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ANTICHOLINERGIC THERAPY IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE: NOVEL MECHANISMS OF ACTION

By

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Abstract:

Introduction:

Because the relationship between pulmonary function and exercise tolerance is highly variable in COPD, other contributing factors were investigated. Physiological factors that contribute to exercise tolerance must contribute to the symptoms limiting exercise, thus the symptoms limiting exercise in COPD and their contributing factors were explored, including an investigation of novel mechanisms to explore the reported tiotropium bromide-mediated improvement in exercise tolerance in COPD.

Methods:

We conducted a retrospective, cross-sectional analysis of 4,424 COPD patients and 4,221 healthy subjects; referred to McMaster University Medical Center for exercise testing. Multiple linear regression, ridge regression, and MANOVA were utilized to determine the factors contributing to exercise tolerance, important symptoms limiting exercise, and factors contributing to dyspnea.

A randomized, double-blind, placebo-controlled, crossover study of 20 COPD subjects was performed. Repeated measures ANOVA was utilized to determine effects of 3 weeks tiotropium bromide vs. 3 weeks placebo on cardiac output and efficiency of gas exchange during exercise.

Results:

MBC, DL_{CO}, and quadriceps strength were the three major, independent, contributors to exercise capacity (MPO = -206.3 + 5.1*Quadriceps Strength + 1.8*MBC + 10.0*DL_{CO}, r^2 = 0.677). MANOVA further illustrated this.

Dyspnea, alone or in equal combination with leg effort, was the most important symptom limiting exercise in COPD. V_E and MBC were the two major, independent, contributors to dyspnea (Dyspnea = 0.95 + 0.08*V_E + -0.01*MBC, r^2 = 0.457). The increase in dyspnea with V_E was much greater than the decrease with MBC.

Tiotropium bromide did not significantly (p = 0.72) improve the efficiency of gas exchange for oxygen, significantly worsened (p = 0.005) the efficiency of gas exchange for carbon dioxide, and did not improve cardiac output.

Conclusion:

We concluded the reported tiotropium bromide-mediated improvement in exercise tolerance in COPD is not mediated through improvements in gas exchange efficiency and/or cardiac output.

<u>Acknowledgements:</u>

Significant accomplishments are rarely achieved without the helpful assistance and involvement of many individuals. My graduate school journey to complete my PhD has certainly provided strong evidence of that simple, yet important fact. Along the way I have been privileged to rely on the assistance of many people, without whom I would not have successfully completed this journey.

Any journey, large or small, begins with an opportunity. My opportunity came when I was offered an interview for a graduate student position by Dr. Gail Gauvreau. When I accepted the position I had little idea of just how challenging and rewarding it was going to be. In the years that followed I learned many important skills, particularly relating to the operation of a successful clinical trial. Dr. Gauvreau shared her extensive experience on the subject and helped me develop the skills and understanding necessary to successfully create and run the clinical trials that made up my thesis work. Dr. Gauvreau's attention to detail always held me to the highest standard with respect to my protocols, record keeping, reports, and presentations. Through her supervision I have gained a strong understanding of what it takes to be successful in a research environment.

Of course this journey required a place to happen, and it has been my privilege to come and work in the research facilities of Dr. Paul O'Byrne. As a member of my academic committee Dr. O'Byrne brought to bear his extensive knowledge and provided incredible insight and always sought to bring out the best in me and my work. His ability to inspire those around him is legendary, and I have reaped the reward of working in his presence.

Having access to great mentors, people who have shaped the way we look at the world, is of the utmost importance when completing a PhD. I have had the distinct honor of having Dr. Norman Jones serve on my academic committee. Dr. Jones' many contributions to field of cardiorespiratory physiology have been monumental. My academic committee meetings were always filled with spirited debate, usually brought on by comments and suggestions made by Dr. Jones. His significant knowledge and experience provided many thought-provoking questions, and always required the very best of me. Serving as my comprehensives advisor, Dr. Jones again provided a wealth of knowledge and experience. Many helpful suggestions about additional directions or factors, things I had overlooked, were always available. His ability to explain concepts and ideas in an easy to understand manner is, I am sure, part of what has made him such a notable figure in this field.

It seems that in the recollection of the events of any great accomplishment, the involvement of one particular person always stands out. For me, that person is the incomparable Dr. Kieran Killian. Equipped with knowledge and experience that only years of practice can bring, matched with an outspoken, no-nonsense personality, Dr. Killian challenged me in ways that were immeasurable. His dedication to learning and understanding, and the expectation of me to do the same was unparalleled. Our often daily discussions helped me to learn the concepts and ideas that were crucial to my success, but these were more than mere conversations. Often starting with a simple question, these discussions quickly grew to encompass years of research and knowledge. Countless pages of spur-of-the-moment, hand-drawn figures, diagrams, and notes would

result. Impromptu database analyses were done on the computer, simply to illustrate a

concept or idea. All of this, for my personal benefit and betterment. When I did things

well, there was acknowledgement, when some things did not go well there was a

veritable hurricane. What I came to learn was that Dr. Killian's tolerance for honest

mistakes was high, while his tolerance for any perceived lack of effort was non-existent.

Dr. Killian held me to a standard that was, at times, well beyond what I thought I could

achieve. Yet I was never alone in my efforts to reach those standards. His tireless

commitment to my success is perhaps the characteristic I will remember the most. More

than the politically-incorrect statements or the often profanity-laden rants, more than the

questions that cut to the very core of what I was discussing, it has been his commitment

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of every day, regardless of past successes, was his way of keeping me focused. Working

with me, literally side-by-side, to help me understand was his method. Few are graced

with the gift to understand in a way that looks beyond the knowledge itself to a higher

level of logic and reasoning. I have had the opportunity to learn from such an individual

in Dr. Killian, and I am the better for it.

  The daily life of a graduate student is often centered on the various tasks

associated with the research being undertaken. Those tasks are often the very

experiments that subsequently generate the all-important "data". But the ability to

perform these experiments, to use all the necessary equipment, requires training. I was

lucky enough to have the opportunity to learn the finer points of pulmonary function and

cardiopulmonary exercise testing from James Kane. Many hours were spent learning the

Perhaps the people I had the greatest interaction with were the other students, techs, and fellows in the lab. Those who like me were pursuing their own goals in medical science. This was a large group of people, each of whom I am glad to have had the opportunity to meet and work with. These are the people I have called my friends throughout my time at McMaster, the people I got to know and the people who made each day great: Eric Farrell, Trudy MacLean, Lena Saleh, Michelle Evans, Rei Fujiki, Tara Strinich, MyLinh Duong, John Brannan, Elaine Keung, Zahida Meghji, Lauren Kitney, Ashley Gluchowski, Benny Dua, Irfaan Remtulla, Nelishah Jiwani, Adrian Cordova, Matt Mistry, Rabyah Murji, Des Murphy, Just van der Lind, Bart Jacobs, Abbey Torek, Steve Smith, Adrian Baatjes, Candice Todd, Haruki Imaoka, Mike Hill, John-Paul Oliveria, Vanessa Byrne, Catie Obminski, Lucie White, Kristen Watson, Takashi Kinoshita, and Brittany Watson.

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Celebrating every achievement, every milestone with me made me feel like I could take on any challenge.

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In truth, everyone I have had the chance to meet while working at McMaster has been a friend. My experiences have been incredible and I count this as one of the great times in my life, one I will remember forever. Thank you to everyone!

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Chronic Obstructive Pulmonary Disease:

Discomfort experienced and associated with the act of breathing (dyspnea) in combination with chronic cough and sputum has been an ageless human problem. In the 1660's Willis identified patients with chronic cough productive of sooty filth with inflated chest walls that could neither fill nor empty properly (Willis, 1695). Post mortem examination disclosed very large lungs that failed to deflate after opening the chest (Baillie, 1799; Bonet, 1679; Morgagni, 1769). Laennec introduced the term "Emphysema" to reflect overinflated lungs with destruction of alveolar walls (Laennec, 1821). With his introduction of the stethoscope, he found that the respiratory murmur due to air entering and leaving the alveoli (vesicular breathing) was reduced in intensity. Using Auenbrugger's percussion these lungs were hyper-resonant. With the evolving understanding of microbial infection and its propensity for the lungs, pulmonary tuberculosis was identified and had to be excluded as causative. By the 1950's effective antibiotics were successful in the treatment of pulmonary tuberculosis and pneumonia. The success of antibiotics in treating acute exacerbations of chronic bronchitis and emphysema was modestly successful (Anthonisen et al., 1987).

In the late 1950's chronic bronchitis with continuous cough and phlegm in combination with dyspnea was recognized as a major problem. In England, chronic bronchitis and emphysema were separately recognized. Chronic bronchitis was believed to predominate. Dyspnea due to the large over inflated lungs ("Emphysema") was a

major problem in North America, and chronic bronchitis was less commonly recognized. At an unpublished Ciba symposium (organized by the Ciba Foundation) held in 1958, the concept of chronic obstructive pulmonary disease (COPD) was born as a means of overcoming the limitations imposed by the lack of ability to measure structural features of the disease in the living subject (Burrows, 1981). COPD was defined by the maximum expired volume over one second (FEV_1) from total lung capacity. This measurement of expiratory airflow was originally introduced by Tiffenau to replace the vital capacity while studying the effects of inhaled acetylcholine in asthmatics (Tiffenau, 1958). Reductions in flow could be logically expected with narrowing or obstruction of airways. This measurement was enthusiastically adopted and popularized by Edward Gaensler in North America, and used by Charles Fletcher to demonstrate the role of cigarette smoke exposure in the London transport workers in one of the earliest prospective controlled trials (Fletcher & Peto, 1977).

Ventilation relative to the maximum breathing capacity was introduced as an objective measure of dyspnea by Means in the 1920's (Means, 1924). The relationship of ventilation to maximum breathing capacity became known as the ventilator index. Dyspnea was the perceptual expression of this relationship. Maximum breathing effort resulted in maximal breathing capacity and maximum dyspnea. Airflow obstruction contributed to dyspnea by increasing the amount of ventilation required to exchange the respiratory gases (oxygen and carbon dioxide) and decreasing the capacity to breath. Inflammation of the airways contributed to bronchitis with cough and sputum production. In order to avoid confusion with asthma, COPD was confined to patients without atopic

disease who showed minimal reversibility of FEV_1 over time or in response to bronchodilators (Burrows, 1981). This exclusion has proven to be problematic and the Dutch rejected this approach from the outset, instead choosing to acknowledge that individuals with allergic diathesis had a greater likelihood of developing severe and serious chronic airflow obstruction; supporting an implied relationship between asthma and COPD (Orie, Sluiter, & DeVries, 1961).

In an early prospective controlled trial carried out in the London transport industry, a slow progressive decline in FEV_1 was seen with age but a further additional decline was confined to smokers (Fletcher & Peto, 1977). The magnitude of the decline in smokers was not significantly related to the frequency of acute exacerbations (assumed to be infective in origin) (Burrows & Earle, 1969; Fletcher & Peto, 1977).

The FEV_1 is the maximum volume that can be expired in one second. The minimum time required to inspire the same volume is determined by the maximum inspiratory flow rate. The FEV_1 divided by the minimum time to expire (i.e. 1 second) and inspire (FEV_1/V_i max) is a measurement of the maximum capacity to breathe. The emergence of the maximum flow volume manoeuvre by Hyatt and Fry fundamentally questioned the reliability of the FEV_1 multiplied by 35-40 as a measurement of the capacity to breathe. Nonetheless, the FEV_1 became a surrogate of ventilator capacity and continues to be widely used as an indicator of disease severity in COPD (see GOLD Classification).

Ventilatory Capacity and its Measurement:

Direct assessment of the ventilatory capacity (capacity to breathe), or maximal breathing capacity (MBC), has utilized the measurement of maximum voluntary ventilation (MVV), since its introduction by Hermannsen in the early 1930s (Freedman, 1970). MVV, expressed in liters per minute, is the maximum volume of gas which the subject can breathe when breathing as deeply and as quickly as possible or when breathing as deeply as possible at a controlled frequency, both tests being performed over a specified period of time (usually 15 or 30 seconds) (Freedman, 1970; Gandevia & Hugh-Jones, 1957). The MVV collected over 15 or 30 seconds cannot be maintained over long periods of time because the respiratory muscles fatigue (Freedman, 1970). When assessed over a time period of 4 minutes, the MVV has been shown to be approximately 72% of that measured over 15 seconds (Freedman, 1970).

Due to the time and discomfort involved with directly assessing the MVV, indirect assessment of the MBC has commonly involved estimation from resting measurements of FEV_1. Multiplying the FEV_1 by 35 was adopted as the preferred method of estimation, though the French suggested using a factor of 37.5 (Gandevia & Hugh-Jones, 1957). While this practice was called into question by Hyatt and Fry, the use of FEV_1 as a surrogate for ventilatory capacity has persisted to this day.

Origins:

The inhalation of smoke produced from the burning of tobacco and other plant-based materials has been a staple in many cultures for thousands of years. Manuscripts of Columbus' journeys to Cuba in the late 1400's give an account of the local peoples lighting and inhaling the smoke from dried leaves of cultivated tobacco (Gilman & Zhou, 2004). Tobacco smoking has been a ceremonial ritual in North and South America as far back as 5,000 BCE (Gilman & Zhou, 2004). Smoking was believed to provide healing powers. The smoke was also thought to provide a source of power to the shaman or healer. Smoking captured the imagination of early Europeans (Gilman & Zhou, 2004). The social elite adopted smoking into their lifestyle and soon it became part of popular culture. The addictive effects of nicotinic cholinergic stimulation contributing to a feeling of well being went unrecognized.   By the late 1880's advancements in transportation, manufacturing, and packaging allowed for the inexpensive widespread sale and usage of tobacco throughout Europe and North America (Gilman & Zhou, 2004). Heavy distribution of cigarettes to soldiers during both World Wars created a huge growth in the usage of cigarettes. Many brought the practice home, introducing it to family and friends. Smoking was adopted by women because of its euphoria, while fitting neatly into the image of the liberated woman.

In 1952, Sir Richard Doll and Austin Bradford Hill, influenced but not convinced by the cross-sectional epidemiological studies implying a causative relationship between smoking and lung cancer, initiated a prospective control study in 42,000 English doctors (Gilman & Zhou, 2004). In a very short space of time this work convinced American and

British public health authorities that smoking was bad for your health. Anti-smoking

campaigns began in the early 1950's. In 1997 the World Health Organization began

working on an international anti-smoking treaty. Robust anti-smoking movements are

now universal.

Cigarette smoke exposure accompanied by particulate matter from air pollution

and occupational exposure are the major contributing factors to COPD (American

Thoracic Society, 2000; Bernstein et al., 2004). There is an increased prevalence of

COPD and respiratory symptoms in urban/high pollution areas (Iversen, Hannaford,

Price, & Godden, 2005; Schikowski et al., 2005; Viegi et al., 1999; Xu et al., 2005).

There is also an increased risk of COPD or respiratory hospitalizations in urban/high

pollution areas (Anderson et al., 1997; Dominici et al., 2006; Yang et al., 2005). The

prevalence of new onset of chronic phlegm increased with traffic intensity and outdoor

NO_2 levels among females (Sunyer et al., 2006). COPD is highly prevalent in women

exposed to smoke from the burning of biomass (Ekici et al., 2005), with similar mortality

to those of tobacco smokers (Ramirez-Venegas et al., 2006). Conversely, reduced

exposure to indoor biomass smoke, through the usage of chimneys, has been shown to

reduce the risk of developing COPD (Chapman, He, Blair, & Lan, 2005). The American

Thoracic Society places the population attributable risk from occupational exposure at

15% (Balmes et al., 2003). Occupational exposure, in combination with smoking,

increases the risk for chronic bronchitis by 160% (Zock et al., 2001). Additionally,

exposure to biological dusts has been associated with increased risk for chronic

obstructive bronchitis, emphysema, and COPD in Australian men and women (Matheson

et al., 2005). The impact of occupational and environmental exposure to particulate matter can be measured using pulmonary function tests. There is a reduction in the maximal attained lung function with increased particulate and gaseous pollutants in individuals from 10-18 years of age (Gauderman et al., 2004). Children living within 500m of a motorway had deficits in 8-year growth of FEV_1 when compared to children greater than 1500m from a motorway, and showed notable reduction in attained lung function at 18 years of age (Gauderman et al., 2007). Mortality rates have also been linked to particulate matter exposure. There was a decrease in respiratory death rates in Dublin, Ireland following a ban on bituminous coals (Clancy, Goodman, Sinclair, & Dockery, 2002). The number of deaths attributed to COPD, caused by airborne particulates, worldwide in 2000 was 318, 000 (Driscoll et al., 2005).

Definitions of Emphysema and Chronic Bronchitis:

The CIBA symposium took place in 1958 and was briefly described by Charles Fletcher in *Thorax* in 1959 (Ciba Guest Symposium, 1959). Chronic bronchitis was defined by increased mucus production (sputum), on most days of the week, lasting at least three months in a given year, for two consecutive years (Ciba Guest Symposium, 1959). Emphysema was defined by alveolar wall destruction and enlarged alveolar spaces (Ciba Guest Symposium, 1959). In 1962, the American Thoracic Society Committee on Diagnostic Standards focused on the FEV_1 and the FEV_1/FVC ratio, using the FEV_1 as a measure of disease severity (Committee on Diagnostic Standards for Nontuberculous Respiratory Diseases, American Thoracic Society, 1962). The

pathological features of COPD were reviewed by Thurlbeck and Hogg with an attempt to establish the structure-function relationship (Thurlbeck & Churg, 1995). Mucus plugging, cellular infiltration, and edema of the small airways, together with the reduction in elastic recoil of the lung, due to alveolar destruction increased expiratory airway resistance resulting in reductions in maximal expiratory airflow. Patients were not uniform, many had emphysema, and many had small airway obstruction; while others had both. Mechanical ventilation of post mortem lungs from patients with COPD showed large pressure drops across the small airways confirming the importance of small airway disease in COPD (Hogg, Macklem, & Thurlbeck, 1967).

Patients with COPD present with dyspnea due to the increased effort required to generate the ventilation needed to meet the metabolic demands of exercise. Expiratory resistance increases with the magnitude of the expiratory effort due to compression of the airways during expiration. The prolonged expiratory time forces the inspiratory muscles to contract with greater velocity, at a shorter length, and with more force contributing to exertional discomfort known as dyspnea. Breathlessness, an unpleasant urge to breathe, arises with acute hypercapnia and to a lesser extent with hypoxemia, and is called respiratory failure. Patients with cardiorespiratory diseases can be classified into:

1. Ventilatory Insufficiency: where dyspnea intensifies as ventilation approaches the capacity to breathe.

2. Respiratory Insufficiency: where an unpleasant urge to breathe intensifies with hypercapnia and hypoxemia ("breathlessness").

3.   Circulatory Insufficiency: where dyspnea and the effort required to drive the peripheral muscles intensify due to congestive heart failure.

4.   Circulatory Insufficiency may also contribute to an inadequate cardiac output to meet metabolic demands: leading to weakness and fatigue of all skeletal muscles including the respiratory muscles. A low cardiac output affects all organs and tissues.

GOLD Classification:

In order to bring greater attention to the prevalence and impact of COPD, scientists in 1998 convinced the US National Heart, Lung, and Blood Institute and the World Health Organization to form the Global Initiative for Chronic Obstructive Lung Disease (GOLD). The main goals of GOLD are to increase awareness and provide help to the millions afflicted by this disease. In 2001, GOLD published its first report, *Global Strategy for the Diagnosis, Management, and Prevention of COPD*. This report brought together the newest information on guidelines and pathological mechanisms of COPD into one document. This document has recently been updated in 2009 and the current GOLD definition of COPD is as follows:

"Chronic Obstructive Lung Disease (COPD) is a preventable and treatable disease with some significant extrapulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive

and associated with abnormal inflammatory response of the lung to noxious

particles or gases (Rabe *et al*, 2007)."

Airflow limitation in COPD results from a combination of small airway disease,

obstructive bronchiolitis, parenchymal destruction, and emphysema; with the contribution

of each varying between patients (Rabe *et al*, 2007). Inflammation leads to the narrowing

and structural changes within the airway, and also causes damage to the lung parenchyma

resulting in decreased elastic recoil (Rabe *et al*, 2007). GOLD has utilized pulmonary

function testing to generate a simple spirometric classification system of disease severity.

Stage I is classed as Mild, and is defined by $FEV_1/FVC < 0.70$ and $FEV_1 \geq 80\%$

predicted (Rabe *et al*, 2007). Stage II is classed as Moderate, defined by $FEV_1/FVC <$

0.70 and $FEV_1 \geq 50$ and $\leq 80\%$ predicted (Rabe *et al*, 2007). Stage III is coined Severe,

with $FEV_1/FVC < 0.70$ and $FEV_1 \geq 30$ and $\leq 50\%$ predicted (Rabe *et al*, 2007). Finally,

Stage IV is classed as Very Severe, with $FEV_1/FVC < 0.70$ and $FEV_1 \leq 30\%$ predicted or

$\leq 50\%$ predicted in combination with chronic respiratory failure (Rabe *et al*, 2007).

