomes is intuitive; however, the practice of relating quality to outcomes has become a prominent strategy

8

and more widely accepted in the past few decades in healthcare delivery (Hammermeister, Shroyer, Sethi, and Grover, 1995; Jennings and Staggers. 1998). The Donabedian model of structure-process-outcome will assist in guiding this research endeavor. This model fosters becoming more attuned to all aspects of health care provision, facilitating a more accurate interpretation of causal relationships between structure, process and outcomes.

Avedis Donabedian was born to an Armenian family in Beirut, Lebanon in 1919 and grew up in Palestine, narrowly escaping the Armenian Holocaust. He followed his father's footsteps in becoming a physician and eventually became the Chief Medical Officer of the American University in Beirut. During his tenure there he became frustrated with the limitations as an administrator and turned his interests to quality health care provision and public health. Following the Second World War, Donabedian moved to the U.S. from Lebanon and joined the faculty at the University of Michigan. It was then that he was commissioned with the task of revising all that had been written on quality assessment and to present it in an organized fashion. At that time formal writings on quality assessment were limited, allowing Donabedian to tackle this task in a reasonable amount of time and autonomy. His efforts gave way to the more formal model of process-structure-outcome phenomenon, or Donabedian's Triad. Donabedian's professional career was dedicated to revising and defining his model and outlining its usefulness for quality assessment and management (Donabedian 1966, 1968, 1982, 1985, 1992).

Elements of quality assessment. Donabedian's triad of concepts (structure, process and outcomes) has been defined in his writings. Structure is conceptually defined in terms of the setting in which care is provided and refers to the organization of health care resources (Donabedian, 1966). Structure also includes demographics, the education of providers, the adequacy of the facilities and equipment, as well as the overall administrative environment. Process is conceptually defined as the actions that constitute healthcare, to include diagnosis,

9

treatment, preventative care, collaboration of services, and patient education (Donabedian, 1982). Processes can also include the technical aspects of how care is delivered, or interpersonal processes of how care is delivered (Donabedian, 1980). Donabedian believes the measurement of process is equivalent to the measurement of quality of care as process contains the most relevant aspects of healthcare provision (Donabedian, 2003). Outcome is conceptually defined as the end result of the process of care to include "those things, either favorable or adverse, in the actual or potential health status of persons, groups, or communities that can be attributed to medical care" (Donabedian, 1985, p.256) and refers to "states or conditions attributable to antecedent health care" (Donabedian, 1992). In short, outcome is the measurable or observable result of structure and process; an assessment as to whether or not a treatment modality offered by providers of an institution affected the health state of the consumer. Outcome goals are to prevent progression of poor health, and to restore and maintain health states. Donabedian, (1992) states that quality outcomes are best achieved when the following attributes are employed: effectiveness (achieving the greatest improvements in health currently achievable by the best care), efficiency (the ability to provide appropriate care without diminishing improvements in health), optimality (achieving a balance between the cost of care and the effects of care on health), acceptability (achieving conformity with patient preferences), legitimacy (achieving conformity to social preferences and ethical subscriptions), and equity (conformity to principles guiding the fair distribution of health care in a population). It is widely agreed upon that defining quality is nearly impossible as each individuals value judgments differ one to the next. However, it also believed that most likely the majority equates quality with what the medical community perceives as quality.

Conceptual relationships. The relationship between structure, process and outcome is antecedent, or causal, in nature. In regards to structure and process, Donabedian states that "the assumption is made that given the proper settings (structure) and instrumentalities (process),

good medical care will follow" and, hence, good and desired outcomes (1966, p. 170). Specific attributes of structure, process and outcomes are considered valid for the purpose of quality assessment only to the extent that causal relationships can be demonstrated, meaning a quality outcome is only valid if it can be directly linked to the structure and/or process (Donabedian, 1992).

Structure-Process-Outcome Model used in research. Kunkel, Rosenqvist and Westerling (2007) implemented the Donabedian model in a qualitative survey research endeavor of 386 hospital departments in Sweden. The goal of this study was to analyze whether process, structure and outcome could be used to describe quality systems and whether or not these components are related. It was concluded that the process-structure-outcome model could be used to describe and evaluate single quality systems (P=0.095) as well as to compare different quality systems. They believe it could also serve as an aid to implement a systematic and evidence based system for working with quality improvements in hospital departments. Statistical analyses showed that Structure correlated strongly with process (0.72) and outcome (0.60).

In a two-year prospective study of 288 acute stroke patients across 11 VA medical centers the structure-process-outcome model was used to examine the quality of health care with regard to stroke patient outcomes. The goal of the project was to determine if structure of care is associated with process of care, and if structure of care is associated with stroke outcomes after adjusting for process. The results demonstrated that both systematic organization (P<0.001) and technological sophistication (P<0.001) were independent predictors of process of care. Overall, the full structure-process-outcome model with patient covariates considered, process of care was statistically associated with 6-month functional outcomes (P<0.01), but structure of care was not

shown to be statistically significant. In summary, process appears to be more significant of an indicator for quality outcomes than process (Hoenig et al., 2002).

Model assumptions and limitations. The structure-process-outcome model holds three major assumptions with regard to validity and usefulness for quality health care assessment: 1) that quality is defined and outcomes are selected, 2) health is a multidimensional concept; and 3) multiple causation.

It can be assumed that multiple definitions of quality exist. Valuation is an individualized phenomenon influenced by many outside forces and variables. According to Donabedian (1982), quality as an opinion implies valuation and is directly related to the attainment of proposed goals and objectives. When an individual's goals and objectives are met congruency of quality is realized.

The "state" of health is multidimensional in that it is comprised of physical, physiologic, psychologic, social and spiritual attributes. The influences on each of these attributes are essentially limitless resulting in extensive outcome possibilities. Intimate understanding of the whole being facilitates the meeting of individual goals and outcomes.

Attempting to discern causes of healthcare outcomes is challenging, as the variables of input are vast. Therefore, relying solely on outcome measures cannot provide complete accuracy to those elements responsible for either positive or negative outcomes. Oversight of structure and process, both within and between collaborative disciplines must be considered.

Structure-Process-Outcome model guiding this study. Current practices for managing advanced stages of COPD include the use of low flow domiciliary oxygen therapy for those individuals who meet prescribing criteria and demonstrate improvement with its use. However, to date minimal research has been performed to assess whether or not chronic exposure to oxygen therapy introduces consequences in terms of oxidative injury. In autopsy studies

12

performed by Petty, Stanford and Neff (1971) and again by Steward, Hood and Block (1975) "suspicious" histological changes were noted for those whom had been receiving long term oxygen therapy. And more recently, two studies performed in 2004 (Phillips et al and Carpagnano et al) assessed for the presence of oxidative stress through a variety of biomarkers, all of which demonstrated significant increases following exposure to low concentrations of oxygen exposure.

Determining the outcome of prescribed LFDO therapy can assist in evaluating the current structure and process at Phelps County Regional Medical Center.

Research Questions

The research questions guiding this study are:

1. Does chronic exposure to LFDO contribute to oxidative stress as evidenced by elevated isofuran levels within the lungs of COPD patients?

2. Does a relationship exist between the FEV_1, FEV_1/FVC ratio, $FEF_{25-75\%}$ and isofuran levels?

3. Is there a correlation between length of smoking history, and length of oxygen exposure and concentration of oxygen to isofuran levels?

Hypotheses

The following hypotheses were formulated to address the research questions:

1. Ha: Chronic exposure to LFDO will demonstrate ongoing oxidative stress in the lungs of COPD patients as evidenced by increased isofuran levels.

2. Ha: A relationship between FEV_1 and isofuran levels will be demonstrated in patients diagnosed with moderate to advanced stages of COPD using LFDO

3. Ha: A relationship between FEV_1/FVC ratios and isofuran levels will be demonstrated in patients diagnosed with moderate to advanced stages of COPD using LFDO.

4. Ha: A relationship between $FEF_{25-75\%}$ and isofuran levels will be demonstrated in patients diagnosed with moderate to advanced stages of COPD using LFDO

5. Ha: A positive correlation between oxygen concentration and isofuran levels will be demonstrated on the day of sampling in patients diagnosed with advanced stages of COPD using LFDO.

6. Ha: A positive correlation between length of oxygen exposure and isofuran levels will be demonstrated in patients diagnosed with advanced stages of COPD using LFDO.

Chapter 2 - Literature Review

Health care provision in America has claimed center stage for over a decade, with escalating costs with lack of insurance and access garnering the greatest attention. There are many contributors to the healthcare dilemma in our country; however, for the purposes of this paper, attention will be given to those aspects dealing with provision of prescriptive therapies of one of the top 5 impacting disease states, COPD.

There is a history in medicine of implementing treatment modalities only later to learn that significant consequences may exist to prescribed therapies, contributing to debilitation and compounding costs. This phenomenon is nearly unavoidable as research limitations are many, but it remains the responsibility of the scientific research community to monitor implemented goal directed therapy.

Many subscribe to the fallacy that more is better. We are a country of indulgence, best demonstrated by an overwhelming epidemic of obesity. While oxygen is absolutely critical to human health and survival, too much, however, can increase morbidity and mortality. While oxygen supplementation is necessary during periods of compromised health states, a notion that more is better is a consequential one. Atmospheric oxygen, approximately 21%, is enough to sustain all human metabolic processes. The belief that oxygen is innocuous introduces unwitting consequence to its recipients. Educating healthcare providers about the full potential of oxygen therapy is imperative.

Oxygen is one of the most widely, and necessarily, prescribed therapies used in this country. This fact obligates those responsible for its prescription to better understand its pathologic potential. An increased understanding of the potential of oxygen exposure may lead to a more rational basis for its clinical use as well as the development of therapeutic measures effective in preventing or diseasing the effects of oxygen toxicity. Donabedian's structure-process-outcome framework can be utilized to guide this research study in assessing the potential of low flow domiciliary oxygen therapy in the advanced stages of COPD. In order to begin, however, to appreciate what is abnormal we must appreciate what is considered "normal."

Normal Respiratory Function

The human respiratory system is comprised of a series of structural pathways and specialized tissues operated by musculature for the purpose of drawing oxygen into the body for delivery to tissues for the manufacturing of energy and the subsequent removal of the waste products of metabolism through exhalation. The structures involved in this process include nasal passages (responsible for warming, humidification, and trapping of particles), trachea, bronchi, and bronchioles, which ultimately terminate into alveolar sacs or alveoli. Alveoli are specialized membranes that are enveloped in pulmonary capillary beds allowing for an intimate relationship of gas exchange between the pulmonary circuit and the vasculature. The inward and outward movement of air in the lungs is made possible by a specialized dome-shaped muscle called the diaphragm, intercostal muscles, as well as the inherent elastic recoil properties of the lungs and chest wall.

Alveoli. Alveoli are the terminal sacs within the pulmonary circuit responsible for gas exchange. The alveoli are comprised of three types of specialized cells: type I and type II pneumocytes and alveolar macrophages. Type I pneumocytes are thin and flat and comprise approximately 97% of the alveolar surface. Their thin (~25mn wide), flat construction allows for

a decreased diffusion distance between the alveoli and the blood. Type II pneumocytes cover approximately 3% of the alveolar surface, account for ~15% of all parenchymal cells and have a variety of functions. These specialized cells contain surfactant phospholipids, surfactant A, B and C, lysosomes, and lysosomal enzymes. The primary function of type II pneumocytes is to synthesize and secrete surfactant. Surfactant is a lipid-protein mixture, which coats the inner walls of the alveoli preventing collapse by decreasing the surface tension. Secondary functions of type II pneumocytes include xenobiotic metabolism, the body's mechanism of neutralizing exogenous chemicals/toxins, achieved through the cytochrome P450 system, regulation of transepithelial ion transport, extracellular matrix protein and growth factors production (to aid in repair), the release of proteinase and proteinase inhibitors (to regulate turnover of alveolar matrix proteins), and the expression of cytokines (to mediate inflammation). Type II pneumocytes also assist in the epithelial repair process by migrating to an area of denudation, proliferation and differentiation into a specific cell type (Aoshiba and Nagai, 2003). Lastly, alveolar macrophages are responsible for host defense, immunological homeostasis and tissue remodeling (Lambrecht, 2006).

Alveolar ventilation. Alveolar ventilation is the essential process of oxygenation, the delivery of oxygen to the tissues, and ventilation, the removal of carbon dioxide via exhalation. Normal alveolar ventilation depends on respiratory rate and tidal volume, the amount of air inhaled and exhaled during periods of normal breathing. For the average individual the tidal volume equals approximately 7ml/kg or toughly 500 ml of air. During normal inspiration the pressure within the lungs is slightly negative due to the contraction of the diaphragm resulting in air movement into the lungs. Alveoli, in the absence of disease, are fully open during inhalation. During exhalation the dynamics contributing to alveolar expansion are lessened and could result in alveolar collapse. Alveoli resist collapse during the exhalation phase as a result of the presence

of surfactant, a surface-active lipoprotein present at the air-water interface producing a decrease in surface tension. Maintaining alveolar integrity during both phases of the breathing cycle results in ongoing alveolar ventilation.

Oxygen. Oxygen is an element belonging to the 16th group, or the Chalcogen group, on the periodic table with the atomic number 8 and an atomic weight of ~16 amu. Oxygen is the most plentiful element on the earth's surface. It is a colorless, odorless gas that comprises approximately 21% of earth's atmosphere. Twenty-one percent of atmospheric oxygen, in the absence of disease or periods of increased demands, fully supports human metabolic processes achieved through cellular respiration. The measurement of oxygen is often referred to in terms of atmospheres absolute, or Ata. Atmospheric oxygen is 0.21 ata at sea level.

Leonardo de Vinci proposed that air consisted of two different gases as it supported both flames and life. While several prior to 1772 had repeatedly prepared oxygen they failed to recognize it as an element. The discovery of oxygen was ultimately credited to Priestly in 1774, achieved by the heating of mercury and lead oxide; Carl Wilhelm Scheele independently reported this finding as well. Like de Vinci, Priestly too recognized the deleterious properties of a compound that could support both life and combustion as he made this warning in a six volume series on the properties of air entitled *Experiments and observations on different kinds of air*,

> *" From the greater strength and vivacity of the flame of a candle, in this pure air,*
>
> *it may be conjectured, that it might be peculiarly salutary to the lungs in certain*
>
> *morbid cases, when the common air would not be sufficient to carry off the*
>
> *phlogistic putrid effluvium fast enough. But, perhaps, we may also infer from*
>
> *these experiments, that though pure dephlogisted air might be very useful as a*
>
> *medicine, it might not be so proper for us in the usually healthy state of the body:*
>
> *for, as a candle burns out much faster in dephlogisticated than in common air, so*

we might, as may be said, live out too fast, and the animal powers be too soon

exhausted in this pure kind of air. A moralist, at least, may say, that the air which

nature has provided for us is a good as we deserve" (Priestly, 1775).

Priestly shared his findings regarding dephlogisticated air with Antoine Lavoisier, a

French colleague known now as the father of modern chemistry, who immediately realized the

significance of this finding. It was Lavoisier who named dephlogisticated air "oxygen," which

means acid producer, and by 1786 was publicly denouncing the phlogiston theory (Acott, 1999).

Thomas Beddoes, an English physician, followed Lavoisier's work and in 1794 published

Considerations on the Medical use and on the production of Factitious Air as a result of

collaboration with James Watt, a Scottish inventor and mechanical engineer. This is the first

recorded publication postulating the use of oxygen as a medical therapy, which Beddoes referred

to as "pneumatic therapy" (Stansfield and Stansfield, 1896). Beddoes opened the Pneumatic

Institute in 1799 primarily to treat those with various forms of consumptive disease, such as

tuberculosis or those stricken by environmental toxins. Watts' expertise in engineering aided

Beddoes in developing purified "aires" for inhaled therapy. The first clinical use of oxygen was

reported in 1868, and considered a treatment for bacterial pneumonia in 1885 (Petty, 2000). The

utilization of oxygen flourished from this point as a therapy.

Cellular respiration. Lungs are the portals of oxygen entry. It is what occurs at the

cellular level within the lungs that sustains life. Cellular respiration is the process of converting

food, oxygen and water to usable physiologic energy. The aerobic chemical reaction $C_6H_{12}O_6 +$

$6O_2 + 6H_2O \rightarrow 12H_2O + 6\ CO_{2\ +}$ heat (or energy) demonstrates this point. The energy produced

as a result is stored in the form of adenosine triphosphate (ATP). The process occurs in three

phases: 1) glycolysis 2) the Citric Acid Cycle and 3) oxidative phosphorylation, or electron

transport. The summation of energy produced during cellular respiration has a maximum yield of 36-38 ATP molecules (Voet and Pratt, 2006).

Glycolysis is the splitting of a six-carbon sugar into two molecules of a three-carbon sugar. As a result of this process two molecules of ATP, two molecules of pyruvic acid and two high-energy electron-carrying molecules of NADH are produced. In the presence of oxygen this is the initial step in cellular respiration. Glycolysis occurs within the cellular cytosol while the remaining processes occur within the mitochondria (Holt, Rinehart and Winston, 2006).

The mitochondria are highly specialized organelles distributed throughout the cytosol of human cells. A double membrane surrounds the mitochondria. These membranes are phospholipid bilayers embedded with proteins. The outermost membrane is smooth and contains the organelle, as well as housing the many proteins that form channels through which a variety of molecules can move in and out as necessary. The innermost layer is folded into cristae for enhancing productivity of cellular respiration through increased surface area, and contains the 5 complexes of necessary membrane proteins: NADH dehydrogenase (Complex I), succinate dehydrogenase (Complex II), cytochrome c reductase (Complex III), cytochrome c oxidase (Complex IV) and ATP synthase (Complex V) (Holt, Rinehart and Winston, 2006).

The citric acid cycle is initiated by the conversion of two molecules of three-carbon sugars to acetyl CoA, an essential coenzyme of cellular respiration. Through a series of intermediate steps several high-energy storing compounds, such as nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD^+), are produced as well as two ATP molecules. The FAD^+ and NAD^+ molecules are reduced as a result of this process to a form that carries the high-energy electrons to the next state, FADH and NADH respectively. The citric acid cycle only occurs in the presence of oxygen; however, it does not use oxygen directly (Holt, Rinehart and Winston, 2006).

The electron transport phase of cellular respiration is the stage that directly requires oxygen. The electron transport "chain" is a series of electron carriers aligned within the mitochondrial membrane. High-energy electrons are passed to oxygen during enzymatically initiated oxidative phosphorylation reactions resulting in a proton gradient and ATP production through the addition of a phosphate to ADP. While oxidative phosphorylation performs the life sustaining process of producing energy it also produces reactive oxygen species, highly bio reactive molecules, that contribute to senescence and disease (Kregal and Zhang, 2006).

Cellular homeostasis. The ability of a structure to regulate its environment to maintain stability is referred to as homeostasis. Cellular homeostasis must be maintained to support organism viability. Programmed cell death, or apoptosis, is a mechanism of cellular preservation and, thus, homeostasis. Carl Vogt was the first to describe this phenomenon in 1842, and continues to evolve. Apoptosis is a multi-step, multi-pathway process that occurs in all cells of the human body. A lack of apoptotic activity is believed responsible for the formation of many types of cancers and autoimmune disorders. Apoptosis occurs through cellular signaling, either intracellularly or extracellularly. Extracellular signals result from the presence of toxins, hormones, growth factors, nitric oxide or cytokines. Intracellular signals include those induced by heat, radiation, nutrient deprivation, viral infection, hypoxia and increases in intracellular calcium concentrations and stress resulting in the binding of nuclear proteins to glucocorticoids. The mitochondria of the cell play a significant role in regulation of apoptosis. The mitochondria can achieve cellular homeostasis through the Bcl-2 family, cytochrome c, caspase and Bax balancing activities. The Bcl-2 family is a group of proteins that regulate outer membrane permeability and can either be pro-apoptotic (Bax, BAD, Bak and Bok) or anti-apoptotic (Bcl-2 proper, Bcl-xL, and Bcl-w). Cytochrome c is a heme protein involved in apoptosis through the

binding of protease activating factor-1. Caspase belongs to the cysteine proteases and are

essential in cellular apoptosis, necrosis and inflammation (Menrad et al., 2010).

Oxygen transport/diffusion. The exchange of oxygen and carbon dioxide between the

lungs blood and tissues is controlled by diffusion and driven by concentration gradients.

Dalton's Law of Partial Pressures illustrates ($P_{total} = P_{gas\ a} + P_{gas\ b} + P_{gas\ c} + etc$) the total pressure

of all gases is equal to the sum of the partial pressures of each gas. The gas diffusion principle

supported by Fick's Law demonstrates that a gas diffuses from an area of higher partial pressures

to an area of lower partial pressures. Venous blood returning to the lungs has a lower partial

pressure of oxygen than the concentration within the alveoli facilitating movement of oxygen

from the alveoli into the blood. Poiseuille's Law describes the relationship between pressure,

flow and radius of communicating structures ($\Delta P = r^4/v$). Poiseuille's law can be applied to the

airways to address impedance of airflow. In the diseased lung, such as emphysema where airway

destruction results in loss of elastic recoil, resistance increases as small airways close during

exhalation. As the radius decreases in diseased airways the pressure required to move the same

amount of air through the small tube increase exponentially making alveolar ventilation less

efficient. When the radii of the airways are reduced by half the resistance is increased sixteen

times.

Finally, transport of oxygen is achieved through the binding of oxygen to the hemoglobin

molecules in the blood. The cardiovascular system is responsible for transporting the

oxygenated hemoglobin molecules in the blood to all the tissues of the body. Alterations in

hemoglobin and/or cardiac output can be deleterious for individuals with airway disease.

Protective mechanisms. The respiratory system has inherent mechanisms to control for

the many particulates and infectious agents that are introduced during inhalation. The nose serves

as a first line defense by trapping particulates over 10 μm in diameter and approximately half of

all particles of 3 μm aerodynamic diameter (referring to behavior vs. actual size) (Rubin, 2011). The airway is lined by epithelium that produces a mucociliary blanket responsible for trapping and ridding the lungs of particles 2-10 μm in diameter. The cilia, finger-like projections on the mucosal wall, move in a direction toward the trachea to facilitate removal. Once particles reach the trachea they are expectorated from the lungs through the act of clearing or coughing.

For particles that find their way into the alveoli, alveolar macrophages (AMs) come to action. Metchnikoff first described the macrophage in the 1800s (Rabinovitch, 1995). Macrophages are one of the main mediators of the immune response exhibiting multiple functions, such as phagocytosis (engulfing and ingestion of foreign substances), secretion of cytokines and chemokines (cell signaling), tumor cytotoxicity, and certain antigen presentation (the capture of antigens and enabling their recognition by T-cells).

Macrophages are greatly distributed throughout the body and are calcium-mediated signaling dependent (Hoyal, et al, 1998). They can be found in the lymphoid organs, liver, lung, gastrointestinal tract, central nervous system, serous cavities, bone, synovium and skin (Monick and Hunninhake, 2002). As one of the major effector cells of the immune system, macrophages are found in abundance at sites where organisms interact with the environment. The immense surface area of the adult human lungs (140 m^2) allows for a significant exposure to pathogens and pollutants on a perpetual basis thereby creating a large reservoir of alveolar macrophages (AMs) (Gwinn and Vallyathan, 2006). The macrophages found in the lungs are compartmentalized in airways, interstitium, and intravascular spaces derived from pulmonary circulating monocytes. Alveolar macrophages are quick responders to many internal and external stimuli, such as interferon, cytokines, viruses, viral particles, bacteria, airborne pathogens and particles and changes in calcium (Hoyal et al, 1998). Alveolar macrophages are derived from bone marrow, undergo a maturation process within the interstitium of the lungs and then enter

the alveolar space. Alveolar macrophages are effective in handling particles with an aerodynamic diameter under 2 μm. Through highly adaptive properties AMs provide protection by identifying offending substances and differentiating to the specific state necessary for pathogen destruction. Through phagocytosis, an AM engulfs a pathogen, which initiates a series of processes that culminate in death of the microbe. During phagocytosis, the phagocytic cell undergoes an increase in glucose and oxygen consumption referred to as respiratory burst, resulting in the production of reactive oxygen species (ROS). Three main processes commence as a result of AM initiation: 1) Oxygen-dependent myeloperoxidase-independent intracellular killing. During phagocytosis, glucose is metabolized via the pentose monophosphate shunt, resulting in the formation of nicotinamide adenine dinucleotide phosphate (NADPH), a coenzyme used in anabolic reactions. Cytochrome B from the granulocyte specific granule combines with and activates plasma membrane NADPH oxidase, a membrane bound enzyme complex found in the membranes of phagosomes used by neutrophils to engulf microorganisms. The activated NADPH oxidase uses oxygen to oxidize the formed NADPH with a resultant production of superoxide anion (O_2-). A portion of the superoxide anion is converted to H_2O_2 plus singlet oxygen by superoxide dismutase. Also, superoxide anion can react with H_2O_2 to form a hydroxyl radical as well as more singlet oxygen. In combination these reactions produce the toxic oxygen compounds superoxide anion (O2-), H_2O_2, singlet oxygen (O_2) and hydroxyl radicals (OH•). Ultimately, while the formation of free radicals is toxic to microbes, when left unchecked they contribute to the derangement of the lipid bilayers of the lungs. 2) Oxygen-dependent myeloperoxidase-dependent intracellular killing. When a particle is absorbed by the phagocyte a vesicle is formed call a phagosome. The phagosomes bind with lysosomes, organelles containing digestive enzymes, within the phagocyte forming phagolysosomes. The binding of these granules with the phagosomes causes release of myeloperoxidase into the phagolysosomes.

Myeloperoxidase utilizes H_2O_2 and halide ions (typically Cl-) to produce highly toxic hypochlorite. Some hypochlorite molecules spontaneously break down to yield singlet oxygen. Together these reactions produce toxic hypochlorite (ClO-) and singlet oxygen (O_2). Hypochlorites are very strong oxidizing agents. 3) Detoxification reactions. Neutrophils, first responder phagocytes, and macrophages are able to protect themselves by detoxifying the toxic oxygen intermediates that they generate. Granulocyte self-protection is achieved in reactions employing the dismutation of superoxide anion to hydrogen peroxide by superoxide dismutase and the conversion of hydrogen peroxide to water by catalase. The respiratory burst in AMs is of significantly greater magnitude than those in other forms of phagocytes, likely due to the life sustaining and important boundary between the outside world and the body they provide (Baldridge, 1932; Bellavite, 1988; Nauseef, 2004; Fang, 2011). All aforementioned processes describe the necessary and life-protecting role of reactive oxygen species.

The susceptibility of the lungs to environmental toxins and the large surface area can result in an overproduction of reactive oxygen species. Antioxidants exist within the lungs to balance ROS activity. The most prevalent antioxidant of the lungs is glutathione. Glutathione is located in the epithelial lining of the lungs and in the lower respiratory tract. It has four major functions, 1) a major endogenous antioxidant produced by the cells participating directly in the neutralization of free radicals and reactive oxygen compounds, as well as maintaining exogenous oxidants, such as vitamins E and C in their reduced form; 2) regulation of the nitric oxide cycle is critical for life but can be consequential if unregulated; 3) involved in biochemical reactions, such as DNA synthesis, prostaglandin synthesis, amino acid transport, and enzyme activation; and 4) iron metabolism.

The protective mechanisms of the human respiratory tract described above are efficient and effective in defending against infection and pathology of most offending agents. There are,

however, many diverse organisms that prove challenging for the defenses of the human respiratory tract, such as adenovirus, respiratory syncytial virus, influenza, measles, pertussis and tuberculosis (Rubin 2011). Similarly, irritant gases derived from pollution and industrial accidents also challenge our respiratory defense systems. Environmental oxidants include ozone and nitrogen oxides, as well as sulfur dioxide. Environmental oxidants, for example, are derived from the suns interaction with automobile exhaust. Sulfur dioxide is produced mainly through the burning of fossil fuels. It is believed that the combination of these environmental conditions, along with cigarette smoke, contributes to pulmonary pathology. For those with existing pulmonary disease, such as chronic obstructive pulmonary disease, these pollutants can produce serious morphologic and functional effects.

Respiratory Mechanics

Respiratory mechanics refer to the principles of compliance, elastance, resistance, impedance, flow, and work of breathing. In respiratory physiology compliance refers ability of a hollow organ to distend and resist recoil to its natural resting position by overcoming transmural pressure (Grinnan and Truwit, 2005). Compliance can be expressed by the following equation: $C = \Delta V/\Delta P$, where C = compliance, and ΔV is the change in volume, ΔP is the change in pressure. The inverse of compliance is elastance ($E \sim 1/C$). During inflation airway pressure is influenced by volume, chest wall and lung compliance, and thoracic resistance to flow. Resistance to flow must be eliminated if compliance is to be measured accurately. This can be achieved by measuring pressure and volume during a period of no flow. As a result, compliance is determined by taking static measurements of the distending pressure at different lung volumes and can be obtained during inflation or deflation. Dynamic compliance can also be calculated. Compliance can be affected by chest wall and/or lung stiffness.

Elastance, or elastic recoil, refers to the lungs ability, or the ease, to snap back after the stretch of inhalation. During expiration the diaphragm relaxes allowing the lungs to recoil and regain the interpleural pressure experienced at rest. The phenomenon occurs due to the elastic fibers in the connective tissue of the lungs in conjunction with the surface tension in the alveoli provided by surfactant (Sherwood, 2007).

Resistance refers to opposition within the respiratory circuit to the inward and/or outward flow of air. Resistance can be expressed by the following equation $R_{aw} = \Delta P/V$, where $\Delta P = P_{atm} - P_{A}$, so that $R_{aw} = P_{atm} - P_{A}/V$ (P_{atm} = atmospheric pressure, P_{A} = Alveolar pressure and V= Volumetric airflow). Determinants of airway resistance, then, are airway diameter and whether flow is turbulent or laminar (Kirkby et al., 2010).

Flow (Q) is simply the movement of air. Flow is dependent on a pressure gradient (ΔP) and is inversely related to resistance of flow (R). This relationship is described by the following equation: $Q = \Delta P/R$. Two types of flow exist within the respiratory circuit: laminar flow and turbulent flow. The more distal the airway the more laminar the flow becomes. Flow is influenced by the rate of flow (V), the airway radius (r), the density of gas (p), and the viscosity of gas (η), while factors, such as length of airways and gas density remain essentially constant. In the airways governed by laminar flow, resistance is related to the radius (r), airway length (l), and gas viscosity (η) through Poiseuille's Law ($R=8\eta l/nr^4$). Poiseuille's law demonstrates the strong relationship of the radius on resistance, showing that the doubling of the radius decreases the resistance by 16-fold (Bock, et al, 2000).