However, cut-off points such as post-bronchodilator FEV_1/FVC ratio < 0.70 or

$FEV_1 < 80, 50,$ or 30% are admitted by GOLD to have been chosen for simplicity and

have no clinical validation (Rabe *et al*, 2007). Furthermore, the overall effect of the

disease on the patient is determined not simply by the level of airflow obstruction, but by

the degree of symptoms experienced; such as breathlessness and decreased exercise

tolerance. Here too, GOLD admits that there is an imperfect relationship between airflow

limitation and the presence of symptoms, stating: "Spirometric staging, therefore, is a

pragmatic approach aimed at practical implementation and should only be regarded as an

educational tool and a general indication to the initial approach to management (Rabe *et al*, 2007)."

Capacity to Exercise:

The capacity to exercise is dependent on the activation of the muscle by the motor cortex, the transmission of this motor command to the alpha motor neurons in the anterior horns of the spinal cord, the activation of the final common pathway, depolarization of the muscle, and electromechanical coupling ultimately resulting in the generation of power by the muscle. Assuming no problems in neuromuscular activation, the capacity to generate power is dependent on the amount of muscle activated. The more muscle activated, the more power is generated. The magnitude of the respiratory support required is set by the amount of muscle and its activation. Limitations in ventilatory, gas exchange, and circulatory support should be expressed relative to the amount of muscle present from the outset.

In 1923 the New York Heart Association grappled with the measurement of the severity of cardiac disease. The net result was that the severity of cardiovascular disease was considered inversely related to the capacity to exercise. The capacity to exercise was estimated by the patient's perception of his or her tolerance. The rugged principle can be equally applied to COPD. Exercise intolerance can be demonstrated by reductions in the maximum capacity to generate power during incremental cycle ergometry, endurance cycling, and walking tests. A formal incremental exercise test to symptom limited capacity allows for the identification of limiting factors.

Ventilatory limitation is inferred when the exercising ventilation approaches the capacity to breathe. Gas exchange limitation is inferred with hypercapnia and/or hypoxemia (respiratory failure). Circulatory limitation is inferred if the cardiac output reaches a maximal level. The rationale for the existence of a maximal cardiac output is based on the plateau of oxygen uptake as exercise increases. Because cardiac output increases with exercise, the assumption that cardiac output is maximal at maximum exercise is tautological. Failure of tissue respiration in the muscle is not synonymous with ventilatory, gas exchange, or circulatory limitation. Lactate production and the demonstration of an oxygen debt, which result from anaerobic energy metabolism, are supportive of tissue respiration failure, occurring when aerobic metabolism alone cannot meet the energy demands of the exercising muscle.

The capacity to exercise is highly variable in patients with COPD.   Many perform at a level greater than expected based on their FEV_1, and others perform poorly (Jones & Killian, 1991).   Patients, in general, exhibit a ventilation proportional to carbon dioxide production where the $P_aCO_2 = Constant \times VCO_2/V_A$. However, hyperventilation and hypoventilation are encountered.   Hence, the ventilation required to meet CO_2 production varies. The mechanical efficiency of gas exchange varies with V/Q relationships of the alveoli and the anatomic dead space (V/Q = infinity). The interplay between metabolic demand, control of breathing, and matching of ventilation to perfusion within the lungs varies (Jones & Killian, 1991). Carbon dioxide production arises to an extent that varies with the substrate oxidized (carbohydrate, fat, or protein) and its excretion increases with the added carbon dioxide from lactate production (Brown,

Wiener, Brown, Marcarelli, & Light, 1985; Jones & Heigenhauser, 1996).   Increases in the dead space to tidal volume ratio, increases in ventilation of poorly perfused alveoli (Jones, 1966), or decreases in arterial oxygen partial pressure all collectively increase the ventilation required at a given power output (Jones & Killian, 2000). Expiratory airflow limitation results in an increase of end expiratory lung volume placing the inspiratory muscles in a shortened state which is less efficient at generating tension, thus requiring more effort (Killian, Inman, & Jones, 1995; O'Donnell, Sanii, Anthonisen, & Younes, 1987). Of equal or perhaps even greater importance is the increase in inspiratory flow rate and the velocity of contraction of the inspiratory muscles due to prolonged expiration (Leblanc et al., 1988). These all collectively require more effort to reach the required ventilation (Leblanc et al., 1988). Optimization patterns are adopted to minimize the effort to breathe and are largely centered around reducing the peak forces (Killian et al., 1995).

Symptoms Limiting Exercise:

Exercise evoked symptoms intensify as the power output of the muscles increases, ultimately becoming too uncomfortable to tolerate and the subjects stop.   These exercise evoked symptoms include the effort required to cycle ("perceived exertion"), the effort required to breathe ("dyspnea"), breathlessness due to respiratory failure, chest pain due to active myocardial ischemia, and claudication pain due to peripheral vascular disease. Patients may terminate exercise because of inflammatory arthritic or muscle pain. The effort required to drive a muscle is critically dependent on the strength of the muscle.

More effort is required to drive a weak muscle than a strong muscle to generate the same

force. In COPD, respiratory muscle strength varies from a maximum inspiratory pressure

(MIP) of less than 10 cmH$_2$O to greater than 150 cmH$_2$O and quadriceps strength from a

maximum force of less than 10kg to greater than 130 kg. The potential contribution of

muscle strength to respiratory and leg effort is substantial and not broadly appreciated

relative to the factors outlined previously. Muscles fatigue readily in the presence of

hypoxia.   Hence, the decrease in arterial oxygen partial pressure and the increase in

arterial carbon dioxide partial pressure, seen during exercise, increase the intensity of

dyspnea reported through indirect effects on central respiratory motor drive (Manning &

Schwartzstein, 1995).

The classic "ventilation-limits-exercise" paradigm has changed due to recognition

that lower limb muscles impacted exercise tolerance in COPD (Maltais and Debigare in

Aliverti et al., 2008; Killian et al., 1992). Lower limb muscles demonstrate atrophy,

reduced strength, increased fatigability, and inefficient metabolism (Aliverti et al., 2008).

In COPD, peripheral muscle dysfunction and reduced muscle strength contribute to

intolerance for exercise (Gosselink, Troosters, & Decramer, 1996; Hamilton, Killian,

Summers, & Jones, 1995; Kim, Mofarrahi, & Hussain, 2008; Maltais et al., 1996).

Individuals with COPD that report leg fatigue as the symptom limiting exercise show

reduced improvements in exercise tolerance after bronchodilation, compared to those

limited by dyspnea (Deschenes, Pepin, Saey, LeBlanc, & Maltais, 2008). The intensity

of dyspnea during exercise, in COPD, is related to increases in ventilation, pleural

pressure relative to inspiratory muscle strength, tidal volume relative to vital capacity,

inspiratory flow relative to maximum flow, and the breathing frequency (Leblanc, Bowie, Summers, Jones, & Killian, 1986). Dyspnea becomes more prevalent as the symptom limiting exercise as the severity of COPD increases (Man et al., 2003).

Relationship between Exercise Tolerance and Measurements of Pulmonary Function and Gas Transfer Capacity:

Evaluation of the severity of disability in COPD currently relies on measurement of pulmonary function test parameters. As previously discussed, classification of disease severity is arbitrarily related to ranges of reduction in FEV_1 and FEV_1/FVC. While objective measurement of exercise tolerance would seem to provide a more suitable assessment, classification of disease severity based on pulmonary function has been used as a surrogate, assuming it is closely related to exercise tolerance.

A number of studies have been conducted since the adoption of pulmonary function-based disease severity classification, in an effort to determine how well pulmonary function measurements predict exercise tolerance in COPD patients. Using linear regression in a study group containing healthy subjects, mild/moderate COPD patients, and severe COPD patients, Pineda and colleges were able to illustrate prediction of treadmill exercise tolerance (maximum oxygen consumption, total external work) from resting FEV_1 with some accuracy (Pineda, Haas, Axen, & Haas, 1984). Using a much larger COPD study subject population, followed by validation in a second COPD population of near-equal size, Carlson and colleges used multiple linear regression to assess the accuracy of prediction of exercise tolerance from measurements of pulmonary

function and gas exchange (Carlson, Ries, & Kaplan, 1991). Here too, reasonable

predictions of treadmill exercise tolerance (peak oxygen uptake) were obtained from

measurements of lung function and gas exchange (Carlson et al., 1991). However, the

variability in the prediction was deemed significant enough to negate its use in individual

patients (Carlson et al., 1991). An analogous study by LoRusso and colleges found

similar results (high variability in prediction negates use) when attempting to predict

cycle ergometer exercise tolerance (maximum oxygen uptake and maximum ventilation)

from measurements of pulmonary function in a COPD population (LoRusso, Belman,

Elashoff, & Koerner, 1993). Direct comparison of exercise tolerance from individuals

with similar FEV_1 has illustrated that the capacity to exercise is highly variable in

patients with COPD.   Many perform at a level greater than expected based on their

FEV_1, and others perform poorly (Jones & Killian, 1991).

Cardiac Output:

Under normal conditions, the cardiac output increases by 5L/min for every

1L/min increase in oxygen consumption by the exercising muscles (Shock & Norris,

1971). In essence the variable controlling the cardiac output during exercise is the

metabolic demand of the exercising muscle.   Preload is determined largely by the venous

return from the exercising muscle and together with contractility (ejection fraction), and

afterload, modifies the cardiac output (Frank, 1895; Patterson & Starling, 1914). While

cardiac output is equal to the product of the heart rate and the stroke volume, both factors

are indirectly controlled by the metabolic demand. As exercise intensity increases,

parasympathetic activity of the autonomic nervous system recedes and sympathetic activity increases, modulating the heart rate and stroke volume (Guyton, Coleman, & Granger, 1972). These are mediated through the effects of epinephrine and acetylcholine, respectively. The strength and mass of the peripheral muscles, and their support by effective gas exchange and perfusion control these autonomic effects by both feedback and feed forward mechanisms (Guyton et al., 1972). Any reduction in cardiac output is accompanied by increased sympathetic activity.

Effects of Exercise on Cardiac Output:

When an individual begins to exercise, descending pathways from motor cortex stimulate the cardiovascular control center within the medulla oblongata. This results in decreased parasympathetic activity and increased sympathetic stimulation, increasing cardiac output and causing selective peripheral vasoconstriction increasing blood flow to the exercising muscle (Sarnoff, Brockman, Gilmore, Linden, & Mitchell, 1960). If the blood flow is inadequate for the metabolic demands of the exercising muscle afferent feedback from the small nerve endings are mediated through the spinothalamic pathways projecting to the brain stem increasing sympathetic stimulation (Sarnoff et al., 1960). While the mechanisms are complex the overall control features are simple. Any compromise to respiration relative to the power that must be generated results in sympathetic stimulation and at the same time a reduction in the responsiveness of the muscle to motor stimulation (i.e. fatigue). The same small afferents mediated through the

spinothalamic tracts also stimulate interneurons in the posterior horns of the cords reducing the responsiveness of the alpha motor neurons driving the muscles.

Ventilation-Perfusion Matching:

Blood flow through the pulmonary circulation delivers deoxygenated blood to the lungs. Here it passes through capillaries which are in close contact with the alveoli of the lung. Carbon dioxide diffuses from the blood into the alveoli and oxygen diffuses from the alveoli into the blood. This system operates best when pulmonary blood flow is directed to well ventilated alveoli; thus the term ventilation-perfusion matching. In order to match blood flow to well ventilated alveoli, the pulmonary vasculature constricts or dilates in response to alveolar PO_2 (Riley & Cournand, 1949). Thus, when alveoli are well ventilated, alveolar PO_2 will be high and the surrounding vasculature will be dilated to allow blood flow past this well ventilated area (Riley & Cournand, 1949). Conversely, when alveoli are poorly ventilated, alveolar PO_2 will be low and the surrounding vasculature will constrict, reducing blood flow to this poorly ventilated area; redirecting it to other more well ventilated alveoli (Riley & Cournand, 1949). This basic principle is what governs the distribution of blood flow throughout the pulmonary vasculature, in order to optimize ventilation-perfusion ratios. Generally, there is enough surface area for gas exchange to occur and the resistance of the pulmonary vasculature is low. In COPD the gas exchange surface is limited by the alveolar volume receiving ventilation (Briscoe, Cree, & Filler, 1960). While lung volume generally increases with COPD, the

communicating lung volume (V_A) decreases as does the gas transfer capacity (K_{CO}) (Briscoe et al., 1960).

Changes in Cardiac Output with COPD:

In the presence of COPD, airflow obstruction occurs creating poorly ventilated alveoli (Briscoe et al., 1960). While blood flow is redirected to ventilated areas, the surface area for blood flow decreases, shortening the time for gas exchange and increasing resistance in the pulmonary vasculature. While, for the most part, this is simply compensated for by increased force of contraction of the right ventricle, if the situation is prolonged and the resistance is great enough, right heart failure will occur ("Cor Pulmonale"). The reduced surface area for gas exchange can lead to oxygen desaturation and increased levels of carbon dioxide in the blood if the residence time of the blood in the alveoli is inadequate to allow equilibrium between the alveolar air and capillary blood.   However, throughout all but the most severe stages of COPD, blood gases and cardiac output appear to be preserved within normal levels (Shock & Norris, 1971). While cardiac output is controlled relative to metabolic demand, the blood flow through the lung may be limited by pulmonary vascular resistance as the severity of COPD increases and as exercise intensity increases. A reduction in cardiac output during exercise may play a role in some subjects with COPD.

Severity of COPD   :

The World Health Organization (WHO):

When representatives met in San Francisco in 1945 to form the United Nations, they recognized the need for a global health organization. The first constitution of the World Health Organization (WHO), the agency of the United Nations responsible for the coordination of international public health, was put into force on April 7, 1948, and consisted of 55 members. Malaria, women's and children's health, tuberculosis, venereal disease, nutrition, and environmental sanitation were its initial priorities (World Health Organization, 2010). More recently, priorities have expanded to include coordinating international efforts to manage infectious diseases, developing and distributing safe vaccines, diagnostic tools, and pharmaceuticals, promoting healthy living and research benefiting the specific needs of the member states.

The WHO is responsible for publishing the International Classification of Diseases (ICD), Annual World Health Report, lists of Essential Medications, and Global Plan of Action on Workers' Health (World Health Organization, 2010). The ICD provides a standardized framework and classification system for diseases. Essential Medications provides nations with what, in the WHO's opinion, should be readily available and affordable with respect to treatments and medications. The annual and monthly publications *(Bulletin of the World Health Organization*) provide updates on WHO projects, latest opinions, and information on priorities.

The World Health Organization and the International Classification of Impairments, Disabilities, and Handicaps (ICIDH):

The International Classification of Impairments, Disabilities, and Handicaps (ICIDH) was introduced in 1980 as a guide to the classification of the consequences of disease. The role of the ICIDH was to provide a better understanding of the consequences of disease; through the introduction of three concepts: impairment, disability, and handicap (World Health Organization (WHO), 1980). These three concepts describe the impact of disease on the body, the person, and the person as a social being. With the prevalence of chronic disease increasing, there is a shift of emphasis to living with illness (Badley, 1993). The WHO recognized that individuals with chronic illness are not adequately served by diagnosis, but require an account for the consequences of their illness; the ICIDH was designed to fulfill this role (World Health Organization (WHO), 1980).

The ICIDH defines impairment as abnormalities in the structure or function of the body or parts of the body at one particular point in time and may be temporary or permanent (World Health Organization (WHO), 1980). Impairment is independent of how the condition developed, and can include genetic abnormalities, results of disease processes, or the consequences of an automobile or work-related accident (Badley, 1993). The term does not imply the presence of disease or that the individual is sick; the individual may not even be aware of its presence (Badley, 1993).

Disability relates to the performance of activities; representing tasks, skills, and behavior (World Health Organization (WHO), 1980). Disability is concerned with the

process by which impairment manifests in everyday life; expressing how performance varies from the normal (Badley, 1993). It includes deficiencies and excesses of customary behavior or activity that can be temporary or permanent, reversible or irreversible, and progressive or regressive (Badley, 1993). The term is concerned with what happens as a result.

Handicap refers to the social aspects of what results from impairment and disability (World Health Organization (WHO), 1980). There are four features of handicap: 1) value is attached to departure from a structural, functional, or performance normal; 2) value is assigned based on cultural normals such that an individual may be handicapped in one group, and not in another; 3) time, place, status, and role are all contributing factors; 4) valuation is usually to the disadvantage of the individual (Badley, 1993). Handicap is characterized by the difference between an individual's performance or status, and what is expected by the social group within which they operate (Badley, 1993). Disadvantage is the result of not meeting the normal expectations of the social group.

The ICIDH contains great detail on the classification of impairment, disability, and handicap; with the goal of providing a standardized framework for classification. In this context, the severity of disease is considered as it affects the body, the individual, and the individual's functioning within their social network.

Differences in Functional Classification Criteria between Cardiology and Respirology:

WHO classifies disease severity based on the level of impairment, disability, and handicap experienced as a result of the disease. Cardiology and Respirology are separate disciplines of medicine that are both fundamentally required to support the oxidative metabolism required to meet the metabolic demands of the various organs and tissues of the body. Surprisingly, they have taken different paths with respect to classification of disease severity.

In the early 1920's, Dr. Paul Dudley White working in Boston collaborated with the New York Association of Cardiac Clinics creating the first classification of heart disease severity (White & Myers, 1921). They grappled with the competing importance of cardiac symptoms/signs, etiology, structure, and function (White & Myers, 1921). Structural changes were the basis of diagnosis in most cases but function was obviously a better index of severity (White & Myers, 1921). Functional severity could be directly expressed by the patient's ability to perform work:

"A. Able to carry on the patient's usual activities.

B. Able to carry on slightly to moderately curtailed activity.

C. Able to carry on only greatly diminished activity.

D. Unable to carry on any activity (without distress) (White & Myers, 1921)."

In 1928, shortly after White's publication, the New York Heart Association (NYHA) published its classification of patients with cardiac disease based on clinical severity and prognosis in *Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels* (The Criteria Committee of the New York Heart Association, 1994). This

publication has undergone revisions since its original publication in 1928, and its ninth

edition was released in 1994 (The Criteria Committee of the New York Heart

Association, 1994). This latest edition breaks down functional capacity into four classes,

each with distinct characteristics:

"**Class I.** Patients with cardiac disease but without resulting limitation of physical

activity. Ordinary physical activity does not cause undue fatigue, palpitation,

dyspnea or anginal pain.

Class II. Patients with cardiac disease resulting in slight limitation of physical

activity. They are comfortable at rest. Ordinary physical activity results in

fatigue, palpitation, dyspnea or anginal pain.

Class III. Patients with cardiac disease resulting in marked limitation of physical

activity. They are comfortable at rest. Less than ordinary activity causes fatigue,

palpitation, dyspnea or anginal pain.

Class IV. Patients with cardiac disease resulting in inability to carry on any

physical activity without discomfort. Symptoms of heart failure or the anginal

syndrome may be present even at rest. If any physical activity is undertaken,

discomfort increases (The Criteria Committee of the New York Heart

Association, 1994)."

This functional classification is based on the physician's assessment of the patient's

history (Selzer & Cohn, 1972). A second component involves an objective assessment,

also broken down into four classes, each of which is distinct:

"**Class A.** No objective evidence of cardiovascular disease.

Class B. Objective evidence of minimal cardiovascular disease.

Class C. Objective evidence of moderately severe cardiovascular disease.

Class D. Objective evidence of severe cardiovascular disease (The Criteria

Committee of the New York Heart Association, 1994)."

Studies correlating objective measurements to subjective functional classification are often dubious, failing to recognize the inherent limitations and deficiencies of the classification (Selzer & Cohn, 1972).

Respiratory physicians have taken an approach to classification of disease severity based on pulmonary function (i.e. impairment). To aid the translation of these original concepts to clinical practice, GOLD has generated a simple spirometric classification system of disease severity, already discussed (*Current Definition of COPD – GOLD Classification*), that has become widely adopted. Subjective assessment of activity limitation associated with breathlessness has also been introduced through the application of the MRC Dyspnea Scale (Mahler & Wells, 1988; Warley, Finnegan, Nicholson, & Laszlo, 1987). Classification is based on the intensity of breathlessness which yields limitation in activity:

"**Class 1**: Not troubled by breathlessness except on strenuous exercise

Class 2: Short of breath when hurrying or walking up a slight hill

Class 3: Walks slower than contemporaries on level ground because of

breathlessness, or has to stop for breath when walking at own pace

Class 4: Stops for breath after walking about 100m or after a few minutes on level

ground

Class 5: Too breathless to leave the house, or breathless when dressing or undressing (Medical Research Council, 1966)"

So while cardiology relies on subjective classification of disease severity, based on disability; respiratory physicians rely on symptoms in combination with the FEV_1. Both classification systems have flaws.

Classifying COPD Based on Exercise:

Classification of the severity of COPD is based on measurements of the FEV_1 (Rabe *et al*, 2007) even though it was noted at the CIBA Symposium in 1958 that objective measures of exercise capacity were "essential" measures, in combination with pulmonary function, for the classification of the severity of chronic obstructive lung diseases (Ciba Guest Symposium, 1959). Subjective exercise capacity is measured using the various dyspnea scales which are all fundamentally based on the exercise intensity required to limit activity.

Hypothesis:

1. Ventilatory capacity, gas transfer capacity, and peripheral skeletal muscle strength independently contribute to maximum power output in COPD.

2. The same relative increase in ventilation and ventilatory capacity are not equal in contributing to dyspnea in COPD (the ventilatory index, V_E/MBC, is not optimal).

3.   The efficiency of gas exchange during exercise in patients with COPD
        increases with tiotropium bromide.

4.   Cardiac output during exercise in patients with COPD increases with
        tiotropium bromide.

Other studies have convincingly shown that tiotropium bromide improves
expiratory flow rates, exertional dyspnea and exercise tolerance, and reduces resistive
and elastic work in subjects with COPD; these were not explored. There have been no
investigations of the effects of anticholinergic bronchodilation on the efficiency of gas
exchange and cardiac output.

We hypothesized that ventilatory capacity, gas transfer capacity, and peripheral
skeletal muscle strength contribute to maximum power output in COPD. Exercising
patients stop exercising when the discomfort associated with continuing becomes
intolerable. The physiological factors contributing to maximum power output must also
logically contribute to the intensity of the effort required to breathe or cycle. Thus we
hypothesized that ventilation and ventilatory capacity contribute to dyspnea in COPD.
We further hypothesized that ventilation and ventilatory capacity would not be equal in
their contribution to dyspnea in COPD. Lastly, we believed that tiotropium bromide
would open previously occluded alveoli, thus increasing the surface area for gas
exchange (reducing physiological deadspace and increasing alveolar ventilation).
Through this decrease in physiological deadspace and increase in alveolar ventilation, the
ventilatory demand during exercise would be decreased, thus reducing dyspnea and

increasing exercise tolerance. Hence, we hypothesized that the efficiency of gas exchange during exercise in COPD is improved with tiotropium bromide. Subsequently, if previously occluded alveoli were opened up to ventilation, the surrounding pulmonary vasculature would open as well, increasing pulmonary blood flow. Hence, we hypothesized that tiotropium bromide increases cardiac output during exercise in COPD.

Research Rationale:

1. A retrospective analysis was conducted on 4,424 patients with COPD to establish the major independent factors contributing to exercise limitation, the symptoms limiting exercise, and the major independent factors contributing to these symptoms. Analysis included: age, height, sex, maximal breathing capacity (MBC), the proximity of the maximum ventilation achieved during exercise to the maximum capacity to breath, the efficiency of gas exchange (V_E/VO_2 & V_E/VCO_2), gas transfer capacity (DL_{CO}, V_A, & K_{CO}), and muscle strength (MIP, MEP, & Quadriceps). Additional models were also explored using spirometric and maximal flow volume relations, hemoglobin, and carboxyhemoglobin.

2. Validation of a new noninvasive method to measure cardiac output using nitrous oxide was compared to a standardized and validated CO_2 rebreathing technique to determine whether we could measure cardiac output in an exercise testing environment in a simple, reproducible manner.

3.   An assessment of novel mechanisms through which tiotropium bromide achieves its beneficial effects was carried out by determining if the efficiency of gas exchange or cardiac output improved after opening the lung with tiotropium bromide. By assessing these variables, a more thorough understanding of the mechanisms of action responsible for yielding the reported improvements in exercise tolerance will be developed.

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Chapter 2 - The major independent factors contributing to maximum power output, the symptoms limiting exercise, and the major independent factors contributing to the symptoms limiting exercise, in COPD.

Introduction:

The capacity to exercise is highly variable in patients with COPD.   Many perform at a level greater than expected based on their FEV_1, and others perform poorly (Jones & Killian, 1991). The classic "ventilation-limits-exercise" paradigm has changed due to recognition that lower limb muscles impacted exercise tolerance in COPD (Maltais and Debigare in Aliverti et al., 2008; Killian et al., 1992). In COPD, peripheral muscle dysfunction and reduced muscle strength contribute to intolerance for exercise (Gosselink, Troosters, & Decramer, 1996; Hamilton, Killian, Summers, & Jones, 1995; Kim, Mofarrahi, & Hussain, 2008; Maltais et al., 1996). The intensity of dyspnea during exercise, in COPD, is related to increases in ventilation, pleural pressure relative to inspiratory muscle strength, tidal volume relative to vital capacity, inspiratory flow relative to maximum flow, and the breathing frequency (Leblanc, Bowie, Summers, Jones, & Killian, 1986). These all collectively contribute to the effort required to breathe. Dyspnea becomes more prevalent as the symptom limiting exercise as the severity of COPD increases (Man et al., 2003).

Exercising patients stop exercising when the discomfort associated with continuing becomes intolerable. The physiological factors contributing to maximum

power output must also logically contribute to the intensity of the effort required to breathe or cycle. In order to explore the independent factors contributing to maximum power output and the effort required to breathe or cycle; linear, forward stepwise multiple linear, and ridge regression analyses were conducted. The objectives of this study were to:

1:     Determine the major independent factors contributing to maximum power output in COPD

2:     Determine the major symptoms limiting exercise in COPD

3:     Determine the major independent factors contributing to the symptoms which limit exercise in COPD

Methods:

Study Population:

For an excess of thirty years the McMaster CardioRespiratory Research Unit has maintained a database of demographic, pulmonary function testing, cardiopulmonary exercise testing, and a pharmacological record for every patient (who provided written informed consent) referred to the McMaster University Medical Center for exercise testing.   A retrospective, cross-sectional analysis was performed on 4,424 patients with COPD, based on smoking history (current or ex-smokers with > 10 pack/years), FEV_1<70% of predicted normal, and FEV_1/FVC ratio < 0.70. (Table 2.1). A population of 4,221 healthy subjects, with no history of smoking or respiratory disease, FEV_1>80% of predicted normal, and FEV_1/FVC ratio > 0.70, were used to determine the relative contributions of the symptoms limiting exercise, to exercise limitation, in a healthy population (Table 2.2).

Pulmonary Function Testing:

Forced vital capacity over a minimum of 6 seconds (FVC), FEV_1, and a maximum inspiratory and expiratory flow volume manoeuvre was measured conforming to American Thoracic Society (ATS) performance recommendations. The FEV_1 is commonly used as a surrogate measure of the maximal breathing capacity where MBC ~ 40 * FEV_1. In this study we exploited the maximal inspiratory and expiratory flow volume loop, introduced by Hyatt and Fry, to measure ventilator capacity. The minimum

time that the tidal volume achieved at maximum exercise ($V_T$max) could be inspired and expired was calculated yielding MBC = $V_T$max * 1/T_{TOT}min. The minimum inspiratory time ($T_i$min) is simply $V_T$max/$V_i$max, where $V_i$max is the maximum rate of inspiration. To overcome the volume dependence of maximum expiratory flow, a flow rate half way between the peak expiratory flow rate (PEFR) and the forced expiratory flow 50% (FEF_{50}) was used to reflect the mean maximum expiratory flow rate. Thus the equation MBC = $V_T$max * 1/T_{TOT}min expanded to become MBC = $V_T$max * 1/($V_T$max/$V_i$max + $V_T$max/((PEFR + FEF_{50})/2))).

Communicating lung volume at total lung capacity was measured using an inert gas (V_A) and gas transfer capacity of the lung (DL_{CO}, K_{CO}) was also measured, according to ATS guidelines. Arterialized capillary blood gases were measured in most, but not all patients.

GOLD Classification:

COPD subjects were classed as GOLD I-IV utilizing the classification provided by the Global Initiative for Chronic Obstructive Lung Disease (Rabe *et al*, 2007).

Quadriceps Muscle Strength:

Quadriceps muscle strength was measured by leg extension using maximum volition effort against a hydraulic resistance device (Hydrafitness Industries, Belton, Texas). The device had six levels of hydraulic resistance. Beginning with the lowest

resistance, subjects made a maximal effort at all six resistance levels or until unable to produce any movement. The positively accelerating nature of the pressure/flow relationship for this device was such that flow reached an approximately constant value as resistance increased. The force generated increased with increasing effort, but velocity of contraction was approximately constant. Maximal peak force at the highest resistance was used as a measure of muscle strength (Hamilton et al., 1995).

Cardiopulmonary Exercise Testing:

Incremental cycle exercise testing was performed to symptom-limited maximum power output (MPO). Before exercise, while seated comfortably on the cycle ergometer, the subject breathed for 2 minutes through the mouthpiece with a noseclip in place and resting measurements were taken. The subject performed 1 minute of loadless pedaling at 50-70 rpm. The work rate increased in increments of 100 kpm at the end of each minute. MPO was defined as the highest work rate maintained for at least 30 seconds. The subject was encouraged to continue to exercise for as long as possible.

Measures made during exercise included VO_2, VCO_2, V_E, V_T, RR, BP, HR, SpO_2, and ECG. Using the modified Borg Scale, subjects rated the intensity of leg effort and breathing effort (dyspnea) at each work load by matching their perceived intensity to the descriptors on the scale (i.e. just noticeable, very slight, slight, moderate, somewhat severe, severe, very severe, very,very severe, and maximal). These descriptors were tagged to numbers from 0-10 for numerical analysis. Subjects were monitored for 10 minutes post-exercise (recovery).

Equipment Maintenance:

Calibration of all equipment occurred daily and a calibration log showing the date, calibration values, barometric pressure, and technician initials was maintained.

Statistical Analyses:

All statistical analyses described below were performed utilizing the computer software program *Statistica* (StatSoft Inc., Tulsa, Oklahoma, USA).

Factors Contributing to Maximum Power Output in COPD:

The relationship between independent contributors age, height, sex, impairment in pulmonary function (FEV_1), maximum breathing capacity (MBC), DL_{CO}, muscle strength, and the ability to exercise (maximum power output), was investigated using linear regression analysis. The independent causative factors responsible for the large residual variability were further explored utilizing forward stepwise multiple linear regression analysis including: age, height, sex, MBC, DL_{CO}, and muscle strength (MIP, MEP, & Quadriceps). Additional models were also explored using spirometric and maximal flow volume relations, hemoglobin, and carboxyhemoglobin.

These analyses were repeated using ridge regression analysis to take into account interdependence of the contributing variables. Ridge regression is a variant of multiple linear regression whose goal is to circumvent the problem of predictor collinearity (variables in the model which are strongly correlated with each other).

Because of the limitations imposed by the multiple linear regression model (Maximum Power Output = A + B*Muscle Strength + C*MBC + D*DL_{CO}) the independent quantitative contribution of the major factors contributing to exercise capacity were further explored using analysis of variance (MANOVA). The major contributing factors were categorized based on increasing magnitude. Results were graphically displayed by plotting the exercise capacity against the contributing factors, each with their corresponding mean and 95% confidence limits. Two and three way analysis allowed a direct assessment of the contribution of the independent contributors at fixed levels of the other independent contributors.

Percentage of Healthy and COPD Subjects Limited by Dyspnea, Leg Effort, or Both:

The symptom which limited exercise was defined as the symptom with the highest Borg score at maximum exercise, for all subjects. Subjects were considered to be equally limited by both dyspnea and leg effort if the Borg scores for each were equal at maximum exercise. Percentage of subjects limited by dyspnea, leg effort, or both were calculated for healthy subjects and COPD subjects GOLD I-IV, as the number limited/total number of subjects in the group.

Factors Contributing to the Symptoms Limiting Exercise in COPD:

The symptoms limiting exercise were identified. The perceptual magnitude of the intensity of leg effort and breathing effort (dyspnea) were measured using the modified Borg scale (0-10). At each work load the subject matched the intensity of each to simple descriptive phrases tagged to numbers for numerical analysis. The symptom with the greatest magnitude at maximum exercise was considered limiting.

Forward stepwise multiple linear regression was performed, to explore the independent contributing factors to the symptoms limiting exercise, in the same manner as described above for maximum power output. Correlation matrix analysis, followed by ridge regression analysis, was performed in the same manner as described above for maximum power output. Because of the limitations imposed by linear additive equations the independent contributors were categorized based on increasing magnitude and MANOVA was performed in the same manner as described above.

Maximal activation of the inspiratory muscles results in maximal flow and maximal inspiratory volume expansion. Maximal activation of the expiratory muscle is not required to achieve maximal expiratory flow. The resultant of respiratory muscle activity is ventilation. Maximal activation of the respiratory muscles results in maximal ventilation. Ventilation relative to maximal represents respiratory muscle effort. Results were graphically displayed by plotting rating of dyspnea against ventilation achieved during exercise and ventilatory capacity, stratifying the population by ventilatory capacity and achieved ventilation, respectively (mean and 95% confidence limits (standard deviation X 1.96)).

Results:

Factors Contributing to Maximum Power Output in COPD:

Maximum power output was significantly related to age, height, sex, MBC, DL_{CO}, and Quadriceps Strength (contributing factors in the model). Pearson r for the linear regression relationships are shown in Table 2.3. The plots are shown in Appendix 1 – Figures A1.1 to A1.5.

Forward stepwise multiple linear regression analysis for the model is shown in Table 2.4. Additional models are illustrated in Appendix 1 – Table A1.1. Three independent factors dominated: Quadriceps Strength, MBC, and DL_{CO}. The linear plots with 95% prediction limits for each are shown (Figures 2.1a, 2.2a, & 2.3a).

Correlation matrix analysis for the model is shown in Table 2.5. Interaction between all variables was seen. Correlation matrix analysis of additional models can be found in Appendix 1 – Table A1.2.

Ridge regression analysis for the model is shown in Table 2.6. Three independent factors dominated: Quadriceps Strength, MBC, and DL_{CO}. Ridge regression analyses of additional models are shown in Appendix 1 – Table A1.3.

Analysis of variance (MANOVA) for the dependant and major independent variables of the model are shown in plots of exercise capacity against Quadriceps Strength, MBC, and DL_{CO}, each with their corresponding mean and 95% confidence limits (Figures 2.4, 2.5, & 2.6). Quadriceps Strength, MBC, and DL_{CO} all contribute independently to maximum power output.

Factors Contributing to the Symptoms Limiting Exercise in COPD:

The percentages of subjects limited by dyspnea, leg effort, or both are shown in Figure 2.7. Dyspnea was the symptom limiting exercise in 11% of healthy subjects, and 13, 18, 32, and 41% in COPD GOLD I-IV. The percentage of individuals limited by dyspnea and by dyspnea in equal combination with leg effort was 51% of healthy subjects and 53, 55, 64, and 69% of COPD subjects GOLD I-IV, respectively.

Forward stepwise multiple linear regression analysis for the model is shown in Table 2.7. Additional models are illustrated in Appendix 1 – Table A1.4. Two independent factors dominated the perceived intensity of dyspnea: ventilation achieved (V_E) and MBC. Ventilation increased with exercise intensity as expected. However, at higher power ventilation progressively increased as maximum power output decreased (Figure 2.8). Maximum power output increased as maximal breathing capacity increased, as expected (Figure 2.9). Dyspnea intensified as exercise tolerance (MPO) decreased (Figure 2.10).

Correlation matrix analysis for the model is shown in Table 2.8. Interaction between all variables was seen. Correlation matrix analysis of additional models can be found in Appendix 1 – Table A1.5.

Ridge regression analysis for the model is shown in Table 2.9. Two independent factors dominated: V_E and MBC. Ridge regression analyses of additional models are shown in Appendix 1 – Table A1.6.

Analysis of variance (MANOVA) for the dependant and major independent variables of the model are shown in plots of dyspnea against ventilation achieved during exercise and ventilatory capacity, stratifying the population by ventilatory capacity and achieved ventilation, respectively (mean and 95% confidence limits (standard deviation X 1.96)) (Figures 2.11a & 2.12a). Ventilation achieved and Maximal Breathing Capacity both contribute independently to dyspnea. Doubling ventilation leads to a greater intensity of dyspnea than halving the ventilatory capacity. The ventilatory index (V_E/MBC) oversimplifies this relationship.

Discussion:

Factors Contributing to Maximum Power Output in COPD:

From the regression analysis, the maximum power output achieved following incremental cycle ergometry to symptom limited capacity was most closely related to the strength of the quadriceps muscle. This can be readily appreciated by the relationship of MPO vs. Quadriceps Strength (Figure 2.1a). Each kilogram increase in Quadriceps Strength resulted in an average increase of 11.7kpm/min in maximum power. With no strength, an individual would be predicted to achieve 159.9kpm/min (intercept), which has no biological meaning (Figure 2.1a). Maximal power increased with Quadriceps Strength in a non-linear manner (MPO = 40*Quadriceps Strength$^{0.75}$, r = 0.740). There was a large variability in MPO for a given level of Quadriceps Strength. At a Quadriceps Strength of 40kg, MPO ranged from 250 to 1000kpm (95% prediction limits) (Figure 2.1b). This large variability clearly indicates that other additional factors are important. During sustained activity, the muscle must be supported by the heart and lungs.

Maximal Breathing Capacity was the second most closely related factor contributing to maximum power output (MPO). This is illustrated by the relationship between MPO and MBC (Figure 2.2a). Each l/min increase in Maximal Breathing Capacity resulted in a 4.9kpm/min increase in maximum power. At zero Maximal Breathing Capacity, the intercept of 117.6kpm/min has no biological meaning (Figure 2.2a). The increase in maximal power with Maximal Breathing Capacity is non-linear (MPO = 115*MBC$^{0.40}$, r = 0.511). There was a large variability in MPO for a given level of Maximal Breathing Capacity. At a MBC of 100l/min, MPO ranged from 200 to

1000kpm (95% prediction limits) (Figure 2.2b). This large variability clearly indicates that additional factors are important.

The ability of the lung to transfer gas (DL_{CO}) was the third important factor which contributed to maximum power output. This can be appreciated by the direct relationship between MPO and DL_{CO} (Figure 2.3a). Each ml/mmHg/min increase in DL_{CO} resulted in a 28.8kpm/min increase in maximum power. At zero gas transfer capacity, the intercept of 110.0kpm/min has no biological meaning (Figure 2.3a). The increase in maximum power with gas transfer capacity is non-linear (MPO = $54*DL_{CO}^{0.85}$, r = 0.684). There was a large variability in MPO for a given level of gas transfer capacity. At a DL_{CO} of 20mL/mmHg/min, MPO ranged from 300 to 1100kpm (95% prediction limits) (Figure 2.3b). This large variability clearly indicates that other contributing factors are important.

From the linear regressions above, it is clear that multiple factors, in combination, contribute to maximum power output. This was explored in a forward stepwise multiple linear regression model. This yielded standardized β scores for the contributions of age, height, sex, quadriceps strength, maximal breathing capacity, and gas transfer capacity to maximum power output, which accounted for 70% of the variability in maximum power output (r^2=0.703) (Table 2.4). Additional models included an array of pulmonary function measurements contributing to the capacity to breathe (maximum flow-volume relationships with a logical estimation of maximal breathing capacity), hemoglobin, carboxyhemoglobin, maximal inspiratory pressure (MIP), and maximal expiratory pressure (MEP) (Appendix 1 – Table A1.1).

With standardized β scores greater than 0.1, the independent factors contributing to maximum power output included: Quadriceps Strength (β=0.373), Maximal Breathing Capacity (β=0.262), and gas transfer capacity (β=0.246) (Table 2.4). While age (β=-0.084), height (β=0.076), and sex (β=-0.032) did contribute significantly, their magnitude was small. The forward stepwise multiple linear regression model yielded the following linear additive equation:

MPO = -233.3 + 5.8*Quadriceps Strength + 1.9*MBC + 10.5*DL_{CO}. Hence, for every kg increase in Quadriceps Strength, MPO increases by 5.8kpm/min. For every l/min increase in MBC, MPO increases by 1.9kpm/min. With every ml/mmHg/min increase in DL_{CO}, MPO increases by 10.5kpm/min. This analysis also generated an intercept of -233.3, suggesting that when Quadriceps Strength, MBC, and DL_{CO} are zero the MPO would be -233.3kpm/min; which is biologically meaningless. The biological increase in MPO with Quadriceps Strength, MBC, and DL_{CO} are not linear. Quadriceps Strength, MBC, and DL_{CO} may be interactive and non linear in their contribution to MPO.

Correlation matrix analysis using the variables from the forward stepwise multiple linear regression model illustrated interactive effects between all the variables (Table 2.5). To minimize the interactive effects, ridge regression analysis was performed using the variables age, height, sex, quadriceps strength, maximal breathing capacity, and gas transfer capacity. This analysis accounted for 68% of the variability in MPO (r^2=0.677) (Table 2.5). Based on the resultant standardized β scores, Quadriceps Strength (β=0.328), Maximal Breathing Capacity (β=0.249), and gas transfer capacity (β=0.233) were still the top three contributing factors to maximum power output (Table 2.6).

Additional correlation matrix and ridge regression models included variables from the additional forward stepwise multiple linear regression models described above (Appendix 1 – Tables A1.2 & A1.3). Age (β=-0.102) and height (β=0.083) contributed to MPO, while the contribution of sex did not reach significance. The magnitude was negligible relative to the top three contributing factors. From the ridge regression:

MPO = -206.3 + 5.1*Quadriceps Strength + 1.8*MBC + 10.0*DL_{CO}. Hence, for every kg increase in Quadriceps Strength, MPO increases by 5.1kpm/min. For every l/min increase in MBC, MPO increases by 1.8kpm/min. With every ml/mmHg/min increase in DL_{CO}, MPO increases by 10.0kpm/min. This analysis also generated an intercept of -206.3, suggesting that when Quadriceps Strength, MBC, and DL_{CO} are zero the MPO would be -206.3kpm/min; which is biologically meaningless. Given that the coefficients (B) for Quadriceps Strength, MBC, and DL_{CO} from the ridge regression (Table 2.6) are nearly identical to those generated from the forward stepwise multiple linear regression (Table 2.4), Quadriceps Strength, MBC, and DL_{CO} are largely independent in their effect on MPO. The continued presence of variability in MPO (~32%), and the possibility that the effects of Quadriceps Strength, MBC, and DL_{CO} on MPO may be non-linear, requires the pursuit of additional contributing factors and consideration beyond linear additive models.