Impedance refers to the resistance of airflow as well the energy required to overcome the elasticity of the lungs and chest wall. Impedance is most accurately assessed through the measurements of the work of breathing (WOB). Work is defined as the product of pressure and volume (W = P x V). Since exhalation is generally a passive process WOB is most often

referring to inhalation. However, diseases processes, such as COPD, that exhibit prolonged expiratory phase, can significantly increase WOB. As WOB increases metabolic demands, increased stress is placed on respiratory muscles, which in turn requires increases in cardiac output. Increases in cardiac output can result in ischemia for those with concomitant cardiac disease. The goal of mechanical ventilation for those in acute respiratory distress is to support the vital organs while reducing the WOB. Once the acute phase of distress is resolved ventilatory support can be weaned until extubation is possible (Grinnan and Truwit, 2005).

Spirometry. The ability to quantify the aforementioned respiratory mechanics of compliance, resistance, elastance, flow and impedance is particularly useful in detecting, diagnosing and managing respiratory health and disease states. Quantification occurs through the use of spirometric measurements. Exposing a potential relationship between spirometric measurements and the presence of deleterious processes, such as oxidative stress, could hold merit in early detection and management.

Spirometry, or the measuring of breathing, enables us to assess functional changes within the respiratory circuit and provide meaning to their numeric value. Spirometry is a tool used to measure various lung volumes, capacities, flow rates, patterns and gas exchange. Collectively theses measurements taken for the purpose of assessment are referred to as pulmonary function tests (PFT). John Hutchinson is credited with the invention of the spirometer in 1846 (Petty, 2002). This early device could only measure vital capacity, the volume of air exhaled after maximal inhalation. The evolution of the spirometer has resulted in the ability to measure nearly every aspect of lung function making this device elemental in the diagnosing and management of COPD and a host of other respiratory ills. It would not be until the 1950's that spirometric measurements would be given their due when Barach and Bickerman (1956) edited a comprehensive text, *Pulmonary Emphysema*, in which Dayman recognized and described flow

volume patterns indicative of expiratory airway collapse consistent with emphysema. In 1956, Hinshaw and Garland would demonstrate a 13.5-liter recording spirometer, showing lung capacity spirograms that demonstrated the airflow limitations seen with emphysema. The sophistication of spirometric assessment grew from then and continues today.

Each phase of the respiratory cycle has a corresponding volume or capacity, and flow rate specific to that given phase. Once adjustments are made for age, gender, height, weight and ethnicity particular changes in volumes, capacities and/or flow patterns can be predictive of pathology. Pulmonary diagnoses can be made when obtained values are compared to a "normal" value. Normal spirometric values vary, depending on environment, culture, gender and physical stature. In order to achieve an accurate assessment of an individual, obtained results needs to be compared to a subset of healthy patients with the same/similar demographics. It should be emphasized that effort is critical when obtaining values for the "normal" and afflicted. All individuals obtaining PFTs should be instructed on the importance of maximum respiratory effort and cooperation to ensure accuracy of test results. Tests are often repeated in triplicate to control for this phenomenon. For those individuals afflicted with COPD respiratory effort is an issue due to the debilitating effects of the disease, including muscle wasting and cachexia. Results obtained are compared to the normal volumes, capacities and flow rates of a similar population (age, gender, height, weight, ethnicity) and given percent of predicted values. Based on the aforementioned demographics a "normal" PFT value is assigned. Once a value is obtained it is compared to the "normal" PFT value for that individual. Pulmonary function values nearest 100% are considered most normal. Generally any value less than 80% of "predicted" normal for said characteristic can be indicative of underlying disease.

The past several decades has brought with it research to determine "normal values" for lung volumes, capacities and flow rates as it relates to specific demographics, and has greatly

facilitated interpretation of the results for accurate diagnosing. The relevant variables in this process are age, gender, body height and habitus, and ethnicity. As it relates to age: as a person ages the natural elasticity of the lung decreases, translating into smaller lung volumes and capacities. Also, aging causes decalcification of the skeletal structure resulting in centimeter changes in the thoracic size and subsequent lung size. In the healthy adult, this does not necessarily impede daily function; however, for those with respiratory disease, this phenomenon can be a confounding cofactor. As it relates to gender: typically the lung volumes and capacities of males exceed those of females even when matched for height and weight. As it relates to body height and habitus: size has a significant impact on lung volumes and capacities, as such predictive values have been developed to account for those sizes outside the range of ideal body weight for given height. Obesity, generally defined as a body mass index (BMI) of ≥ 30 kg/m^2, can often appear during pulmonary function testing as a restrictive disorder due to the physical impediment placed on the chest wall by the chest and abdominal weight. Standing height is considered the most important predictor of lung function and is related, to some degree, to the other demographics that influence forced vital capacity (FVC) values (the forced exhalation following maximal inhalation), such as weight, presence of disease, scarring, etc. (Cotes, 1993). Diseases that affect stature, such as kyphosis, scoliosis, osteoporosis, congestive heart failure, tuberculosis, etc., will predictably decrease lung capacities. Age also has a confounding influence on the FVC as aging often results in further loss of muscle mass resulting in the displacement of the diaphragm cephalad causing concomitant decreases in lung volumes and capacities. As it relates to ethnicity: different ethnic origins have inherent differences in lung volumes and capacities, as such spirometric software has been adjusted to address the related differences. These differences appear to be primarily a result of stature and metabolic indices specific to a given culture.

Pulmonary function testing can be used for the screening of obstructive or restrictive lung disease states, documenting obstructive or restrictive lung disease progression, evaluating therapeutic effectiveness, as a tool for weaning mechanical ventilation, as well as evaluating patients prior to surgery, especially if patients are > 60 years old, have known pulmonary disease, are obese, have a history of smoking, cough or wheezing, been exposed to prolonged surgical times or are undergoing abdominal or thoracic surgeries. (http://www2.nau.edu/~daa/lecture/pft.htm).

The ability to quantify spirometric measurements aids in assessing lung function. Spirometric measurements are obtained with a relatively simple apparatus by which a patient inhales and exhales into a mouthpiece. The force of these two efforts is measured by a differential pressure transducer, converting the volume and rate of breathing over a specified amount of time into a quantitative value. Parameters, such as rate of breathing and ventilation, vital capacity (the maximum amount of air exhaled after maximum inhalation), tidal volume (the amount of air exchanged during normal breathing), and variations of expiratory volumes (FEV), vital capacity from a maximally forced expiratory effort (FVC), peak expiratory flow rates (PEFR), forced expiratory flow rates (FEF), and maximal voluntary ventilation (MVV), and many others, can be determined. As these values are plotted on a graph inferences can be made regarding pulmonary functionality.

The forced expired volume in 1 second (FEV1), FVC, and their ratio to each other (FEV_1/FVC) are often initially evaluated to assess lung function. The FEV1 is the volume of air that can be forcibly exhaled in one second after maximal inhalation and is valuable in assessing for obstructive disease. Average values for FEV_1 in healthy people depend mainly on gender and age. Results between 80% and 120% of the average predicted value or ~ 75-80% of their vital capacity (volume of air following maximal inhalation) are considered normal. If the FEV1 value

31

is low (<80%) compared to FEV$_1$% predicted in the normal population in the presence of maximal effort obstructive disease is highly suggested. The diagnosis of restrictive vs obstructive disease is further refined when expressed in terms of a proportion to the FVC. A low FEV1 (<75%) in conjunction with a low FEV$_1$/FVC ratio of (70%) is indicative of obstructive disease. The FEV1 for the patient with restrictive disease will be lower as well but proportional to the FVC and, therefore, if both the FEV1 and the FEV1/FVC ratio is 85% or greater of predicted normal, a diagnosis of restrictive disease is highly suggested.

Spirometric results consistent with obstructive disease, such as chronic bronchitis and emphysema, include changes in the FVC. The forced vital capacity is the amount of air that can be maximally and forcibly expelled from the lungs after maximal inhalation. For the patient with obstructive disease expired volumes will be less than normal and require longer amounts of time to achieve. Spirometric results consistent with restrictive disease, or emphysema, include changes in the FVC as well; however, the resultant decrease in expired volume is due to a reduced ability to maximally inhale normal volume due to the mechanical limitations as a result of the loss of alveolar elastic wall integrity inherent with the disease. Since both chronic bronchitis and emphysema result in smaller FVC volumes FVC is not a stand-alone diagnostic indicator of either disease. Concern does not typically occur until the FVC result in 80-85% of the predicted normal values. Delineations can occur with a post-bronchodilator FVC measurement. Improvements in the FVC following a bronchodilator suggest obstructive disease, while no change suggests restrictive disease.

Detection of pulmonary changes consistent with COPD can be made with spirometry before debilitating stages develop. According to the National Lung Health Education Program, all persons aged 45 or older who currently smoke, or even those who have quit smoking, should have spirometric testing (Ferguson et al., 2000). Additionally, those individuals who present

with symptoms of a chronic cough, sputum production, wheezing or dyspnea that is inconsistent with the activity being performed should undergo spirometric testing (Ferguson, et al., 2000; Celi and MacNee, 2004).

COPD

Chronic obstructive pulmonary disease (COPD) is a progressive disorder resulting in irreversible lung tissue changes. Approximately 14 million Americans have COPD and an estimated 40 million cases are believed to be undiagnosed (CDC, 2008). COPD is currently the fourth leading cause of death and projected to be the 3rd leading cause of death by 2020 (Doherty et al., 2007). COPD kills approximately 120,000 Americans annually (Mannino et al., 2002). Normal tidal breathing for the average adult occurs approximately 17,000 times per day. Depending upon the stage of the disease the effort to take those 17,000 breaths increases, resulting in fatigue, anxiety and general discomfort. As the disease progresses microscopic changes occur to the multiple organ systems within the body. Identifying a method to detect the early presence of COPD to thwart its progression is ideal; an effort that would improve quality of life and lessen the economic burden on the afflicted individual, their family and the healthcare system.

Descriptions of COPD can be traced back to 1679 in Bonet's description of the "voluminous" lungs (Bonet, 1679). In 1769 Morgagni described 19 cases in which the lungs were "turgid" or dull with air. Baille, an early pathologist, utilized the investigation of post mortem human tissue to better understand the pathology that may have claimed lives. In his 1795 manuscript, *The morbid anatomy of some of the most important parts of the human body,* Baille described the morbid appearance of each organ and correlated autopsy reports with case histories. Included in this compilation is the first documented observed physical description of the emphysematous lung.

Badham first offered clinical understanding of COPD in 1814 when he used the word "catarrh" to refer to the classic symptoms of COPD, cough and hyper secretion of mucus. He noted this condition to be disabling. Laennec (1821), a clinician, pathologist and inventor, eloquently describes emphysema in his *Treatise of diseases of the chest*, noting that emphysematous lungs were hyper inflated. Laennec dissected patients he followed during the course of their illness. Part of his assessment was the utilization of a stethoscope, his own invention. He described his auscultative assessments in *A treatise on the disease of chest and on mediate auscultation* and provided this anecdote,

> "*The disease which I designate by this title is very little known and has not hitherto been correctly described by any author. I for a long time thought it very uncommon, because I had observed only a few cases of it: but since I have made use of the stethoscope, I have verified its existence as well on the living as the dead subject, and am led to consider it as by no means infrequent. I consider many cases of asthma, usually deemed nervous, as depending on this cause. The chief reason of this affection having been so completely overlooked is, that it is in some sort of merely the exaggeration of the natural condition of the viscous.*"

Laennec also reported that upon autopsy it was not unusual to find that the lungs did not collapse for those with symptomatic disease but rather filled up the chest cavity completely on either side of the heart. Further inspection would show that the lungs were full of air while the bronchus was filled with mucus. This description appears to describe both bronchitis and emphysema.

The 1950's ushered in significant contributions to the understanding of COPD and its effects on related organs. Dickerson Richards, Nobel Laureate, described the relationship

between a diseased pulmonary circuit leading and right sided heart failure, or cor pulmonale; Cherniack described the diseases effect on the acid-base system; Menelee and Callaway described pulmonary function test results as it related to the emphsymic patient (Petty, 2006).

In an effort to develop a comprehensive and accurate definition of the disease two historical meetings took place: The CIBA Guest symposium in 1959 and the American Thoracic Society Committee on Diagnostic Standards in 1962. The definition provided by the American Thoracic Society (ATS) is the most widely accepted definition and states, "a diagnosis of chronic bronchitis can be made with the presence of a chronic cough lasting at least three months for at least two years; and that the diagnosis of emphysema can be made when evidence of enlarged alveolar spaces and loss of alveolar walls is made."

These ambiguous definitions prompted continued research in order to better understand optimal recognition, diagnosis and management. In 1998 a group of committed scientists encouraged the US National Heart, Lung and Blood Institute and the World Health Organization to come together to combat COPD. This group is now formally recognized as the Global Initiative for Obstructive Lung Disease, or GOLD.

Global initiative for obstructive lung disease: GOLD. The Global Initiative for Obstructive Lung Disease (GOLD), a group of dedicated health care providers, continually work with healthcare professionals and public health officials to raise COPD awareness and to assist in improving treatment of the disease worldwide.

The first task undertaken by GOLD was to prepare a consensus report, *Global Strategy for the Diagnosis, Management and Prevention of COPD*, published in 2001. Revisions are continually made to reflect ongoing research (GOLD 2006).

With the goal to provide an individual with the appropriate management therapy, GOLD developed a classification of COPD by dividing it into 4 stages: mild, moderate, severe and very

severe. Certain normal values have been assigned as it relates to age, gender, height, weight and

ethnicity. The ratio is used to diagnose obstructive and restrictive disease. Table 1 defines the

stages in terms of pulmonary function test results.

Table 1

Stages of COPD

Stage I	Mild COPD	FEV1/FVC<0.70	$FEV_1 \geq 80\%$ normal
Stage II	Moderate COPD	FEV1/FVC<0.70	FEV_1 50-79% normal
Stage III	Severe COPD	FEV1/FVC<0.70	FEV_1 30-49% normal
Stage IV	Very Severe COPD	FEV1/FVC<0.70	FEV_1 <30% normal, or <50% normal with chronic respiratory failure present

Stages of COPD. In an effort to accurately assess and appreciate an individual's stage on

the COPD spectrum a series of stages are defined. During Stage I of COPD there may be only

mild airflow obstruction with no awareness of functional decline. Parenchymal, or lung tissue,

changes are typically insidious leaving patients unaware that lung damage is occurring.

Treatment is rarely sought during this stage. Airflow worsens during Stage II as parenchymal

damage worsens and symptoms become increasingly detectable, particularly shortness of breath

upon exertion and cough with sputum production. It is typically during this stage that patients

become aware of the presence of the disease. Upon entering Stage III, or the severe phase of the

disease, the patient experiences airflow obstruction that significantly worsens, shortness of

breath occurs with less and less exertion, and COPD exacerbations becoming more frequent. It

is during this stage that people begin to battle constant fatigue. Stage IV may lead to chronic

respiratory failure, the inability of the lungs to effectively oxygenate and/or ventilate, cor

pulmonale and death (Leader 2011). To better understand the stages of COPD it is necessary to understand the physics of the pulmonary circuit and the concomitant changes.

In a review performed by Tuder, et al (2006) a literature search ranging from 1949 to 2005 revealed that 40,309 papers were published on COPD, in which 1637 involved the worked "inflammation" and 187 involved "protease" or "matrix." Forty-six papers directly addressed inflammation and protease imbalance. It is important to consider these factors when formulating an understanding about the pathology that results in emphysema and chronic bronchitis.

When considering the mechanics of airflow the pulmonary circuit is inherently affected by tubular diameter and pressure changes, two variables directly impacted by inflammation and matrix changes within the pulmonary infrastructure. As a result reduced airway diameters produce increases in resistance, whereas loss of elastic recoil results in diminished pressure on exhalation. Airway narrowing occurs in chronic bronchitis or asthma, whereas emphysema exhibits a loss of elastic recoil, resulting in decreased alveolar ventilation and air trapping.

Emphysema. Emphysema is characterized by the loss of alveolar wall integrity resulting in increases in work of breathing in response to increases in compliance and increases in airway resistance. Airway mutagens, such as cigarette smoke or environmental pollutants incite apoptosis, oxidative stress and a protease/antiprotease imbalance resulting in a breakdown in alveolar infrastructure (Tuder, et al., 2006). Progressive deterioration of wall integrity ultimately manifests as loss of elastic recoil. Many alveolar sacs will merge into large singular bleb-like sacs and will lose their pulmonary capillary architecture. The subsequent inability of alveolar recoil results in outflow obstruction; air becomes trapped in the alveoli resulting in poor gas exchange (Petty, 2006). Stagnation of air movement means decreased oxygen supply and retention of carbon dioxide. Low oxygen states within the body are compensated by increasing hemoglobin concentrations and through hyperventilation.

As more and more alveoli break down hyperventilation is no longer an effective compensatory mechanism and worsening hypoxia ensues. Hypoxic vasoconstriction, the rerouting of blood to functional alveoli, occurs as the body's last resort to restore equilibrium. Chronic hypoxic vasoconstriction can result in pulmonary hypertension, or high blood pressure, within the pulmonary vascular circuitry. A significant consequence of pulmonary hypertension is right-sided heart failure, or cor pulmonale. The right ventricle must overcome the increased pulmonary pressure to maintain perfusion pressure. In an already hypoxic environment, the heart is vulnerable to increased metabolic demands. The accessory musculature of the respiratory system experience increased oxygen demands as a result of increased work of breathing to compensate, placing even greater strain on the heart and lungs.

While there are some genetic predispositions associated with emphysema, such as alpha-1 antitrypsin disorder, it is most directly linked to smoking. Most individuals diagnosed with emphysema are over 60 years old and present with a long history of smoking, exertional dyspnea with use of accessory muscles, tachypnea with a prolonged expiratory phase, minimal nonproductive cough, and weight loss and cachexia. Cachexia presents a vicious cycle as the malnourishment contributes to ongoing weakness that in turn contributes to ongoing anorexia. Those individuals who suffer from advanced stages of the disease may require low flow domiciliary oxygen therapy (LFDO) therapy to assist in ameliorating symptoms demonstrating the properties of Dalton's Law of partial pressure, supporting diffusion through an increase in the partial pressure of oxygen, and Poiseuille's Law of increased rates of flow by air being forcibly delivered into the nasal passages and down the airways.

Chronic bronchitis. Chronic bronchitis is characterized by inflammation of the bronchi resulting in a copious production of thick mucus, hypertrophy of mucous glands and recurrent respiratory infections. The mucus produced in this disorder, as well as the chronically inflamed

bronchioles, creates a physical obstruction to airflow as well as a constant predisposition to pulmonary infections. Patients who are diagnosed with chronic bronchitis have exhibited symptoms for greater than three months in a year for two consecutive years in a row. Cough and sputum production are initially more severe in the winter; however, as the disease progresses, the productive cough remains present. The chronic obstruction to airflow produced by COPD states predisposes these patients to low oxygen blood states, or hypoxia, as well as high carbon dioxide blood states, or hypercarbia (Chiang et al., 2002).

Chronic hypercarbia places the COPD patient at risk of cardiovascular and central nervous system compromise (Petty 2006). Blood CO_2 levels are responsible for communicating with the respiratory center in the brain to determine rate and depth of breathing. When CO_2 levels are chronically elevated respiratory center receptors become desensitized, thus, dependence on oxygen levels for respiratory stimulation ensues. Although hypercarbia is a compensatory mechanism to manage respiratory stimulation, elevated levels of CO_2 can produce anxiety, irritability, confusion, stupor, coma, hypertension, hypotension, dysrhythmias and death (Petty, 2006).

Patients in advanced stages of chronic bronchitis are vulnerable to opportunistic infections. Low oxygen states, the presence of mucous and compromised respiratory immune systems puts this patient population at a particular risk for bacterial infections, particularly *Hemophilus influenza and S. pneumonia* (Murphy, 2000). Chronic hypoxemia predisposes the COPD patient to a wide range of complications including metabolic and electrolyte disturbances, erythrocytosis or polycythemia and, perhaps the most detrimental, pulmonary hypertension and right sided heart failure, or cor pulmonale (Chaouat, 2008). The development of cor pulmonale is often accompanied with cyanosis and edema giving the afflicted patient a blue and swollen appearance, oftentimes referred to as the "blue bloater." Many patients in the advanced stages of

the disease are placed on LFDO in an effort to provide them with needed support to maintain independence and functionality. Oxygenation is impaired in this patient population. Optimizing oxygenation through LFDO via a nasal cannula has significantly improved symptomatology and activities of daily living (ADL's). Patients with advanced COPD often become easily fatigued and short of breath. Domiciliary oxygen therapy can boost energy levels and lessen labored breathing by increasing oxygen concentration in the lungs and blood (Croxton et al., 2006; Sandland, 2008).

Hypoxia. Hypoxia is the state of insufficient oxygen delivery necessary to support cellular respiration, and cannot be long tolerated. Mechanisms of hypoxia/hypoxemia include hypoventilation, low inspired oxygen, anemia, right to left shunt, and perfusion/ventilation mismatching (V/Q mismatch) (Osborne, 2000). There are several causes for hypoventilation: drugs that depress the CNS, such as narcotics, anxiolytics and sleep aids, trauma to the brainstem, abnormal spinal cord pathways, and brain stem motor neuron disease, such as amyotrophic lateral sclerosis (ALS) (Phukan, et al., 2007); diseases affecting the respiratory muscles, such as Guillain-Barre' syndrome; diseases of the neuromuscular junction, such as Myasthenia Gravis; abnormalities of the chest wall, such as scoliosis (Laghi and Tobin, 2003); and upper airway obstruction, such as epiglottitis. Low inspired oxygen can be a result of altitude changes or medical mismanagement for a dependent patient. A right to left shunt refers to phenomena where a "rerouting" of blood results in reduced oxygenation of the blood. This phenomenon can occur through an anatomic shunt, such as a coronary sinus, where unoxygenated blood drains into the left heart and mixes with saturated blood. A physiologic shunt refers to disease states by which blood cannot come into communication with gases within the alveoli due to filling of the alveolar spaces as seen with pulmonary edema, pneumonia, etc. Ventilation/perfusion (V/Q mismatch) refers to the disparity between alveoli that are well

ventilated but poorly perfused, or poorly ventilated and well perfused. This phenomenon can occur from something as benign as positioning to the presence of atelectasis. Diffusion impairment refers to interruptions in the rate of diffusion of the gases across the alveolar membrane. This phenomenon can occur with a variety of interstitial lung diseases. Anemia results in hypoxemia due to the absence of sufficient oxygen carrying capacity in the form of hemoglobin. Anemia is prevalent in such processes as kidney disease, uterine fibroid disease and trauma.

Compensatory mechanisms. In the presence of acute low oxygen states, or cellular hypoxia, a series of mitochondrial protective mechanisms will occur to maintain cellular homeostasis. Likely considered the most important rate-limiting step in mitochondrial oxygen consumption is the decrease in cytochrome c oxidase activity. Cytochrome c oxidase is responsible for converting molecular oxygen into two molecules of water. The mitochondria, the site responsible for initiating biochemical processes that determine cell survival or death, will maintain ATP production temporarily via anaerobic glycolysis. The untoward effects of the decreased cytochrome c oxidase activity and anaerobic glycolysis is a backlog of protons at all the redox points along the electron transport chain and the production of pyruvate respectively, both of which will ultimately result in the build up of cellular acids and eminent cell death (Galkin et al., 2009, Wheaton and Chandel, 2011).

In the acute phase of hypoxia the release of cytochrome c oxidase allows for the adaptation to altered oxygen concentrations through the inhibition of proton transportation, thus decreasing the membrane potential. The decrease in ATP production and a hyper permeable state of the inner mitochondria is a result of the release calcium and cytochrome c through the Bcl-2-associated X (BAX) system, a process that can also lead to caspase activation and programmed cellular death (apoptosis).

The maladaptive response of cytochrome c initiated cell death in the presence of prolonged hypoxia converts to an adaptive process over time by the inhibition of BAX and the generation of nitric oxide. Nitric oxide binds to a reduced form of cytochrome a3 in the mitochondria, the same binding site of oxygen, resulting in inhibited cytochrome c activity and thus reducing oxygen consumption. Furthermore, the presence of nitric oxide changes the membrane potential due to the increased production of reactive oxygen species (ROS). Oxygen free radicals, through superoxide dismutase (SOD), are converted to H_2O_2, which exerts a protective influence on cellular enzymes, such as the kinase of monophosphate adenine (AMPK) resulting in cellular energy homeostasis. (Mungai, 2011).

In the presence of chronic low oxygen states cells will continue to adapt through oxygen-dependent enzymes: the hypoxia inducible factor (HIF)-prolyl and asparaginyl hydroxylases, prolyl hydroxylases domain (PHDs), and factor inhibiting HIF (FIF). The result of chronic hypoxia is a "pooling" of PHDs and an over activation of the three PHD isoforms (HIF1α, HIF2α and HIF3α), mediated by an increase in intracellular oxygen availability (Ginouves, 2008). The desensitization of HIFα triggers a negative feedback mechanism that results in cellular protection against cell death.

Cigarette smoking. Tobacco use is the leading preventable cause of death globally, claiming 5 millions deaths annually and a projected 8 million annually by 2030 (WHO, 2011). A reported 480,000 people die annually in the U.S, with 41,000 of those from exposure to second hand smoke (US Dept of Health & Human Resources 2013 Report). Cigarette smoke has been implicated as the single most significant contributor to the development of COPD. Cigarette smoke induces two particular processes within the lung tissue that contribute to structural changes associated with COPD: inflammatory responses and oxidative stress. It is, therefore,

important to consider the implications and role smoking in the development of COPD in order to better develop appropriate strategies of prevention, treatment, and cure.

In a study by Fletcher and Peto (1977) it was projected that 15-20% of those who smoke would be vulnerable to the long-term deleterious effects of smoking and would subsequently develop airflow limitations. The GOLD 2005 Report from the NHLBI/WHO workshop reported that cigarette smoke-induced airway inflammation is considered to be an important variable in the pathogenic process of COPD.

When toxicants, such as cigarette smoke, are breathed into the lungs, particles are trapped in the alveoli and initiate an inflammatory cascade that includes the activation of neutrophils. Neutrophils contain serine elastase and other proteases that serve to catabolize proteins resulting in the breakdown of the alveolar infrastructure. Neutrophils also contribute to the development of reactive oxygen species (ROS), otherwise known as free radicals. Reactive oxygen species overwhelm lung antioxidant defenses, a situation confounded by cigarette smoke induced decreases in glutathione, the lungs antioxidant defense, and oxidative stress ensues.

Cigarette smoke has been linked to alterations in glutathione (GSH), an intra and extracellular antioxidant present in the lungs (Rahman and MacNee, 1999). Disabling the body's inherent protective antioxidant defenses invites unchecked oxidative reactions, especially in the presence of increased ROS. Ultimately, this imbalance results in lung parenchymal damage causing irreversible alterations in the lipid bilayers induced by oxidative stress.

Cigarette smoke contains ROS with considerable oxidative power, which serves as direct toxins as well as precursors to other toxins. The primary free radicals, or ROS, in cigarette smoke are superoxide (O_2-•), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2) (Pryor, 1997). Smoking also interferes with α_1-antitrypsin activity, a protease inhibitor, by oxidizing MET[358] and MET [351] residues to methionine sulfoxide (Johnson and Travis, 1979) interrupting

the check and balance system for elastase activity within the lungs (Janoff et al., 1979; Hill et al., 2000; Sullivan et al., 2005). The combination of increases in elastase activity introduced by neutrophils, ROS generation compounded with the decreased ability of α_1-antitrypsin activity to control for of these processes can lead to extensive wall destruction.

According to a study by Tuder, et al (2006) cigarette smoke houses over 5000 offending chemicals. They purport that each puff of cigarette smoke contains ~ 10^{15} free radicals in the gas phase and 10^{18} free radicals per gram of tar, and includes potent oxidants such as hydrogen peroxide, hydroxyl anion, and organic radicals. Chronic exposure to these deleterious toxicants result in the disruption of the normal functions of the pneumocyte type I and II cells leading to increases in epithelial permeability, decreases in surfactant production, and inappropriate generation of cytokines leading to the initiation of chronic inflammation (Aoshiba and Nagai, 2003). Nitrogen oxide and superoxide radicals are short-lived oxygen free radicals that readily react to form peroxynitrites. Peroxynitrites are anions with a chemical formula of $OONO^-$ that are oxidizing agents, making it a threat to surrounding cells including DNA and other proteins as they trigger cellular responses ranging from cell signaling to oxidative injury, inducing cellular necrosis and apoptosis. Peroxynitrite generation has been implicated in such disease processes as stroke, myocardial infarction, chronic heart failure, diabetes, circulatory shock, chronic inflammatory diseases, cancer, and neurodegenerative disorders (Pacher, Beckman and Liaudet, 2007).

Cigarette smoke contains two different forms of free radicals, one in the tar phase and one in the gas phase. The tar phase contains the long-lived hydroquinones that undergo a redox-cycle to form superoxide radicals and hydrogen peroxides via semiquinones. Semiquinones are highly reactive free radicals formed by the removal of one hydrogen atom with its electron during the process of dehydrogenation of a hydroquinone to quinone. This phenomenon results

in persistent oxidative stress. (Pryor and Stone 1993; Cross et al., 1997; Nakayama 1989) The gas phase of cigarette smoke contains small oxygen and carbon-centered radicals that are more reactive than those in the tar phase. It is thought that these free radicals contribute to the inactivation of α_1-antitrypsin proteinase inhibitor (Church and Pryor, 1985).

It has been reported that 85-90% of those diagnosed with chronic bronchitis or emphysema are smokers, occurring in less than 5% of nonsmokers, and that moderate to severe emphysema is rare in nonsmokers (ALA COPD Fact Sheet, 2011). According to the CDC, smoking is the most important preventable cause of death in the United States and is responsible for over 480,000 deaths each year, with 41,000 of those deaths related to second-hand smoke. Long-term cigarette smoking is a key contributor to heart disease, peripheral vascular disease, diabetes, chronic lung disease and strokes, as well as a variety of cancers. Significant focus should be represented in the structure and process of healthcare delivery systems in order to affect the best possible outcomes with regard to reducing smoking and its wide array of implications.

COPD treatment. Diseased lungs impact the overall human experience. As such a multimodal treatment regimen is employed. The treatment and management of COPD aims to 1) improve pulmonary function; 2) decrease symptoms; 3) improve exercise tolerance; 4) decrease exacerbations; and, 5) reduce mortality (WHO, 2013). Simultaneous approaches often optimize the health state of those afflicted to achieve these goals. Depending on where the individual falls on the spectrum of the disease dictates how much quality is added back to their life with management of their disease (Scuirba, 2008).

The global initiative for chronic obstructive lung disease (GOLD) guidelines recommend a staged approach to the management of COPD; however, the American Thoracic Society (ATS) and European Respiratory Society (ERS) suggest the continuum of symptoms and FEV_1 should

dictate treatment regimens. All agree, however, that smoking cessation is first and foremost to any level of recovery.

Smoking cessation is critical in significantly slowing the progression of the disease and exercise improves overall functionality of respiratory mechanics and oxygen delivery (Shea and Martinez, 2009). Smoking cessation is the only disease modifying therapy for management of mild to moderate COPD (Ind, 2005). It has been demonstrated that smoking cessation slows the accelerated decline of the FEV_1 (Fletcher and Peto, 1977; Simmons et al., 2005). According to the Lung Health Study the rate of decline in FEV_1 was reduced by ~50% of those who permanently quit smoking (Anthonisen et al., 1994). Kanner et al., (2001) reported that those with COPD who quit smoking experienced fewer exacerbations of their illness.

Respiratory infections are always a significant threat to those with COPD. Those with chronic bronchitis are particularly vulnerable due to the persistent presence of inflammatory mucus exudates. As a result microbial pathogens harbor in the lower respiratory tract, disrupt ciliary function, trigger enhanced mucus secretion, and further damage the epithelium (Sethi, 2000). Recent research suggests the addition of daily antibiotics can significantly reduce the incidence of acute microbial induced exacerbations and improve the quality of life for COPD sufferers. The New England Journal of Medicine (2011) published the results of a randomized clinical trial including 1577 participants, 570 received azithromycin and 572 received a placebo for 1 year. The treatment group reported a significant ($P<0.01$) reduction in acute exacerbations. A small portion of the participants reported small decrements of hearing loss with azithromycin prophylaxis.

Once the disease has progressed to advanced stages, acute exacerbations of symptoms are often managed with supplemental oxygen; inhaled beta agonists, long acting beta agonists (LABA) and anticholinergics to aid in bronchodilation; corticosteroids to reduce inflammation;

and antibiotics to treat infections (Hunter and King, 2001). Approximately one half of patients diagnosed with COPD have high concentrations of bacteria in their lower airways (Rave, et al., 2007; Sethi, et al., 2002). The most frequently offending bacteria include Streptococcus pneumonia, Hemophilus influenza, Moraxella catarrhalis, Mycoplasma pneumonia, as well as a variety of viruses (Rave, et al., 2007; Sethi, et al., 2002). It has been suggested that the use of antibiotics for those with moderate or severe disease with COPD exacerbations have a reduced risk of treatment failure and death (Ram, et al., 2006).

For patients who cannot be managed successfully with medication therapy, surgery may be warranted. Three types of surgery are offered to patients with end-stage COPD suffering from severe symptoms: bullectomy for bullae that compress on more viable tissue; lung volume reduction surgery (LVRS) to removed ineffective and diseased lung to allow more effective tissue to function; or a lung transplant. Patients <65 years old with end-stage lung disease in the absence of other significant disease could be considered for transplantation. Chronic stable COPD patients who are hypoxemic often require LFDO to assist in maintaining activities of daily living (Tiep and Carter, 2008).

Low-flow domiciliary oxygen. Low flow domiciliary oxygen therapy has long been in use as a means of ameliorating respiratory distress and shortness of breath for those afflicted with chronic lung disease. While the medical community is very aware of the risks associated with exposure to high concentrations of oxygen, even for relatively short periods of time, the research is sparse regarding to long-term exposure to low flows of oxygen. Understanding this dichotomous drug is the responsibility of the healthcare providers.

Following his 1913 scientific expedition to the summit of 14,210-foot Pike's Peak in Colorado, the venerable British physiologist, John Haldane wrote, "partial anoxia means not a mere slowing down of life, but progressive, and perhaps irreparable damage to human structure"

(Haldane, 1919). Haldane went on to predict that someday the use of oxygen therapy would become commonplace in hospitals. In 1956 Cotes and Gibson of the UK were providing ambulatory patients with supplemental oxygen via portable cylinders. Increased walking time and improved arterial saturation with the administration of supplemental oxygen was noted (Cotes and Gibson, 1956). In 1958, Alvan Barach was credited to be the first in the U.S. to develop transfillable oxygen cylinders, as well as inspiring the advent and continued evolution of LFDO (Petty, McCoy and Doherty 2005).

In 1980, a major randomized clinical trial took place to determine the effects of nocturnal oxygen therapy treatment. The study enrolled 203 COPD patients with a blood oxygen level <50mmHg. The results showed a significant difference (P<.01) in survival outcomes between those patients on continuous oxygen therapy (COT) and those on nocturnal oxygen therapy (NOT) only (Timms, et al., 1981).

In 2006 T.J. Ringbaek completed a mixed methods analysis of the domiciliary oxygen research from 16 different countries between the years of 1994 and 2000 in an effort to better understand the types of home therapies offered, the adherence to guidelines, the effects of continuous oxygen therapy (COT), the presence of predictors of survival and hospitalizations of COPD patients on COT, as well as the effect of nocturnal COT as it related to hypoxemia. Over 17,000 people with advanced COPD were analyzed in this study. Results from this analysis demonstrated the following: the prevalence of patients on domiciliary oxygen was greater when prescribed by a general practitioner vs. a pulmonologist; adherence to oxygen therapy was greater when prescribed by a pulmonologist; the incidence of prescribed domiciliary oxygen therapy increased between 1995 and 2000 in all evaluated countries; most patients had mobile liquid oxygen systems; the median survival time of all COPD patients who started domiciliary

oxygen therapy between 1995 and 1999 was 1.27 years (95% CI; 1.20-1.34) longer than those compared to those with same diagnosis and no domiciliary oxygen therapy.

Consistently research demonstrates that symptoms of those utilizing LFDO improve activities of daily living (ADLs), as well as ameliorate shortness of breath; however, there is no evidence that lung functionality improves or that life is lengthened (Strom, 1993; Dubois, 1994; Croxton and Bailey, 2006).

The American Thoracic Society has provided the following criteria for the implementation of LFDO therapy as outlined in Table two.

Table 2

LFDO Prescription Guidelines

PaO$_2$ mmHg	Sa, O$_2$ %	LFDO	Qualifying Condition
≤ 55	≤ 88	Absolute	None
55-59	89	Relative with qualifier	cor pulmonale, polycythemia > 55%, History of edema
> 60	> 90	None except with qualifier	Exercise desaturation Sleep desaturation not corrected by CPAP Lung disease with severe dyspnea responding to O$_2$

PaO$_2$: arterial oxygen tension, SaO$_2$: arterial oxygen saturation; CPAP: continuous positive airway pressure; O$_2$: oxygen.

The multiple factors that contribute to the morbidity and mortality of COPD have confounded finding a cure. It remains, however, the responsibility of the research community to better understand the disease and treatment therapies to bring us nearer to a cure. A step in that direction includes a better understanding of the dichotomous drug, oxygen. Understanding the

biochemical risks and benefits of oxygen therapy could result in the discovery of control for those qualities that contribute to pathology.

Based on information already understood about oxygen utilization, therapeutic guidelines have been developed. According to the ATS the goal of LFDO therapy is to optimize ventilation/perfusion (V/Q) matching as a means of correcting hypoxemia, particularly after an acute exacerbation of symptoms. Prior to initiating LFDO an arterial blood gas (ABG) should be obtained after breathing room air for 30 minutes. An ABG result will provide insight into arterial oxygen saturation as well as provide information on the presence of hypercarbia, or respiratory acidosis. Ventilation/perfusion mismatching can also be calculated by the alveolar-arterial gradient. The ATS recommends oxygen therapy for 24 hours/day with ambulatory capability. Exceptions to continuous administration include patients who: 1) are incapable or unwilling to be mobile; 2) only require oxygen during sleep; 3) require oxygen only during exercise. Liters of flow are gauged by the ABG result, and can be adjusted according to exertional needs. Flow rates should be adjusted to a target SpO_2 of $\geq$ 90% during periods of rest. This value should be calibrated with the initial ABG to assess relationship between SpO_2 and PaO_2. During sleep flow rates can be determined by two strategies: 1) the flow can be increased 1 L/min above the daytime resting prescription; or 2) nocturnal polysomnography or nocturnal pulse oximetry can be performed to support a more accurate prescription. The latter is especially important for those patients with cor pulmonale. During periods of exertion flow rates goals should be to maintain a $PaO_2 > 60$mmHg or $SaO_2 > 90$%. Increasing overnight prescription of oxygen flow rates by 1 L/min above the day was challenged in a study by Nisbet et al, (2006) stating overnight desaturation was not an issue if daytime flow rates were used during sleep.

Consequences of oxygen therapy. While many treatment interventions are effective at addressing and improving specific symptoms, oftentimes the treatment can predispose a patient

to undesired consequences. As a supplemental therapy that is prescribed to nearly 800,000 individuals annually at an estimated cost of 1.8 billion dollars it is necessary to appreciate any consequences that occur as a result of LFDO (Kim, et al., 2008).

It is well understood that high concentrations of oxygen therapy for relatively short periods of time can result in lung pathology. Chronic exposure of lung tissue to moderate oxygen concentrations can create pathologic changes such as bronchopulmonary dysplasia (BPD) and fibrotic scarring resulting in deficiencies in ventilation and oxygenation (Chen, 2007). In experimental BPD models, ROS-induced damage stemming from exposure to supraatmospheric oxygen tensions manifested in pulmonary epithelial DNA oxidation, accumulation of HO, lipid peroxidation, and protein oxidation (Liao et al., 2006; Auten, Whorton and Mason, 2003; Luo, et al., 1999; Vozzelli, 2004). Human BPD studies strongly support a role for ROS mediated damage (Ballard, et al., 2008). Plasma-3nitotyrosine, a marker for ONOO- formation, and protein carbonyls, a marker of protein oxidation, are elevated in premature newborns at the highest risk for developing BPD. ROS may inactivate antioxidant enzymes, with oxidized or nitrated proteins critical to lung function (Martin and Walsh, 2004). Retinopathy of prematurity (ROP) implicates oxygen therapy as central to its pathology. According to a study by Chen and Smith (2007) excessive oxygen contributes to ROP through regulation of vascular growth factor suppression. It is also speculated that due to decreased levels of antioxidants in the premature retina allows for oxygen radicals to accumulate resulting in retinal pathogenesis (Sira, Nissenkorn & Kremer, 1988).

A standard for directly assessing lung pathology was through the collection and evaluation of bronchoalveolar lavage (BAL). In a study by Davis, et al (1983) volunteers were exposed to 50% oxygen for 17 hours after which increased amounts of albumin were measured in their BAL, demonstrating a pulmonary-capillary membrane compromise. In 1986, Griffith et

al, conducted a study that yielded similar results in when individuals were exposed to concentrations of 30-50% oxygen for 45 hour with dose dependent increase in albumin in the BAL. In the same study, to measure lung epithelial permeability, inhaled technetium-labeled diethyentriamine pentaacetate was assessed; increases were detected in individuals exposed to 50% oxygen.

Historically it was believed that only high concentrations of oxygen were offensive to lung parenchyma, however, more recent research demonstrated concentrations as low as 40% have been implicated as well (Nagatomo, et al., 2012). The 1970's offered the "60%" rule, delivering oxygen at 60% or less as an acceptable guideline for management of hypoxemia, after previous studies of higher concentrations of oxygen clearly demonstrated pathologic consequences. This practice has been essentially unchallenged due to ethical considerations. Results from animal studies have provided significant insight into the deleterious effects of oxygen exposure (Martin and Kachel, 1989); however, in 1989 a study using cultured human pulmonary artery endothelial cells it was demonstrated that cytotoxicity occurred as early as 8 hours following exposure to 0.95 FiO2, and after 48 hours of exposure to concentrations as low as 60% (Martin and Kachel, 1989).

Oxygen toxicity causes damage to the lungs through a series of progressive stages. Initially, epithelial integrity is interrupted resulting in transudate in the alveoli; subsequently, capillary damage results in exudate between the alveolar sacs leading to fibrosis of the type I epithelial cells (Campbell, 2002). It is well documented in the literature that interstitial pulmonary changes occur after exposure to elevated concentrations of oxygen; however, there is a paucity of research to addresses the consequences associated with chronic exposure to low concentrations of oxygen.

An exhaustive Medline data search regarding the risks and benefits of long-term oxygen therapy revealed 14 studies assessing the value of dating from 1970 to 2010. Multiple variables were employed with only four studies clearly addressing the presence of an oxidative injury.

In 1971, Petty, Stanford and Neff evaluated 20 patients diagnosed with severe COPD who were placed on continuous oxygen therapy to correct hypoxemia to a goal of a P_aO_2 between 50 and 75mm Hg. Multiple blood gases were drawn until desired range was achieved, which usually resulted in 1-2 L of oxygen per nasal cannula. Survival ranged between 7 and 61 months, with 14 patients dying after an average of 26.7 months. Upon autopsy it was noted that 6 of the deceased showed exudative or proliferative tissue injury commensurate with oxygen toxicity. Survival was not shortened in those with histologic changes, but quality of life was not defined. Five patients went on to live an average of 42.4 months with continued excellent benefit. In 1975, Steward, Hood and Block performed a similar study that evaluated 12 patients receiving portable oxygen therapy for a mean period of 25.2 months. The overall impression of the researchers was that oxygen therapy was beneficial. The variables evaluated were pulmonary function testing, electrocardiogram and cor pulmonale, and hospital admissions. Decline in pulmonary function tests was not demonstrated, an electrocardiogram exhibited an improvement in cor pulmonale in 5 patients, and a reduction in hospitalizations for respiratory illnesses. Six study participants died during the study period demonstrating a 56% survival rate for the remainder. However, five of the 6 deceased presented with histologic changes consistent with oxygen toxicity upon autopsy.

A study published in 2004 in the European Respiratory Journal by Phillips et al, aimed to determine if short-term exposure to a low concentration of supplemental oxygen (28%) had an induced oxidative stress in nonhypoxic subjects. Breath methylated alkane contour (BMAC) was the marker utilized in this study. Thirty-one healthy subjects were recruited for this study and

served as the control and treatment groups in a pre and posttest design. Exhaled breath was collected prior to oxygen exposure and then after exposed to 28% oxygen at 2L/min via nasal cannula for 30 minutes while at rest. The results showed a significant ($p<0.05$) increased in the oxidative stress biomarker, BMAC.

A similar study performed in 2004 by Carpagnano et al, recruited 23 healthy subjects and 23 subjects with COPD. The control group consisted of 5 healthy subjects and 5 COPD subjects who breathed ambient air through a facemask for one hour; the treatment group consisted of 18 healthy subjects and 18 COPD subjects (7 of which were receiving domiciliary oxygen treatment) and exposed them to 28% oxygen via a facemask for one hour. The exhaled breath was collected from both groups and compared for the presence and quantification of 8-isoprostane and interleukin-6, biomarkers of oxidative stress and inflammation. The outcomes of this study showed a significant increase ($p<0.05$) in both biomarkers following exposure to one hour of 28% oxygen.

Oxygen is one of the most widely used therapeutic agents and is a considered a drug in the truest sense of the word as it has specific biochemical actions, a distinct range of effective doses, and a well defined consequence at higher doses; however, a therapeutic range as it relates to dose and time, has not been established. The cost of use is low, yet it has been reported that in many hospitals the annual expenditure of oxygen therapy exceeds those of most other high-profile agents (Bitterman, 2009). Sufficient evidence-based data does not exist to address the potential of this dichotomous drug.

Hyperoxia. Oxygen is so central to human existence that it was once intuitive to believe it incapable of being dangerous, let alone lethal. As such, oxygen was/is administered liberally within hospital settings, used as a pretreatment prior to deep sea diving or piloting an aircraft, and even offered at oxygen bars for patrons to imbibe in its protective and healing powers. The

dangers of prolonged exposure to high concentrations of oxygen are now very well documented. Oxygen is no longer considered innocuous but rather as a formidable and dichotomous drug that can give life or take it away. To not adhere to meticulous therapeutic protocols would be medical negligence. Oxygen continues to be one of the most widely used therapeutic agents but the margin of safety for various conditions remains to be clearly defined.

Hyperoxia is the term used to describe the presence of excessive oxygen in the lungs and vasculature for delivery to the tissues of the body. This can occur when inspired oxygen is delivered at higher than necessary concentrations or at higher than atmospheric pressure, as with positive pressure ventilation or hyperbaric chambers, and increased fractions of inspired concentrations. The standard atmosphere is unit of pressure and is defined as being equal to 101.325 kPa or 760 mmHg. The pressure of oxygen of inspired air at sea level is 0.21 atmospheres absolute, or Ata (Saltzman, 1973). Oxygen exposure at 1 atm of pressure, the lung is the most severely damaged as pulmonary tissue PO_2 is the highest in the body. Since pulmonary tissue PO_2 is directly determined by the alveolar PO_2 arterial hypoxemia does not delay the development of pulmonary oxygen toxicity at 1 atm (Nunn, 1987).

While prolonged periods of hyperoxia can cause damage to various structures of the body, such as the central nervous system (CNS), the retina, hemolysis of erythrocytes, myocardium, the pulmonary structures are particularly vulnerable to the consequences of prolonged periods of high-pressure ventilation and increased fractions of inspired oxygen as they are directly exposed to the offending variables without buffer (Yusa et al., (1987); Gu et al., 2003; Mengal and Kann, (1966); Sen et al., (2006). Individuals exposed to 1.0 Ata of oxygen for 24 hours will display discernible symptoms of respiratory distress (Saltzman, 1973; Klein, 1990; Miller et al., 1970). If inspired pressures exceed 2.5 Ata, as possible in a hyperbaric chamber, neurologic symptoms, such as confusions and coma, will occur abruptly, but are reversible;

however, pulmonary toxicity is more insidious and, therefore, less reversible (Behnke, 1935). As early as 1899 J.L. Smith was able to demonstrate that animals exposed to uninterrupted exposure to one Ata of oxygen for several days would experience respiratory distress, hypoxia and even death. Upon necropsy these animals displayed severe parenchymal damage.

Paul Bert (1943), a French physiologist, first described central nervous system toxicity in his publication *Barometric pressure: Researches in Experimental Physiology*. CNS toxicity is often referred to, as the "Paul Bert effect." In his research he showed oxygen was toxic to insects, arachnids, myriapods, molluscs, earthworms, fungi, germinating seeds, birds, etc. Bert was able to demonstrate that the deleterious effects of oxygen exposure were a result of increases in partial pressure vs. concentration, a theory that was essentially unchallenged for decades. In more recent years a biochemical process resulting in the production of oxygen metabolites has been implicated as the likely culprit in oxygen toxicity (Klein, 1990).

Oxygen toxicity. The mechanism of oxygen toxicity at the molecular level is now attributable to free radical interactions with cellular components. As a naturally occurring phenomenon with physiologic functions, their highly reactive nature makes them formidable when left unchecked. The oxygen molecule is normally susceptible to univalent reduction reactions within the cell to form a superoxide anion and hydrogen peroxide. While both of these compounds can have direct toxic effects, they interact to form more dangerous species through a process called the Fenton reaction, a reaction resulting in the formation of radicals from nonenzymatic processes. Due to the deleterious potential of free radicals, or reactive oxygen species, a complex and layered defense of enzyme systems and low-molecular weight radical scavengers exists.

It is difficult to fully appreciate the whole process of oxygen toxicity in humans for obvious ethical reasons. As a result, most studies conducted regarding this deleterious process

56

have utilized animal models, including ultrastructural morphometry, or the measurement of an external form. Human studies consist of autopsies following a severe illness requiring high concentrations of oxygen, usually delivered by high-pressure ventilation. In animal models researchers have been able to track oxygen toxicity from the mild to severe stages; however, only the end stages have been studied in humans during autopsy. Fortunately, relevant relationships exist between the animal and human studies with regard to morphologic changes (Lambertson, 1971; Crapo, et al., (1980); Crapo, 1986; Kapanci, et al., (1969); Thet & Parra, (1986); Kapanci, et al., (1972). In most species, exposure to 100% oxygen at 1 atm for 24-72 hours is associated with the initial phase of injury in all species.

It has been proposed that the alveolar destruction that occurs as a result of oxygen toxicity is compromised of multiple processes: apoptosis, oxidative stress, and protease antiprotease imbalance. In the pages that follow these mechanisms will be addressed in an effort to better understand the cascade of events that result in COPD.

During periods of hyperoxic pathologic conditions, a large influx of ROS is produced, disrupting the balance between oxidants and antioxidants, making surrounding tissues vulnerable to pathologic changes. Exposure time, atmospheric pressure, and fraction of inspired oxygen (FiO_2) determine the cumulative oxygen dose predisposing toxicity (Mach, et al., 2010).

Pulmonary capillary endothelial and alveolar epithelial cells are targets for ROS. The earliest morphologic changes seen in the initial, or inflammatory, phase involve subtle changes in endothelial cell structure, which result in pericapillary accumulation of fluid. If left unchecked interaction can result in excessive edema, alveolar flooding, hemorrhage, and collagen, elastin, and hyaline membrane deposits (Yee, et al., 2006; Kan, Lee and Kim, 2005; Pagano and Barazzone-Argiroffo, 2003). The initial phase is associated with, or rapidly followed by, accumulation of thrombocytes, macrophages and neutrophils in the lungs accompanied by the

release of soluble mediators of inflammation resulting in ongoing edema and cellular signaling

(de los Santos, et al., (1987); Barry & Crapo, (1985); Fox, et al., (1981); Rinaldo, et al., (1988);

Forman, York & Fisher, (1960); Ozawa, et al., (1988)). The hemoglobin-oxygen buffering

mechanisms fail when the P_AO_2 reaches a critical level and the tissue PO_2 rises above hundreds

of mmHg. At high levels of oxygen, protective endogenous antioxidant enzyme systems become

overwhelmed by ROS resulting in cell death (Mantell, et al., (1999).

Pulmonary oxygen toxicity can be explained occurs in 5 phases: 1) Initiation: during this

phase there are increases in production of toxic oxygen metabolites, depletion of antioxidant

stores, decreases in flow of tracheal mucus lending to infection and no obvious evidence of lung

injury; 2) Inflammation: during the inflammation phase damage occurs to the pulmonary

capillary endothelial lining, increases in inflammatory mediators rush to site of insult and the

development of pulmonary edema ensues; 3) Destruction: during the destruction phase there is

amplified damage to the endothelial lining with the accumulation of platelets, neutrophils, a

continued release of inflammatory mediators, and this is the phase most associated with

mortality; 4) Proliferation: the proliferation phase becomes evident after prolonged exposure to

oxygen concentrations between 60-85% with hypertrophy occurring to the remaining pulmonary

capillary endothelial cells, increases in monocytes, increases in type II pneumocyte activity, or

surfactant production; 5) Fibrosis: permanent lung damage occurs during this phase as collagen

deposition occurs in the lung interstitium, increases in the thickness of the pulmonary interstitial

space and increases in interstitial fibrosis (Mensack and Murtaugh, 1999).

The pulmonary epithelial surface is vulnerable to a destructive inflammatory response.

Inflammation damages the alveolar capillary barrier leading to impaired gas exchange and

edema. Reactive oxygen species induces pulmonary secretion of chemo attractants, and

cytokines stimulate macrophage and monocytes mobilization and accumulation into the lungs,

58

leading to further ROS production, extending injury. As highly reduced cell layers become increasingly oxidized and levels of antioxidants fall, ROS-induced activation of signal transduction pathways regulates cellular responses of adaptation, repair or cell death by apoptosis, oncosis, or necrosis (Romashko, et al., 2003; Kannan, et al., 2006).

Cellular response to ROS signaling occurs as a result of mitogen-activated protein kinase (MAPK), toll-like receptor 4 (TLR4), signal transducers and activators of transcription (STAT), and nuclear factor kappa beta (NFkß) communicating with the DNA of the cell and eliciting a response. The MAPK pathway regulates cell death genes, stress and transformation and growth regulation. The TLR4, STAT pathways are associated with survival gene expression and antioxidant response. The NFkß pathway is an up-stream signal for inflammation and survival genes: antioxidant enzymes, Bcl-2, hemeoxygenases, and heat shock proteins (Mach, et al., 2010).

Oxidative stress. Oxidative stress occurs as a result of the cumulative effects of an excess of reactive oxygen species (ROS). Prolonged exposure to higher than normal concentrations and partial pressures of oxygen than the lungs are normally exposed to will result in the production of ROS (Fridovich, 1998). Conditions that precipitate oxidative stress through different ROS producing mechanisms include pneumonias, sepsis, acid aspiration, hyperoxia, high-pressure ventilation, pulmonary contusion, reperfusion injury and/or bleomyocin exposure (Matute-Bello, Frevert and Martin, 2008). When the body is unable to control for an excess of these highly bioactive intermediates that result from these pathologic processes significant cellular damage and pathology can ensue. Ironically, the precipitating conditions typically require prolonged oxygen exposure as a support therapy placing them at an even greater risk of oxidative damage.

Research regarding high concentration oxygen exposure in healthy patients has not demonstrated permanent results when exposure was less than 24 hours. In a 1970 study performed by van der Water et al, nine young healthy men were exposed to 100% normobaric oxygen for 6-12 hours. Following this exposure the alveolar-arterial O_2 gradient, pulmonary artery pressure, total pulmonary resistance, cardiac output, pulmonary vascular volume, and CXR were evaluated with no pathologic findings noted. However, unpublished research conducted on exceptionally fit pilots that routinely inspired 100% oxygen immediately pre flight suggests the pilots experienced symptoms consistent with inflammatory lung injury, such as cough, substernal chest pain and burning. Pulmonary function tests in this group indicated that pathologic changes had occurred in those who had participated in repeated, pre-flight, short-term inspiration of high oxygen concentrations (Vacchiano, personal communication).

The symptoms primarily observed in conscious patients with oxygen toxicity is tracheobronchial irritation resulting in a cough and substernal discomfort. The onset of symptoms of tracheal irritation occur ~4-22 hours after the start of oxygen exposure (Comroe et al., 1945). According to Sackner et al, (1975) these symptoms precede changes in pulmonary function tests.

Historically, the most commonly used index of oxygen toxicity in humans has been vital capacity (VC) (Comroe, et al., (1945); Caldwell, et al., (1966). Vital capacity is measured as the total volume of expired air from the lungs following maximal inhalation. Early respiratory physiologists reported that subjects who were suspected to have suffered oxygen poisoning had concomitant decreases in their VC (Comroe et al., 1945; Caldwell et al., 1966, Clark and Lambertsen, 1971). However, more current research has abandoned this index as a primary indicator as too many variables confound accuracy.

Hyperoxic lung damage. Oxygen exposure at concentrations greater than 50%

administered between 4 and 22 hours can induce lung injury between (Bitterman, 2009) via two

mechanisms: 1) the formation of cytotoxic oxygen free radicals; and 2) absorption atelectasis

(Carvalho et al., 1998). The formation and activity of oxygen free radicals is the topic of interest

for this research study and, therefore, will be addressed exclusively.

Most research addressing the subject of pulmonary oxygen toxicity has been performed

almost exclusively on animal models for obvious ethical reasons. Research performed on

humans is typically carried out during autopsy (Klein, 1990). In most species, exposure to 100%

at one atm for a period of 24-72 hours results in the initial phase of lung injury. Normally this

phase displays no outward symptoms of injury but is characterized by increases in production of

oxygen metabolites, such as reactive oxygen species, through the recruitment and activation of

neutrophils (Jamieson et al, 1986; Rinaldo et al., 1988, Vacchiano et al., 1997; Wheaton and

Chandel, 2011). The presence of neutrophils in the lungs is commensurate with parenchymal

damage (Crapo et al., 1980; De los Santos et al., 1987, Steinberg, K.P. et al., 1994; Reutershan et

al., 2005). Neutrophils release inflammatory mediators, which, in this environment, result in the

production of reactive oxygen species via various oxidases (Zmiljewski et al., 2008; Wheaton

and Chandel, 2011). The ROS also mediate increased mucous production, decreased ciliary

function, fibroblast injury, gene expression of pro-inflammatory mediators (TNG-a, IL-8, IL-1,

NO), and diminished pulmonary mechanics and lung repair (Barnes, 1990; Barnes, 1996;

Keatings et al., 1996; Barnes, 2004; Cross, et al., 1994; Macnee and Rahman, 1999).

The major cellular mediators in pulmonary oxygen toxicity are considered alveolar

macrophages (AMs), neutrophils (PMNs), and vascular endothelial cells (VECs) (Vacchiano,

Osborne and Temple, 1997). The consequence of cytotoxic free radical activity is fibrotic

changes to the parenchyma. Parenchymal fibrosis results in disrupted wall integrity and

impaired gas exchange. Alveolar macrophages have been implicated as the initial metabolic source producing reactive oxygen species (Vacchiano, Osborne, and Temple, 1997; Grommes and Soehnlein, 2011).

The discontinuation of hyperoxic exposure can result in three ways: 1) Type II pneumocytes proliferate to begin restructuring of the alveoli; 2) fibroblast proliferation that may lead to interstitial fibrosis, and 3) development of pulmonary hypertension as a result of the derangement of large and small arterial walls (Coflesky, et al., (1987); Shaffer, et al., (1987).

The role of oxidative stress is now widely recognized as a key component of airway inflammation in a variety of pulmonary ailments (Riedl and Nel, 2008). Through the excessive production of ROS structural cellular damage occurs through the oxidation of proteins, lipids and DNA (Riedl and Nel, 2008). Continued attention needs to be given to this pervasive process as it undermines the human infrastructure making it vulnerable to disease and discomfort.