In order to explore the contribution of the major factors to MPO independent of any forced model, MANOVA was performed, using the mean values for categories (based on increasing magnitude) of Quadriceps Strength (<20, 20-40, 40-60, & >60kg),

MBC (<40, 40-80, 80-120, >120l/min), and DL_{CO} (<10, 10-20, 20-30, >30mL/mmHg/min).

By individually plotting maximum power output against each of the contributing factors, using mean values for categories of increasing magnitude, a two-dimensional analysis based on actual mean values from the population was done. By determining, through visual analysis, the slope of the relationship between maximum power output and each of the contributing factors, a more realistic magnitude of effect for each factor was determined. These relationships were derived from plots of MPO vs. Quadriceps Strength (Figure 2.4), MBC (Figure 2.5), and DL_{CO} (Figure 2.6). The slope of the relationship between MPO and Quadriceps Strength was found to be 11.25. Thus, an increase in Quadriceps Strength of 1kg results in an increase in MPO of 11.25kpm/min. The slope of the relationship between MPO and MBC was found to be 4.4. Thus, an increase in MBC of 1l/min results in an increase in MPO of 4.4kpm/min. The slope of the relationship between MPO and DL_{CO} was found to be 27.5. An increase in DL_{CO} of 1ml/mmHg/min results in an increase in MPO of 27.5kpm/min. These relationships are very similar to those generated from the linear regression analyses of MPO vs. Quadriceps Strength (slope = 11.7), MBC (slope = 4.9), and DLCO (slope = 28.8).

To further assess the independent nature with which the contributing factors influence maximum power output MANOVA was performed, using the mean values for categories (based on increasing magnitude) of Quadriceps Strength (<20, 20-40, 40-60, & >60kg), MBC (<40, 40-80, 80-120, >120l/min), and DL_{CO} (<10, 10-20, 20-30, >30mL/mmHg/min).

MPO was plotted against categories of MBC stratifying the population by categories of DL_{CO} (Figure 2.13) and Quadriceps Strength (Figure 2.14) (mean and 95% confidence limits). MPO was also plotted against categories of DL_{CO}, stratifying by categories of Quadriceps Strength (Figure 2.15) (mean and 95% confidence limits). In this three-dimensional way, if the line patterns produced by the stratification have the same slope, then the effects of the x-axis factor and the stratification factor on MPO are independent.

This yielded plots which illustrated that MBC contributed to MPO, independent of DL_{CO} (Figure 2.13) and Quadriceps Strength (Figure 2.14); given that the direct relationship pattern (slope of line pattern) remained no matter what the DL_{CO} or Quadriceps Strength was. Additionally, DL_{CO} was seen to contribute to MPO, independent of MBC (Figure 2.13) and Quadriceps Strength (Figure 2.15); also showing a direct relationship pattern that remained unchanged with stratification. Finally, the same plots illustrated that Quadriceps Strength was directly related to MPO and was independent of MBC (Figure 2.14) and DL_{CO} (Figure 2.15); given there was no change in the pattern seen. Illustrated in this way, with the standard deviation and 95% confidence limits for the mean of each x-axis variable category, there was residual variability in the MPO. Yet the overall direct relationship between MPO and any of the top three contributing factors, and their independent nature, was still seen indicating their independent roles in contributing to MPO.

To appreciate the independent effects of Quadriceps Strength, MBC, and DL_{CO} on MPO, a single plot of MPO against all three independent contributors was generated

(Figure 2.16). This plot illustrates the increase in MPO that occurs with increases in Quadriceps Strength, MBC, and DL_{CO}, respectively. It also highlights that at low muscle strength, there are no individuals with a high DL_{CO}. Conversely, at high muscle strength, there are no people with low DL_{CO} or MBC.

Contributing Factors to the Symptoms Limiting Exercise in COPD:

Exercising patients stop exercising when the discomfort associated with continuing becomes intolerable. The intensity of breathing effort, leg effort, or both are the common symptoms limiting exercise. The physiological factors contributing to maximum power output must also logically contribute to the intensity of the effort required to breathe or cycle. In order to explore the independent factors contributing to the effort required to breathe or cycle, a manner similar to that utilized to explore the factors contributing to maximum power output was used.

Dyspnea, either alone or in equal combination with leg effort, was the commonest symptom limiting exercise (Figure 2.7). Dyspnea was the symptom limiting exercise in 11% of healthy subjects, and 13, 18, 32, and 41% in COPD GOLD I-IV. The percentage of individuals limited by dyspnea and by dyspnea in equal combination with leg effort included 51% of healthy subjects and 53, 55, 64, and 69% of COPD subjects GOLD I-IV, respectively. Dyspnea, alone and in combination with leg effort, accounts for the majority of symptom limitation in individuals with COPD at all severity levels.

Dyspnea intensifies as power output increases and systematically as the capacity to generate power decreases, limiting exercise tolerance (Figure 2.17). The ventilation required to maintain power systematically increases with the metabolic demands of exercise. The maximal breathing capacity decreases with the severity of COPD. The ventilation relative to the capacity to breathe varies with the intensity of exercise and the severity of COPD. Individuals with a high exercise tolerance reached a higher maximal intensity of dyspnea. However, the difference in dyspnea at maximum exercise between the lowest and highest exercise tolerance (MPO) groups was 2 Borg units (Figure 2.17). All muscles fatigue with sustained repetitive contractions as the forces increase relative to their capacity. The time to fatigue, while dependent on the force/force capacity ratio, is also dependent on the respiratory support of the muscle. Fatigue, regardless of its contributing factors, is perceptually manifest as an increase in the motor effort required to sustain activity over time. The subject is also aware of evolving weakness. The exercising quadriceps must logically fatigue at a greater rate as the strength of the muscle decreases. This results in an increased intensity of leg effort at any given power during exercise as the strength of the muscle decreases (Figure 2.18). While it is true that individuals with a high exercise tolerance reach a higher maximal intensity of leg effort, the difference in leg effort at maximum exercise between the lowest and highest exercise tolerance (MPO) groups is only 2 Borg units (Figure 2.18). Respiratory muscles must also fatigue at an increased rate as their strength decreases. The power generated by the legs is controlled during cycle ergometry. The power generated by the respiratory muscles increases with ventilation and with the forces opposing their contraction. With

COPD these are more complex mechanical relationships. Nonetheless, dyspnea

(breathing effort) competes with leg effort and one or both ultimately become the

symptom limiting exercise (Figure 2.7). Maximal respiratory muscle effort produces

maximal ventilation. The ventilation at maximum exercise relative to the maximal

breathing capacity reflects respiratory muscle effort to a limited extent. Maximum

respiratory effort results in maximal ventilation such that V_E/MBC reflects relative

breathing effort. This simple relationship has long been used as an index of dyspnea.

However, this relationship does not take into account the increase in effort required to

sustain the same ventilation over time (i.e. fatigue). The factors contributing to fatigue

are more complex.

Forward stepwise multiple linear regression illustrated that the V_E

($\beta = 0.75$) and the MBC ($\beta = -0.21$) were the two most important contributing factors to

dyspnea; with the analysis accounting for 51% of the variability in dyspnea ($r^2 = 0.512$)

(Table 2.7). Ventilation increased with exercise intensity as expected. However, at

higher powers, ventilation progressively increased as maximum power output decreased

(Figure 2.8). Maximum power output increased as maximal breathing capacity increased,

as expected (Figure 2.9). Dyspnea intensified as exercise tolerance decreased (Figure

2.10). Taken together, the resulting relationship sees dyspnea increase as ventilation

increases and ventilatory capacity decreases, which supports the findings of the forward

stepwise multiple linear regression analysis. Correlation matrix analysis illustrated

interactive effects between the contributing factors (Table 2.8). Ridge regression analysis

showed that the V_E ($\beta = 0.67$) and the MBC ($\beta = -0.16$) were still the two most important

contributing factors to dyspnea, with the analysis accounting for 46% of the variability in

dyspnea

($r^2 = 0.457$) (Table 2.9). Additional forward stepwise multiple linear regression,

correlation matrix, and ridge regression models were explored, but yielded similar results

(Appendix 1 – Tables A1.4, A1.5, & A1.6).

Given the difference in magnitude of the standardized β for V_E and MBC (Table

2.9), further assessment of whether improvement in ventilatory capacity would have the

same magnitude of effect on improving dyspnea as a reduction in the ventilation achieved

during exercise was conducted. Dyspnea was plotted against V_E, stratifying the

population based on MBC (Figure 2.11a) and against MBC, stratifying the population

based on V_E (Figure 2.12a). When assessing the effects of V_E on dyspnea, analysis

focused on the level of dyspnea for an MBC of 50 to 70L/min at a V_E of 10 to 30L/min

and 30 to 50L/min (Figure 2.11b). By this assessment, dyspnea at a $V_E \sim 20$L/min was $\sim$

0.8 and increased to ~ 3.6 at a $V_E \sim 40$L/min. This doubling of V_E resulted in a 4.5 fold

increase in dyspnea.

Conversely, when assessing the effects of MBC on dyspnea, analysis focused on

the level of dyspnea for a V_E of 30 to 50L/min at an MBC of 30 to 50L/min and 70 to

90L/min (Figure 2.12b). By this assessment, dyspnea at an MBC ~ 80L/min was ~ 3.1

and increased to ~ 4.2 at an MBC ~ 40L/min. This halving of MBC resulted in a 1.4 fold

increase in dyspnea.

The effect of doubling ventilation achieved during exercise, a 4.5 fold increase in

dyspnea, was far greater than the effect of halving the ventilatory capacity, which yielded

a 1.4 fold increase in dyspnea. Assessment based on a doubling of ventilation and a halving of ventilatory capacity illustrates the effect of an equal relative magnitude change of each on dyspnea. Comparison of the effects on dyspnea using an equal absolute change in both MBC and V_E (say 5 L/min) would be biased, illustrating a greater effect of V_E, simply due to the fact that the absolute change would represent a larger percentage increase in V_E vs. MBC. This visual analysis is confirmed by the linear regression equations (Dyspnea = A + B*V_E + C*MBC) generated from the forward stepwise multiple linear regression and ridge regression models that were utilized to determine the factors contributing to dyspnea (Tables 2.7 & 2.9). If the effects of V_E and MBC were equal in magnitude in their effect on dyspnea, the standardized β scores for each would be equal (though opposite in sign (+/-)). This is clearly not the case as can be seen in Tables 2.7 and 2.9. Together, this strongly illustrates the unequal effects of V_E and MBC on dyspnea, and that reductions in ventilation during exercise, by the same relative degree as improvements in ventilatory capacity, yield a much more pronounced decrease in dyspnea.

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Tables:

Table 2.1. *Demographics of COPD population.*

Descriptive Statistics					
	n	Mean	Minimum	Maximum	Std.Dev.
Age (years)	4424	63.68	35.00	91.00	11.00
Gender (% male)	4424	70.00			
Height (m)	4424	1.70	1.41	2.05	0.10
Weight (kg)	4424	77.25	32.00	175.00	16.55
BMI (kg/m^2)	4424	26.72	13.15	53.71	4.77
FEV$_1$ (L)	4424	1.87	0.30	4.80	0.75
FEV$_1$ % Predicted	4424	60.53	11.91	119.66	18.52
VC (L)	4424	3.05	0.50	6.90	1.03
VC % Predicted	4424	76.81	16.61	157.07	18.29
FEV$_1$/VC	4424	0.61	0.10	0.70	0.09
MBC (L/min)	4424	110.93	20.48	263.97	42.15
DL$_{CO}$ (mL/mmHg/min)	4424	18.86	3.10	52.60	7.07
K$_{CO}$	4424	3.68	0.57	8.50	1.05
V$_A$ (L/min)	4424	5.18	1.00	9.60	1.44
MPO (kpm)	4424	650.04	100.00	2200.00	293.63
Quad Strength (kg)	4424	42.00	2.00	134.00	18.85

BMI = body mass index, FEV$_1$ = forced expired volume in one second, VC = vital capacity, MBC = maximal breathing capacity, DL$_{CO}$ = diffusing capacity of carbon monoxide, K$_{CO}$ = transfer coefficient for carbon monoxide, V$_A$ = alveolar ventilation, MPO = maximum power output, Quad Strength = strength of quadriceps muscle

Table 2.2. *Demographics of health, normal population.*

Descriptive Statistics					
	n	Mean	Minimum	Maximum	Std.Dev.
Age (years)	4221	55.90	35.00	87.00	10.43
Gender (% male)	4221	71.00			
Height (m)	4221	1.70	1.40	1.98	0.09
Weight (kg)	4221	80.45	41.00	180.00	14.91
BMI (kg/m^2)	4221	27.91	17.30	60.84	4.37
FEV$_1$ (L)	4221	3.12	1.30	5.80	0.71
FEV$_1$ % Predicted	4221	104.55	80.01	167.55	13.72
VC (L)	4221	3.86	1.60	7.70	0.88
VC % Predicted	4221	104.04	80.00	164.95	13.06
FEV$_1$/VC	4221	80.93	70.00	130.00	5.48
MBC (L/min)	4221	179.21	11.63	361.41	47.88
DL$_{CO}$ (mL/mmHg/min)	4221	26.31	12.80	47.80	5.76
K$_{CO}$	4221	4.45	2.30	7.70	0.80
V$_A$ (L/min)	4221	5.98	2.10	10.20	1.12
MPO (kpm)	4221	955.30	400.00	2200.00	268.41
Quad Strength (kg)	4221	52.76	0.50	418.20	20.36

BMI = body mass index, FEV$_1$ = forced expired volume in one second, VC = vital capacity, MBC = maximal breathing capacity, DL$_{CO}$ = diffusing capacity of carbon monoxide, K$_{CO}$ = transfer coefficient for carbon monoxide, V$_A$ = alveolar ventilation, MPO = maximum power output, Quad Strength = strength of quadriceps muscle

Table 2.3. *Linear regression relationships (Pearson r) for MPO and potential contributing factors in COPD population.*

	Correlations		
	Means	**Std.Dev.**	**Pearson r (MPO (kpm))**
Age (years)	63.03	10.48	-0.4567
Gender (% male)	65.00		0.4630
Height (m)	1.70	0.10	0.5237
FEV_1 (L)	1.95	0.79	0.7946
VC (L)	3.19	1.10	0.7893
FEV_1/VC	0.61	0.08	0.3196
PEFR (L/min)	5.05	2.10	0.7936
FEF_{50} (L/min)	1.40	0.80	0.6599
V_I Maximum (L)	4.20	1.45	0.6654
MBC (L/min)	107.76	40.23	0.7826
MIP (mmHg)	69.12	24.51	0.6425
MEP (mmHg)	111.57	39.39	0.6570
Hb (g/dL)	13.93	1.58	0.3942
HbCO (g/dL)	2.03	1.57	-0.0497
DL_{CO} (mL/mmHg/min)	18.09	6.96	0.7678
K_{CO}	3.80	1.14	0.2266
V_A (L/min)	4.85	1.48	0.7220
Quad Strength (kg)	46.09	22.02	0.8183
S_AO_2 Maximum (%)	95.85	1.76	0.3816

All Pearson r correlations in the table were significant ($p < 0.05$). FEV_1 = forced expired volume in one second, VC = vital capacity, PEFR = peak expiratory flow rate, FEF_{50} = forced expiratory flow 50%, V_I Maximum = maximum inspiratory volume, MBC = maximal breathing capacity, MIP = maximum inspiratory pressure, MEP = maximum expiratory pressure, Hb = hemoglobin, HbCO = carboxyhemoglobin, DL_{CO} = diffusing capacity of carbon monoxide, K_{CO} = transfer coefficient for carbon monoxide, V_A = alveolar ventilation, Quad Strength = strength of quadriceps muscle, S_AO_2 Maximum = maximum arterial oxygen saturation

Table 2.4. *Forward stepwise multiple linear regression showing the independent contributors to MPO.*

	Beta	Std.Err. of Beta	B	Std.Err. of B	t(3165)	p-level
Regression Summary for Dependent Variable: MPO R= .83837033 R²= .70286480 Adjusted R²= .70230151 p<0.0000 Std.Error of estimate: 160.92						
Intercept			-233.301	73.09939	-3.19155	0.001429
Quad Strength (kg)	0.373250	0.014474	5.810	0.22531	25.78785	0.000000
DL_{CO} (mL/mmHg/min)	0.246046	0.014293	10.539	0.61222	17.21486	0.000000
MBC (L/min)	0.262445	0.014437	1.886	0.10376	18.17860	0.000000
Age (years)	-0.083876	0.011861	-2.165	0.30613	-7.07177	0.000000
Height (cm)	0.075812	0.014636	235.176	45.40242	5.17980	0.000000
Gender (% male)	-0.032298	0.014052	-20.929	9.10588	-2.29841	0.021603

Quad Strength = strength of quadriceps muscle, DLCO = diffusing capacity of carbon monoxide, MBC = maximal breathing capacity

Table 2.5. *Correlation matrix analysis of contributing factors to MPO.*

	Correlations							
	Age	**Gender**	**Height**	**MBC**	**MIP**	**MEP**	**DL$_{CO}$**	**Quad Strength**
Age (years)	1.00	0.02	-0.15*	-0.35*	-0.37*	-0.23*	-0.44*	-0.41*
Gender (% male)	0.02*	1.00	0.67*	0.45*	0.37*	0.42*	0.41*	0.51*
Height (cm)	-0.15*	0.67*	1.00	0.56*	0.39*	0.36*	0.50*	0.58*
MBC (L/min)	-0.35*	0.45*	0.56*	1.00	0.62*	0.50*	0.67*	0.62*
MIP (mmHg)	-0.37*	0.37*	0.39*	0.62*	1.00	0.68*	0.50*	0.62*
MEP (mmHg)	-0.23*	0.42*	0.36*	0.50*	0.68*	1.00	0.39*	0.59*
DL$_{CO}$ (mL/mmHg/min)	-0.44*	0.41*	0.50*	0.67*	0.50*	0.39*	1.00	0.59*
Quad Strength (kg)	-0.41*	0.51*	0.58*	0.62*	0.62*	0.59*	0.59*	1.00

* = significant at $p < 0.05$, MBC = maximal breathing capacity, MIP = maximum inspiratory pressure, MEP = maximum expiratory pressure, DL$_{CO}$ = diffusing capacity for carbon monoxide, Quad Strength = strength of the quadriceps muscle

Table 2.6. *Ridge regression analysis showing the independent contributors to MPO.*

	Beta	Std. Err. of Beta	B	Std. Err. of B	t(3166)	p-level
Ridge Regression Summary for Dependent Variable: MPO R= .82267239 R²= .67678986 Adjusted R²= .67627942 p < 0.0000 Std. Error of estimate: 167.81						
Intercept			-206.299	59.14511	-3.48801	0.000493
Quad Strength (kg)	0.327862	0.013077	5.104	0.20356	25.07216	0.000000
DL$_{CO}$ (mL/mmHg/min)	0.233332	0.013181	9.995	0.56460	17.70223	0.000000
MBC (L/min)	0.248786	0.013304	1.788	0.09562	18.70039	0.000000
Age (years)	-0.102346	0.010904	-2.642	0.28144	-9.38613	0.000000
Height (cm)	0.083349	0.012159	258.556	37.71910	6.85479	0.000000

Quad Strength = strength of quadriceps muscle, DL$_{CO}$ = diffusing capacity for carbon monoxide, MBC = maximal breathing capacity

Table 2.7. *Forward stepwise multiple linear regression showing the independent contributors to Dyspnea.*

	b*	Std. Err. of b*	b	Std. Err. of b	t(29144)	p-value
Regression Summary: Dyspnea R= .71548694 R²= .51192156 Adjusted R²= .51183782 p < 0.0000 Std. Error of estimate: 1.6750						
Intercept			0.454656	0.254587	1.7859	0.074133
V_E (L/min)	0.753971	0.004352	0.083894	0.000484	173.2325	0.000000
MBC (L/min)	-0.209446	0.006311	-0.013314	0.000401	-33.1884	0.000000
Gender (% male)	-0.057449	0.005859	-0.297767	0.030367	-9.8056	0.000000
Age (years)	-0.044163	0.005018	-0.007348	0.000835	-8.8004	0.000000
Height (cm)	0.016919	0.006242	0.004367	0.001611	2.7107	0.006719

V_E = minute ventilation, MBC = maximal breathing capacity

Table 2.8. *Correlation matrix analysis of contributing factors to Dyspnea.*

Correlations				
	Height	**Age**	**V$_E$**	**MBC**
Height (cm)	1.000000	-0.182560*	0.592967*	0.606712*
Age (years)	-0.182560*	1.000000	-0.427766*	-0.521884*
V$_E$ (L/min)	0.592967*	-0.427766*	1.000000	0.762060*
MBC (L/min)	0.606712*	-0.521884*	0.762060*	1.000000

* = significant at $p < 0.05$, V$_E$ = minute ventilation, MBC = maximal breathing capacity

Table 2.9. *Ridge regression analysis showing the independent contributors to Dyspnea.*

	b*	Std. Err. of b*	b	Std. Err. of b	t(29145)	p-value
Ridge Regression Summary: Dyspnea R= .67629899 R²= .45738032 Adjusted R²= .45730585 p < 0.0000 Std. Error of estimate: 1.7661						
Intercept			0.950348	0.067063	14.1710	0.000000
V$_E$ (L/min)	0.674185	0.004331	0.075016	0.000482	155.6536	0.000000
MBC (L/min)	-0.157199	0.005409	-0.009993	0.000344	-29.0618	0.000000
Gender (% male)	-0.046335	0.004819	-0.240164	0.024975	-9.6160	0.000000
Age (years)	-0.030620	0.004789	-0.005094	0.000797	-6.3932	0.000000