Acute Respiratory Distress Syndrome. Ashbaugh et al, (1967) were the first to describe and report a syndrome with a set of pulmonary pathologies that presented with such symptoms as diffuse lung infiltrates and respiratory failure and referred to as adult respiratory distress syndrome (ARDS). During that time those who identified this syndrome included cases of battle trauma, severe sepsis, pancreatitis, and massive transfusions. Following ongoing research, Ashbaugh and Petty (1971) refined their definition to include clinical features of severe dyspnea, cyanosis refractory to oxygen therapy, decreases in pulmonary compliance, atelectasis, pulmonary vascular congestion and hemorrhage, pulmonary edema and hyaline membranes at autopsy.

By 1988 a 4-point physiologic quantification system was introduced to stratify lung injury: 1) level of positive end expiratory pressure (PEEP); 2) P/F Ratio (ratio of arterial oxygen concentration to the fraction of inspired oxygen; 3) static lung compliance; and 4) degree of

pulmonary infiltrations on chest x-ray (CXR). Other considerations with this point system

included the examination of nonpulmonary organ dysfunction (Murray et al., 1988).

Shortcomings, however, existed with this definition as it did not clearly delineate ARDS from

cardiogenic pulmonary edema or offer predictive outcomes. In 1994 the American-European

Consensus Conference Committee (AECC) developed the now widely accepted definition of this

collection of phenomenon and changed the name to acute respiratory distress syndrome to reflect

that all are vulnerable to the possibility of experiencing this pathologic process.

The current definition of ARDS is as follows: acute onset, bilateral pulmonary infiltrates

on CXR, pulmonary capillary wedge pressure (PCWP) <18 mmHg, P/F ratio <200. Adult

respiratory distress syndrome occurs in four stages: exudative (acute phase), proliferative,

fibrotic and recovery, if recovery is possible. Management of ARDS includes oxygen,

mechanical ventilation, fluid therapy and a variety of medications to combat and support the

various manifestations associated with the pathology. Ventilatory management, to include

elevated oxygen concentrations, serves a dichotomous role. The patient will not survive without

positive pressure ventilation and increases in supplemental oxygen, however, these treatments

introduce potentially irreversible sequelae. Higher concentrations of inspired oxygen at

atmospheric pressure are associated with pulmonary oxygen toxicity.

Reactive oxygen species. Reactive oxygen species (ROS) are free radicals containing

oxygen in multiple forms to include oxygen ions, peroxides, superoxides, dioxylgenals and

ozone. Free radicals are atoms, molecules or ions characterized by unpaired electrons in their

outer orbits, making them highly reactive. Oxygen free radicals are continuously being produced

intracellularly by oxidation-reduction reactions (Southorn and Powis, 1988).

Normal cellular metabolic processes are the major source of ROS production, with

electron transport chain of the mitochondria the largest contributor. ROS generation also occurs

endogenously during activation of circulating inflammatory cells or phagocytes, or exogenously due to interactions with noxious chemicals (Kirkham and Rahman, 2006).

It should be understood that ROS have essential homeostatic responsibilities, such as regulation in signal transduction cascades, cellular phosphorylation, gene expression, and DNA synthesis (Upham and Trosko, 2009). Specific roles of ROS are dependent upon location, neighbors and timing (Auten and Davis, 2009). Of particular value, as it relates to this study, is the role of ROS in the antimicrobial activity of phagocytic cells for host defense (Fang, 2011). In contrast, however, in an unregulated environment, all components of the cell (lipid, protein and DNA) are vulnerable to irreparable changes (Bowler and Crapo, 2002). The lipid bi-layer is particularly vulnerable to the activity of oxygen free radicals due to the presence of multiple double bonds lending to increased reactivity. The oxidative degradation of lipids is referred to as lipid peroxidation.

Due to the unique molecular structure of oxygen and its abundance within cells oxygen has powerful potential. It readily accepts free electrons generated by normal oxidative metabolism within the cell, producing ROS. Processes causing uncoupling of electron transport can further enhance ROS production. However, other entities, such as the endoplasmic reticulum-bound enzyme systems, cytoplasmic enzyme systems, and the surface of the plasma membrane contribute. Contributions by multiple enzyme systems, such as the cytochrome P450 monoxygenase system, xanthine oxioreductase system, nitric oxide synthases, and several others involved in the inflammatory process (cyclooxygenase and lipoxygenase), can also increase the generation of ROS. While several forms of ROS are produced, concern of over the production of the more toxic radicals, such as HO•, especially in the presence of reduced transition metals such as iron, exists. Also, important to note, rapidly reacts with nitric oxide to form peroxynitrite

(ONOO-), a strong nitrating and oxidizing compound. These highly reactive species can react with membrane lipids to cause more complex radicals through the initiation of lipid peroxidation, the non-enzymatic degradation of lipids.

The endogenous production of O_2-• arises from NADPH oxidases (NOX 1-3) at low levels in the smooth muscle and vascular endothelium, with dual oxidases 1,2, and 4 in the epithelial cells. Reactive oxygen species have an important role in regulating nitric oxide availability, influencing airway and vascular reactivity (Auten and Davis, 2009).

Free radical induced oxidative stress has been implicated in many disease processes such as cardiovascular, pulmonary, autoimmune, inherited metabolic disorders as well as cancers and aging. However, the lungs and pulmonary vasculature are particularly vulnerable to the effects of free radical mediated tissue injury due to the increased presence of unsaturated fatty acids (the substrate for lipid peroxidation) and exposure to higher concentrations of oxygen than any other organ in the body (Torres, 2004).

Numerous pathways are initiated when stress is experienced leading to free radical production. A cascade of free radical activity leads to lipid peroxidation, protein oxidation, DNA damage, all of which contribute to pathology and cell death (Kappus and Sies, 1981; Bowler and Crapo, 2002).

Reduction-oxidation reaction. Reduction-oxidation, or redox, reactions are those that refer to chemical reactions in which atoms have their oxidation state changed. Oxidation state refers to the charge an atom would have if all bonds to atoms of different elements were 100% ionic, the chemical bond created by the attraction of opposite charges. The increase in the oxidation state of an atom as a result of a chemical reaction with a net loss of electrons is referred to as oxidation. The decrease in the oxidation state of an atom as a result of a chemical reaction with a net gain in electrons is referred to as reduction (Voet, Voet and Pratt, 2006).

Metabolic redox reactions within the body can result in the formation of molecules that are missing electrons. When a system is overwhelmed and there is an imbalance of antioxidants present new molecules are formed. In an effort to neutralize, these highly reactive and unstable molecules (or free radicals) will react with the first available source, such as DNA, lipid membranes, often resulting in irreparable damage and cell death (Kappus and Sies, 1981).

The direct effects of ROS on signaling pathways include redox-sensitive transcription factors, such as HIF, Nrf-2, and Nf-κB, as well as through direct effects through the inactivation of NO-based signaling (Auten and Davis, 2010).

To demonstrate the pathologic potential of unchecked ROS an example is provided. The developing retina is especially sensitive to ROS-mediated damage, contributing to retinopathy of prematurity in newborns. Vascular growth into the developing posterior retina is normally driven by redox-sensitive pathways that upregulate vascular endothelial growth factor (VEGF). Following birth, the neonate experiences increases in oxygen tension that suppresses VEGF. In the premature infant the suppression of VEGF in conjunction with altered autoregulation of retinal blood flow, as well as a deficiency in antioxidants, results in cessation of angiogenic budding, while apoptosis of developing vessels occurs secondary to the formation of ROS and nitrogen species (Gu, et al., 2002; Gu, et al., 2003; Saugstad, 2006).

Antioxidant systems. All aerobic organisms have enzymatic and non-enzymatic cellular defenses, collectively referred to as antioxidants, to maintain cellular homeostasis. Regulation of ROS activity is provided by antioxidants (Bowler and Crapo, 2002; Southorn and Powis, 1988).

Antioxidants are both water-soluble and lipid soluble. Water-soluble antioxidants generally react with oxidants in the cell cytosol and the blood plasma. Lipid soluble antioxidants protect cell membranes from lipid peroxidation. With regard to function there are four main classifications: 1) Preventative, which suppress the formation of ROS, 2) radical scavenging,

which suppress chain initiation and/or halt propagation reactions, 3) repair and restore, such as proteases, and 4) those that allow for adaptation that occurs when the signal for the production and reactions of ROS induces oxidant formation and transport (Rahman, Biswas & Kode, 2006).

The enzymatic antioxidants include superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione S-transferase and thioredoxin (Kirkham and Rahman, 2006). The non-enzymatic antioxidants include low molecular weight compounds, such as glutathione, urate, ascorbate, α-tocopherol, bilirubin, and lipoic acid, and high molecular weight compounds, such as the proteins albumin and transferrin.

Glutathione (GSH), a tripeptide thiol, is the primary intra and extracellular antioxidant in the lungs and is markedly up regulated in the presence of oxidative stress (Barnes, 2004; Rahman and MacNee, 2000; Deneke and Fanburg, 1989; Meister and Anderson, 1983). Alterations in alveolar and lung GSH metabolism are recognized as a central feature of many inflammatory lung diseases, such as COPD (Rahman and MacNee, 1999).

A delicate balance must be maintained between ROS production and the antioxidant defenses to ensure cellular stability. The balance is disturbed by conditions of hyperoxia, inflammation, or ischemia-reperfusion, or in the presence of impaired antioxidant defenses, such as seen with smoking (Auten and Davis, 2009). Mechanisms of ROS-related cell death include direct injury to proteins, lipids, and nucleic acids. For example, nitrosylation and protein oxidation impair a variety of enzymatic process and growth factors leading to cellular dysfunction (Stadtman and Levine, 200). Lipid peroxidation has been linked to cell death through the effects on cellular phospholipids through the activation of sphingomyelinase and the release of ceramide, which result in apoptosis (Fruhwirth and Hermetter, 2008). Nucleic acid oxidation has been linked with physiologic and premature aging as well as DNA strand breaks,

which leads to necrosis and/or maladaptive apoptosis (Auten, Whorten and Mason, 2002). The ability of the cell to handle changes determines whether or not it is adaptive or maladaptive.

In the proper location and concentrations, ROS can function as second messengers and activate multiple signal transduction pathways within the cell, such as growth factors, cytokines and calcium signaling. Possibly through the production of lipid peroxide intermediates, ROS can activate protein kinases, which phosphorylates and releases to Bcl-2-related proteins sequestered within cells (Parinandi, et al., 2003; Lei and Davis, 2003). The release of these key proteins can activate the Bax system resulting in apoptosis.

Significantly high levels of O2- are generated by NOX in phagocytes, such as the neutrophils and microphages (~1000-fold higher than nonphagocytic cells) (Auten and Davis, 2009). Blocking neutrophil influx induced by hyperoxia resulted in mitigating oxidative DNA damage, HO$\bullet$ formation, and O$_2$-$\bullet$ accumulation, while simultaneously enhancing alveolar development (Auten, Whorton and Mason, 2002; Belik, et al., 2004; Auten et al., 2001; Ning et al., 2006). It has been demonstrated that when the NADPH oxidases are genetically ablated reduction in pulmonary ROS accumulation occurs; however, this has not been proven to be protective as accompanying inflammation ensues (Gao et al., 2002; Yao et al., 2008).

Lipid Metabolism

Lipid metabolism can occur from both enzymatic and nonenzymatic processes. In the increased presence of reactive oxygen species enzymatically driven lipid metabolic pathways may be bypassed for the nonenzymatic lipid peroxidation pathway (Voet, Voet and Pratt, 2006).

Enzymatic lipid metabolism. Enzymatic lipid peroxidation occurs when cyclooxygenase and lipoxygenase control the peroxidation of various fatty acid substrates. These cyclooxygenase derived fatty acid substrates include prostaglandins. Enzyme generated products mediate specific bioactivities via receptor-dependent pathways that are under tight regulation.

Cyclooxygenase is the precursor to prostaglandin synthesis, and are pro-inflammatory. Non-steroidal anti-inflammatory drugs (NSAIDS), such as ibuprofen or naproxen, are cyclooxygenase (COX) inhibitors, antagonizing prostaglandin synthesis, thwarting inflammation and relieving associated pain perception.

Non-enzymatic lipid metabolism. An overabundance of ROS can lend to non-enzymatic lipid peroxidation, the metabolism of fatty acids, primarily arachidonic acid, resulting in the oxidative degradation of lipids. This is a process by which free radicals readily bind with electrons of the lipids in cell membranes resulting in cellular damage (Repetto, Semprine and Boveris, 2012). Since this process does not involve cyclooxygenase no current therapies are available to ameliorate the inflammatory response caused by these mediators.

Lipid peroxidation most readily affects polyunsaturated fatty acids due to the presence of multiple double bonds that are positioned between methylene groups and highly reactive hydrogen atoms. Products of ROS initiated non-enzymatic lipid peroxidation are proinflammatory prostanoids, which can generate more radicals that are capable of releasing damaging enzymes (Auten and Davis, 2010). Radical reactions consist of three main steps: initiation, propagation, and termination (Repetto, Semprine and Boveris, 2012).

Initiation is the step in which a fatty acid radical is produced. Of these the most notable initiators in living cells are reactive oxygen species, such as $OH\bullet$ and HO_2, which readily bind with a hydrogen atom to form water and a fatty acid radical (Porter, Caldwell and Mills, 1995).

Propagation is the step by which the newly formed unstable fatty acid radical readily binds with molecular oxygen to form an unstable peroxyl-fatty acid radical. This process continues, or propagates, producing a different fatty acid radical and lipid peroxide, or cyclic peroxide if it reacts with itself (Porter, Caldwell and Mills, 1995).

Termination occurs when two radicals react to form a non-radical. This can only occur when the concentration of radical species is high enough to potentiate the interaction between two radicals. When a radical reacts with a non-radical the interaction will always produce a radical, thus the chain reaction phenomenon. Organisms have evolved to protect themselves by speeding up termination through scavenging free radicals with antioxidants, such as vitamin E (α-tocopherol), as well as the enzymes superoxide dismutase, catalase and peroxidase (Porter, Caldwell and Mills, 1995).

The location the initial hydrogen atom abstraction takes place along the carbon chain determines the by-product produced. By-products of ROS generated non-enzymatic lipid peroxidation include isoprostane compounds, which are non-classical eicosanoids, such as 8-Iso-PGF2α, a biomarker now used extensively to assess for the presence of oxidative stress in a variety of conditions. In conditions where increased oxygen tensions exist, the formation of isofurans is favored over 8-Iso-PGF2α (Fessel, et al., 2002). The isofurans are a more sensitive indicator of oxidative injury. These biomarkers can be detected in multiple media: serum, urine and exhaled breath.

Eicosanoids

Eicosanoids are oxidative derivatives of 20-carbon chain essential fatty acids (EFA), specifically omega-3 (ω -3) and omega-6 (ω-6), which are primarily responsible for immune and inflammatory responses as well as serving as central nervous system messengers. Eicosanoids are comprised of four main families: prostaglandins, prostacyclins, leukotrienes and thromboxanes, all of which have bioactive subcategories. Prostaglandins, prostacyclins and thromboxanes comprise a group called prostanoids. Eicosanoids are not stored in cells but rather are synthesized in response to hormonal stimulation. Synthesis occurs through a series of steps. Initially, a fatty acid substrate, such as arachidonic acid, is released from cellular phospholipids

via phospholipase A_2; the free fatty acids are then acted upon by either cyclooxygenase 1 or 2 (COX1 or COX2) forming a hydroperoxide. The newly formed hydroperoxide is then reduced through a peroxidase reaction resulting in PGH2, an unstable intermediate from which all other prostanoids are derived via various enzymatic reactions (Voet, Voet and Pratt, 2006).

Prostanoids. Prostanoids are biologically active lipids formed from cyclooxygenases or prostaglandin synthases acting on the 20-carbon EFA chain by adding two oxygen molecules as two peroxide linkages and forming a 5-carbon ring approximately midway in the structure. Prostanoids are comprised of prostaglandins, prostacyclins and thromboxanes, all of which have a specific role with regard to the immune/inflammatory response and homeostasis. Prostanoid activity is achieved through autocrine and paracrine stimulation, meaning they exert their activities through cellular signaling among same cells and adjacent cells, respectively. Homeostatic functions of prostanoids include cytoprotection of gastric mucosa, regulatory influence on the renal, cardiovascular, and pulmonary physiology, protein metabolism and gestation and parturition regulation; however, they are involved in pathologic processes, such as inflammation, cardiovascular disease, cancer, dementia and Alzheimer's as well as aging to name a few (http://lipidlibrary.aocs.org/Lipids/eicprost/file.pdf).

Prostaglandins. Prostaglandins were identified in the 1930's and named so as they were thought to originate in the prostate gland. It was discovered by the 1960's that prostaglandins exist in nearly all cells of the human body as a result of the biosynthesis of specific EFAs. While cellular concentrations of prostaglandins are low-level, measuring only in nanomoles, their biologic activities are profound.

An important chemical distinction of prostaglandins is the 5-carbon ring positioned between the 8 and 12 carbons. Dependent upon subgroups, the prostaglandin family is comprised of at least 11 variations: PGA, PGB, PGC, PGD, PGE, PGFα, PGG, PGH, PGI, PGJ,

71

and PGK. Further distinctions can be made by the absence or presence of double bonds, denoted by the subscript 1, 2, or 3. Typically, double bonds are located with the alkyl substituents. The number of double bonds is directly related to the fatty acid precursor. For example, PGE_1, PGE_2 and PGE_3 are derived from linoleic, arachidonic and eicosapentaenoic acids respectively (http://lipidlibrary.aocs.org/lipids/eicprost/file.pdf). Greek subscripts α and β are used with the PGF series to describe the stereochemistry of the hydroxyl group on carbon 9 (http://lipidlibrary.aocs.org/Lipids/eicprost/file.pdf).

Thromboxanes. Thromboxanes are lipid molecules in the eicosanoid family responsible for clot formation and vasoconstriction following endothelial trauma. The two major thromboxanes are thromboxane A_2 and B_2. Platelets contain an enzyme, thromboxane-synthase A, which converts arachidonic acid into thromboxane. Thromboxane promotes platelet aggregation, facilitated by vasoconstriction (Voet, Voet and Pratt, 2006).

Prostacyclins. Prostacyclins are lipid molecules and, though considered independent mediators, in eicosanoid nomenclature are referred to as PGI_2, derived from ω-6 arachidonic acid, and are, therefore, grouped with prostanoids. Prostacyclins are produced in endothelial cells from prostaglandin H_2 (PGH_2) via prostacyclin synthase. The primary function of prostacyclin in the inflammatory cascade is inhibition of platelet aggregation and vasodilation, balancing thromboxane activity and resulting in homeostasis. Unopposed thromboxane activity could result in significant vascular consequence (Voet, Voet and Pratt, 2006).

Non-classical Eicosanoids

Isoprostanes. Isoprostanes (IsoPs) are prostaglandin F2-like compounds formed *in vivo* in abundance as a result of non-enzymatic lipid peroxidation of EFAs, primarily arachidonic acid, in the presence of oxidative stress. Reactive oxygen species attack the fatty acid, prostaglandin, resulting in the chemically altered compound with a prostane ring, making up a

series of isomers named F2-isoprostane. Isoprostanes possess powerful inflammatory, vasoconstrictive and hyperalgesic properties capable of significant pathology. These compounds were discovered as a result of reevaluating plasma samples from health subjects stored at -20C for several months, while the peak times were the same on the chromatogram the peak levels had increased by ~100-fold. This occurred as a result of autoxidation, a non-enzymatic free radical metabolism, of plasma lipids during storage. Further investigation confirmed these compounds to be isomeric to PGs and possess an F-type cyclopentane (prostane) ring, leading to the nomenclature F2-isoprostanes (Morrow, et al., 1990).

Since that time over a thousand studies have been performed using the 8-isoprostane as a biomarker of oxidative stress and are considered accurate markers of lipid peroxidation in both animal and human models of oxidative stress with the isoprostane 8-iso-PGF2α consistent with pulmonary oxidative stress are (Barnes, 2006) during periods of normoxia and the isofurans (IsoF) in circumstances where elevated oxygen tension levels are present (Roberts et al, 2004). The production of isoprostanes is self-limiting making isofurans a better biomarker of oxidative stress in the presence of hyperoxia.

Isofurans. Isofurans, like isoprostanes, are a result of non-enzymatic free radical metabolism of arachidonic acid but are differentiated by a substituted tetrahydrofuran ring. IsoPs and IsoFs share a carbon-centered radical as an intermediate. The generation of IsoPs at the carbon-centered radical undergoes a 5-exo cyclization to form a cyclopentane ring; IsoFs are generated when the carbon-centered radical interacts with molecular oxygen, which is why IsoF formation is favored during periods of increased oxygen tension. Elevated oxygen concentrations, greater than 21%, favor isofuran over isoprostane production, such as during periods of hyperoxic therapies or with disorders of the mitochondria where oxygen utilization is

impaired (Roberts et al., 2004). This unique differentiation allows for more sensitive evaluation of pathologic processes meeting these criteria.

In a study by Fessel et al, (2002) steps were taken to determine the mechanism of formation of IsoPs compared to IsoFs when assessing for hyperoxic induced oxidative stress, as noted with hyperoxia-induced acute lung injury, retinopathy of prematurity, etc. In the pathway for IsoP formation a carbon-centered radical must undergo intramolecular rearrangement to form a cyclopentane ring. Competition from molecular oxygen in this reaction prevents the formation of an IsoP. The researchers presumed this limited IsoP formation at higher oxygen concentrations. Fessel and colleagues hypothesized that an independent pathway would be modulated by higher oxygen tensions. To test the hypothesis they measured IsoP and IsoF formation *in vitro* from the oxidation of arachidonic acid in the presence of increasing oxygen tensions. It was concluded that IsoPs and IsoFs increased comparably up to 21% oxygen as oxidation requires the presence of oxygen; however, IsoF formation continued to increase significantly as oxygen tension increased to 100% with a ratio of ~6:1 IsoF to IsoP formation. Further experiments were then undertaken to assess for *in vivo* IsoF formation. Using the well-established liver oxidant injury animal model CCl_4-treated rats was utilized to assess for IsoF formation. The results showed much lower levels of IsoFs than IsoPs. Concerned that this may be due to the fact that liver tissue has a low oxygen tension they opted to repeat the experiment in rat kidney and brain tissue. These results were 2-2.3 fold higher than the IsoFs measured in the liver. Finally, the researchers wanted to look specifically at the formation of IsoFs vs. IsoPs in the lungs following high concentrations of oxygen exposure. After 3 hours of 100% oxygen exposure there was no significant difference between IsoP levels between the treatment and control groups; however, IsoFs increased 5-fold.

To date eight regioisomers of IsoFs have been identified from which 256 enantiomerically pure IsoFs can be formed (Fessel et al., 2002). An IsoF-type compound has been identified as an enzymatic oxidation product of arachidonic acid and has been synthesized in the lab (Pace-Asciak, 1971; Bild et al., 1978; Just and Oh, 1981).

In order to provide clarity, standard rules for chemical nomenclature have been applied and conform to prostaglandin conventions. Isofurans are abbreviated to IsoFs, consistent with their biosynthetic relative the IsoPs. The IsoFs have been divided into eight classes based on the position of the isofuran ring and the alkene of the allylic alcohol. Consistent with prostaglandin standards, the C-1 will always be the carboxylate carbon; the number of the first carbon of the ring then will denote the position of the isofuran. Consistent with organic natural-product nomenclature, the position of the alkene of the allylic alcohol is represented by the Δ symbol followed by a superscripted number of the first carbon of the alkene. Eight families of IsoFs are recognized. These families are further subdivided and differentiated based on two considerations: the relationship of the alkyl groups of the tetrahydrofuran ring (syn vs. anti), and the relationship of the ring hydroxyl to the adjacent alkyl group (cis vs. trans) resulting in 32 differentiated subgroups, each comprised of eight enantiomerically –pure diastereomers.

F2-IsoPs and IsoFs can be quantified in a variety of human and animal fluids and tissues. The detection and measurement of these molecules in plasma and urine demonstrate global oxidative stress in vivo while quantification of F2-IsoPs and IsoFs in a tissue sample or in a specific fluid such as exhaled breath condensate represents local, organ-specific oxidative stress. Currently the only methodology used to detect and quantify isofurans is the GC/MS. A "standard" per se is not available but rather the retention times generated from the oxidation of arachidonic acid are considered consistent with the presence of isofuran (Roberts & Fessel, 2004).

Exhaled Breath Condensate

It is a goal of the healthcare community to discover safe and effective methods of assessing for the presence and/or severity of disease processes with the least amount of risk and discomfort to the patient. For those with debilitating respiratory diseases, traditional means of collecting tissue samples could exacerbate distress states. The advent of exhaled breath analysis has opened the door to a safe, effective and non-invasive analysis of some significant disease states.

Exhaled breath is the air exhaled from the lungs and consists primarily of water vapor with particles of aerosolized airway-lining fluid (ALF). Aerosolized airway-lining fluid is comprised of volatile and non-volatile compounds. Many of these compounds can be indicative of a variety of physiologic or pathologic process occurring within the body.

The concept of evaluating exhaled breath dates back to 400 B.C. when Hippocrates, the "father of medicine" described *fetor oris* and *fetor hepaticus* as a symptoms of disease states (Duveen and Klickstein, 1955). In the mid 1800's, Nebelthau determined the presence of acetone in the breath of diabetics (Hubbard, 1920). In 1874, Francis Anstie, an English physician, spent a significant portion of his career trying to better understand the effects of alcohol on the body. As such he was the first to demonstrate that alcohol could be collected into a rubber football bladder from the exhaled breath of one whom had been imbibing.

In 1927, Dr. Emily Bogen, a practicing physician in Los Angeles during the time of Prohibition felt compelled to accurately diagnose the presence of alcohol in those persons involved in car collisions, which were on the rise at that time, to avoid wrongfully accusing and punishing the innocent. As a result, she developed a breath-collecting device, similar to Dr. Anstie's, to measure the presence and degree of alcohol for those persons involved in car accidents (Bogen, 1927). During that same year in Chicago, Illinois, McNalley, a coroner-

chemist, invented a breathalizer that evaluated changes in color of air moving through chemicals in water (Popular Science, 1927).

In 1938 Dr. Rolla Harger, a professor of biochemistry and toxicology at Indiana University, developed the Drunkometer, considered the first roadside breath-testing device to be used by police. The exhaled breath would pass over and interact with potassium permanganate resulting in a color change. The degree of color change was directly related to the degree of intoxication. In 1954 Robert Borkenstein, an Indiana police captain, was credited with developing a more practical and portable device that does not require recalibration, allowing for ease of use.

In the1970's Linus Pauling opened wide the field of breath analysis when he demonstrated over 250 identifiable substances in exhaled breath via a gas-liquid partition chromoatgraphy mass spectrometry (Dweik and Amann, 2008; Corradi and Mutti, 2012). Over 80 organic compounds have been identified as directly related to just smoking (Fillpiak, 2012). Chromatography and spectrometry expanding the exploration of exhaled breath for detection and measurement of compounds and the subsequent development of exhaled breath collection devices and assays. As a result of an ongoing evolution of this technology over 5000 compounds can be measured in exhaled breath to date.

Although it had been established for centuries that chemical evidence exists, and is measurable, in exhaled breath and that exhaled breath is the greatest source of insensible water loss, the use of EBC to assess for physiologic and pathologic processes did not occur until 1980 when researchers from the former Soviet Union began to publish reports of its use. Sidorenko et al (1980) published an article entitled *Surface-active properties of the exhaled air condensate, a new method of studying lung function.* However, it would be well into the 90's before the United States would begin to explore the value of EBC.

Collection of exhaled breath condensate is a non-invasive method of collecting aerosolized air particles from the lungs in order to assess for the presence of ongoing pathology. EBC contains a variety of biomarkers including hydrogen peroxide, ammonia, leukotrienes, nitrogen oxides, peptides, cytokines, isoprostanes and isofurans that represent such processes as airway and lung redox pathology, acid-base imbalances, degree and type of inflammation in acute and chronic asthma, COPD, adult respiratory distress syndrome, occupational disease, cancers and cystic fibrosis (Hunt 2002; Horvath et al, 2009).

Respiratory diseases as well as the prescribed therapeutic regimens influence the degree of biomarker concentration obtained (Horvath 2005). While precise assessment of individual solute concentrations cannot be made it can provide useful information when concentrations differ between health and disease states (Hunt 2007).

EBC collection devices. Several commercially available collection devices have been developed to procure the exhaled breath condensate for biomarker analysis; however, the concepts of obtaining a collectate are the same amongst them. The exhaled air passes through a condensing unit resulting in the accumulation of fluid, or condensate. Care to avoid salivary contamination should be always be taken. Approximately 1-3 milliliters of collectate are required to guarantee ability to assay in duplicate or triplicate. In general it requires ~10-15 minutes of tidal breathing to acquire this volume. During normal tidal breathing the levels of aerosolized particles ranges between 0.1-0.4 particles/cm^3 with a mean diameter of 0.3μm. The amount of aerosolized particles from the respiratory tract is dependent upon airway velocity and surface tension within the extracellular lining fluid (Mutlu et al., 2001).

The most widely used EBC collection devices utilized in the United States are the EcoScreen® and the RTube™. Both devices collect exhaled breath through tidal breathing into a mouthpiece where the exhalant is guided into a cooling chamber for condensation. The

78

EcoScreen® is a large nonportable device that requires access to an electrical outlet to operate the cooling chamber. The EcoScreen® requires 30 minutes to reach the cooling standard, in between patient cleaning of the mouthpiece and condensing chamber, accessing the cooling chamber (oftentimes requires some thawing for removal from chamber), and seven minutes for sample collection. The RTube™ does not require cleaning of its parts as the device is single use and disposable; multiple samples can be obtained with single patient use and issues with thawing sample for storage are not encountered. While the EcoScreen® is better able to maintain a constant temperature than the RTube™ the portability and ease of use of the RTube™ makes this device extremely advantageous. These features make the RTube™ the device of choice for this research project.

Concerns, such has condensing temperatures and adherence to the collection unit wall, have been raised regarding factors introduced by the collection devices that may alter the composition and, therefore, measurability of biomarkers in the condensate. In a study by Czebe et al (2008) three condensing units were evaluated to determine the efficacy of the EcoScreen®, RTube™ and Anacon condensers as well as to test the influence of condensing temperature. The results of this research effort demonstrated that condenser type influences sample pH, total protein and leukotriene concentrations with the samples from the EcoScreen® being more alkaline and contained more protein while the RTube™ and Anacon correlated with regard to condensing temperature affects on pH but not protein content. The researchers concluded that adherence of the biomarkers to condenser surface and condensing temperature may play a role in biomarker variability.

Montuschi (2007) evaluated the EcoScreen®, EcoScreen II®, and the RTube™ with regard to methodological differences. Each poses benefits and limitations. The EcoScreen® and EcoScreen II® are able to maintain temperatures to preserve the sample until analysis can occur.

The EcoScreen® offers an added benefit of respiratory parameters measurability. The EcoScreen® II has the ability to collect EBC derived from the airways or the alveoli at the same time. This may allow for the study the origin of biomarkers in the lung compartments.

The RTube™ offers the advantage of portability making collection possible nearly anywhere; the device also prevents salivary contamination (Hunt, 2002) and has a condensing sleeve that can be cooled to any temperature. Limitations of the process of using this device include maintaining temperature during storage and travel; for those compounds that are chemically unstable this can present a problem.

Exhaled breath condensate pH is typically low in patients with a variety of diseases inducing airway inflammation, such as cystic fibrosis, COPD, asthma, etc., (Dupont et al, 2006; Hunt et al, 2000; Borrill et al., 2005; Ojoo et al., 2005). In a comparative study performed by Koczulla et al., 2009, in which results were repeated on two different days comparing the EcoScreen® and the RTube™ for pH analysis results showed no significant differences (P=0.754). The results of the study demonstrated repeatability and reproducibility in healthy controls, COPD and asthma patients as well as subjects experiencing a common cold. In a similar study by Prieto et al, 2007, significantly higher pH values in EBC were noted when obtained with the RTube™ compared to the EcoScreen®.

Rosias et al (2006) evaluated condensers to determine if the properties of the condensing units interacted with eicosanoids and proteins resulting in values that were at the lower limits of detection. Albumin and 8-IsoP were evaluated for adherence to five different condensing unit coatings: silicone, glass, aluminum, polypropylene and Teflon. Results of this study showed that *in vitro* silicone and glass coatings yielded a higher recovery of both albumin and 8-IsoP. *In vivo* the same was true for 8-IsoP with regard to silicone and glass while albumin was only detectable in glass.

According to a follow up study by Rosias et al, (2008) on biomarker reproducibility in EBC, an optimized glass condenser provided more EBC volume and biomarker detection; however, biomarker reproducibility in the EBC of healthy adults was not influenced by condenser type (glass, silicone, EcoScreen® or optimized glass). With regard to bioactive lipids it has been demonstrated that polypropylene condensers should be considered to avoid the concern of absorption that can occur with others (Mutlu, et al., 2001).

In a letter to the editors, Sapey et al, (2008) provided five challenges to the interpretation of the findings in the 2008 Rosias study. Sapey and colleagues questioned the validity of basing the coefficient of variation (CV) on the mean of spiked samples stating such methods artificially lowers the CV and, therefore, does not provide a true representation of the inherent variability of measuring mediators near the lower levels of detection of said assay. Secondly, they contend that the use of CV is not always the ideal way to express variability stating, "When mean values are low, CV values can be abnormally high." Thirdly, the Rosias study stated a "matrix effect is seen when samples are spiked with 10 pg/ml of mediator but not at 100 pg/ml; Sapey and colleagues contend that spikes of these concentrations would mask a matrix effect. Fourthly, they did not find it sensible to address other sources of variability when the assay itself has high variability; and finally, Sapey and colleagues took issue with the lack of definition of a lower limit of quantification for any assay as they believe that the lower limit of quantification is central to the measurement of mediators as well as adhering to strict methodologies as it pertains to a given biomarker.

Standard for assessing alveolar lining. Historically, pathologic processes occurring within the lower respiratory tract were evaluated by invasive methods such as bronchoalvealor lavage (BAL), sputum induction and bronchoscopy. Bronchoscopy is used to visualize the proximal and distal airways; to control alveolar hemorrhage; sampling of fluids; collecting

81

tissues through biopsy; identifying tumors; exploring lesions; investigating hilar lesions; assessing mechanical difficulties; inspecting the nasopharynx and larynx; and removal of foreign bodies, etc., (Stocks, et al., 2005). The BAL requires the instillation of saline into the smaller airways to collect ALF for assessment. The invasive nature of these maneuvers typically requires sedation and, though rare, carries the risk of hypoxia, dysrhythmias, pneumothorax, bleeding, infection, bronchospasm, laryngospasm, fever and even death (Zimmerman and Klein, 1997; Morrow and Agent, 2001). The advent of exhaled breath collection allows for non-invasive sampling making these ideal collection devices in a fragile patient subset (Montuschi, 2007). No research supports any such risks to patients utilizing exhaled breath condensing devices.

Comparing EBC biomarker concentrations to established techniques, such as BAL, could be useful in establishing standards and interpreting results. However, to date few studies exist that compare the two collection media. Interpreting BAL has its own limitations, such as the large range of normal values for each substance being measured resulting in a lack of sensitivity in detecting disease. Additionally, abnormalities detected in BAL fluid are rarely related to a specific interstitial lung disease. The BAL is fraught with false positive and negative results. The actual technique utilized to collect BAL may result in tissue damage confounding the results of the collectate (Leroy et al., 2008).

In a study by Jackson et al. (2007) 48 subjects participated in a study to compare EBC values to BAL values for 8-IsoP, nitrogen oxides, pH, hydrogen peroxide, total protein, phospholipids and keratin. The results demonstrated significantly higher concentrations of 8-IsoP, nitrogen oxides and higher pH (P<0.001) in EBC. No difference was demonstrated between EBC and BAL with regard to hydrogen peroxide concentration. Total protein was noted to be significantly higher in BAL (P<0.001). Phospholipids were also higher in EBC, but no significance for keratin was detected. No significant correlation was noted between EBC and

BAL for any of the biomarkers listed. However, in a study by Piotrowski et al, (2007), a positive correlation (r=0.68) was demonstrated between BAL and EBC 8-isoprostane and LTB4.

In that same year Piotrowski et al, evaluated 28 patients diagnosed with sarcoidosis and compared the results to a control of 17 healthy subjects. Enzyme-linked immunoassay with utilized to detect 8-IsoPs in EBC and BAL, as well as a cell count, percentage, and number and activity of cells. Results demonstrated a significant elevation of 8-IsoP in the EBC of the sarcoidosis group and a positive correlation between EBC and BAL media (r = 0.68, P<0.0001) with regard to EBC 8-IsoPs and BAL CysLT may support a possible prognostic value.

Methodological considerations for EBC collection. In 2005 the American Thoracic Society (ATS) and European Respiratory Society (ERS) Task force on EBC convened to develop guidelines for the collection and measurement of exhaled biomarkers. Since that time several reports (Grob, et al., (2008); Davis, et al., (2012); and Rosias, 2012) have been published, however, with little to add to the original report. Due to the inherent nature of the biomarker molecule being assessed, the varying storage practices and the specimen assessment tool (assays vs mass spectrometry) a singular methodology for EBC collection and biomarker detection has been elusive. Provided below are areas considered to have potential influence on the collection, maintenance, concentration and detection of biomarkers in exhaled breath.

Duration and temperature of condensate collection. Generally, 10 minutes of tidal breathing will produce 1-3 mL of EBC. Considerations may need to be made for individuals with shortness of breath and fatigue easily. According to the report, no differences could be determined in biomarker concentrations of H2O2, nitrite/nitrate, 8-isoprostane, adenosine and MDA in studies utilizing 10, 15 or 20 minute collection periods for EBC sampling.

Gessner et al, (2001) demonstrated that not only was EBC volume linearly related to the volume expired, but also to the total protein and urea content. This finding suggests that these

accumulated in the collection device by a similar mechanism as expired water vapor, but is not necessarily accurate for all substances present. Therefore, Gessner and colleagues suggest that maybe a volume target should be the goal rather than an amount of time.

Condensation is best achieved when breathing into a cooled collection device. Note should be taken that the act of breathing through a device will inherently increase its temperature. The solubility of volatile mediators may be influenced by temperature. Collection temperatures should be maintained and recorded.

Ambient air. Ambient air may contain molecules that may influence EBC composition through the following mechanisms: 1) directly contribute to EBC levels; 2) react and, therefore, change or consume molecules trapped in EBC; and 3) lead to inflammatory and biochemical changes in the airway that are reflected in EBC composition.

Breathing pattern. When assessing for mediators in individuals with respiratory disease, breathing patterns were considered. According to the ATS/ERS Task Force "breathing pattern" differences did not contribute to significant differences in mediator levels. However, in a report by Montuschi (2007) flow dependence maybe an indicator of where biomarkers originate, in the airways or the alveoli. Concentrations of EBC biomolecules dependent upon expiratory flow rates are believed to originate from the airways (Schleiss, et al., 2000). In contrast, the lack of flow dependence would indicate that a biomolecule is mainly derived from the alveolar region where flow has a minor role (Schleiss, 2000). Hydrogen peroxide is flow rate dependent (Schleiss, et al, 2000) whereas 8-isoprostane would primarily be detected from activity in the alveoli (Montuschi, et al., 2003; Carpenter, et al., 1998).

Airway health and lung function. Exhaled breath condensate volume does not depend on lung function parameters including FEV_1 and FVC in either normal subjects or in those

diagnosed with COPD. Currently there is no supporting evidence that changes in airway health cause any difference in mediator release or dilution of EBC.

Age, gender, height and weight. The largest amount of information available on biomarkers that affect the EBC is on H_2O_2. This mediator does not seem to be age dependent in children but does express higher concentrations in older than younger adults. This is likely attributable to senescence as well as any pathology. Body height and weight do not seem to have a relationship to EBC volume and H_2O_2; however, it is recommended that further research be conducted using other biomarkers. A study performed by Basu, et al, (2009) performed in three countries, Sweden, Poland and Italy, it was demonstrated a slight increased in isoprostane production in men when compared to women. No studies were identified that linked height and weight to changes in biomarker associated with lipid peroxidation.

Food and drink. To date there is no established data that links volatile mediator concentrations to food and/or drink consumption. However, no systematic studies have been performed. For the purposes of this study those individuals who ingest antioxidant supplements will be blocked when performing statistical analyses.

Circadian rhythm. Time of day appears to be relevant for H2O2 in the EBC of normal subjects and those with COPD. Further research studies are recommended to determine if this translates to biomarkers consistent with lipid peroxidation.

Tobacco smoking. Information regarding smoking is only available on cigarette smoking, not pipe, cigar or e-cigarette smoking. The effects of EBC biomarkers regarding the absence or presence of a filter, or specific brands have not been evaluated. Both acute and chronic smoking causes increases in H2O2, isoprostane, nitrate and nitrotyrosin levels, as well as increases in chemotactic activity. In patients with COPD, no difference was observed in the mean EBC H2O2 and 8-isoprostant levels between smokers and non-smokers (Horvath, 2005). The

ATS/ERS recommend study participants refrain from smoking for at least three hours prior to EBC analysis. Studies on the influence of continued smoking in the presence of diagnosed COPD do not demonstrate significant increases 8-isoprostane levels following acute smoking consumption (Montuschi, et al., 2000).

Systemic disease. The Task Force deems it important to consider the potential effect of systemic diseases, including extrapulmonary diseases, with regard to EBC mediators. In the report prepared by Horvath (2005) it was stated that 20-fold increases in EBC H_2O_2 levels were detected in uremic patients.

Current research suggests the synthesis of isofuran occurs during periods of increased cellular oxygen concentrations. Clearly this occurs as a result of delivery of supplemental oxygen therapy, but also can occur as a result of pathology that damages the mitochondria. In a report by Fessel et al, (2004) it was demonstrated that mice exposed to 100% oxygen conditions esterified isofurans were detected in the lungs, whereas isoprostane levels remained the same. It is theorized that diseases with mitochondrial dysfunction cellular tensions of oxygen increase due to the diminished capacity of the mitochondria to consume oxygen. This notion bore out in a study that discovered that isofurans, but not isoprostanes, are significantly increased in the substantia nigra in brains from patients with Parkinson's and Lewey body disease (Fessel et al, 2003). Other mitochondrial/neurodegenerative diseases that should be considered would include Alzheimer's, amyotrophic lateral sclerosis, and Huntington's disease.

The origin of biomarker synthesis remains a confounding variable in EBC research. Further research is recommended in this area. Developing methodologies that tightly address this conundrum are recommended. For purposes of this study all systemic diseases will be recorded.

Salivary contamination. Saliva contains many of the mediators present in lower airways. Care needs to be taken to avoid sample contamination. Many devices include a saliva trap to

control for this confounding phenomenon. The RTube™ offers a saliva trap and is the device selected for this project. Many studies have been conducted regarding potential of sample contamination to which the inclusion of a saliva trap has all but eliminated the concern.

Medications. While no study was identified addressing a relationship between steroid use and biomarker suppression the use of steroids will be noted for subjects participating in this study. Asthmatic patients have been a specific population used to address the potential of influence of ingested or inhaled steroids on 8-isoprostane production. Several studies demonstrate that despite treatment with oral or high doses of inhaled glucocorticoids patient with severe asthma have the highest concentrations of 8-isoprostane in EBC, indicating that this marker is relatively resistant to glucocorticoids, inhaled or ingested (Montuschi et al., 1999). Kostikas et al, (2002) reported a similar finding when comparing steroid naïve patients to those receiving inhaled doses. A study by Antczak et al, (2001) aspirin-tolerant asthma demonstrated in increase in 8-isoprostane following treatment of inhaled glucocorticoids. This finding demonstrates the possibility of a phenotype influence on biomarker response. The use of antioxidant supplements will be recorded; however, a study by Green and McElvaney (2009) the use of a variety of antioxidant therapies, none of which resulted in a reduction of exhaled 8-isoprostane. A history of bleomycin therapy will not result in exclusion from study even though it has inherent consequences to the lungs. The lack of research does not preclude the possibility of influence on biomarker production and therefore will be addressed during statistical testing.

Condensate storage. Some mediators, such as H_2O_2, are unstable at room temperature and therefore require immediate frozen storage. However, the isoprostanes are stable and less vulnerable to issues of timing. However, for purposes of this study samples will be immediately placed on dry ice in a cooler with a goal of -70°C.

8-isoprostane. The 8-isoprostane biomolecule is considered stable in exhaled breath condensate. Sample reproducibility is achieved with enzyme-linked kits with detection limits of 3.9 pg-ml. The assay was validated directly by gas chromatography/mass-spectrometry demonstrating a high correlation between added known amounts of the biomarker. GC/MS is considered the most sensitive method of detection and quantification of 8-isoprostane (Maloney et al, 2005). Disease states that influence 8-isoprostane concentrations in EBC are asthma, ARDS, pulmonary sarcoidosis, obstructive sleep apnea, cystic fibrosis, as well as healthy adults following exposure to ozone-inhalation (Horvath, 2005).

Isofuran. Isofuran is a biosynthetic relative of the isoprostane. It has been detected and quantified in blood, urine, plasma, CSF and exhaled breath condensate. Currently the GC/MS has been the only method utilized to detect and quantify its presence.

Safety concerns. The collection device utilized in this study will be the RTube™, a disposable device, which will eliminate any concern of contamination from one subject to the next. There are no reported risks associated with use of the device.

Content and compounds in EBC. Depending on the existing pathology respective compounds can be present within the alveoli when collected by a variety of collection devices made of glass, polystyrene, or polypropylene (see Table 3) (Mutlu et al., 2001). Compounds within EBC are detected and measured via liquid/gas chromatography and are most commonly quantified via mass spectrometry or enzyme-linked immunoassays. EBC does not currently represent all of the compounds detected in ALF; however, as assays or tools of measurement become more sensitive and a better understanding of the ionic composition of lung lining is gained this non-invasive test could prove invaluable (Hunt 2002).

Table 3

Compounds found in EBC

Pathology	Compound
Cigarette Smoking	H2O2, 8-Isoprostane
COPD	H2O2, 8-Isoprostane, serotonin, cytokines (IL-1, sIL-2R, TNF-α
Asthma	H2O2, 8-Isoprostane, nitrotyrosine thiobarbituric acid-reactive products, leukotrienes, pH
Chronic bronchitis	Leukotrienes
Bronchiectasis	H2O2
Cystic Fibrosis	H2O2, nitrite, 8-Isoprostane, IL-8
ALI/ARDS	H2O2, 8-Isoprostane, PGE_3

EBC Measurement

Several methods are used to measure the presence of compounds within exhaled breath condensate, to include gas or liquid chromatography, mass spectrometry, and enzyme-linked immunoassays.

An assay is "the analysis of a substance or mixture to determine its constituents or their level" (Webster online dictionary, 2013). Specific assays have been developed to detect and quantify the presence of target compounds within a mixture. Due to the inherent reactivity of biological assays, chemical analysis can become complex creating a challenge for controlling variability. This unpredictability can create challenges when interpreting the data. As such, care and attention must be given during testing so that variance is avoided.

Reproducibility of EBC Collection Methods

A method is considered reliable when the results are reproducible. In a study by van Beurden et al, (2002) 20 stable COPD patients breathed into a condensing device twice for a period of 10 minutes each time. Hydrogen peroxide was measured following collection and then again after freezing at -70° C for a period of 10, 20 and 40 days. The study concluded that the exhaled air volume and condensate volumes were strongly correlated with reproducible H2O2 even on samples stored for 40 days.

In a pilot study performed by Hoffmeyer et al, (2007) the methodology of EBC sampling and processing was evaluated to measure field assessments. Sixteen healthy adults were evaluated for four days over two consecutive weeks to determine the effects of sample collection (time vs. volume, with vs. without nose clip) and sample processing on volume, pH, NO, and LTB_4 Within-day, between-day, and between-week reproducibility were assessed. Results demonstrated that no significant differences existed with utilization of a nose clip; storage at 4-6°C for 24 hours had not significant effects on NO and LTB_4 concentrations but did result in increases in pH. The study result demonstrated "good" within and between-day reproducibility; however, between-week showed elevated results on the Mondays of each week.

Compound Detection and Measurement

Enzyme-linked immunosorbant assay (ELISA). The enzyme-linked immunosorbant assay (ELISA) is a type of assay that allows for the detection of a substance in a liquid or wet sample (Lequin, 2005). Perlmann and Engvall of Sweden developed the ELISA technique in 1971. The ELISA evolved from the radioactive-immunoassay (RIA) developed by Berson and Yalow in 1960 when evaluating endogenous plasma insulin. Radioactive labeling became very popular; however, growing concern of personnel over-exposure to radioactive materials, as well as the need to build and maintain safe appropriate laboratories and waste management, prompted

change. Initially, weaker radioactive iodine, iodine-125, was developed to address these concerns. However, the European RadioimmunoAssay Club (ERIAC) held meetings in the 1970's to approach the issue; it was during this time that the idea of using enzyme labels was born. Skepticism of how such a large and bulky molecule could attach to an antigen or antibody without sterically impeding the necessary chemical reaction was addressed through careful planning and execution of experiments. In 1971 Pearlman and Engvall published their first paper utilizing the ELISA by measuring rabbit serum IgG with alkaline phosphatase as the biomarker label.

Essentially, all microbial species have at least one antigen that is unique. As a result these antigens can be purified and used to produce specific monoclonal antibodies. Both the antibodies and the purified antigens can then be used as a diagnostic tool. The ELISA is such a diagnostic tool. There are two forms of this assay: 1) the direct ELISA uses monocolonal antibodies to detect the presence of a specific antigen in a sample; and 2) the indirect ELISA is used to determine the presence of a specific antibody in a sample.

Concerns expressed by Il'yasova et al, regarding the use of ELISA when quantifying isoprostanes is the cross-reactivity of the polyclonal antibodies used to bind with the IsoPs will bind to other similar molecules resulting in a potentially inflated quantification. They also purport that other biological impurities in the sample can interfere with antibody binding with the ELISA analysis, which impacts specificity, etc.

Mass spectrometry. Mass spectrometry (MS) is a technique that measures the mass-to-charge ratio of charged particles by separating them according to their masses. This technique is used to determine the masses of particles, the elemental composition of a sample or molecule, as well as revealing the chemical structures of molecules achieved by ionizing chemical compounds to generate charged molecules, ultimately resulting in mass-to-charge ratios. A mass

91

spectrometer consists of three modules: an ion source, a mass analyzer and a detector. The ion source converts gas phase molecules into ions. The mass analyzer sorts the ions by their masses through the use of an electromagnetic field. The detector measures the value of an indicator quality providing the raw data for calculating amounts of each ion present.

Mass spectrometry generally follows the following steps:

1. Loading a sample into the MS to undergo vaporization

2. Sample ionization through electron beam impaction

3. Ion separation by electromagnetic fields

4. Ion detection

5. Ion signal processing through mass spectra

A mass spectrometer has both qualitative and quantitative capabilities, such as identifying unknown compounds, determining the isotopic composition of elements in a molecule, determining the structure of a compound, quantifying the amount of a compound in a sample or studying the fundamentals of gas phase ion chemistry (Sparkman, 2000).

Chromatography. Chromatography is a method of physically separating mixtures for the purpose of identifying compounds and is achieved through two phases: mobile and stationary. The mobile phase consists of dissolving the sample mixture into a fluid. The resulting solution is then delivered into a holding structure with another predetermined mixture and becomes fixed in place, or the stationary phase. The compounds within the mobile mixture travel at varying speeds depending on their inherent properties, causing a separation. Differential partitioning of the compounds occurs between the mobile and the stationary phase, resulting in measurability of the compounds present in the mixture. Chromatography can either be used as a purification process for further analysis or for the analysis of smaller amounts of material to

determine relative proportions of analytes. Chromatography techniques include gas and liquid media.

Many techniques of chromatography can be employed to achieve separation, such as displacement, planar, paper, and thin layer chromatography. There is also column chromatography in which the stationary bed is within a tube or column. Sample identification is determined by the interaction/retention times occurring between the particles of the mobile phase and the content lining the column. Chromatography that employs techniques by physical state of mobile phase is called gas-liquid chromatography (GLC). Gas chromatography (GC) is based on a partition-equilibrium of an analyte between a solid stationary phase and a mobile gas phase. Liquid chromatography (LC) is a separation technique in which the mobile phase is a liquid rather than a gas.

High performance liquid chromatography. High performance liquid chromatography (HPLC) is an improved form of column chromatography. Rather than a mobile phase solvent dripping through a column under gravity the HPLC utilizes high pressure, up to 400 atmospheres, resulting in a much faster process. The HPLC also utilizes much smaller particle sizes for the column packing material giving it a much larger surface area for interaction between the stationary phase and the mobile phase for enhanced separation of the components of the mixture. The methods employed by the HPLC are highly automated and extremely sensitive. Measurable compounds include amino acids, proteins, nucleic acids, hydrocarbons, carbohydrates, drugs, terpenoids, pesticides, antibiotics, steroids, metal-organic species, as well as a variety of inorganic substances.

Gas chromatography. Gas chromatography is an instrument used to separate chemicals in a complex sample for the purposes of identification and quantification. Often the gas chromatograph is used in conjunction with mass spectrometry to achieve a much finer degree of

substance identification than can be achieved when used in isolation. Using these techniques in tandem reduces the likelihood of error, as it is very unlikely that two different molecules will behave in the same manner in both a gas chromatograph and a mass spectrometer.

Gas chromatographic techniques vaporize liquid samples as a means of separating them based on boiling points. Vaporization of the compound can occur without inciting decomposition of the compound. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture. In some situations GC can be used to identify a compound.

Gas chromatography is principally similar to column chromatography and HPLC with some notable differences. Separation of compounds is carried out between a liquid stationary phase and a gas mobile phase, rather than a solid stationary phase and liquid mobile phase used with column and HPLC techniques. The column through which a gas travels is located in an oven where the temperature of the gas can be controlled. GC uses the boiling points of compounds as a means of detecting their presence and identification.

Gas chromatography analysis requires a predetermined volume of analyte be injected into the entrance of a column. As the carrier sweeps the analyte through the column movement is inhibited by the adsorption of the analyte molecules by either the column walls or the packing materials. The rate of movement along the column is dependent upon the strength of adsorption. The total time it takes to travel the length of the column is referred to as the retention time. A detector is located at the distal end of the column and measures the outlet stream from the column. The time it takes each component to teach the outline and the concentration of that component can be determined. Substances are generally identified in the order they elute from the column and by the retention time of the analyte in the column.

For purposes of this study only gas chromatography-mass spectrometry will be addressed, as this will be the technique employed to detect and quantify isofurans in exhaled breath condensate.

Physical components. The following are specific components are required to carry out the individual functions of gas chromatography and mass spectrometry. Autosamplers: to introduce a sample automatically into the inlet of the analyzer. Automatic, rather than manual, insertion enhances reproducibility and maximizes time.

Inlets: provides the means to introduce a sample into a continuous flow of carrier gas. Detectors: component responsible for detecting the presence of the desired compound. The most commonly used detectors are the flame ionization detector (EID) and the thermal conductivity detector (TCD). Both are considered sensitive to a wide range of components, and both work over a wide range of concentrations.

Chromatogram: A detector responds to the presence of an analyte placed at the end of the column, the signal is plotted as a function of time (or of volume of the added mobile phase), and a series of peaks is obtained and plotted. This is referred to as a chromatogram. The position of peaks on the time axis may serve to identify the components of the sample; the areas under the peak provide a quantitative measure of the amount of each component. Once the analyte is injected into the system the time it takes to peak once it reaches the detector is referred to as the retention time.

Individual analyte particles during migration through the column will undergo thousands of transfers between the mobile and stationary phases. The amount of time spent in either phase is initially highly irregular and depends upon the thermal energy gained during the process to reverse the transfer. The residence time spent in each phase may be transitory or long. The particle is only eluted during the mobile phase contributing to irregular migration times. The

95

consequence of random individual processes is a symmetric spread of velocities around the mean value, which represents the behavior of the average and most common particle. The breadth of a band (time phenomenon) increases as it moves down the column due to the time allowed for spreading to occur. As a result the zone breadth is directly related to the residence time in the column and inversely related to the velocity at which the mobile phase flows.

Experiments must occur to enhance separation and improve particle resolution. Chromatographic separation is optimized once the components of a mixture are separated cleanly with minimum expenditure. Optimization experiments are aimed at either reducing zone broadening or altering relative migration rates of the components. Column resolution is a quantitative measure of the columns ability to separate two analytes. Quantitative analysis is the comparison of either the height or the area of the analyte peak with that of one or more standards. Analysis based on peak height can be achieved by connecting the base lines, on either side of the peak, with a straight line then measuring the perpendicular distance from this line to the peak. Peak heights are inversely related to peak widths. Therefore, accurate peak heights are only accurate if variations in column conditions do not alter the peak widths during the period required to obtain chromatograms for sample and standards. Variables that must be closely controlled for are column temperature, eluent flow rate, and the rate of sample injection. The effect of sample injection rate is particularly critical for early peaks of a chromatogram. Analysis based on peak areas are not affected by column temperature, eluent flow rate, or the rate of injection and, as a result, are a more satisfactory analytical parameter than peak heights.

Calibration occurs through the preparation of standards for comparison. Chromatograms for the standards are then obtained and peak heights or peak areas are plotted as a function of concentration. The major source of error in this process is the uncertainty of the sample volume and can be the rate of injection. The volumes for this process are small (~1μL); uncertainties

related to the injection of a reproducible volume of this size with a microsyringe may amount to several percent relative. This issue can be handled through the use of a rotate sample valve.

The utilization of internal standards enhances precision. With this process, a carefully measured quantity of an internal substance is introduced into each standard and sample, and the ration of analyte to internal standard peak areas or heights serves as the analytical parameter. However, in order for this method to be successful it is imperative that the internal standard peak be well separated from the peaks of all other components of the sample. The standard peak should be close to the analyte peak. A suitable internal standard will yield precisions better than 1% relative.

There are over 5000 identifiable substances in exhaled breath. As a result, the combination of gas chromatography and mass spectrometry provide an ideal mechanism for detection and quantification of IsoP's and IsoF's in exhaled breath. For purposes of this study GC/MS methodology will be discussed for the detection and quantification of isofuran.

Method development. Currently there are no commercially available kits or standards for the measurement of isofurans. The labs at Vanderbilt University have developed and retain exclusive right to the process of detecting and quantifying the presence of isofuran. Since this research study is questioning the potential of chronic exposure to supraatmospheric concentrations of oxygen in inducing oxidative stress, isofuran is the only appropriate biomarker identified to answer that question. Therefore, Vanderbilt University labs will be employed to analyze the exhaled breath condensate collected for this study.

Data Analysis

The objective of this study is to determine if LFDO as a treatment modality in COPD superimposes oxidative lung damage. The specific aims of this study are 1) assess for isofuran in the EBC of COPD patients on LFDO; 2) determine if a correlation exists between standard

diagnostic spirometry (FEV_1/FVC ratio) and exhaled breath condensate (EBC) biomarker (isofuran); 3) determine if relationships exist between age, gender, height, weight, length of smoking history, length and concentration of oxygen exposure and EBC biomarkers (isofuran levels).

Following a data collection period necessary to achieve an adequately powered sample statistical analyses utilizing a Student's t-test will be utilized to assess mean differences in exhaled isofuran levels between the active control and active treatment groups. Significant mean differences in isofuran levels between the groups will begin to address the potential of chronic exposure to low flows of domiciliary oxygen as a mechanism of in oxidative stress Significance will be set at a P-value of <0.05, a confidence interval of 95 and an α= 0.5 to reduce the occurrence of a Type I error, detecting an effect that does not exist.

Next, linear regression analyses will be performed to determine if a relationship exists between the study variables (age, gender, height, weight, ethnicity, smoking history, length of time and concentration of oxygen therapy, FEV_1, FEV_1/FVC, $FEF_{25-75\%}$ and isofuran levels). Should a relationship be detected correlational testing, utilizing the Pearson's *r,* will be performed to determine the strength of the relationship. As it relates to obstructive disease the FEV_1, FVC and the FEV_1/FVC ratio are standard to assessing the stage of COPD. It is well understood that the results of spirometric testing vary with effort and may produce inconsistent values as a result. Therefore, participants will be instructed to maximize effort for these specific tests to ensure accuracy. It is the desire of this researcher to determine if the isofuran biomarker has a future in predicting degree of obstructive disease states.