V$_E$ = minute ventilation, MBC = maximal breathing capacity

Figures:
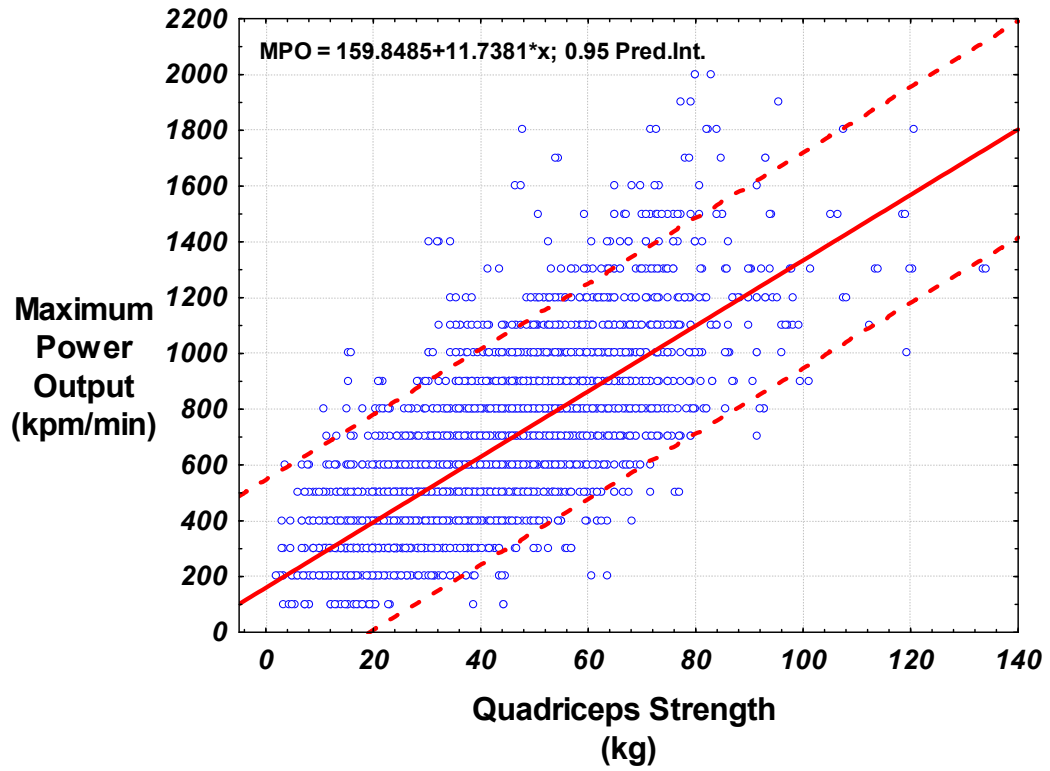
Figure 2.1a. *Maximum power output (MPO) achieved based on exercising muscle strength.*
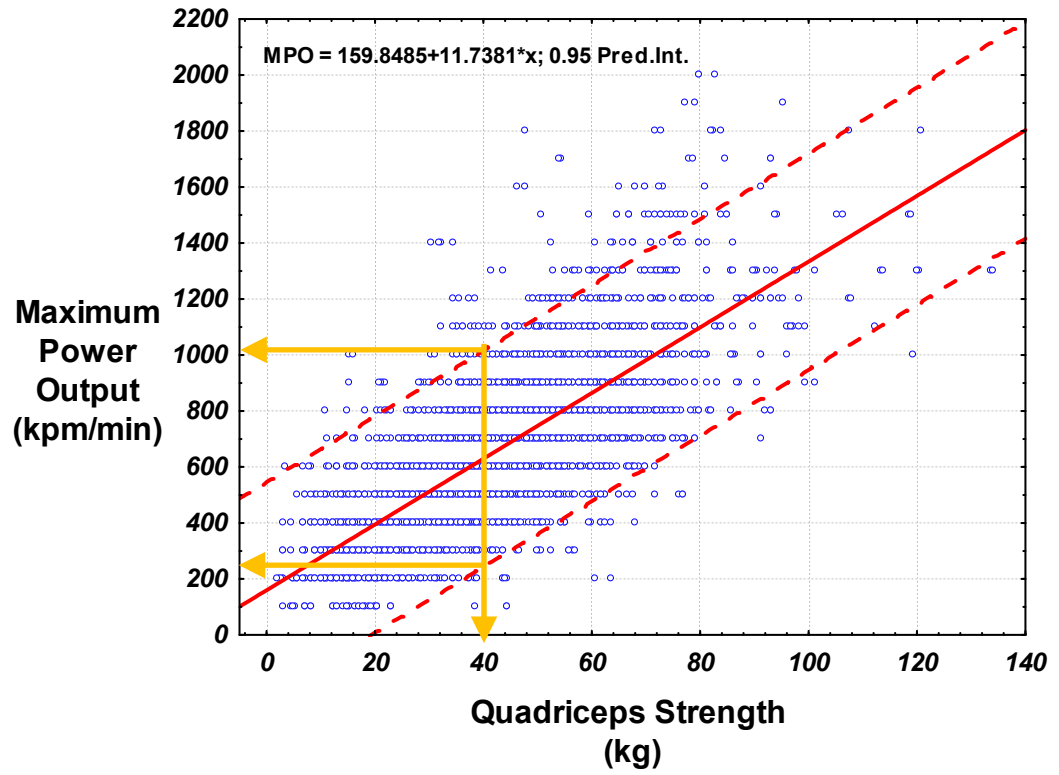
Figure 2.1b. *Variability in maximum power output (MPO) for a given exercising muscle strength.*
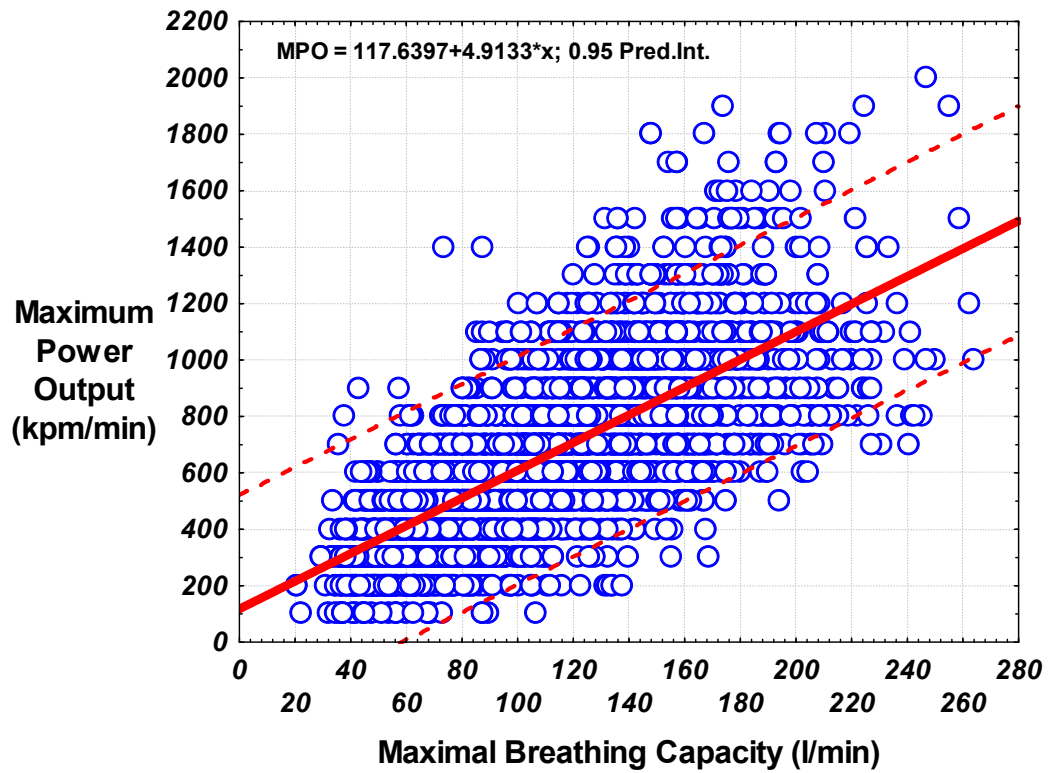
Figure 2.2a. *Maximum power output (MPO) achieved based on ventilatory capacity.*
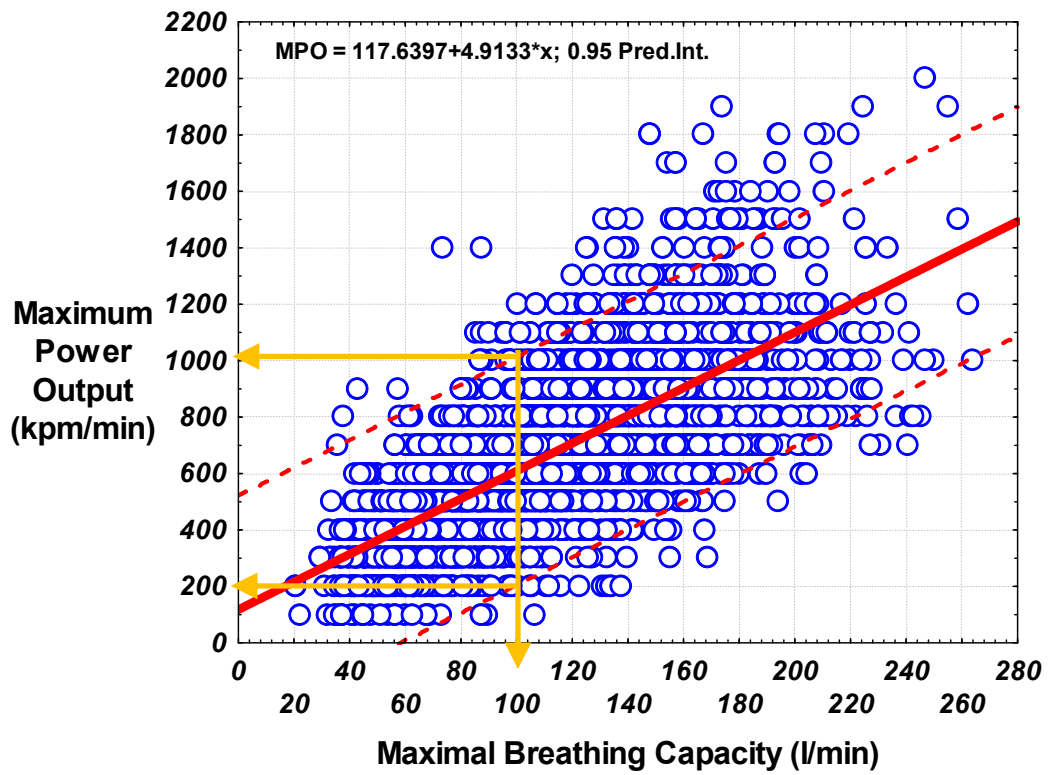
Figure 2.2b. *Variability in maximum power output (MPO) for a given ventilatory capacity.*
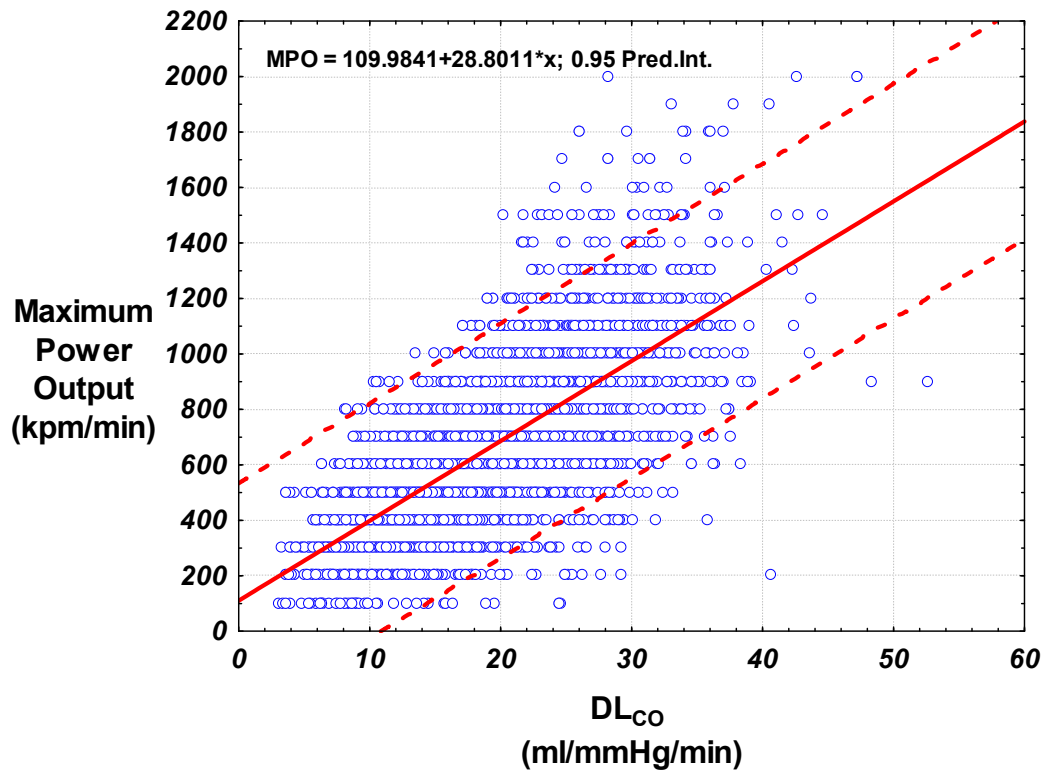
Figure 2.3a. *Maximum power output (MPO) achieved based on diffusion capacity of the lung (DL$_{CO}$).*
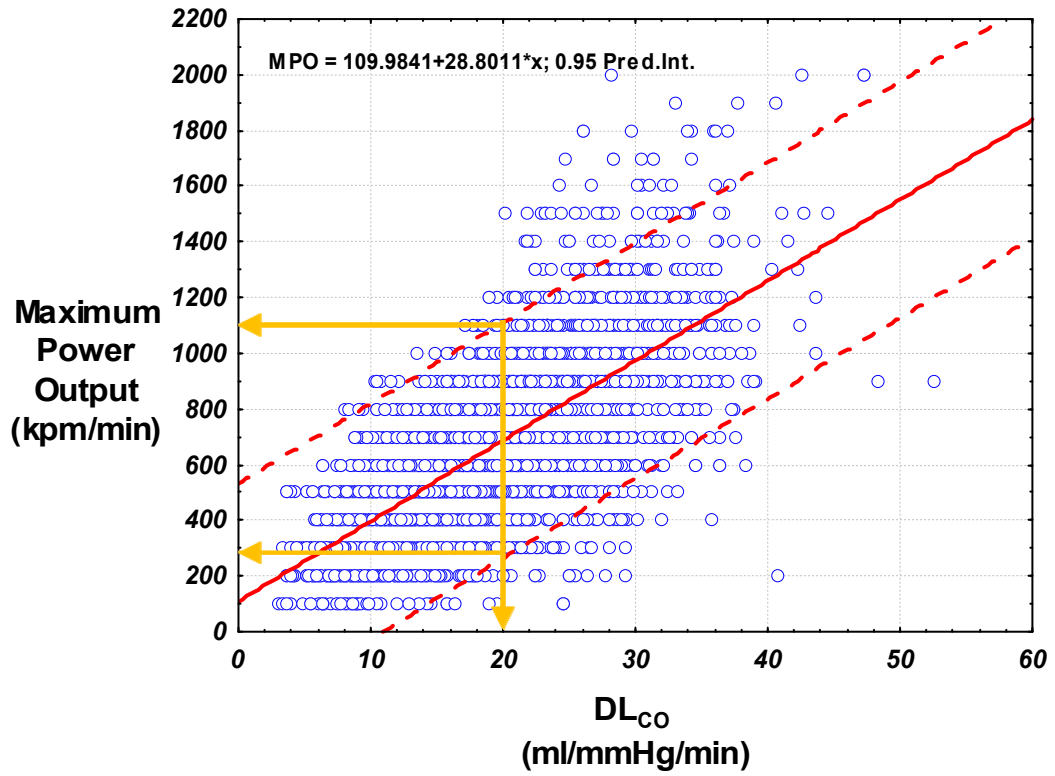
Figure 2.3b. *Variability in maximum power output (MPO) for a given diffusion capacity of the lung (DL$_{CO}$).*
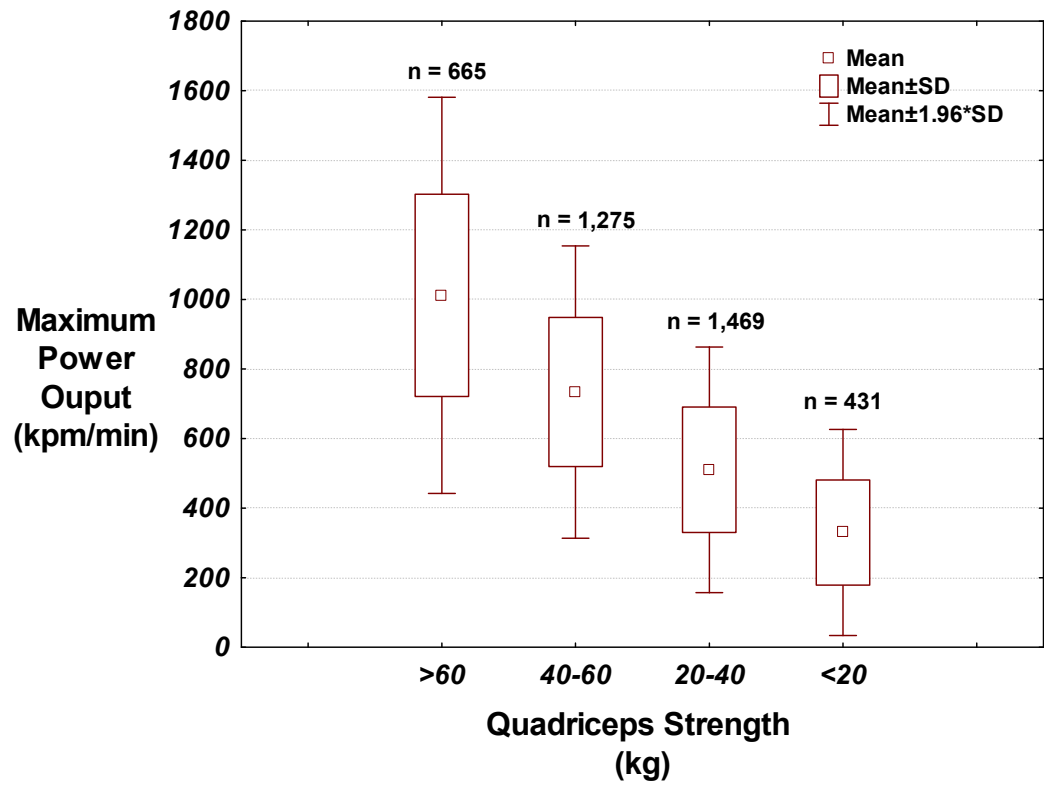
Figure 2.4. *Maximum power output achieved, expressed as mean with standard deviation and 95% confidence limits, based on quartiles of exercising muscle strength.*
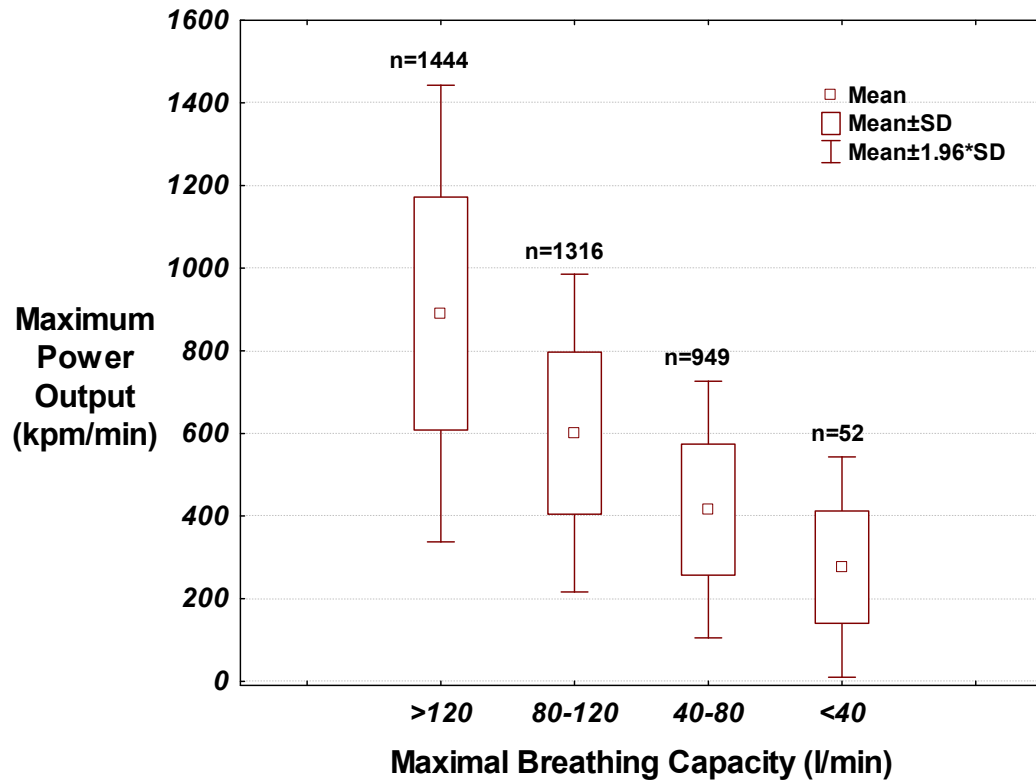
Figure 2.5. *Maximum power output achieved, expressed as mean with standard deviation and 95% confidence limits, based on quartiles of ventilatory capacity.*
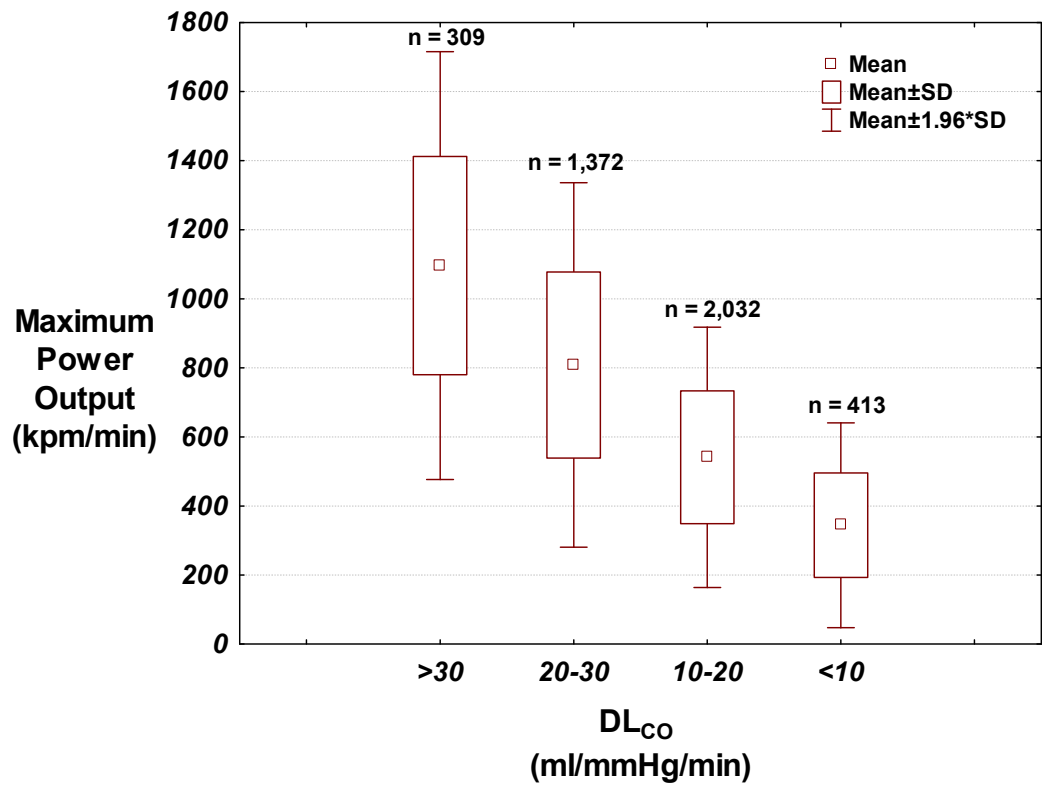