Chapter 3 – Methodology

Purpose

Chronic obstructive pulmonary disease in an advanced state often requires oxygen supplementation to ameliorate distress. It is well established that prolonged exposure to increased oxygen tensions contributes to oxidative stress and subsequent tissue damage. The purpose of this study is determine if the level of a specific biomarker of oxidative stress is elevated in the presence of exposure to low flow domiciliary oxygen (LFDO) in patients with COPD.

Research Questions

The research questions guiding this study are:

1. Does chronic exposure to LFDO contribute to oxidative stress as evidenced by elevated isofuran levels within the lungs of COPD patients?

2. Does a relationship exist between FEV1, FEV1/FVC, and FEF25-75% isofuran levels?

3. Is there a correlation between length of oxygen exposure and concentration of oxygen to isofuran levels?

Donabedian's Structure-Process-Outcome Model

Donabedian states, "the assumption is made that given the proper settings (structure) and instrumentalities (process), good medical care will follow" and, therefore, good and desired outcomes. Quality outcomes are only valid if it can be directly linked to the structure and/or

process (Donabedien, 1966). The results of the planned methodology and data collection will render results that will allow us to assess whether the structure in which care is provided, and the treatment modalities it prescribes to their patient's results in desirable outcomes. Consistent with the Donabedian process-structure-outcome model, the purpose of outcomes research is to determine if healthcare modalities and/or treatment management optimize patient and societal end results (Clancy and Eisenberg, 1998). With this in mind the following hypotheses were developed.

Hypotheses

The following hypotheses were formulated in response to findings within the literature review:

1. Ha: Chronic exposure to LFDO will demonstrate ongoing oxidative stress in the lungs of COPD patients as evidenced by increased isofuran levels.

2. Ha: A relationship between FEV1 and isofuran levels will be demonstrated in patients diagnosed with moderate to advanced stages of COPD using LFDO

3. Ha: A relationship between FEV1/FVC ratios and isofuran levels will be demonstrated in patients diagnosed with moderate to advanced stages of COPD using LFDO.

4. Ha: A relationship between FEF25-75% and isofuran levels will be demonstrated in patients diagnosed with moderate to advanced stages of COPD using LFDO

5. Ha: A positive correlation between oxygen concentration and isofuran levels will be demonstrated on the day of sampling in patients diagnosed with advanced stages of COPD using LFDO.

6. Ha: A positive correlation between length of oxygen exposure and isofuran levels will be demonstrated in patients diagnosed with advanced stages of COPD using LFDO.

This chapter will describe the research design, study setting, health care providers, sample selection process, sample characteristics, instruments, data collection procedures, and statistical analyses employed to address the research questions guiding this study.

Research Design

This research study utilized a non-experimental cross-sectional prospective data collection design to assess for the presence of oxidative stress for those individuals diagnosed with advanced stages of COPD requiring LFDO therapy. The active control group will consist of individuals diagnosed with advanced stages of COPD and managed with the standard therapeutic agents predetermined by their physician with the exception of LFDO therapy. The study did not impact the care or management of the study participants as no manipulation occurred to an already prescribed treatment plan. No risks are imposed on the patient as a result of this study. No patient identifying information will leave the confines of the clinic in which care is provided to the study participant. A coding system will be utilized to identify samples to relevant study variables.

Noted in the writings of Donabedian, healthcare provision variables are antecedents to healthcare outcomes and, therefore, study variables such as administrative demographics and mission, patient demographics, healthcare provider variables, pulmonary function test results, medicinal regimen, and isofuran biomarker measurements will be considered when evaluating outcomes. Those health care providers whose services impact patient care and process will be described. Patient demographics, such as age, gender, ethnicity, past and current smoking history are potentially confounding variables.

Independent and dependent variables. The treatment (independent) variable in this study is LFDO; the effect (dependent) variable is oxidative stress, represented by the biomarker isofuran. Also being assessed for are relationships between the independent variables of FEV1,

FVC, FEV1/FVC, FEF25-75, length of oxygen exposure (hrs/day and years), concentration of

oxygen therapy (L/min), smoking history and isofuran levels (see Table 4).

Table 4

Study Variables

Variable	Variable	Measurement	Statistical Analysis	Unit of Measure
LFDO	IV	Continuous	t-test	L/min
Length of Oxygen Exposure	DV	Continuous	Linear Regression Pearson's r	Hrs/day Years
Concentration of Oxygen Therapy	DV	Continuous	Linear Regression Pearson's r	Liters/minute
Length of Smoking History	DV	Continuous	Linear Regression Pearson's r	years
Isofuran	IV/DV	Continuous	Linear Regression 1-tailed Student's t-test	Pg/ml
FEV_1	DV	Continuous	Linear Regression Pearson's r	L/sec
FVC	DV	Continuous	Linear Regression Pearson's r	L/sec
FEV_1/FVC	DV	Continuous	Linear Regression Pearson's r	L/sec
$FEF_{25-75\%}$	DV	Continuous	Linear Regression Pearson's r	L/sec

Study setting. The setting for this study is a moderately sized medical center in Midwestern United States. This 236-bed facility offers acute care services as well as obstetrics, pediatrics, oncology services, long term care, skilled nursing facility, inpatient rehabilitation, outpatient clinic services and psychiatric treatment. The ethnic breakdown of the town of the medical center was as follows, with overall U.S. statistics in parentheses: Males: 55% (50%), Females: 45% (50%); Average age: 26 years (36.8); Caucasian: 84.8% (72.4%), Asian: 5.7% (4.8), African-American: 4% (12.6%), Hispanic: 2.6% (16.3%); two or more races: 2.4% (2.9%). The demographics between this Midwestern town and the United States were similar with the exception of average age and Hispanic population. Patients diagnosed with COPD and receiving services from an outpatient clinic at Phelps County Regional Medical Center will be recruited for participation in the study.

Population and Recruitment Strategy

In collaboration with Dr. James, Chief Medical Officer at Phelps County Regional Medical Center, a convenience sampling will be utilized to recruit participants. A review of patient records will be made to identify those patients with advanced stages of COPD, both receiving and not receiving LFDO as qualifiers. As qualifying patients are identified on site PCRMC staff to determine interest in participating in the study will approach them. Once interest has been solicited I will explain the study and provide a study participation outline. Informed consent will be obtained prior to sample collection. The population of interest for this study is those COPD patients with advanced symptoms of COPD confirmed by pulmonary lung volume measurements, specifically an FEV1/FVC ratio of < 70%, indicating obstructive disease of predicted normal volume for age, gender, height and weight. The goal of recruitment is to create closely matched comparison groups to avoid confounding variables. A convenience sample of patients with COPD and managed on an outpatient basis from the dates of March 10, 2015 to

May 7, 2015 was utilized. Group AC, or the active control group, consisting of those COPD patients diagnosed with advanced COPD but not receiving supplemental oxygen therapy, for Group AT, or the active treatment group, consisting of those COPD patients diagnosed with advanced disease requiring supplemental oxygen therapy.

Upon arrival to the outpatient clinic patients were provided a detailed explanation of the study outlining participation requirements including study goals and exhaled breath collection requirements as well as another opportunity to have any questions answered.

Inclusion/Exclusion Criteria

Eligibility for study participation was guided by inclusion/exclusion criteria. Inclusion/exclusion criteria served to control for variability and to aid in creating equivalent groups.

Inclusion criteria for the study were as follows:

1. >18 years of age

2. Mentally competent to provide informed consent (not currently under the care of a physician for such disabilities that preclude ability to consent).

3. Outpatient status

4. Diagnosed with advanced stage COPD as evidenced by spirometric FEV1 <70% of predicted normal for age and gender, height and weight, smoking history

5. With or without supplemental oxygen >6 hours/day depending on group assignment

Exclusion criteria for the study were as follows:

1. < 18 or > 89 years of age

2. Pregnant women

3. Mentally incapable of providing informed consent (currently under the care of a physician for such disabilities that preclude ability to consent).

4. Refusal to participate

5. Active, systemic, oral or nasal infections

6. Patients diagnosed with amyotrophic lateral sclerosis, Huntington's, Alzheimer's, Parkinson's or Lewey body disease.

Data Collection Procedures

Following Institutional Review Board (IRB) approval from the participating research institutions, Virginia Commonwealth University and Phelps County Regional Medical Center, study participants will be assigned to either an active control group or an active study group based on long-term low flow domiciliary oxygen therapy utilization. The active control group, Group AC, will consist of patients diagnosed with moderate to advanced stage COPD not receiving supplemental oxygen therapy; the active treatment group, Group AT, will consist of patients diagnosed with moderate to advanced COPD receiving supplemental oxygen therapy for a minimum of 6 hours/day. According to the American Thoracic Society guidelines, once criteria for oxygen therapy has been met the recommendation for oxygen therapy is for 24 hour use; however, in an effort to capture enough study participants a minimum oxygen usage has been of 6 hours has been selected, as supported by the Nocturnal Oxygen Treatment Trial and the Medical Research Council study.

Data Collection and Analysis Instruments

The following instruments and chemicals will be utilized for this study:

1. Futuremed Discovery II Spirometer™

2. The Rtube™ EBC collection device (Respiratory Research, Inc., manufacturer)

3. Gas chromatograph/Mass spectrometry

a. Capillary Gas chromatography column (DB-1701. Agilent: Cat. No. 21512067)

b. Gas chromatograph/mass Spectrometer (with capabilities for negative-ion chemical ionization (NICI) mass spectrometry. (Agilent 5973)

4. Isofuran quantification methods (owned by Vanderbilt University Labs)

Collection of exhaled breath condensate (EBC) is a noninvasive means of obtaining samples from lungs in order to assess pathologic processes. Due to the portability and ease of use the EBC devices can be used in a variety of settings, such has hospitals, clinics and even in the home. For the purposes of this study, a commercially manufactured, FDA approved EBC device, the Rtube™, will be used for collecting and storing as the EBC sample can be easily removed for storage and transport. The collection tube resides within an aluminum sleeve that is cooled by placing it in a freezer maintained at a temperature of -70°. The sleeve results in the condensation and collection of the exhaled gas. General guidelines for EBC collection suggest 10-15 minutes of normal tidal breathing as sufficient for collecting ~3mls of condensate; however, the diminished capacity of individuals with COPD may result in an increased time requirement to achieve the same volume. Also, in an effort to ameliorate fatigue during the EBC collection phase patients may be allowed to maintain nasal cannula oxygen delivery.

Pulmonary Function Testing

Phelps County Regional Medical Center population recruitment target sites include, but are not limited to, the Internal Medicine Clinic and the Cardiology clinic. Neither location participates in routine pulmonary function testing. Participants identified for study inclusion require current pulmonary function testing for comparison to same day IsoF levels. PFTs will be obtained using the portable Discovery II Spirometer™ by Futuremed. The Discovery II Spirometer ™ measure parameters include the parameters targeted for this study, FEV1, FVC,

FEV1/FVC and FEF25-75. The device has a range volume of 12 liters, flow of 0.03-20 l/s, a flow resistance of <0.70 cm H_2O/l/s @ 12 l/s, and an accuracy of ± 2%.

EBC Sample Collection and Preparation

Once group assignment is determined, consistent with the most current guidelines offered by the European Respiratory Society (ERS) and the American Thoracic Society study, with respect to determining a FEV1/FVC ratio of <70% predicted value, participants will be instructed to breath into an exhaled breath collection device, the Rtube™, with the following methods employed:

1. Normal tidal breathing into a mouthpiece attached to a collection chamber wrapped by a cooling sleeve for approximately 10-15 minutes, or until ~2-3 milliliters of condensate will be obtained. Sleeves will have been cooled over night in a -70° freezer maximizes analyte collection volume. The cooling sleeve will be wrapped with a protective cover.

2. Utilization of a nose clip will be employed for those who can tolerate it and/or required its use for adequate sample retrieval.

3. Utilization of a saliva trap to minimize salivary contamination of the analyte.

4. Storage of analyte in a -70° freezer until analysis can be made. Samples cannot be frozen, thawed and refrozen.

Approximately 5-15 minutes of normal breathing into the device should result in a volume adequate for measuring exhaled biomarkers. Nose clips can be utilized to minimize escape of exhaled air through the nose; however, compliance is of concern because they can be perceived as uncomfortable. Utilization of a saliva trap is necessary to minimize salivary contamination of the analyte; however, salivary contaminants have not proven to have significant

effects on the biomarkers of interest (Hutterman et al., 2011). A cooling sleeve is placed around the collection chamber to facilitate condensation.

The Rtube™ is designed to provide maximum sample collection with minimal time. Exhaled air is directed through a one-way valve and into a cooled collection chamber where vapors, aerosols and moisture in the breath condense along the protected walls. The one-way valve is then used as a plunger that collects droplets stuck to the inside wall. The samples are conveniently stored and shipped in the collection sleeve. No risks are introduced to the study participant as a result of breathing into the condensate collector.

The clinic staff and the PI will share in the responsibility of overseeing adherence to collection techniques and timing. Samples will be labeled to identify group membership. Samples will be logged with identifying code along with the relevant demographics of age, gender, race, height, weight, LFDO concentration, and oxygen exposure timeline. Samples will be securely sealed in transport coolers at a temperature of -70° C for transport to lab until analysis can be made.

Laboratory Analysis

The labs at Vanderbilt University will be employed to analyze the exhaled breath condensate, as there currently are no commercially available isofuran standards available for public acquisition. The technique utilized by Vanderbilt University to detect the presence and quantification of isofuran in exhaled breath is through GC/MS, currently the only method established for detecting and quantifying isofurans. Therefore, the methods employed to perform this test are described below.

Gas chromatography permits the separation of closely related components of complex mixtures. The GC method is noted for its sensitivity, adaptability to accurate quantitative determinations, its suitability for separating nonvolatile species or thermally fragile species, and

its widespread applicability to substances that are of prime interest to the industry, science and the public.

Chromatography methods occur in two phases: mobile and stationary. In column chromatography, which will be used in this study, the stationary phase is held in a narrow, coiled, heated tube through which the mobile phase travels. As the mobile phase comes in contact with the stationary phase and separation of compounds occurs. This separation results in the ability to identify and quantify targeted substances. The use of mass spectrometry adds sensitivity and specificity as it identifies the compounds based on molecular weight.

Sensitivity is achieved by the GC/MS technique through the ability to resolve the peaks. Resolution occurs by adjusting flow rates and mobile phase composition. Adjusting flow rates allows for the separation of peaks for accurate identification. Mobile phase packing allows for the separation of polar from non-polar compounds, thus enhancing sensitivity. When using the GC/MS for detection of both IsoPs and IsoFs quantification can only occur when they are in the free acid form. In tissues a plasma arachidonic acid oxidation products are generally esterified in glycerophospholipids. The glycerophospholipids must be subjected to alkaline hydrolysis to release the compounds into the free fatty acid form to ready them for GC/MS analysis (Milne, et al., 2013).

The GC/MS methodology is considered robust and has been used for over 20 years. According to Milne et al (2013) "this particular method offers the lowest limit of quantification of any reported mass spectrometric methodology for IsoPs." Due to the fact that these particular molecules are measured in terms of picograms (or similar derivations) in plasma, CSF, EBC and other biological fluids in which low levels of IsoPs are found the GC/MS is ideal as a means of detection and quantification of these groups of molecules. The low limit of detection for isoprostane is 5 pg/ml.

For the purposes of compound identification many reagents may be employed, as well as the equipment necessary to perform the functions. A detection and quantification standard for isofuran is not currently available to the public for purchase and use. Researchers at Vanderbilt University discovered isofuran and the methods for its detection and quantification the list provided are those reagents commonly used when identifying by-products of non-enzymatic lipid peroxidation, such as the IsoPs, at the Vanderbilt Laboratory. The following agents have been provided regarding the identification process for IsoPs, which can be applied to the identification of IsoFs with the delineating difference being the eluting times.

Instrumentation. The following instruments were used to identify and quantify IsoF:

- Capillary gas chromatography column (DB-1701, Agilent)

- Gas chromatograph/mass spectrometry (GC/MS, with capabilities for negative-ion chemical ionization (NICI) mass spectrometry)

While many different means can be utilized to detect the presence and amount of IsoPs, such as LC/MS and immunoassays, only GC/MS has been identified as accurately detecting and measuring IsoFs. Identifying and quantifying the F_2-IsoPs by mass spectrometry has advantages over immunoassay procedures, such as the ELISA. While the ELISA methodology is cost effective and provides quality analysis, the antibodies used to bind to the F_2-IsoPs may exhibit cross-reactivity with many other molecules similar in structure, confounding the result. Also, biological impurities can interfere with antibody binding. Mass spectrometry offers high sensitivity and specificity resulting in enhanced detection and accuracy of desired compounds.

Once specimens arrive in the lab GC/MS analysis will be performed in triplicate to gain accurate results of the isofuran biomarker. The three results will be averaged in an effort to most accurately represent the results. The results then will be logged into a data bank to include group membership, age, gender, race, LFDO concentration utilized, and oxygen exposure timeline.

Data Analysis

The objective of this study is to determine if LFDO as a treatment modality in COPD superimposes oxidative lung damage. The specific aims of this study are 1) assess for isofuran in the EBC of COPD patients on LFDO; 2) determine if there is a correlation between standard diagnostic spirometry and exhaled breath condensate (EBC) biomarker (isofuran) levels; and 3) determine if relationships exist between duration and concentration of oxygen exposure and EBC biomarker isofuran levels.

Following a thorough literature review evaluating methodologies described in this paper for this study it was determined that a large effect size exists between control and treatment groups regarding the generation of isoprostanes, consistent with oxidative stress. Since isofurans are derived from isoprostanes the assumption holds that the effect of the treatment, LFDO, will result in a large effect size regarding isofuran production.

To determine the significance of isofuran in the EBC of the active treatment group, severe stage COPD patients on LFDO, compared to the active control, severe stage COPD patients not receiving LFDO, a Student's t-test will be performed, with the assumptions of bivariate independent variables, a continuous dependent variable, independent observations of the dependent variable, and normal distribution being met. A power analysis was performed utilizing the software G*Power Analysis for a 1-tail t-test to compare group means of the active control and active treatment groups with a $\alpha=0.5$ to control for Type I errors and a $\beta= 0.80$ to control for Type II errors with an effect size of 0.8, and generated an n = 52.

Pulmonary function testing includes a variety of assessments that provide for the specific interpretation of their results, such as changes in lung volumes and capacities, respiratory flow patterns and rates, as well as diffusion capacity, or the exchange of nutrients between the alveoli and the capillary beds. In an effort to better assess where oxidative stress exerts its derangement

111

it is necessary to determine a relationship between the presence of oxidative stress and the parameters most affected by its presence. This study will directly assess for the relationship between isofuran, a validated biomarker of oxidative stress, and the standard diagnostic pulmonary function parameter of FEV1 and FEV1/FVC. However, all spirometry parameters will be collected and evaluated as a mechanism of discovery and future evaluation. Linear regression will be performed to determine if a relationship between FEV1/FVC exists, and the strength of that relationship determined with correlation analysis. Assumptions of linear regression include linearity and additivity, statistical independence of the errors, homoscedasticity of the errors and normality of the error distribution will be met. If a relationship is identified through linear regression a Pearson's r will be utilized to assess for strength of the relationship between the demographic independent variables and the dependent variable, isofuran. Considerations may have to be made when assessing isofuran levels and the age demographic as senescence can result in diminished antioxidant ability.

In order to better understand the impact of LFDO on COPD patients with regard to ongoing oxidative stress relationships between duration of oxygen therapy (hours/day of use) and concentration of oxygen (L/min flow) need to be explored. Linear regression will be performed to determine if a relationship between length of exposure and concentration of oxygen relates to isofuran levels. Should a relationship be identified the strength of that relationship will be determined through correlation analysis, the Pearson's r. Assumptions of linear regression include linearity and additivity, statistical independence of the errors, homoscedasticity of the errors and normality of the error distribution will be met.

Chapter 4 – Results

Introduction

The purpose of this study was to determine if oxidative stress is occurring in the lungs of individuals with COPD as a result of chronic exposure to low flow domiciliary oxygen (LFDO) therapy as evidenced by a specific biomarker, isofuran (IsoF). A methodology was developed to address this phenomenon and statistical analyses employed to interpret the findings. Each result will be preceded by the research question it intended to address.

A power analysis, utilizing scientific Graph Pad Prism 6.0 software, revealed a sample size of 52 necessary to sufficiently power this study. A convenience sample of 54 subjects was identified at Phelps County Regional Medical Center, collected from March 12, 2015 to May 7, 2015 that met study inclusion criteria. Two subjects were removed from the active treatment (AT) group due to insufficient exhaled breath condensate sample volume necessary to detect and quantify IsoF. The active control (AC) group consisted of 26 participants, 15 males (58%) and 11 females (42%); and 26 participants in the active treatment, 10 males (38%) and 16 females (62%) individuals all of whom had an FEV1/FVC <0.70 and FEV1 <80% of predicted normal (See Table 5). The AT group utilized LFDO for a minimum of 7 hrs/day. No current research exists regarding risks of prescription LDFO therapy, therefore, duration of oxygen (hrs/day) exposure did not impact study inclusion. All study participants were Caucasian.

Table 5

Study Participant Demographics

	Control (COPD no Oxygen)	Treatment (COPD with Oxygen)	P value
Number	26	26	
Age (years)	62 ± 29	65± 16.5	0.3655
Male/Female	15/11	10/16	0.1717
Height (inches)	66 ± 5	65 ± 7.5	0.3079
Weight (lbs)	190 ± 87.5	217 ± 119	0.0900
Caucasian	26	26	
Smoking History	22/26	22/26	
Current Smokers	13/26	0/26	

*Age, height and weight are reported as the mean ± standard deviation

Study participants enrolled in the study from the cardiology clinic received

pulmonary function testing the day of data collection by the PI utilizing the Discovery II

Spirometer™. Study participants enrolled in the pulmonary function lab received

pulmonary function testing the day of data collection by certified respiratory therapists.

Vmax Encore Software v21-2A by CareFusion is utilized by the PCRMC to interpret

pulmonary function test results. A *t*-test was performed to compare group means between

the AC (COPD without LFDO) and the AT (COPD with LFDO) (see Table 6).

Table 6

Study Participant Spirometry Results

	Control (COPD no Oxygen) Mean + SEM	COPD (w/ Oxygen) Mean + SEM	P value
FEV1	60.19 ± 2.73	60.08 ± 3.92	0.9819
FVC	75.81 ± 3.67	70.42 ± 4.60	0.3615
FEV1/FVC	64.63 ± 2.86	68.38 ± 2.81	0.3544
FEF25-75	34.93 ± 3.99	51.38 ± 6.06	0.0265

It was the intention of this study to create closely matched study groups with

regard to age, gender, height, weight, ethnicity and the spirometric tests FEV1, FVC,

FEV1/FVC and FEF25-75, (with the notable exception of LFDO therapy) to enhance

interpretability of the results. Student's *t*-tests were performed to compare group means

for each parameter. No significant differences were noted between any of the parameters

in the AC and AT groups with the exception of the FEF25-75 results, which

demonstrated a P = 0.0265. This difference warrants future investigation.

The following research questions were addressed through statistical analyses.

Research Question 1

Does chronic exposure to LFDO contribute to oxidative stress as evidenced by elevated

isofuran levels within the lungs of COPD patients?

Using the statistical software package, Graph Pad Prism 6, a one-tailed *t*-test was

performed to determine the mean difference in exhaled breath IsoF levels between the

AC and AT groups. The descriptive statistics are provided below (see Table 7).

Significance was set at a P value <0.05, an α=0.05, with a 95% CI. The results

demonstrated a t=1.61, with df=50, and a nonsignificant P = 0.056 with a SEM 35.81±

4.91 of IsoFs in the active control, and a SEM 51.35±8.27 of IsoFs in the treatment group

(see Table 8). The R^2 value of 0.049 suggests that only 4.9% of the variance could be

explained by LFDO therapy. An F test to compare variances was performed resulting in a

P=0.0115, indicating significant differences in variances between groups. An unpaired *t*-

test with a Welch's correction for unequal standard deviations was performed generating

a nonsignificant P=0.0569 (see Table 9, Figure 1). Based on these findings it cannot be

supported that LFDO therapy results in statistically significant increases in exhaled

breath IsoF levels in COPD patients.

Table 7
Descriptive Statistics

	N	Mean	Standard Deviation	Std Error Mean
Active Control IsoFs	26	35.81	25.062	4.908
Active Treatment IsoFs	26	51.35	42.170	8.270

Table 8
Unpaired One-Tailed t-test comparing group means, 95% Confidence Interval

One-Tailed *t*-test table	Isofuran Mean ±SEM	df	Difference between the Mean ±SEM	P value
Control Group	35.81±4.91	25	15.54±9.62	P = 0.0562
Treatment group	51.35±8.27	25		

Table 9
Unpaired One-tailed t-test with Welch's correction

One-Tailed *t*-test table	Isofuran Mean ±SEM	t	df	Difference between the Mean ±SEM	P value
Control Group	35.81±4.91	1.616	40.67	15.54±9.62	P = 0.0569
Treatment group	51.35±8.27				
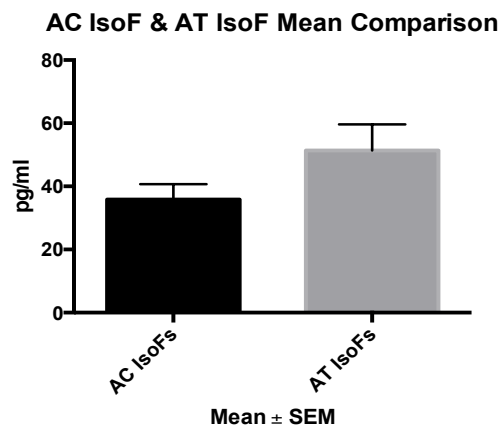
Figure 1: AC IsoF & AT IsoF Means Comparison

The *t*-test descriptive statistics revealed an AC group standard deviation (SD) of 25.03 and an AT SD of 42.17. Clearly, a larger SD is noted in the AT group, meaning a wider dispersion of obtained values. A between groups data dispersion comparison was made (see Table 10). Evidenced by the dispersion data a significant difference is noted between the maximum values obtained between the groups. No a priori knowledge existed suggesting cause for exclusion of the 181pg/ml sample obtained and, therefore, the result remained.

Table 10
Between Groups Data Dispersion

	AC pg/ml	AT pg/ml
Minimum	10.0	11.0
25% Percentile	16.75	18.75
Median	27.5	33.5
75% Percentile	44.0	79.25
Maximum	96.0	181.0

The mean AC group exhaled breath IsoF level was 35.82 pg/ml with a one SD range of 10.78 to 60.84 pg/ml, and a relatively small SEM (4.91) suggesting less variation in the AC group when compared to the AT group. The AT group mean exhaled breath isofuran level was 51.35 pg/ml with a one SD range of 9.18 to 93.52 pg/ml, a comparatively larger SEM (8.27), suggesting more unexplained variance in the AT group than in the AC group.

Research Question 2

Does a relationship exist between FEV1, FVC, FEV1/FVC, FEF25-75% and isofuran levels?

The following relationships were explored through simple linear regression utilizing the statistical software package SPSS, version 23. An analysis of the statistical

117

results, including a Pearson's *r* correlation, coefficients, model summary and ANOVA is provided. Tables, graphs and figures may accompany the explanation to enhance clarity of the findings. It is the intention of the PI to determine the clinical application of IsoF as replacement for standard pulmonary function testing. Therefore, in the following analyses IsoF is the situated as the predictor variable, while the PFTs are the outcome variables. The results of the linear regression analysis are reported below.

A simple linear regression was calculated to determine if a relationship exists between FEV1 in COPD patients receiving LFDO therapy and exhaled breath isofuran levels. A line of best-fit analysis revealed a relatively flat slope of -0.07314 to 0.3125 and a goodness of fit R^2=0.075 indicating a very weak correlation to the best-fit line (see Figure 2), an assessment supported the B value revealing that every 1 unit of increase in isofuran would only generate a 0.132 unit of change in the FEV1. According to the R^2 coefficient of determination only 7.5% of the model variation could be explained by exhaled breath IsoF levels, leaving approximately 92.5% of the variance unexplained. A model summary was performed revealing a Pearson correlation (r =0.274) (see Table 11), synonymous with the Beta coefficient (see Table 12), demonstrating a weak relationship between IsoF levels and FEV1. A subsequent ANOVA analysis of the model summary was performed with a resulting nonsignificant P=0.176, confirming that isofuran levels do not correlate to FEV1 values (see Table 13). In summary, no significant correlation was established between IsoF levels from individuals with COPD on LFDO to FEV1.