Figure 2.6. *Maximum power output achieved, expressed as mean with standard deviation and 95% confidence limits, based on quartiles of lung diffusion capacity.*
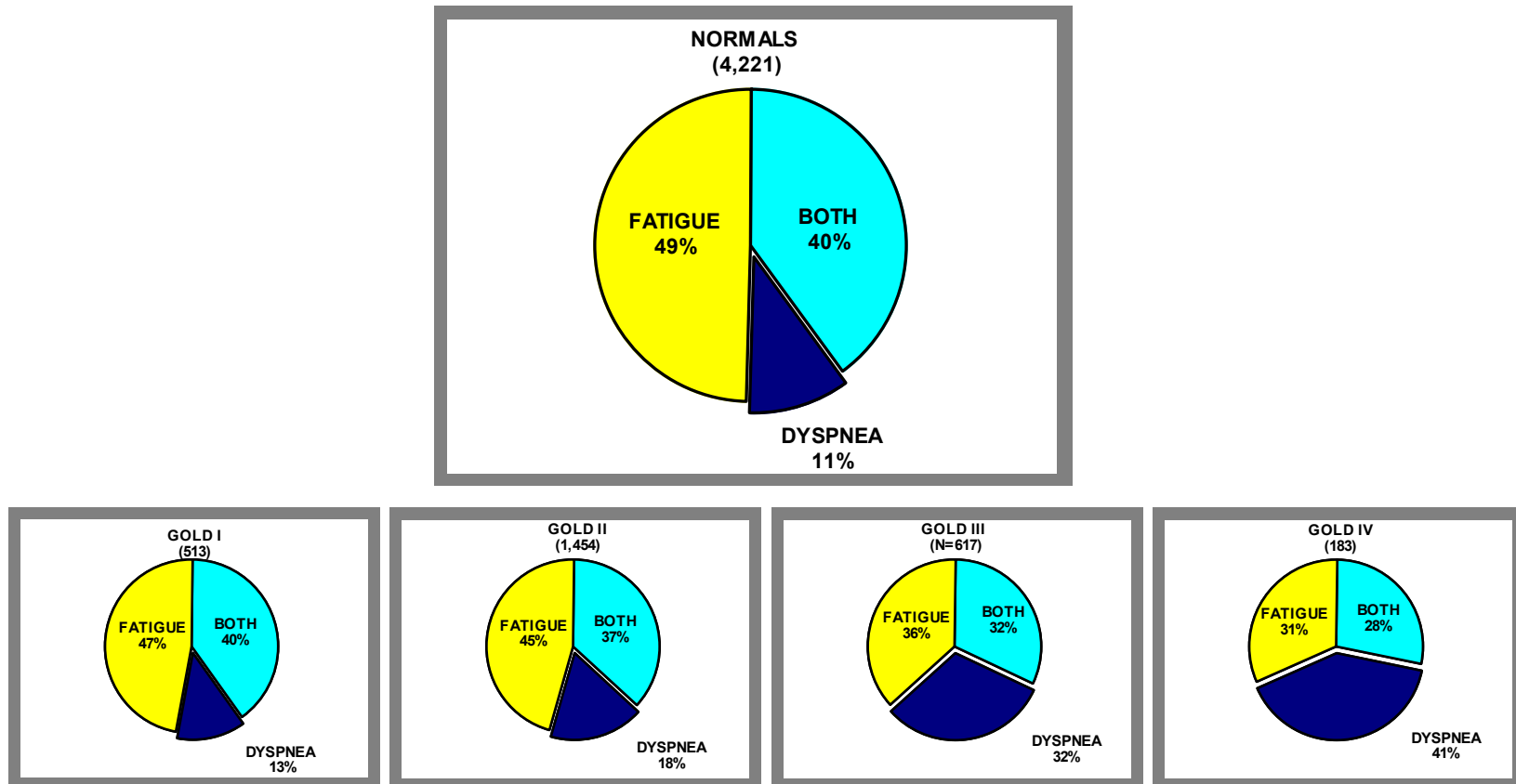
Figure 2.7. *Percentage of individuals whose exercise is limited by leg fatigue, dyspnea, or both in health and increasing severity of COPD.*
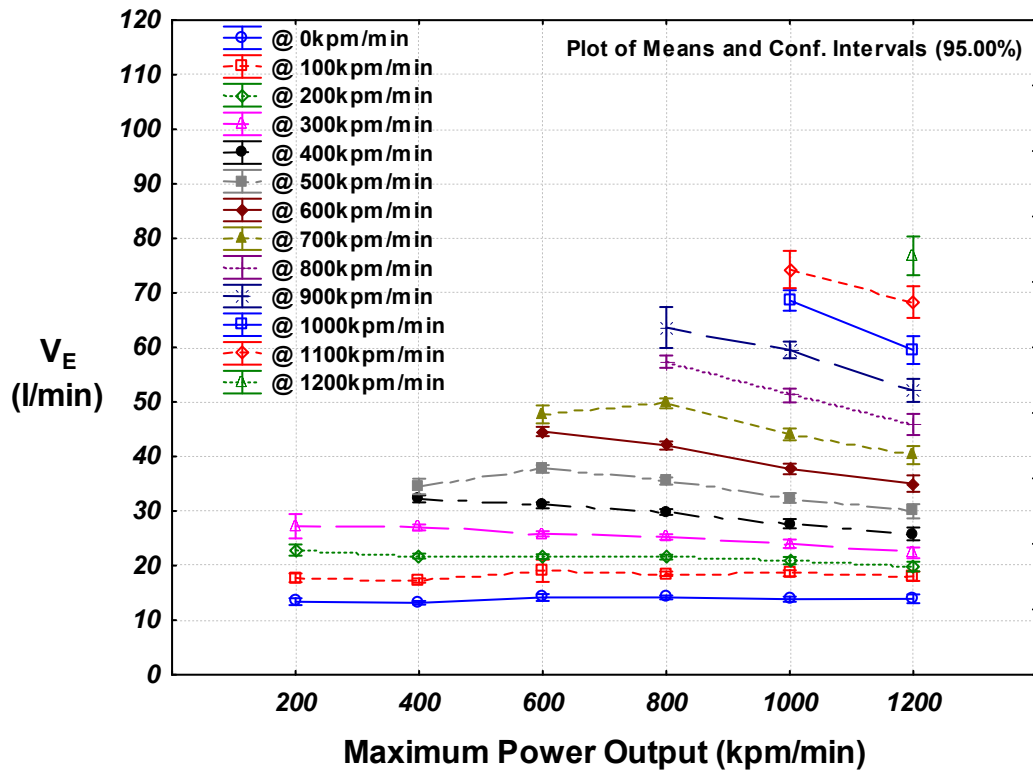
Figure 2.8. *The effects of exercise tolerance on ventilation (V_E) during increasing intensities of exercise.*
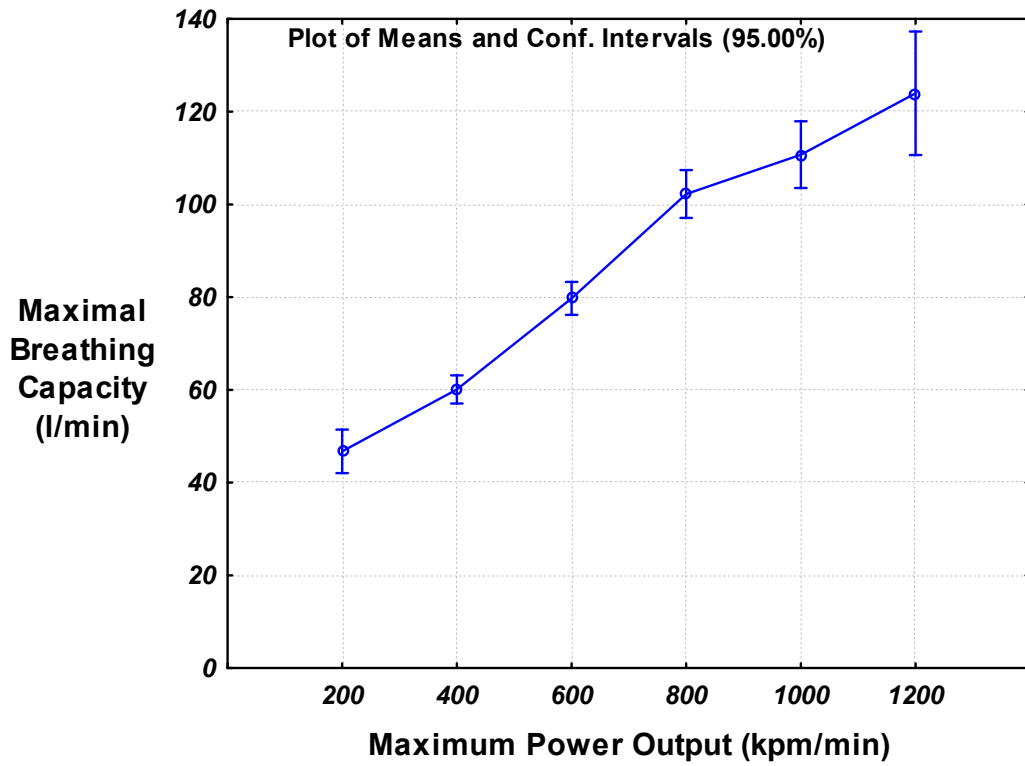
Figure 2.9. *Changes in ventilatory capacity as exercise tolerance increases.*
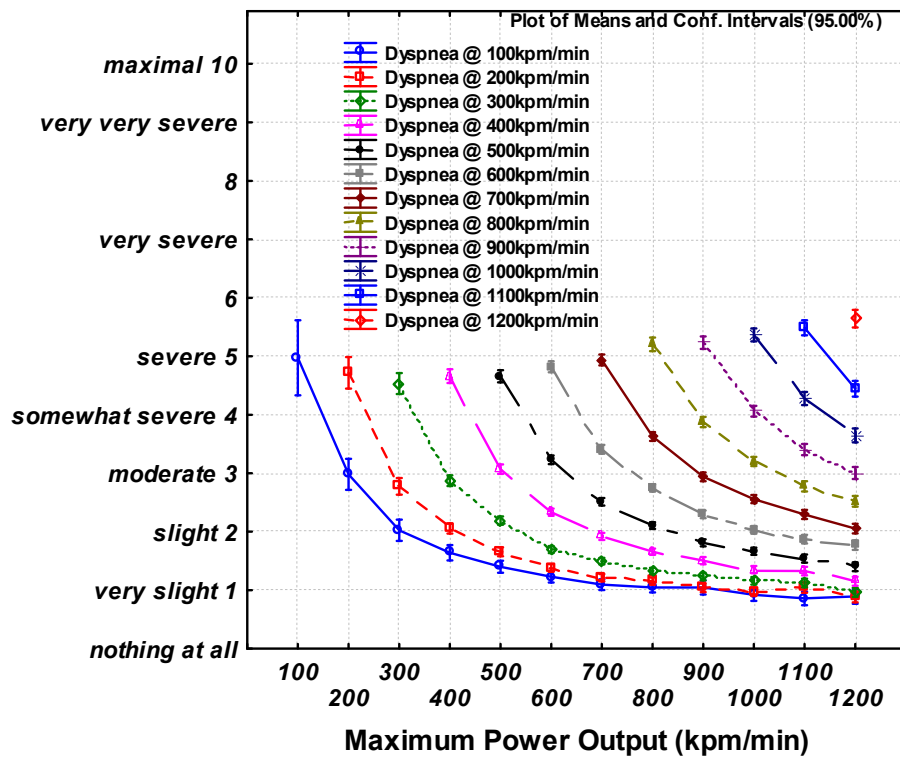
Figure 2.10. *The effects of exercise tolerance on dyspnea (Borg scale rating) during increasing intensities of exercise.*
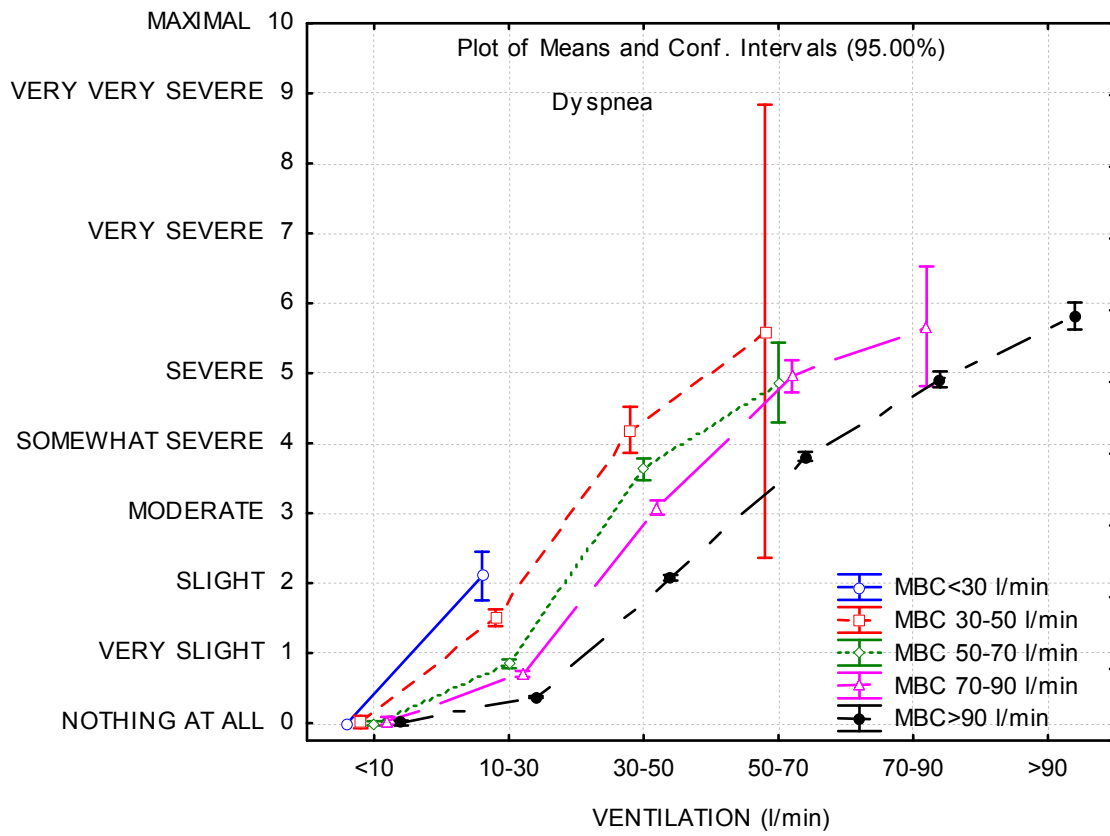
Figure 2.11a. *The effects of achieved ventilation on dyspnea (Borg scale rating) at different levels of ventilatory capacity (MBC).*
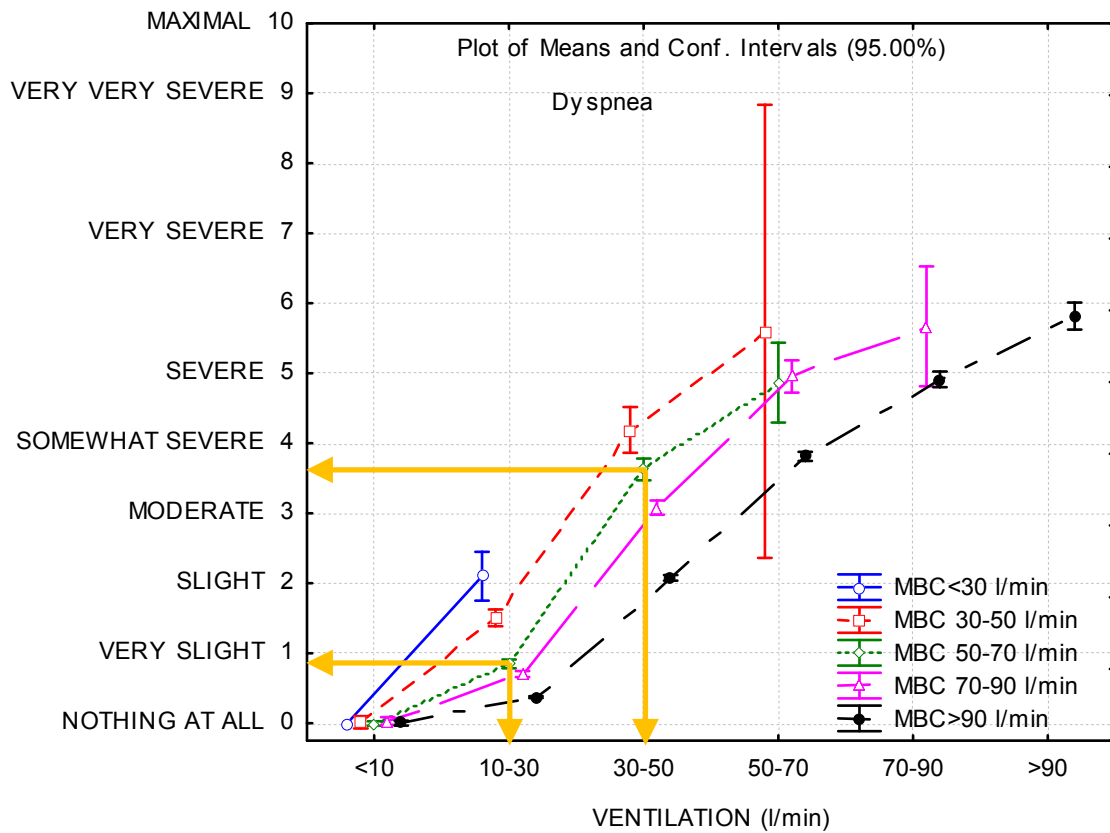
Figure 2.11b. *The effects of doubling achieved ventilation on dyspnea (Borg scale rating) for a given level of ventilatory capacity (MBC).*
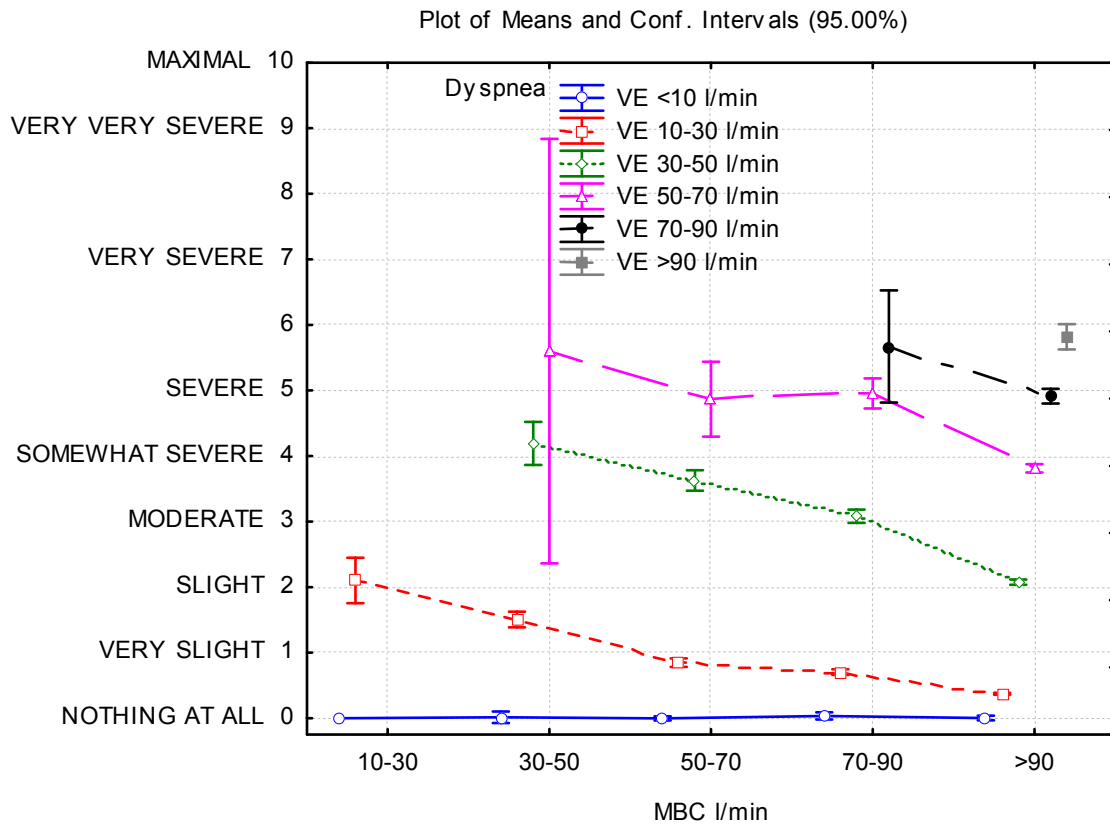
Figure 2.12a. *The effects of ventilatory capacity (MBC) on dyspnea (Borg scale rating) at different levels of achieved ventilation (V_E).*
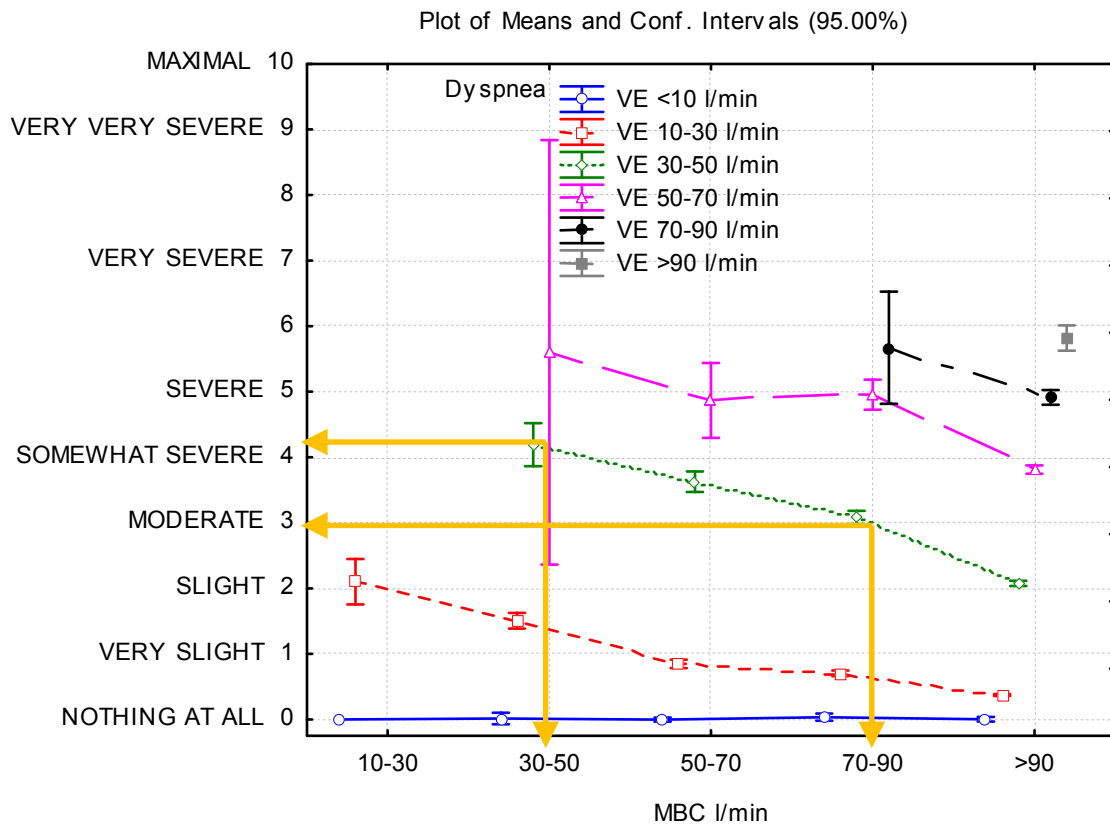
Figure 2.12b. *The effects of halving ventilatory capacity (MBC) on dyspnea (Borg scale rating) for a given level of achieved ventilation (V_E).*
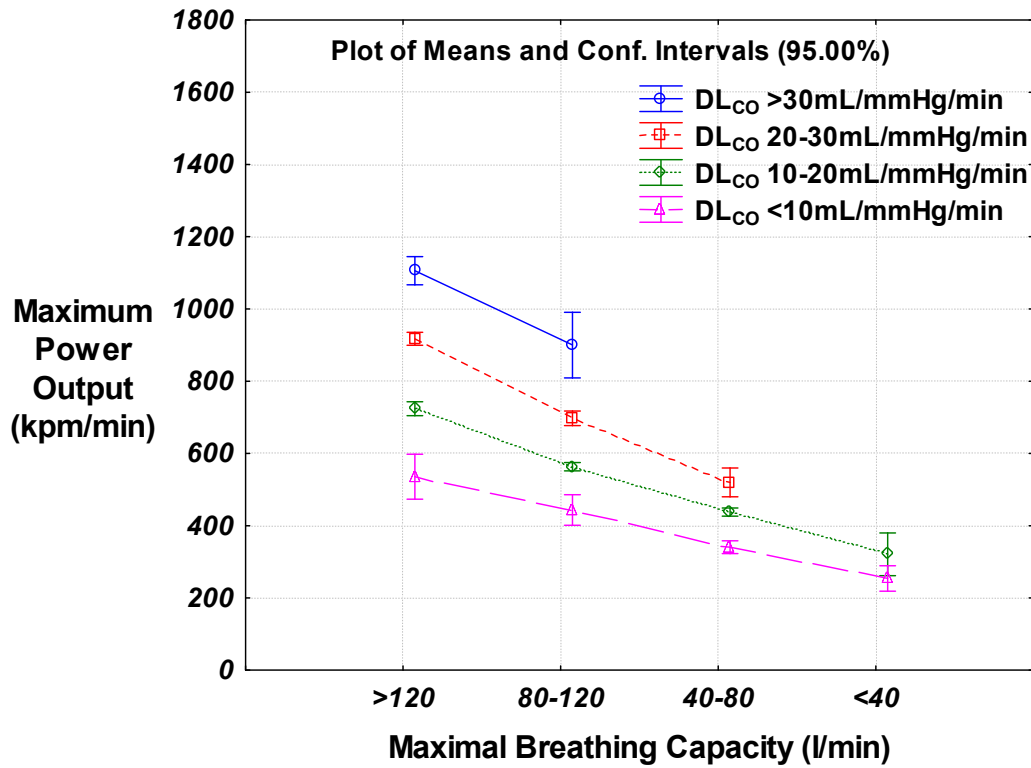
Figure 2.13. *The independent effects of diffusion capacity (DL_{CO}) and maximal breathing capacity (MBC) on maximum power output.*
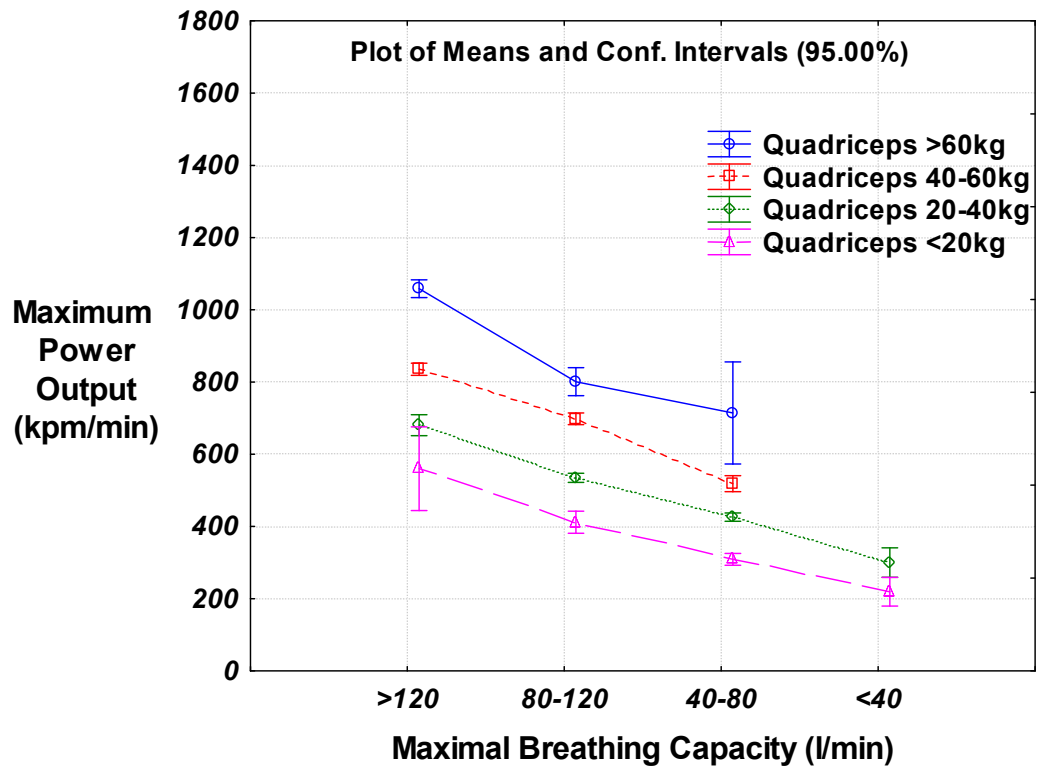
Figure 2.14. *The independent effects of quadriceps strength and maximal breathing capacity on maximum power output.*
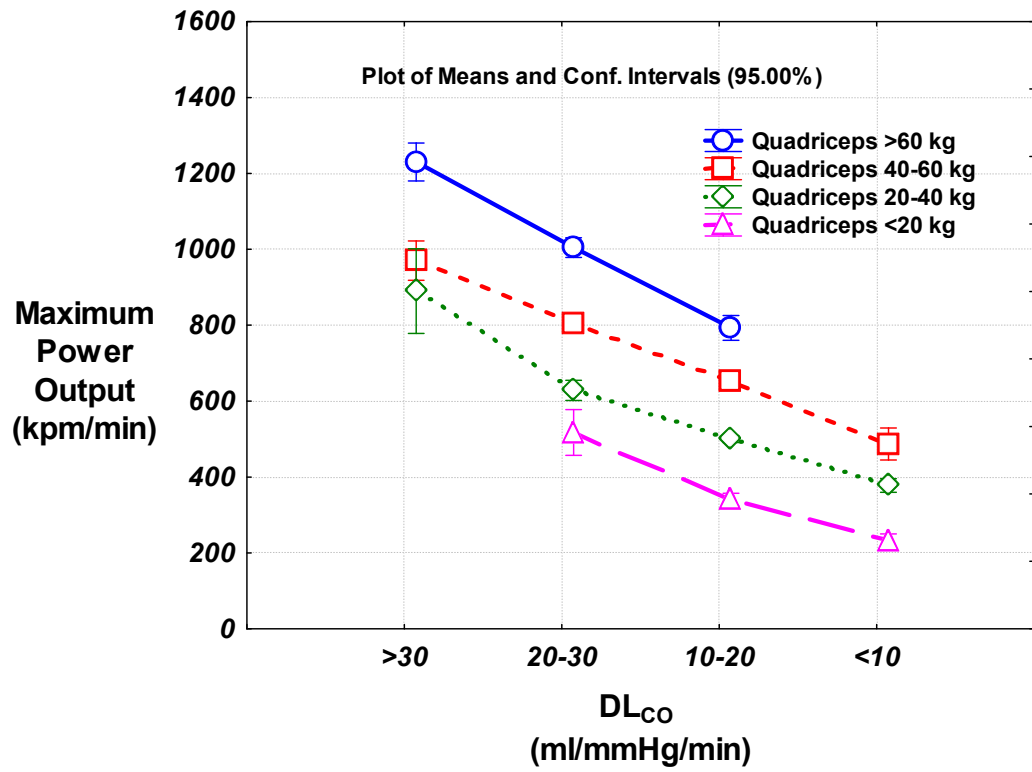
Figure 2.15. *The independent effects of quadriceps strength and diffusion capacity (DL$_{CO}$) of the lung on maximum power output.*
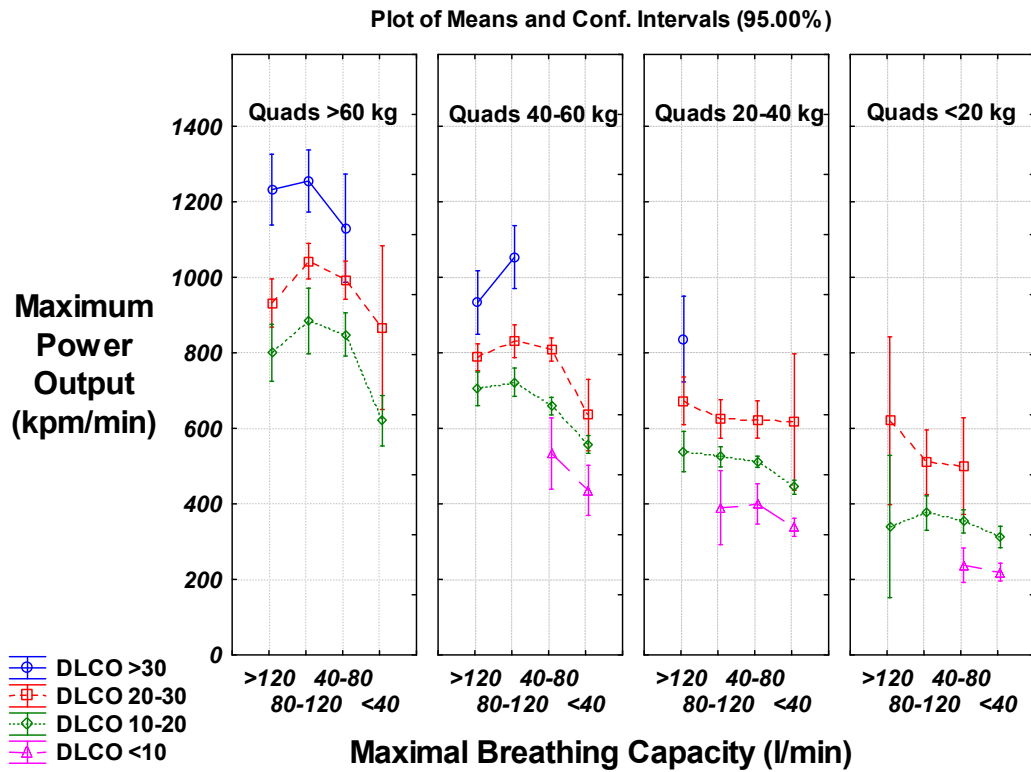
Figure 2.16. *The independent effects of quadriceps strength (Quads), maximal breathing capacity, and gas transfer capacity (DLCO) on maximum power output.*
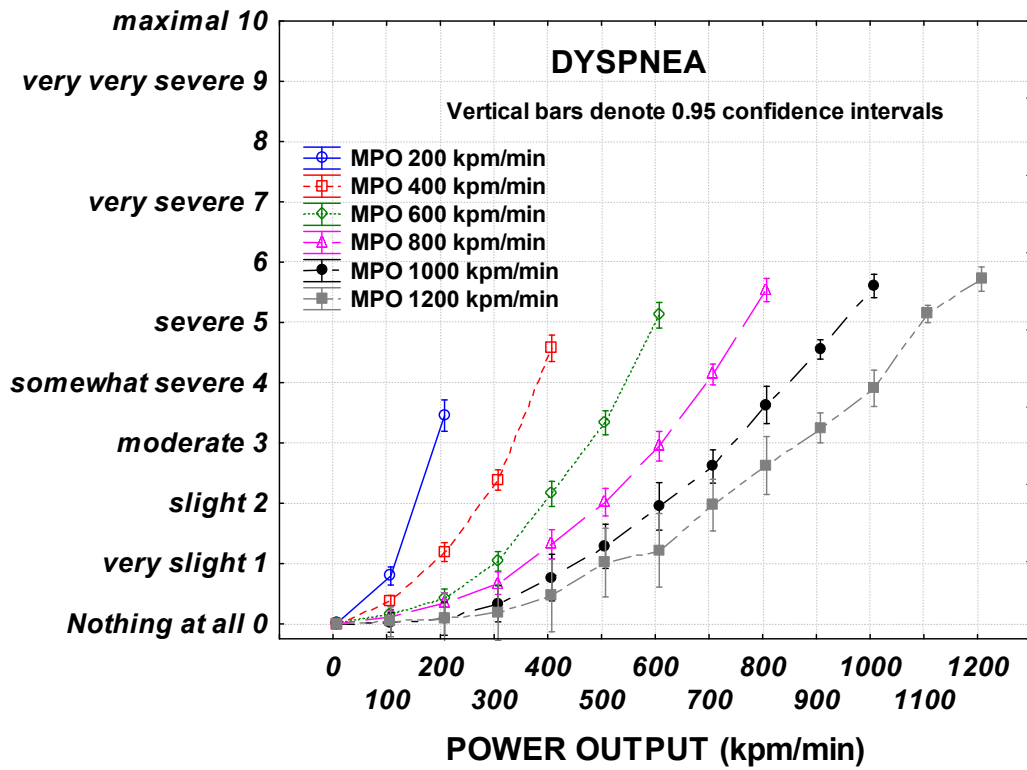
Figure 2.17. *Effects of exercise tolerance (MPO) on the intensity of dyspnea (Borg scale rating) reported at the end of endurance cycling at increasing power outputs.*
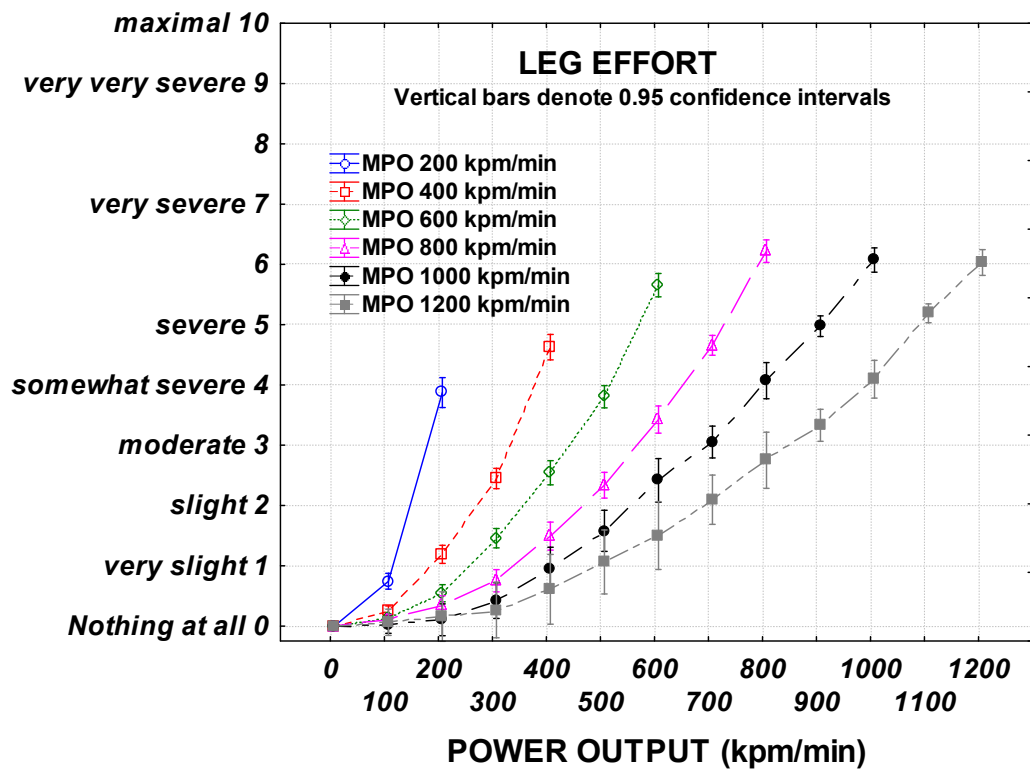
Figure 2.18. *Effects of exercise tolerance (MPO) on the intensity of leg effort (Borg scale rating) reported at the end of endurance cycling at increasing power outputs.*