AT IsoFs & AT FEV1

Figure 2: AT IsoFs & AT FEV1

Table 11
AT IsoF and FEV1 Model Summary

Model	R	R Square	Adjusted R Square	Change Statistics		
				R Square Change	F Change	df1
1	.274	.075	.036	.075	1.941	1

Table 12
AT IsoF and FEV1 Regression Coefficients

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig
	B	Std. Error	Beta		
1 (Constant)	54.147	6.244		1.941	.176
Active Treatment IsoF	.132	.095	.274		

Table 13
AT IsoF and FEV1 ANOVA for Regression Analysis

Model	Sum of Squares	df	Mean Squares	F	Sig
1 Regression	774.173	1	774.173	1.941	.176
Residual	9571.673	24	398.820		
Total	10345.846	25			

119

A simple linear regression was calculated to determine if a relationship exists between IsoF levels and the FVC in COPD patients receiving LFDO therapy. A line of best-fit analysis revealed a relatively flat slope of -0.1937-0.2732 and a goodness of fit $R^2=0.001$ indicating a very weak correlation to the best-fit line (see Figure 3), assessment supported by the B value revealing that every 1 unit of increase in IsoF levels would only generate a 0.015 unit of change in the FVC. According to the R^2 coefficient of determination only 0.1% of the model variation could be explained by exhaled breath IsoF levels, leaving approximately 99.9% of the variance unexplained. A model summary was performed revealing a Pearson correlation (r =0.028) (see Table 14), synonymous with the Beta coefficient (see Table 15), demonstrating a weak relationship between isofuran levels and FVC. A subsequent ANOVA analysis of the model summary was performed showing no statistical difference (P=0.892), confirming that IsoF levels do not correlate to FVC values (see Table 16). No significant correlation was established between IsoF levels from individuals with COPD on LFDO to FVC.
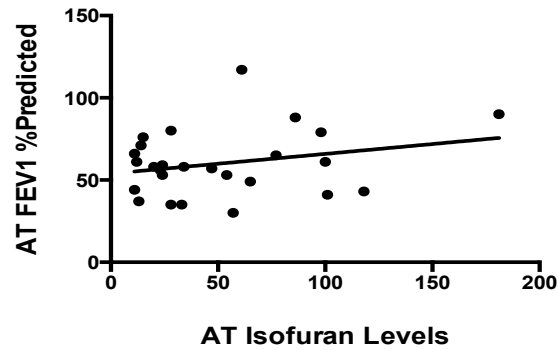
AT IsoFs & AT FVC

Figure 3: AT IsoFs & AT FVC

Table 14
AT IsoF and FVC Model Summary

Model	R	R Square	Adjusted R Square	Change Statistics		
				R Square Change	F Change	df1
1	.028	.001	-0.041	.001	0.019	1

Table 15
AT IsoF and FVC Regression Coefficients

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig
	B	Std. Error	Beta		
1 (Constant)	69.212	7.396		9.358	.000
Active Treatment IsoF	.015	.112	.028		.892

Table 16
AT IsoF and FVC ANOVA for Regression Analysis

Model	Sum of Squares	df	Mean Squares	F	Sig
1 Regression	10.462	1	10.462	.019	.892
Residual	13429.538	24	559.564		
Total	13440.000	25			

A simple linear regression was calculated to determine if a relationship exists between FEV1/FVC in COPD patients receiving LFDO therapy and exhaled breath IsoF levels. A line of best-fit analysis revealed a relatively flat slope of -0.08833-0.1949 and a goodness of fit R^2=0.051 indicating a very weak correlation to the best-fit line (see Figure 4), an assessment supported the B value revealing that for every 1 unit of increase in IsoF levels would only generate a 0.076 unit of change in the FEV1/FVC. According to the R^2 coefficient of determination only 5.1 % of the model variation could be explained by exhaled breath IsoF levels, leaving approximately 94.1% of the variance unexplained. A model summary was performed revealing a Pearson correlation (r

AT IsoFs & AT FEV1/FVC

Figure 4: AT IsoFs & AT FEV1/FVC

=0.227) (see Table 17), synonymous with the Beta coefficient (see Table 18),

demonstrating a weak relationship between IsoF levels and FEV1/FVC. A subsequent

ANOVA analysis of the model summary was performed showing no statistical difference

(P=0.265), confirming that IsoF levels do not correlate to FEV1/FVC values (see Table

19). No significant correlation was established between exhaled breath IsoF levels from
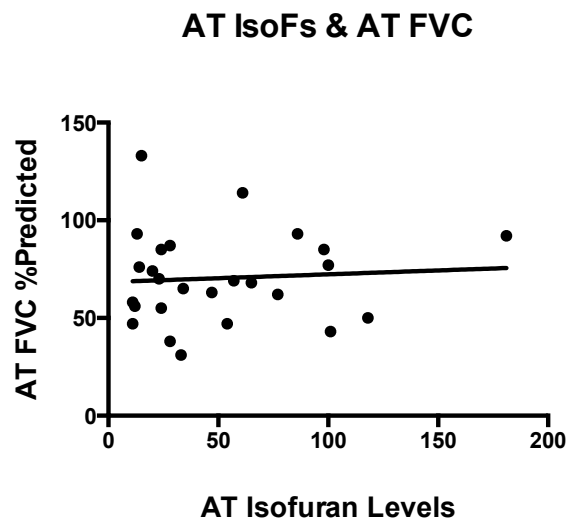
individuals with COPD on LFDO to FEV1/FVC.

Table 17
AT IsoF and FEV1/FVC Model Summary

Model	R	R Square	Adjusted R Square	Change Statistics		
				R Square Change	F Change	df1
1	.227	.051	0.012	.051	1.302	1

Table 18
AT IsoF and FEV1/FVC Regression Coefficients

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig
	B	Std. Error	Beta		
1 (Constant)	64.091	4.399		14.570	.000
Active Treatment IsoF	.076	.067	.227	1.141	.265

122

Table 19

AT IsoF and FEV1/FVC ANOVA for Regression Analysis

Model		Sum of Squares	df	Mean Squares	F	Sig
1	Regression	275.732	1	257.732	1.302	.265
	Residual	4750.268	24	197.928		
	Total	5008.000	25			

A simple linear regression was calculated to determine if a relationship exists between FEF25-75% in COPD patients receiving LFDO therapy and exhaled breath IsoF levels. A line of best-fit analysis revealed a relatively flat slope of -0.1784-0.4298 and a goodness of fit R^2=0.038 indicating a very weak correlation to the best-fit line (see Figure 5), an assessment supported the B value revealing that for every 1 unit of increase in IsoF levels would only generate a 0.146 unit of change in the FEF25-75. According to the R^2 coefficient of determination only 3.8 % of the model variation could be explained by exhaled breath IsoF levels, leaving approximately 96.2% of the variance unexplained. A mod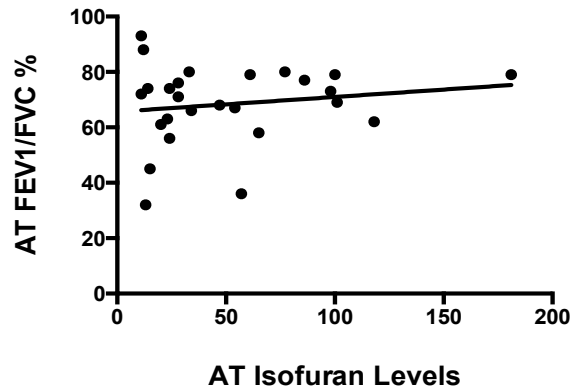el summary was performed revealing a Pearson correlation r =0.194 (see Table 20), synonymous with the Beta coefficient (see Table 21), demonstrating a weak relationship between IsoF levels and FEF25-75%. A subsequent ANOVA analysis of the model summary was performed showing no statistical difference (P=0.265), confirming that IsoF levels do not predict FEF25-75% values (see Table 22). While significant between group differences were noted on pulmonary function test FEF25-75 parameters no significant between group differences were noted with regard to exhaled breath IsoF. No significant correlation was established between IsoF levels from individuals with COPD on LFDO to FEF25-75.
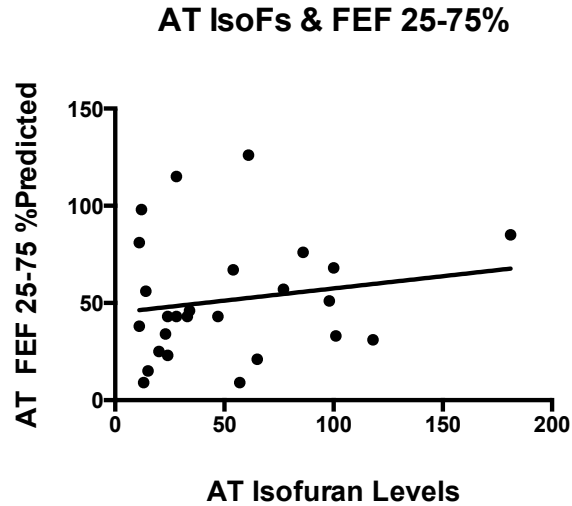
AT IsoFs & FEF 25-75%

Figure 5: AT IsoFs & FEF25-75

Table 20
AT IsoF and FEF25-75% Model Summary

Model	R	R Square	Adjusted R Square	R Square Change	F Change	df1
				Change Statistics		
1	.194	.038	-.003	.038	.935	1

Table 21
AT IsoF and FEF25-75% Regression Coefficients

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig
	B	Std. Error	Beta		
1 (Constant)	44.710	9.934		4.501	.000
Active Treatment IsoF	.146	.151	.194	.967	.343

Table 22
AT IsoF and FEF25-75% ANOVA for Regression Analysis

Model	Sum of Squares	df	Mean Squares	F	Sig
1 Regression	943.994	1	943.994	.935	.343
Residual	24224.045	24	1009.335		
Total	25168.038	25			

124

Research Question 3

Is there a correlation between length of smoking history, and length of oxygen exposure and concentration of oxygen to isofuran levels?

The following relationships were explored through simple linear regression utilizing the statistical software package SPSS, version 23. An analysis of the statistical results, including a Pearson correlation, coefficients, model summary and ANOVA is provided. Tables, graphs and figures may accompany the explanation to enhance clarity of the findings. The results of the linear regression analysis were as follows:

A simple linear regression was calculated to determine if a relationship exists between length of smoking history (yrs) in COPD patients receiving LFDO therapy and exhaled breath IsoF levels. A line of best-fit analysis revealed a relatively flat slope of -1304-0.2243, and a goodness of fit $R^2 = 0.012$ indicating a very weak correlation to the best-fit line (see Figure 6), an assessment supported the B value revealing that every 1 unit of increase in IsoF levels would only generate a 0.047 unit of change in the smoking history. According to the R^2 coefficient of determination only 1.2% of the model variation could be explained by exhaled breath IsoF levels, leaving approximately 98.8% of the variance unexplained. The model summary revealed a Pearson correlation r =0.111 (see Table 23), synonymous with the Beta coefficient (see Table 24), demonstrating a weak relationship between IsoF levels and length of smoking history. A subsequent ANOVA analysis of the model summary was performed showing no statistical difference (P=0.590), confirming that IsoF levels are not significantly correlated with length of smoking history (see Table 25). Not all individuals in the AT group had a history of smoking. Four individuals reported having never smoked. No significant correlation was

AT IsoFs & Length of Smoking History
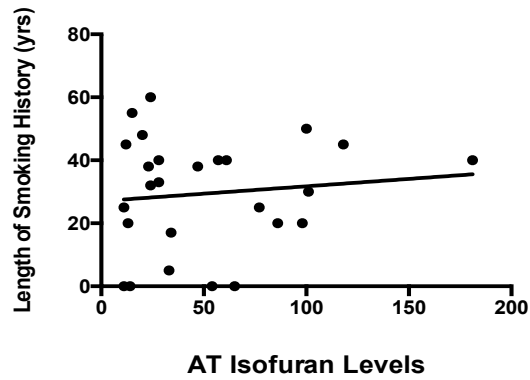
Figure 6: AT IsoFs & Length of Smoking History

Table 23
AT IsoF and Length of Smoking History Model Summary

Model	R	R Square	Adjusted R Square	Change Statistics		
				R Square Change	F Change	df1
1	.111	.012	-.029	.012	.299	1

Table 24
AT IsoF and Length of Smoking History Regression Coefficients

	Unstandardized Coefficients		Standardized Coefficients		
Model	B	Std. Error	Beta	t	Sig
1 (Constant)	27.049	5.665		4.775	.000
Active Treatment IsoF	.047	.086	.111	.547	.590

Table 25
AT IsoF and Length of Smoking History ANOVA for Regression Analysis

Model	Sum of Squares	df	Mean Squares	F	Sig
1 Regression	98.144	1	98.144	.299	.590
Residual	7878.317	24	328.263		
Total	7976.462	25			

established between IsoF levels from individuals with COPD on LFDO to length of

smoking history (yrs).

A simple linear regression was calculated to determine if a relationship exists

between length of daily oxygen exposure (hours/day) in COPD patients receiving LFDO

therapy and exhaled breath IsoF levels. A line of best-fit analyses revealed a relatively

flat slope of -0.03538-0.09946 and a goodness of fit R^2=0.039, indicating a very weak

correlation to the best-fit line (see Figure 7), an assessment supported the B value

revealing that every 1 unit of increase in IsoF levels would only generate a 0.032 unit of

change in the length of oxygen therapy (hrs/day). According to the R^2 coefficient of

determination only 3.9% of the model variation could be explained by exhaled breath

IsoF levels, leaving approximately 96.1% of the variance unexplained. A model summary

was performed revealing a Pearson correlation r =0.196 (see Table 26), synonymous with

the Beta coefficient (see Table 27), demonstrating a weak relationship between IsoF

levels and length of daily oxygen exposure (hours/day). A subsequent ANOVA analysis

of the model summary was performed showing no statistical difference (P=0.590),

confirming that IsoF levels are not correlated to length of daily oxygen exposure (hrs)
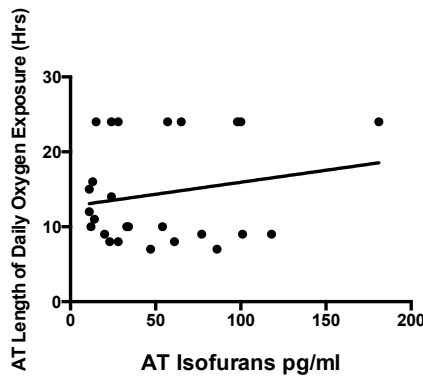
AT IsoFs & AT Length of Daily Oxygen Exposure (Hrs)

Figure 7: AT IsoFs & AT Length of Daily Oxygen Exposure (hrs)

Table 26

AT IsoF and Length of Daily Oxygen Exposure (hrs) Model Summary

Model	R	R Square	Adjusted R Square	Change Statistics		
				R Square Change	F Change	df1
1	.196	.039	-.002	.039	.962	1

Table 27

AT IsoF and Length of Daily Oxygen Exposure (hrs) Regression Coefficients

	Unstandardized Coefficients		Standardized Coefficients		
Model	B	Std. Error	Beta	t	Sig
1 (Constant)	12.739	2.154		5.916	.000
Active Treatment IsoF	.032	.033	.196	.547	.336

(see Table 28). No significant correlation was established between isofuran levels from

individuals with COPD on LFDO to length of daily oxygen exposure (hrs).

Table 28

AT IsoF and Length of Daily Oxygen Exposure (hrs) ANOVA for Regression Analysis

Model	Sum of Squares	df	Mean Squares	F	Sig
1 Regression	45.646	1	45.646	.962	.336
Residual	1138.508	24	47.438		
Total	1184.154	25			

A simple linear regression was calculated to determine if a relationship exists

between oxygen delivery (L/min) in COPD patients receiving LFDO therapy and exhaled

breath IsoF levels. A line of best-fit analysis revealed a relatively flat slope of -0.003896

-0.007623 and a goodness of fit $R^2=0.018$ indicating a very weak correlation to the best-

fit line (see Figure 8), an assessment supported the B value revealing that every 1 unit of

increase in IsoF levels would only generate a 0.002 unit of change in the oxygen delivery

(L/min). According to the R^2 coefficient of determination only 1.8% of the model

AT IsoFs & AT Oxygen Concentration
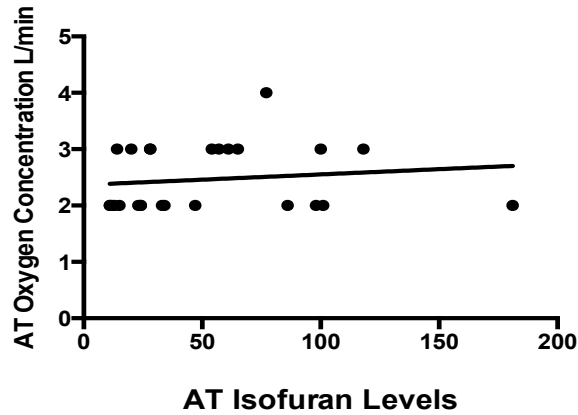
Figure 8: AT IsoFs & AT Oxygen Delivery (L/min)

variation could be explained by exhaled breath IsoF levels, leaving approximately 98.2%

of the variance unexplained. A model summary was performed revealing a Pearson

correlation r =0.135 (see Table 29), synonymous with the Beta coefficient (see Table 30),

demonstrating a weak relationship between IsoF levels and oxygen delivery (L/min). A

subsequent ANOVA analysis of the model summary was performed showing no

statistical difference (P=0.511), confirming that IsoF levels are not correlated to oxygen

delivery (L/min) (see Table 31). No significant correlation was established between IsoF

levels from individuals with COPD on LFDO to oxygen delivery (L/min).

Table 29

AT IsoF and Oxygen Delivery (L/min) Model Summary

Model	R	R Square	Adjusted R Square	Change Statistics		
				R Square Change	F Change	df1
1	.135	.018	-.023	.018	.446	1

Table 30

AT IsoF and Oxygen Delivery (L/min) Regression Coefficients

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig
1	(Constant)	2.366	.184		12.861	.000
	Active Treatment IsoF	.002	.003	.135	.668	.511

Table 31

AT IsoF and Oxygen Delivery (L/min) ANOVA for Regression Analysis

Model		Sum of Squares	df	Mean Squares	F	Sig
1	Regression	.154	1	.154	.446	.511
	Residual	8.307	24	.346		
	Total	8.462	25			

A simple linear regression was calculated to determine if a relationship exists between duration of oxygen therapy (yrs) in COPD patients receiving LFDO therapy and exhaled breath IsoF levels. A line of best-fit analysis revealed a relatively flat slope of 0.06711 -0.01327 and a goodness of fit $R^2 = 0.074$ indicating a very weak correlation to the best-fit line (see Figure 9), an assessment supported the B value revealing that every 1 unit of increase in IsoF levels would only generate a -0.027 unit of change in the duration of oxygen therapy (yrs). According to the R^2 coefficient of determination only 7.4% of the model variation could be explained by exhaled breath IsoF levels, leaving approximately 92.3% of the variance unexplained. A model summary was performed revealing a Pearson correlation r =0.272 (see Table 32), synonymous with the Beta coefficient (see Table 33), demonstrating a weak relationship between IsoF levels and duration of oxygen therapy (yrs). A subsequent ANOVA analysis of the model summary was performed showing no statistical difference (P=0.180), confirming that IsoF levels are not correlated to duration of oxygen delivery (L/min) (see Table 34). No significant

130

AT IsoFs & AT Length of Oxygen Therapy (yrs)
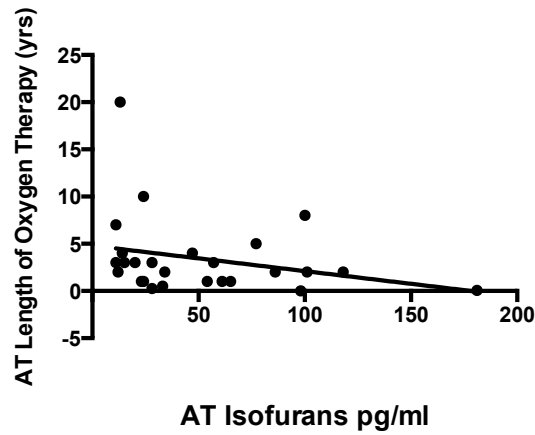
Figure 9: AT IsoFs & AT Length of Oxygen Therapy (yrs)

Table 32

AT IsoF and Duration of Oxygen Therapy (yrs) Model Summary

Model	R	R Square	Adjusted R Square	Change Statistics		
				R Square Change	F Change	df1
1	.272	.074	.035	.074	.1.911	1

Table 33

AT IsoF and Duration of Oxygen Therapy (yrs) Regression Coefficients

	Unstandardized Coefficients		Standardized Coefficients		
Model	B	Std. Error	Beta	t	Sig
1 (Constant)	4.799	1.284		3.738	.000
Active Treatment IsoF	-.027	.019	-.272	-1.382	.180

Table 34

AT IsoF and Duration of Oxygen Therapy (yrs) ANOVA for Regression Analysis

Model	Sum of Squares	df	Mean Squares	F	Sig
1 Regression	32.215	1	32.215	1.911	.180
Residual	404.610	24	16.859		
Total	436.825	25			

131

correlation was established between isofuran levels from individuals with COPD on LFDO to duration of oxygen therapy (yrs).

Secondary Findings

In an effort to better understand whether "years" of exposure of LFDO influenced the IsoF levels, "time" (years of exposure) was subdivided into three categories: 0-2 years (n=14), 3-6 years (n=8), and 7-20 years (n=4) (see Figure 10). An ANOVA was performed to compare group means between the three established time frames. A P value was set at a significance level of 0.05 at a CI 95%. The results generated were means of 65.57, 33.63 and 37.00 respectively, with a $P = 0.1795$ (see Table 35). In summary, "time" in terms of years of exposure showed no statistical significance with regard to exhaled breath IsoF levels in the lungs of COPD on LFDO therapy.

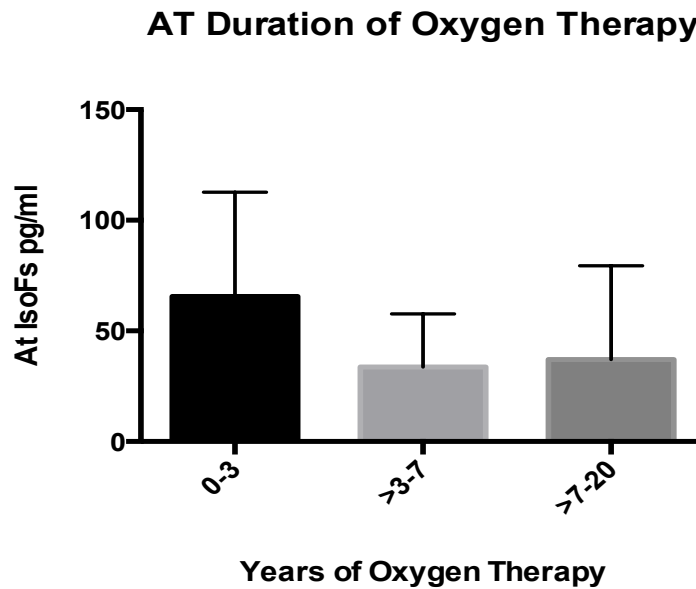**AT Duration of Oxygen Therapy**

Figure 10: AT Duration of Oxygen Therapy (yrs)

Table 35

One-way Analysis of Variance for Years of Oxygen Exposure

ANOVA table	SS	DF	MS	F(DFn, DFd)	P value
Treatment (between columns)	6169	2	3084	F (2,23) = 1.853	P = 0.1795
Residual (within columns)	38289	23	1665		
Total	44458	25			

Chapter 5 – Summary & Discussion

Introduction

This chapter summarizes the results of this research study and provides discussion regarding the findings. The results were examined in the context of the literature review and the theoretical framework guiding this study. Description of the data collection site and population demographics is presented first with the integration of the theoretical framework to follow. Quantitative results are discussed as they relate to the individual research questions and the theoretical framework. A methodological review will be presented. Finally, limitations, implications of the study and suggestions for future research are presented.

Purpose

The purpose of this study was to elucidate whether or not LFDO contributes to ongoing oxidative stress in the lungs of COPD patients. Supplemental oxygen is a known inducer of oxidative stress. Oxidative stress is a central mechanism in the pathologic processes resulting in the parenchymal changes in the lungs commensurate with chronic bronchitis and emphysema. It is understood that oxygen therapy clearly improves symptomatic dyspnea associated with the disease in individuals with COPD; however, the medical research community would be remiss to ignore the nocuous potential of chronic oxygen exposure.

Synthesis of Quantitative Results

No research to date has been conducted utilizing IsoF as a marker of oxidative stress in the lungs of COPD patients. Actually, no research has been conducted utilizing any biomarker to determine if LFDO presents an oxidative risk. This study is foundational in that regard. The results of this study provide benchmark information about baseline IsoF levels in the COPD population subset, with and without LFDO. Isofuran is a biosynthetic relative to the validated oxidative stress biomarker, IsoP, whose formation is favored in the presence of elevated oxygen tensions. This particular sensitivity to oxygen tension makes it an ideal biomarker to examine the potential of oxygen therapy. A simple first step approach to uncovering this potential was to create closely matched groups with regard to age, gender, COPD diagnosis and ethnicity and to compare the mean IsoF levels between the active control (no LFDO) and treatment groups (with LFDO).

Research Question 1. The first research question asks if chronic exposure to LFDO contributes to oxidative stress in the lungs of COPD patients as evidenced by elevated exhaled breath IsoF levels**.** The resulting study groups demonstrated no statistically significant demographic differences, which serves to minimize threats to internal validity. No statistical difference in mean IsoF levels was determined between the AC and AT groups (P=0.057); therefore, the null hypothesis was accepted.

While inferences may not be drawn from this result, questions are raised. What is responsible for IsoF levels greater than one standard deviation from the mean in four participants in the control group and five participants in the treatment group? Are there other factors, such as concomitant comorbidities, that contribute to IsoF formation detectable in exhaled breath? How long does it take for IsoF levels to return to basal following cessation of therapy? Is IsoF an

appropriate biomarker to address supplemental oxygen as a contributor of oxidative stress? Does length or concentration of oxygen exposure explain these variations? The latter question will be addressed in a subsequent section; however, regarding relevant comorbidities, little information has been established in exhaled breath biomarker research implicating known sources of confounding IsoF results with the exception of those with known mitochondrial diseases, such as amyotrophic lateral sclerosis (ALS), Alzheimer's, Lewy body and Parkinson's diseases (Fessel et al., 2003). Even then, what is understood about the significant presence of IsoF in these diseases was tested in the substantia nigra of individuals diagnosed with Parkinson's and Lewy body disease, not exhaled breath. As a result, minimal exclusions were imposed except for those diagnosed with ALS, Alzheimer's, Lewy body and Parkinson's diseases.

When considering significant sources of increased tissue oxygen tension alveolar macrophages (AMs) must be included, as they responsible for producing large quantities of ROS. According to Barnes (2004) the conditions imposed by AM activation in the lungs leads to a cascade of processes that "together could account for all of the pathophysiological features of COPD." A mechanism of defense incited by AMs is respiratory burst, a marked increase in superoxide (an oxygen radical) to serve as a bactericide. Vacchiano et al (1997) demonstrated in rat culture a mean 8-IsoP macrophage production of 14 ± 1 pg/ml when exposed to air, and a 25 ± 2 pg/ml when exposed to 100% oxygen. This evidence supports macrophage contributions to increased oxidant load, and, subsequently, oxidative stress when unchecked. Relevant to note in the same study, however, are the findings that show no increase from baseline in mean macrophage production of 8-IsoP following exposure to 50% oxygen. This finding supports that concentrations $\leq 50\%$ FiO_2 do not contribute to ongoing oxidative stress at normal partial pressures (at sea level or 1 ATA). When compared to the current study, it suggests this IsoF

levels obtained are likely a result of the original disease process and not the addition of LFDO therapy as no study participants were exposed to >36% at 1 ATA.

A significant challenge, with regard to accurately interpreting the results, in the current study is that no data exists defining baseline exhaled breath IsoF levels in healthy subjects or in individuals diagnosed with COPD. Only three studies to date have employed exhaled breath IsoF as a biomarker of oxidative stress, all of which utilized different study groups (refer to Table 36). The three studies are presented below with comparisons made in order to discern any existing inferences between them.

A study by Dworski et al (2009) demonstrated a basal exhaled breath IsoF level of 3.9 ± 3.3 pg/ml (mean $\pm$ SD) in known human allergic asthmatics. The study demonstrated that asthmatics who were exposed to an inhaled antigen challenge (IAC) showed an increase in exhaled breath IsoFs at all time points, with significance being reached at 2 h (P=0.036) and 4 h (P=0.025) post challenge (7.7 ± 6.7 and 6.8 ± 4.7 pg/ml respectively). While the population for the current study (adults with COPD) differed from the Dworski study (children with asthma) it is the only published study utilizing exhaled breath as a medium for which to evaluate and compare the results of the oxidative stress biomarker, IsoF. The mean exhaled breath IsoF level for the control group in the Dworski study (3.9 pg/ml) was significantly lower than that the mean obtained in the active control group in the current study (35.8 pg/ml). The disparity in control means may reflect an overall disparity in disease states of the participants: adults with advanced COPD compared to asthmatic children in a quiescent state.

In an unpublished study by Vacchiano (2005) the EBC IsoF levels of naval pilots who were exposed to 100% oxygen intermittently during high altitude flight over the course of their flying careers were compared to age, gender and smoking matched naval pilot candidates

(control group) whom had never been exposed to supplemental oxygen (personal

communication). The control group mean EBC IsoF levels obtained from the pilot candidates

was reported to be 3.1 ± 3.5 SD pg/ml. The results obtained are very similar to the control mean

obtained in the Dworski study, 3.9 ± 3.3 SD pg/ml, suggesting they may be reflective of basal

IsoF levels in healthy lungs. The AC group (COPD patients without LFDO) mean EBC IsoF

value obtained in the current study of 35.8 ± 25.0 SD represents diseased lungs. The oxygen

exposed pilot group in the Vacchiano study generated a mean EBC IsoF level of 38.8 ± 116.5 SD

(15.86 SEM) pg/ml, similar to the AC group EBC IsoF values obtained in the current study (see

Table 36). In addition, there was a further marked increase in EBC IsoF levels, from their

already increased baseline levels, in the actively flying pilot group following a training flight

during which 100% oxygen was inspired for an average of 1.5 hours. It was reported in the

personal communication that the oxygen exposed pilots percent (%) predicted FVC and FEV_1

values were lower than expected for the entire aviator group at 3 measurement periods (preflight

and 2 post-flight periods). There was also a statistically significant decrease compared to

preflight baseline in both % predicted FVC and FEV_1 at the 1st post flight measurement period in

aviators with 2200 to 3500 hours of total flight time breathing 100% oxygen. This suggests that

this group of aviators had developed signs of restrictive lung disease during the course of their

careers. It can be speculated that chronic intermittent high concentration supplemental oxygen

exposure is central to that development. The control group for the current study and the treatment

group for the Vacchiano study reflect basal IsoF levels in diseased lungs. Both have incurred

lung damage but from different instigators of oxidative stress. The COPD patients have

developed their disease due to first or second hand smoke, or environmental pollutants while the

pilots appear to have incurred lung damage as a result of inspiring sufficient supplemental

138

Table 36.

Exhaled Breath Isofuran Studies

Study	N	Control IsoF Mean ± SD pg/ml	Treatment IsoF Mean ± SD pg/ml	Population
Dworski (2009)	27	3.9 ± 3.3	7.7 ± 6.7 6.8 ± 4.7	Children with human allergic asthma
Vacchiano (2005)	48	3.1 ± 3.5	38.8 ± 116.5	Naval pilot candidates Naval pilots
Stulce (2015)	52	35.8 ± 25.0	51.4 ± 42.2	COPD w/o LFDO COPD with LFDO

oxygen required to achieve a blood oxygen partial pressure to maintain normal cognitive

function at high altitude. Explanations for the increased exhaled breath IsoF mean (51.4 ± 42.2)

in the current study treatment group remains unclear; however, they follow a similar trend as in

the Vacchiano study, increasing from baseline following oxygen therapy, possibly indicating that

supplemental oxygen may be responsible for the observed increases in exhaled breath IsoF.

As reported in the literature review, no medications are known to impact IsoP formation

in humans, and, presumably then IsoF, as it is a biosynthetic relative of IsoP would similarly not

be affected. Corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs) and acetylsalicylic

acid (ASA) inhibit prostaglandin synthesis via the cyclooxygenase pathway. Prostanoid isomers,

such as the isoprostanes, are produced as a result of non-enzymatic processes and, therefore,

corticosteroids, NSAIDs and ASA presumably have no significant impact on their formation.

However, it has been demonstrated that ASA significantly reduced 8-IsoP production by primary

cultures of rat alveolar macrophages under laboratory conditions but has not been substantiated

in human studies (Vacchiano, Osborne and Tempel, 1997). This finding suggests an alternative

mechanism of ASA in regard to ameliorating oxidant load, such as scavenging of reactive oxygen species (ROS) or through the NADPH oxidase inhibition, the enzyme responsible for the production of superoxide as a result of respiratory burst. In the current study ASA use did not preclude study participation. Medication lists were reviewed as a method of confirming co-morbidities, however, no subjects were excluded due to medication use. The six participants in the active control group that reported daily low dose aspirin use generated an IsoF range of 15-42 pg/ml; in the active treatment group the six participants reporting daily low dose aspirin use generated an exhaled breath IsoF range of 11-77pg/ml. The AC IsoF group mean ± SEM was 35.81 ± 4.92; the AT group mean ± SEM was 51.35 ± 8.27. Daily aspirin use did not appear to exert a negative influence on IsoF synthesis in either group, suggesting that ASA may not have ROS scavenger or NADPH oxidase inhibitor capabilities in this population subset likely due to the degree of lung pathology.

In summary, the lack of significant findings in this study are a result of 1) the methodology employed was not suitable to address the research questions; 2) IsoF, a biomarker influenced by multiple sources, lacks specificity with regard to source, rendering it unsuitable for purposes of evaluating LFDO as an instigator of oxidative stress; or 3) LFDO at concentrations less than 36% do not contribute to ongoing oxidative stress in the lungs of individuals of COPD. The contribution of this study to the large gap in literature on IsoF as a biomarker of oxidative stress is, however, as a benchmark with which to compare future research. The comparison of the three studies (Dworski, Vacchiano, Stulce) begins to shed light on baseline EBC IsoF in a variety of populations. Exhaled breath biomarker analysis is in its infancy, with exhaled breath IsoF analysis nearly non-existent. A recent PubMed search reported 692 research studies since 1992 utilizing "exhaled breath" as a search term; of those, 58 studies were conducted in 2015. A

140

PubMed search utilizing "isofuran" as the search term yielded 21 studies, only one of which evaluated exhaled breath as the medium for IsoF analysis. The current study may provide insight into the scope of its value.

Research Question 2. The second research question asks whether or not a relationship exists between FEV1, FVC, FEV1/FVC, FEF25-75% and IsoF levels. The noninvasive nature of exhaled breath condensate collection makes it an ideal method for the assessment of pathologic processes occurring in individuals in fragile health states, such as those with advanced COPD. In light of this concept, multiple studies have been conducted to correlate exhaled breath biomarkers to those pulmonary function test parameters consistent with diagnosing and quantifying disease states of individuals with COPD, but without real success. Until this study none had attempted to correlate exhaled breath IsoF levels to standard diagnostic spirometry, such as FEV1, FVC, FEV1/FVC, and FEF25-75%.

To address this question current PFTs were obtained to compare and correlate to exhaled breath IsoF levels to assess for clinical application. In order to obtain accurate PFT results maximal effort is required, and often times multiple maneuvers, all of which can be very challenging for the individual with COPD, particularly those with advanced disease. If an actual correlation between a validated exhaled breath biomarker and pulmonary function parameters could be identified, individuals could theoretically be spared from this rigorous PFT process. IsoF was selected as the predictor variable with the intention of replacing conventional pulmonary function testing.

A linear regression statistical model was employed to explore possible relationships between FEV1, FVC, FEV1/FVC, and FEF25-75% and exhaled breath IsoF levels. No significant relationships were revealed between any of the tested pulmonary function parameters

and exhaled breath IsoF. As mentioned, multiple studies have been performed to determine if a correlation exists between exhaled breath oxidative biomarkers and pulmonary function test results without success with the exception of a study conducted in 2010 by Carraro et al, which evaluated 20 children diagnosed with asthma, comparing their exhaled breath 8-IsoPs to FEV1/FVC and FEF25-75% parameters to identify whether or not a correlation exists. The analysis demonstrated that 8-IsoP in the exhaled breath condensate was significantly increased in children with problematic asthma (p=0.05) with a negative correlation to both the FEV1/FVC and FEF25-75% (r= -0.5), and both statistically significant (P=0.03). Although these findings did not utilize IsoF as the oxidative stress biomarker, it is closely related to 8-IsoP, suggesting a potential relationship could be established in the same setting. As such, support for continued research and refinement in determining whether or not exhaled breath biomarker analysis as a noninvasive means of predicting disease states has a place as a diagnostic tool should continue. While no correlation was established in the current study between IsoF and FEF25-75% (r=0.194, P=0.34) it is of interest to note a significant difference in FEF25-75% existed between the AC and AT groups (P=0.0265). Two methods were employed to collect PFT data, the Discovery II Spirometer™ conducted by the PI, and in the PFT lab conducted by certified respiratory therapists. Error may have been introduced as a result; however, no significant differences in the remaining test parameters were noted.

In summary, studies have failed to consistently correlate standard spirometric testing with validated oxidative stress biomarkers. The current study also failed to identify a correlation between FEV1, FVC, FEV1/FVC and FEF25-75% parameters and the oxidative stress biomarker, IsoF. However, the ideal noninvasive conditions offered by exhaled breath analysis to individuals with advanced lung disease make it desirable as a diagnostic and prognostic tool.

Exhaled breath biomarker analysis is still in the early stages; as more research is conducted and methodologies improved there may come a time when correlations can be made between exhaled breath biomarkers and current standard diagnostic testing.

Research Question 3. The third research question asks whether or not exhaled breath IsoF levels were correlated to length of oxygen exposure, smoking history and/or concentration of oxygen delivery (L/min). Understanding the relationships between the relevant variables of time of exposure and concentration of oxygen delivery to IsoF levels could provide crucial insight into the potential of LFDO as a mechanism of ongoing oxidative stress. It is intuitive that time would be a relevant factor in promoting oxidative stress as longer exposure provides more opportunity to incite oxidative stress. However, in the current study, chronicity did not result in significant elevations in exhaled breath IsoF levels. In fact, an individual who had been on oxygen for 20 years generated an exhaled breath IsoF level of 13 pg/ml, one of the lowest values obtained in the study, while the individual generating the highest exhaled breath IsoF level (181 pg/ml) reported oxygen use for only 3 weeks. Perhaps the crucial assessment period, with regard to "time", is at the initiation or introduction of LFDO therapy.

It is also intuitive to believe that dose (or concentration) would influence IsoF production, as more of the offending substance is available to incite pathology. The relevance of concentration may exist, however, with the higher concentrations/doses. There are many studies implicating high concentrations ($\geq 50\%$) of supplemental oxygen exposure to oxidative stress, however, there are no studies evaluating low flow/concentration (L/min) of oxygen therapy for individuals on LFDO therapy as a mechanism of oxidative stress. The L/min flows of oxygen therapy reported in the current study were between 2-4 L/min (28-36% concentration). Two L/min was the lowest reported concentration of oxygen therapy in the current study, it generated

both the lowest and the highest IsoF values at 11 pg/ml after LFDO 12 hrs/day for 3 years and 181 pg/ml with LFDO 24 hrs/day for 3 weeks. The single participant reporting 4 L/min of daily oxygen use (9 hrs/day for 5 years) generated an exhaled breath IsoF of 77 pg/ml. The disparity in the range in IsoF compared to concentration suggests LFDO does not influence oxidative stress.

It is well established, particularly in diving and aviation literature, that oxygen is toxic when breathed at a partial pressure in excess of 0.4 ATA (40% O2 at atmospheric pressure) for a sufficient period of time. As partial pressures exceed 0.4 ATA, oxidant load increases and pathology ensues. Nagatomo et al (2012) exposed male Wistar rats to 14.4%, 20.9%, 35.5%, 39.8%, 62.5% and 82.2% oxygen at 1 ATA for 24 hours. Derivatives of reactive oxygen metabolites (dROMs) in plasma were utilized as an index of oxidative stress. It was concluded that 14.4%, 20.9% and 35.5% oxygen did not affect dROM levels; however, rats exposed to 39.8% 62.5% and 82.2% oxygen had significant ($p < 0.05$) increases in dROMs, with the highest level in the 82.2% oxygen exposure group. Vacchiano et al (1997) had a similar finding with no significant increase in alveolar macrophage mean concentration of 8-IsoP at 50% oxygen exposure at 1 ATA. These studies demonstrate safe oxygen therapy administration <40% oxygen at 1 ATA. With regard to the current study, these findings support LFDO concentrations ranging from 28-36% as a safe therapy. Concentrations this low minimally impact blood oxygen partial pressures, particularly in the presence of diseased lungs with a decreased diffusion capacity.

There is evidence, however, that brief exposure (30-60 minutes) to low concentrations of oxygen (28%) results in significant increases ($p < 0.05$; $p < 0.001$) in validated exhaled breath oxidative stress biomarkers (BMAC; 8-Isoprostane and IL-6) in healthy adults (Phillips, 2003; Carpagnano, 2004). The target population in the Phillips study was exclusively healthy adults while the Carpagnano included both healthy adults and adults with COPD. Both studies

144

employed a pre and post EBC analysis after breathing ambient air and 28% supplemental

oxygen. While these time points (30-60 minutes) do not represent chronicity they do address the

concept "low concentrations/flow" as a mechanism of oxidative stress. The results from the

Phillips and Carpagnano studies are in conflict with what is currently understood about minimal

oxidant load occurring as a result of concentrations <40% at sea level. With regard to the current

study, there may be some similarities to those reporting brief or sporadic exposure generating the

highest results. The individual reporting sporadic use in the two months prior to sample

collection generated an exhaled breath IsoF of 148 pg/ml. The individual reporting three weeks

of 24 hrs/day 2L/min of oxygen use reported 181 pg/ml. These results, along with those reported

in the Phillips and Carpagnano study, support investigating oxidative stress associated with an

introductory phase of LFDO therapy, and subsequently, the mechanisms responsible for return to

baseline in its continued presence.

To explore the concept of "time" further to determine if IsoF levels were influenced by

length of exposure in terms of years a one-way analysis of variance (ANOVA) was performed.

Lengths of exposure were defined as 0-2 years (n=14), 3-6 years (n=8) and 7-20 years (n=4).

These range bins were determined to meet the criteria necessary to perform an ANOVA.

Significance differences between groups was not found (P = 0.1795), meaning time in terms of

years of exposure did not implicate LFDO as a mechanism of oxidative stress. Consistently the

variables of potential influence on oxidative stress have not been substantiated in this study.

Again, indicating that the IsoF levels obtained in the study are likely a result of preexisting

diseases, not LFDO.

In summary, with what is currently understood regarding IsoF as a biomarker sensitive to

elevated tissue oxygen tensions it could not be demonstrated that concentration of oxygen

delivery (L/min), or duration of therapy in terms of hours/day or years predictably influenced its production. Low flow domiciliary oxygen prescription flow rates fall between 1-5 L/min (24-44%). It has been substantiated that concentrations < 40% oxygen delivery at 1 ATA are safe. The current study had no participants utilizing greater than 36%; therefore, it would not be expect to observe an elevation in IsoF. There may be evidence suggesting oxidative stress may occur during an initiation phase of oxygen therapy. As more is understood regarding the variables influencing oxidative stress, and hence the biomarkers of oxidative stress, the more specific methodologies can be developed and employed, bringing healthcare provision closer to quality outcomes. Until then, the current study suggests that LFDO does not contribute to ongoing oxidative stress, but rather that the IsoF levels are a reflection of disease.

Theoretical Framework

The overarching goal, as it relates to health sciences research, is patient safety. An organized and methodological approach is ideal when attempting to determine if practice modalities/treatments are optimal in promoting, maintaining and restoring health. Donabedian's Structure-Process-Outcome framework postulates that if the institutional "structure" and "process" are constructed and conducted in the appropriate way then quality outcomes will certainly follow. To assess whether LFDO therapy is appropriately prescribed and managed at Phelps County Regional Medical Center the Structure-Process-Outcome model was utilized.

Qualitative Observations. Donabedian's model of Structure-Process-Outcome provides a framework by which to evaluate the effectiveness and/or quality of healthcare. "Structure" refers to the context in which care is delivered, such as hospital buildings, administration, staff, economic resources and equipment. Phelps Country Regional Medical Center is active in and supportive of research as a mechanism by which to provide those they serve with the most

appropriate and effective care. The Chief Medical Officer at PCRMC supported this research

endeavor in the form of allowing the PI access to all areas of the hospital that offered and

managed the care of individuals with COPD in an effort to better understand any potential risks

associated with the use of prescribed LFDO therapy as a treatment modality. This level of

engagement demonstrated the administration's attitude and commitment toward providing

quality care.

Areas accessed for this research project included the cardiology, internal medicine, and

the pulmonary function clinics. The clinics were appropriately staffed with licensed practical

nurses, registered nurses, and nurse practitioners, registered physical therapists, and certified

respiratory therapists and physicians. All areas were equipped with emergency equipment and

protocols to address untoward medical emergencies. The cardiac and internal medicine clinics

were housed in the Medical Office Building directly attached to the hospital, while the

pulmonary function lab was housed within the hospital itself. The location of the clinics

facilitated ease of transport and quick access to acute care. The "structure" aspect, which

promotes quality outcomes, was sufficient.

"Process" refers to the sum of all interactions between patients and providers throughout

the delivery spectrum that translates to healthcare. It is during the "process" phase that diagnoses

are made, treatments are formulated and delivered, and patient education takes place. The

manner in which care is delivered significantly influences patient satisfaction and perception of

quality. The "process" of delivery at PCRMC is collaborative between disciplines per protocol.

Phelps County is committed to the evolution of improved communication to enhance outcomes.

Witness to this process was observed firsthand during the data collection phase. This assessment

was not only made as a primary observer, but also as a result as the consistent feedback obtained from client surveys of the respective clinics as part of their quality assurance commitment.

"Outcome" refers to those measurable or discernable changes in health status, behavior, or knowledge as well as overall patient satisfaction and health-related quality of life improvements. Improving health states is the primary goal of healthcare; therefore, assessing outcome with regard to this central parameter is necessary. Health "state" can be defined broadly or narrowly. With regard to this study, a very specific assessment of a prescribed treatment was utilized to determine the "outcome" of said treatment. The benefits of LFDO therapy are well understood; however, until recently, there had not been a specific mechanism by which to assess for pathology related to LFDO. Utilizing new techniques to measure IsoF allowed us to examine the outcome of a prescribed treatment of LFDO offered by PCRMC to their constituents.

In summary, according to Donabedian, outcome is conceptually defined as the end result of the process of care to include "those things, either favorable or adverse, in the actual or potential health status of persons, groups, or communities that can be attributed to medical care" (Donabedian, 1985, p.256). Based on the results of this study, when utilizing IsoF as an outcome measurement of LFDO, quality can be assumed, as ongoing oxidative stress as a result of LFDO therapy was not substantiated.

Methodologic Review

The final inquiry for this study is whether or not the methodology employed is suitable for future research. The response is a mixed yes and no. The exhaled breath condensate collection protocol utilized in the current study is consistent with recommendations by the American Thoracic Society and European Respiratory Society (ATS/ERS), and consistent with the protocol at Vanderbilt Eicosanoid Core labs, currently the only known lab in the United

States equipped to identify and quantify IsoFs. Therefore, no changes are currently indicated with regard to the actual sample collection, storage and analysis methodology.

Refinement of inclusion/exclusion criteria for participant selection is required. Greater insight is warranted regarding the impact acquired mitochondrial disease and alveolar macrophages contributes to increased tissue oxygen tensions and, thus, IsoF formation. Until this is realized it will be difficult to precisely assess exogenous oxygen as the mitigator of oxidative stress when utilizing the IsoF biomarker.

Confounding study results circle around the highest IsoF values obtained (181 and 148 pg/ml) by individuals reporting either less than a month or sporadic use of LFDO in the two months prior to data collection. Is it a function of time? Are there compensatory mechanisms that explain the lack of findings? Are there particular disease processes that result in greater IsoF synthesis? As a result of these, and other questions, a methodology addressing these confounders requires development.

Exhaled breath condensate analysis was included in the methodology in the current study to evaluate LFDO as a mechanism of ongoing oxidative stress due to its noninvasive low risk profile. Historical approaches to diagnosing and prognosing pulmonary disease states include bronchoscopy with bronchoalveolar lavage and/or biopsy. Risks associated with the procedure vary depending on the overall health state of the individual. Individuals with COPD who are chronically hypercarbic with symptomatic shortness of breath are at risk for post procedure ventilatory support (Rand et al, 2013). As COPD progresses even mild insults can result in exacerbations. Exhaled breath condensate collection as an approach of assessing disease states in this fragile population makes it desirable.

Limitations

A significant limitation of this study was the lack of a standardized protocol for exhaled breath condensate collection. Due to the inherent nature of the individual biomarker compounds being assessed, the varying storage practices and the specimen assessment tool (assays vs. mass spectrometry) makes establishing a singular methodology for exhaled breath condensate collection and biomarker detection challenging. As a result, the ATS/ERS have only developed recommendations versus a standardized protocol. Recently, Mastrigt et al (2015) conducted an extensive literature search utilizing the Medline, Embase and PubMed databases on the analysis and applications of volatile organic compounds (VOCs) in exhaled breath (EB) and EBC on children. They retrieved 1165 papers, nine of which contained original data on VOCs in EB and 84 on biomarkers in EBC. Following an analysis of the studies obtained it was concluded that, while EBC analysis adds value to the diagnostic process and follow-up care of individuals with respiratory disease, the lack of standardization of collection methods and analysis techniques impedes the introduction of EBC use into clinical practice. This assessment is consistent with those determined by Horvath et al in 2005, Montuschi in 2007 and supported by the ATS/ERS. After approximately 13 years of EBC research clinical application has not been achieved. The results of the current study did not substantiate clinical use of EBC analysis with regard to either LFDO as an instigator of oxidative stress or as a substitute for pulmonary function testing.

Currently no studies have been conducted that define basal levels of exhaled breath IsoF in individuals diagnosed with COPD, making the inability to interpret the obtained results is a crucial limitation. Not only are there no exhaled breath basal IsoF levels established in individuals with COPD, there are no exhaled breath IsoF levels established in healthy individuals

either. While this is considered a significant limitation of this study, the obtained results will aid in establishing that benchmark knowledge for future studies to compare.

Isofuran production is currently understood to occur in response to elevated oxygen tensions (Roberts et al, 2004). Elevated oxygen tension levels within the body can occur from both endogenous and exogenous sources. This study aimed to discern if oxidative stress was occurring as a result of prescribed LFDO therapy, an exogenous source of reactive oxygen species production. Endogenous sources of molecular oxygen can result from genetic or acquired mitochondrial diseases, such as amyotrophic lateral sclerosis (ALS), Huntington's, Alzheimer's, Lewy body and Parkinson's diseases (Fessel et al, 2003). Study candidates were surveyed for these particular diseases with none reported or observed. However, other more common diseases that are linked to mitochondrial dysfunction, such as diabetes and hypertension, were not controlled for.

Acquired mitochondrial dysfunction occurs as a result of adverse effects from drugs, infections or other environmental factors (Ellinas and Frost, 2011). Diseases of the mitochondria appear to cause the most damage to cells of the brain, heart, liver, skeletal muscles, kidney and the endocrine and respiratory systems (Ellinas and Frost, 2011). The most common co-morbidities reported by the participants in this study were COPD (100%), hypertension (87%), diabetes (40%), coronary artery disease (56%), and hyperlipidemia (56%). Oxidative stress is central to many of these disease processes with acquired mitochondrial dysfunction as a contributing factor. It is unknown at this time to what degree, if any, these disease processes contribute to elevated IsoF levels as a result of liberated molecular oxygen from disrupted mitochondria. The limited understanding of contributing sources of elevated tissue oxygen

tensions make it challenging to interpret which source, endogenous or exogenous, is responsible for and to what degree of IsoF synthesis in this study.

A limitation of biomarker analysis is the lack of specificity with regard to which tissue it represents. It has not been established that biomarker levels are exclusive to specific disease processes when obtained via blood, plasma or urine. It is speculated that compounds collected in exhaled breath condensate are representative of the alveolar lining, but, it does not necessarily preclude the possibility that portions of a particular biomarker aren't representative of other systemic oxidative stress processes. As a result, it is recommended that exhaled breath analysis of oxidative stress should not occur in the presence of an active infection, specifically sepsis and pneumonia. No individuals with either current or recent pneumonias or other systemic infections were considered for study inclusion. Candidates were excluded for active oral infections and current use of antibiotics. Those who reported current antibiotic therapy were excluded for concern of lingering inflammatory processes that might contribute to an oxidant load and confound the result.

Limitations of this study with regard to PFT testing were the mixed-provider mixed-tool methodology. Multiple clinic sites (Cardiology Clinic, Internal Medicine Clinic, Pulmonary Rehab Clinic, Pulmonary Function Lab) were available for recruiting participants. Only the PFT lab provided same day PFT testing; all other recruitment sites required obtaining PFT utilizing a portable spirometer. This mixed provider approach utilizing two different tools introduced a potential measurement error, a threat to internal validity. Ensuring consistent effort provided by study participants during PFT testing outside of the pulmonary function lab was difficult. The portable Discovery II™ spirometer, while considered sensitive and accurate, lacked the sophistication of the software employed in the PFT lab. The lack of previous experience with

administering pulmonary function testing by the PI introduced an initial limitation; however, proficiency was realized early on.

The sample size generated for this study was based on an effect size determined through examining 8-IsoP research. Isofuran may have a smaller effect size requiring a larger N with which to power a study. A larger sample size would allow for the detection of patterns in comorbidities as it relates to IsoF levels as well as possible explanation of the significant outliers in the active treatment group.

Recommendations for Future Research

While known genetic mitochondrial diseases (Lewy body, Alzheimer's and Parkinson's Diseases) were excluded from this study, current research implicates mitochondrial pathology in many of the same diseases correlated to oxidative stress, such as diabetes and hypertension, etc. Acquired mitochondrial mutations manifest within the human in the form of cellular senescence, such as aging, heart disease, and diabetes, etc. Cytochrome c oxidase is responsible inside the mitochondria for converting molecular oxygen to two molecules of water. In the presence of cytochrome c oxidase mutations this feature is retarded and molecular oxygen goes unchecked, contributing to oxidative stress within the cell. Future research to aid in a better understanding the relationships between mitochondrial disease, cytochrome c oxidase mutations and IsoF production could lead to an enhanced understanding of the origin of IsoF synthesis and validating it as a useful tool in oxidative stress research.

It is of interest to consider that IsoF concentrations may decline at some undefined point in time following initiation of LFDO. It would be of value to know the mechanism(s) responsible for stabilizing isofuran synthesis following an "introductory phase" of therapy. Tolerance or adaptation within the body manifests in many different ways. Mechanisms of systemic

tolerance/adaptation, such as endogenous free radical scavenging systems activity, up and down regulation, or potential reductions in cytokine activity, as a mechanism of stabilizing IsoF levels following prolonged LFDO use, should be explored.

Identified contributors of increased oxidant load include mitochondrial disease, AM activity and supplemental oxygen. With what is currently understood about IsoF there is no way of discerning which source of ROS is responsible for IsoF synthesis. Currently, over 250 isomers of IsoFs have been identified. Continued research in delineating these isomers could provide insight into their unique qualities regarding specificity and sensitivity to specific processes/diseases. Until then, in light of what is currently known, IsoF is not a sensitive biomarker of supplemental oxygen therapy.

A methodology recommendation would be to utilize a longitudinal repeated measures pre-post test design. Develop a study design that targets individuals being prescribed LFDO, obtaining baseline IsoF levels prior to LFDO initiation, then again at predetermined time points following initiation (24 hours after initiation, then at 1, 2, 3, 5 and 6 months). A determination could be made pending the need for continued IsoF evaluation of IsoF. Such an approach would be better able to identify whether or not supplemental LFDO is a mechanism of ongoing oxidative stress.

Summary

The purpose of this chapter was to present the results of this study in such a way that was meaningful to researchers and healthcare providers. It was intended to encourage the reader to consider the implications of traditional therapies/practices employed. This study did not demonstrate LFDO as a mechanism of ongoing oxidative stress; however, as more is learned about the variables contributing to IsoF synthesis we may be able to optimize its unique feature

of oxygen tension specificity in gaining greater insight into oxidative stress and related diseases, specifically COPD.

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Appendix A

Low-flow domiciliary oxygen therapy as a mechanism of ongoing oxidative stress in
COPD patients
Study Participant Data Collection Sheet
Active Control Group ID #_____   Active Treatment Group ID #_____

Age: _____

Gender: M or F

Height: (in feet and inches): _____

Weight: (in pounds): _____

Ethnicity: _____

Current Smoker?   Y   N

How long ago did you quit? _____

How many years did you smoke? _____

How many packs per day did you smoke? _____

Do you have alpha-1 antitrypsin deficiency? Y   N

Are you currently on oxygen therapy at home? Y   N

If yes, how long have you been on home therapy? _____

How much oxygen (liters per minute) are you on? _____

Are you currently receiving antibiotics? Y   N

If yes, do you currently have a nose or mouth infection?   Y   N

Please provide a complete list of medications you take daily:

Appendix B

Sample Storage Data Sheet

Sample ID#: _____

Sample Collection Date: _____ Sample Collection Time: _____

Cooler Temperature: _____

Ship Date: _____

Comments:
_____

_____

Appendix C

Informed Consent

<u>Title of Research:</u> Chronic exposure to low flow home oxygen therapy as a mechanism of oxidative stress.

Investigators: Jill Stulce, MSN, CRNA

<u>Informed Consent</u>
Before you agree to take part in this study, it is important that you read the following description of the study. This paper describes the purpose, methods, benefits, and risks of the study. It is always your right to change your mind and decide not to be in the study at any time.

<u>Purpose of Research</u>
To determine if home oxygen therapy contributes to ongoing lung inflammation for people diagnosed with COPD.

<u>Explanation of Procedures</u>
This is a study that compares the exhaled breath of individuals diagnosed with COPD receiving home oxygen therapy to those individuals diagnosed with COPD not receiving oxygen therapy to determine if oxidative stress is occurring within the lungs as a result.

There will be two groups of patients. The groups include 1) patients diagnosed with advanced COPD not receiving home oxygen therapy; and 2) patients diagnosed with advanced COPD receiving home oxygen therapy. If you are selected to participate in this study you will be assigned to one of these two groups. Group selection will be decided by use of home oxygen therapy. A PhD candidate from Virginia Commonwealth University is conducting this study.

The care given to the patient will not be changed in any way other than the collection exhaled breath into a collecting device, which will take approximately 10 minutes. The patient will be asked to breath into a spirometry device to determine lung function. Information needed for the study will be collected from the principle investigator, project manager or staff. This information will include but not be limited to age, height, weight, ethnicity, smoking status and history, oxygen therapy history.
Participants Initials:_____

Appendix D

Informed Consent

<u>Title of Research:</u> Chronic exposure to low flow home oxygen therapy as a mechanism of oxidative stress

Investigators: Jill Stulce, MSN, CRNA

<u>Risks</u>
No risks are associated with study participation.

<u>Benefits</u>
There may be no direct advantage to being in this study. Information from this study may help make the care better for future patients.

<u>Participant Selection</u>
We are asking only those individuals who have been diagnosed with COPD who may or may not be on oxygen therapy.

<u>Withdrawal Without Prejudice</u>
You do not have to be in this research study. You can agree to be in the study now and change your mind later. Your decision will not affect your care.

<u>Confidentiality</u>
Information collected will remain private. The information collected from your chart may be printed for scientific purposes. This information includes the your medication list, age, height, weight, ethnicity, pulmonary function test results, smoking status and history, oxygen therapy history. Your identity will not be revealed. The researchers will be the only ones with access to the study information.

<u>Costs and/or Payment to Subject for Participation in Research</u>
There will be no costs for taking part in the study. Also, you will not be paid to take part in this study.

Participant's Initials:_____

Appendix E

Informed Consent

<u>Title of Research:</u> Chronic exposure to low flow home oxygen therapy as a mechanism of oxidative stress

Investigators: Jill Stulce, PhDc, CRNA

<u>Questions</u>
Any questions regarding study participation should be made directly to Jill Stulce, PhDc, CRNA at 314-691-1310.

<u>Agreement</u>
This agreement states that you have received a copy of this informed consent. Your signature below says that you agree to participate in this study.

_____   _____
Signature of Participant   Date

_____
Participant Name (printed)

_____   _____
Signature of Researcher or Anesthesia Provider   Date

Appendix F

Exhaled Breath Collection Instruction Sheet

To better understand processes occurring within the lungs of COPD patients receiving oxygen therapy we are asking you to participate in a research project by providing your exhaled breath for analysis. Below is a list of what is required by you to ensure success in this evaluation.

 Thank you for your participation.

1. Rinse mouth prior to introducing RTube™ mouthpiece into mouth.
2. Secure lips around mouthpiece and breath in and out through your mouth
3. Approximately 10 minutes is required to obtain an adequate amount of exhaled breath condensate for analysis

Vita

Jill Stulce was born in the city of St. Louis in 1965 and grew up in a suburb of the city, Ferguson, Missouri. She earned a Bachelors of Science in Nursing degree from the University of Missouri, Columbia, in 1988, a Masters of Science in Nursing with a Nurse Anesthesia Specialization from Southern Illinois University, Edwardsville in 1998 and a Ph.D. in Health Related Sciences from Virginia Commonwealth in 2016. She has taught for the Nurse Anesthesia Department at Webster University since 2006 and currently serves as Chair of the department.