Chapter 3 - Effects of Tiotropium Bromide on Ventilatory Efficiency and Cardiac Output During Exercise in COPD

Introduction:

Rationale:

Introduced in the 1960s, maximal flow volume manoeuvres are now routinely used in most pulmonary function laboratories. Maximal breathing capacity is the product of a tidal volume multiplied by the maximum rate it can be inspired and expired. The capacity to breathe can be measured by taking the tidal volume (V_T) at maximum exercise and calculating the minimum time required for inspiration and expiration using the limiting flows. Maximal expiratory flow rate declines with expiration. The mean maximal expiratory flow constraint lies between peak expiratory flow and flow rates at 50% of the expired vital capacity. Maximum inspiratory flow rate is generally maintained over most of inspiration. Improvements in flow rates allow for better ventilation of open alveolar units and may also lead to opening of previously closed alveolar units. The effort required to breathe and dyspnea both decrease when the capacity and efficiency of gas exchange improve minimizing the stress on the respiratory muscles and their propensity to fatigue.

Improving alveolar ventilation and opening previously closed alveoli should reduce hypoxic and hypercapnic vasoconstriction, reducing the resistance to pulmonary blood flow and thereby increasing total pulmonary blood flow. All the cardiac output must traverse the lung, and improvements in pulmonary blood flow may translate into

improvements in overall cardiac output. The effort required to drive the peripheral

skeletal and respiratory muscles is dependent on the work performed and is dependent on

the perfusion of these muscles with oxygenated blood with a normal acid base status.

More effort is required to drive muscles that are inadequately perfused and/or

oxygenated. These effects have been broadly acknowledged for several hundred years

but their exploration in a standardized setting has been neglected. Measurement of the

uptake of soluble inert gases has allowed for the measurement of pulmonary blood flow

($\approx$cardiac output) during a simple re-breathing manoeuvre over 15 seconds. This

approach provides a non-invasive alternative to the measurement of mixed venous gases

(Innocor, Innovision, Denmark).

A study was conducted to explore the feasibility of measuring gas exchange

efficiency (V_E/VO_2 & V_E/VCO_2) and non-invasive cardiac output during exercise in

healthy subjects. The details of this study can be found in the Appendix 3. The study

supported the feasibility and reliability of collecting measurements of gas exchange

efficiency in combination with non-invasive cardiac output during exercise in a

population of healthy subjects (Appendix 3). This provided support for the investigation

of the effects of tiotropium bromide on gas exchange efficiency and cardiac output during

exercise in COPD.

Anticholinergic bronchodilators have proven effects on exertional dyspnea

(O'Donnell et al., 2004), exercise duration (Casaburi, Kukafka, Cooper, Witek, & Kesten,

2005), and on respiratory mechanics of subjects with COPD (O'Donnell & Webb, 2005).

The effects of these medications on the efficiency of gas exchange and cardiac output

have not been addressed. With the advent of new technology, it is possible to measure the efficiency of gas exchange and cardiac output in patients with COPD.

The gas exchange efficiency of the lungs is often impaired in COPD (Jones and Berman, 1984). It has already been established that, due to a high deadspace tidal volume ratio (V_D/V_T), the ventilation required to meet the metabolic demand (V_E/VCO_2) is elevated in patients with COPD (Jones and Killian, 1991). Furthermore, the V_E/VO_2 and V_E/VCO_2 both increase as the severity of COPD (GOLD classification) increases. This is the logical result of the V/Q mismatching and increased physiological deadspace, resulting from damaged and obstructed alveoli (and damaged alveolar capillaries), that is observed in COPD (Wagner, 1991).

Bronchodilation with tiotropium bromide could result in opening and ventilation of previously occluded alveoli in COPD. This would yield an increase in alveolar ventilation and a reduction in physiological deadspace. Hence, the surface area for gas exchange, to which the ventilation is exposed, would be increased. This would provide the potential for more gas to be exchanged from a given volume of ventilation (V_E/VO_2 & V_E/VCO_2 would decrease), reducing the ventilatory demand.

Dyspnea was already shown to be the important symptom limiting exercise in COPD, and ventilation is a major contributing factor to the intensity of dyspnea (Chapter 2). Thus, if tiotropium bromide is able to reduce the ventilatory demand during exercise, as described above, dyspnea would consequently be reduced. As such, an increase in exercise tolerance would manifest. This rationale provides a novel mechanism for the

tiotropium bromide-mediated improvement in exercise tolerance that has been reported in COPD patients (Casaburi, Kukafka, Cooper, Witek, & Kesten, 2005).

Additionally, increased alveolar ventilation, via opening of previously occluded alveoli, could result in vasodilation of the surrounding pulmonary vasculature. This would yield reduced resistance and an increase in pulmonary blood flow; and potentially cardiac output. An improved cardiac output would increase the support of working muscle with oxygenated blood, reducing fatigue and the motor drive required to drive the respiratory (dyspnea) and peripheral skeletal (perceived leg effort during cycle ergometer exercise) muscles.

Pharmacological Treatment of COPD:
Role of Acetylcholine:

In 1914, Dale and Loewi discovered that acetylcholine was a neurotransmitter. This neurotransmitter is found in the peripheral, autonomic, and central nervous systems. Acetylcholine is generated within neurons via choline acetyltransferases and is degraded by acetylcholinesterases. Acetylcholine acts on two receptor classes, nicotinic acetylcholine receptors (nAChR) and muscarinic acetylcholine receptors (mAChR). The nAChR class are ligand gated ion channels containing five subunits arranged around a central membrane-spanning pore; arranged like staves around a barrel, and are blocked by curare. The nAChR family consists of three branches: muscle type, neuronal type that bind α bungarotoxin, and neuronal type that do not bind α bungarotoxin. The nAChR family of receptors are found on motor endplates, autonomic ganglia, and in the central

nervous system. At the neuromuscular junction, two α subunits are combined with up to four other types of subunit (β, γ, δ, & ε), in the ratio 2α:β:ε:δ. They function to amplify the small motor neuron current to trigger an action potential in the muscle, ensuring neuromuscular transmission (Lindstrom, 1997). Neuronal nAChRs typically differ from those of muscle. The neuronal, α bungarotoxin binding, type are composed of combinations of α2, α3, α4, or α6 subunits with β2 or β4 subunits (Lindstrom, 1997). The α4β2 type account for over 90% of high-affinity nAChRs in the brain and modulate the release of many neurotransmitters (Lindstrom, 1997). The neuronal, non-α bungarotoxin binding, type are composed of combinations of α7, α8, or α9 subunits (Lindstrom, 1997). They show a rapid rate of depolarization and a high level of selectivity for calcium (Lindstrom, 1997). Rapid desensitization limits their ability to respond to sustained stimulation. Their calcium selectivity allows calcium to function as a second messenger, affecting many cellular functions (Lindstrom, 1997).

The mAChR class are G protein-coupled receptors that activate other ionic channels via a second messenger cascade. Each subunit contains approximately 20 amino acids spanning the membrane. Within this class there are five main types identified as M1-M5. Subunit composition is highly variable across tissues, but they are all blocked by atropine. M1 receptors excite postganglionic nerves in exocrine glands and the central nervous system (Eglen, 2006). They are bound to G proteins that upregulate phospholipase C, inositol triphosphate, and intracellular calcium (Eglen, 2006). M2 receptors cause a decrease in cAMP, leading to various inhibitory effects (Eglen, 2006). This subtype slows heart rate by slowing the speed of depolarization,

reducing the contractile force of the atria, and reducing the conduction velocity of the AV node; while having no effects on the contractile force of the ventricles (Eglen, 2006). The M3 receptor subtype is coupled to specific G proteins (Gq) and increase intracellular calcium typically causing constriction of the smooth muscle of blood vessels and the lungs (bronchoconstriction) (Eglen, 2006). Activation of M3 receptors on vascular endothelial cells causes increased synthesis of nitric oxide causing relaxation, explaining the paradoxical effect of parasympathomimetics on vascular tone and bronchiolar tone (Eglen, 2006). M3 receptors are also found on many glands (salivary glands), stimulating secretion through upregulation of phospholipase C, inositol triphosphate, and intracellular calcium (Eglen, 2006). M4 receptors are found in the central nervous system and are associated with specific G proteins (G_i), decreasing cAMP in cells and producing inhibitory effects (Eglen, 2006). Lastly, M5 receptors are coupled with specific G proteins (G_q), causing upregulation of phospholipase C, inositol triphosphate, and intracellular calcium as a signalling pathway (Eglen, 2006). This subtype is found on dopaminergic neurons in the ventral tegmental area of the brain, which is associated with rewarding hypothalamic stimulation (Eglen, 2006).

Tiotropium Bromide:

Tiotropium bromide is a once-daily, inhaled anticholinergic for the treatment of COPD. This is the most popular first-line treatment for COPD. Tiotropium bromide is a non-selective muscarinic receptor antagonist. However, it mainly acts on M3 type mAChRs. Acetylcholine acts on M3 type muscarinic receptors to cause smooth muscle

contraction in the airways through a G_q-coupled increase in intracellular calcium. M3

type receptors are also located on many glands stimulating secretion in salivary glands

and the stomach. Blockade of M3-mediated effects with tiotropium bromide yields

bronchodilation, and a dry mouth (side effect). There are other effective drug treatments

in COPD. The mechanisms of their therapeutic effects are addressed in Appendix 2.

Metaanalysis of prospective randomized controlled trials using tiotropium

bromide for the treatment of COPD have shown improved spirometry, quality of life, and

exercise tolerance (Barr, Bourbeau, Camargo, & Ram, 2006). The metaanalysis included

nine randomized control trials with a total of 8002 patients. In COPD tiotropium

bromide treatment has been shown to improve FEV_1 by 190±10 to 210±10mL and FVC

by 420±20 to 510±20mL (mean±SEM) (Casaburi et al., 2002). The number of patients

with exacerbations were reduced by 14% and health-related quality of life scores were

improved in those receiving tiotropium bromide (Casaburi et al., 2002). Tiotropium

bromide decreases hyperinflation with reductions in functional residual capacity (FRC) of

0.41±0.08L and reductions in residual volume (RV) of 0.51±0.10L (mean±SE)

(O'Donnell et al., 2004). Tiotropium bromide has been shown to improve exercise

endurance time by 121±191 seconds and reduce dyspnea from 6.2±2.4 to 5.2±2.3 at the

same intensity and duration of exercise (mean±SE) (O'Donnell et al., 2004).

The statistically significant improvements reported for FEV_1 and FVC are based

on 518 patients (Barr et al., 2006; Casaburi et al., 2002). The improvements in FRC, RV,

exercise endurance time, and dyspnea are based on 96 subjects treated with tiotropium

bromide, compared to 91 placebo treated subjects (Barr et al., 2006; O'Donnell et al.,

2004). However, these improvements were not universally observed in all subjects.

With the relatively small effect sizes and wide range of variability for FEV_1 and FVC,

with and without tiotropium bromide, a small randomized control trial with cross-over in

20 patients would not be expected to necessarily reach statistical significance. A small

study of 20 COPD patients with a cross-over design would be expected to include both

individuals whose exercise tolerance improves (responders), and whose do not (non-

responders), following treatment with tiotropium bromide. The presence of both a

responder and non-responder population would allow for investigation into what

characteristics may be different between individuals who respond to tiotropium bromide

treatment and those who do not. This might allow us to effectively customize treatment

to the individual and perhaps identify novel mechanisms through which the improvement

is mediated.

Hypothesis:

        The working hypothesis was that the efficiency of gas exchange and cardiac

output increase during exercise in patients with COPD receiving tiotropium bromide.

Other studies have shown that tiotropium bromide improves expiratory flow rates,

exertional dyspnea and exercise tolerance, and reduces resistive and elastic work in

subjects with COPD; these were not explored. There have been no investigations of the

effects of anticholinergic bronchodilation on the efficiency of gas exchange and cardiac

output.

This study aimed to investigate these novel mechanisms of action of tiotropium bromide. Efficiency of gas exchange was evaluated through assessments of metabolic demand (VO_2), cardiac output (Q), and ventilation (V_E) in overall terms (i.e. Q/VO_2, V_E/VO_2).

Methods:

Study Design:

This randomized, double-blind, placebo-controlled, crossover study focused on specific and novel potential effects of tiotropium bromide in a group of 20 subjects with COPD. Patients were treated as outpatients with a minimum of 7 visits over a period of approximately 12-14 weeks (Figure 3.1).

After informed consent, patients attended an initial screening visit (Visit 1) for review of medical history, clinical assessment, and complete pulmonary function testing (plethysmography and spirometry). A symptom-limited incremental cycle exercise test, with measurement of incremental and peak VO_2, VCO_2, V_E, V_T, respiratory frequency, heart rate (HR), oxyhemoglobin saturation by pulse oximetry (SpO_2), and modified Borg score for dyspnea and leg effort was also performed at screening, as well as measurements of airway responses to salbutamol.

Patients who met the eligibility requirements (see *Study Population and Sub-Population*) were randomized to treatment with tiotropium bromide or placebo. Double-blind medication was dispensed in HandiHalers to be taken once daily in the morning for 21 days. On days when patients attended the lab for a visit, study medication dose for the day was taken at the lab at the beginning of the visit. All testing proceeded 30 minutes after study medication administration. Patients reported to the laboratory for two separate treatment periods with a washout of 4 weeks between treatment periods. The patient returned used medication capsules for confirmation of medication compliance. Safety was assessed by examining adverse events (AEs), resting and exercise electro

cardio grams (ECGs), routine laboratory tests, and vital signs. The study specific

breakdown, including the details of each visit, can be found in Table 3.1.

Sample Size, Study Population and Sub-Population:

Sample size for the study was calculated based on detecting a 20% change in

V_E/VO_2 and cardiac output. A sample size of 20 generated a power level of >0.86 at all

levels of exercise intensity for V_E/VO_2, and a power level of >0.84 at all levels of

exercise intensity for cardiac output.

20 patients (14 males, 6 females) with COPD were enrolled.   Administration of

short acting beta-agonists as rescue medication was allowed at any time during the study.

Patients abstained from rescue salbutamol for at least 8 hours before pulmonary function

testing and exercise tests. The following medication was allowed for control of acute

exacerbations as medically necessary during the study:

a) Salbutamol inhalation aerosol via metered dose inhaler (MDI) as occasion

required (p.r.n), provided by the laboratory.

b) One temporary increase in dose or addition of oral steroids while the patient was

not taking study medication. Treatment periods did not begin within seven days

of the last administered dose of an increase or addition.

The following medications were allowed if stabilized for at least six weeks prior to Visit

1 and throughout the study:

a) Long acting inhaled beta-agonist (patients abstained from long acting inhaled beta-agonists for at least 24 hours before pulmonary function testing in study visits).

b) Inhaled steroids at stable doses

c) Fixed combination products of long acting inhaled beta-agonists and inhaled steroids (patients abstained from long acting inhaled beta- agonists for at least 24 hours before pulmonary function testing in study visits).

d) Oral steroid medication at stable doses (less than or equal to 10 mg/d prednisone or prednisone equivalent).

e) Mucolytic agents not containing bronchodilators.

f) Leukotriene modifiers

g) Cromolyn or nedocromil

h) Theophyllines

i) Patients permitted to take medication prescribed for other conditions provided that the medications were not specifically restricted.

The following medications were not allowed during the study:

a) All anticholinergic agents, including inhaled ipratropium/ fenoterol combination, ipratropium/ salbutamol combination, tiotropium bromide (except for study medication), or nasal ipratropium.

b) Oral beta-agonists.

c) Inhaled short acting beta-adrenergics other than the supplied rescue medication (salbutamol MDI) were not allowed during the study.

All subjects were 37 years of age or older, with a cigarette smoking history of >10 pack years (Pack Years = [Number of cigarettes/day X years of smoking]/20). They all had a physician's diagnosis of COPD. All subjects conformed to the following spirometric criteria (measured at screening: Visit 1): FEV_1 < 80% predicted normal and FEV_1/VC <70% predicted normal. Subjects with significant diseases other than COPD, or who had a myocardial infarction, cardiac arrhythmia, or respiratory infection in the 6 months prior to enrollment were excluded from this study. Any subjects who required daily oxygen therapy, had active tuberculosis, or who were enrolled in a pulmonary rehabilitation program within 4 weeks of enrollment were excluded. Specific demographics for the subject population are presented in Table 3.2.

Subsequently, enrolled subjects that showed any improvement in constant load exercise endurance time (exercise tolerance) measured at visits 4 and 7, following tiotropium bromide treatment, were classed as Responders (7 males, 4 females). Those that showed no improvement in endurance time with tiotropium bromide were classed as Non-Responders (7 males, 2 females). This provided the two groups for sub-group analysis. The specific demographics for the Responder and Non-Responder sub-populations are presented in Tables 3.3 and 3.4.

Final Study Enrolment Status:

For this study 32 subjects were screened for enrolment, 4 failed screening criteria, 6 declined to participate, and 22 were enrolled (Figure 3.2). Due to severe adverse events (respiratory infections requiring antibiotic therapy, not related to study or study drugs), 2

subjects were withdrawn from the study. Upon final study completion, 20 subjects successfully finished all study procedures and visits (Figure 3.2).

Study Objectives:

The primary objective of this study was to demonstrate if improvements in the efficiency of gas exchange or cardiac output occurred during exercise in COPD subjects treated with tiotropium bromide. Failing this, was there an improvement in the efficiency of gas exchange or cardiac output during exercise in those that had improved exercise tolerance with tiotropium bromide?

Primary Endpoints:

The primary endpoints were efficiency of gas exchange and cardiac output, at rest and during steady state exercise, before and after 3 weeks treatment with tiotropium bromide compared to placebo.

Secondary Endpoints:

Secondary efficacy parameters were intensity of dyspnea and leg effort during steady state exercise, exercise endurance capacity, and ventilatory capacity. Also included were endpoints used for safety assessments including all adverse events, vital signs on each study visit, and physical examination during screening and termination visits.

Spirometry and Body Plethysmography:

Forced vital capacity over a minimum of 6 seconds (FVC), FEV_1, and a maximum inspiratory and expiratory flow volume manoeuvre were measured conforming to American Thoracic Society (ATS) minimal performance recommendations (Miller et al., 2005). Equipment was calibrated prior to pulmonary function testing. Calibration results were maintained in a calibration log. Lung volumes were measured with a pressure body plethysmograph (body box), according to the guidelines from the American Thoracic Society (Wanger et al., 2005). Calibration of the body plethysmograph occurred daily and a calibration log showing the date, calibration values, barometric pressure, and technician initials was maintained. For study measurements, at least three (with no more than 8) separate measurements were obtained and the three acceptable measurements agreed within 5% of the average of the three measurements.

Stage One Exercise Tests:

At Visit 1 (screening) an incremental cycle exercise test was performed to a symptom-limited maximum (maximum power output, MPO). The initial work rate was set at 0 watts. The subject performed 1 minute of loadless pedaling at 50-70 rpm prior to increasing the work rate. The subject was encouraged to continue to exercise for as long as possible at this pedaling frequency, with the work rate increased in increments of 15 watts at the end of each minute. MPO was defined as the highest work rate that was maintained for at least 30 seconds.

At visits 2, 3, 5, and 6, exercise was performed on a cycle ergometer consisting of 6 minutes of loadless pedaling, followed by 6 minutes at 1/3 MPO and 6 minutes at 2/3 MPO (total 18 minutes).

Before exercise, while seated comfortably on the cycle ergometer, the subject breathed for 2 minutes through the mouthpiece with a noseclip in place while metabolic measurements were taken. Measures of ventilation (VO_2, VCO_2, V_E, V_T, and RR) and circulation (BP, HR and SpO_2) were monitored from rest to maximum exercise. If a sustained drop in SpO_2 below 80% was observed at Visit 1 (per Investigator's discretion), the test was discontinued and the patient was ineligible for the study. Using the modified Borg Scale, subjects rated the intensity of leg effort and dyspnea at each work load. At the third and fifth minute of loadless, 1/3 MPO, and 2/3 MPO exercise, the subjects had their cardiac output measured (see below). Subjects were monitored post-exercise for safety.

Constant Load Exercise Tests at 80%MPO:

Measurements during constant work load endurance exercise (noted as 4/5 MPO on all figures), performed at visits 4 and 7, were identical to those for stage one exercise tests. Additional procedures that were specific to constant-load testing are outlined below.

Before exercise, while seated comfortably on the cycle ergometer, the subject breathed for 2 minutes through the mouthpiece with a nose-clip in place while metabolic and safety measurements were taken. The initial work rate was set at 0 watts (loadless

pedaling) for a 6 minute warm-up. The work rate was then increased to 80% (noted as 4/5

MPO in all figures) of the maximum work rate (MPO) determined during the stage one

test at Visit 1. The subject was encouraged to continue exercising for as long as possible

at a pedaling frequency of 50-70 rpm. At the end of exercise, the time of exercise was

recorded (minutes and seconds). Endurance time was equal to the time from the start of

loaded pedaling to the end of exercise. Measures of exercise ventilation (VO_2, VCO_2,

V_E, V_T, and RR) and circulation (BP, HR and SpO_2) were monitored throughout testing.

Subjects were monitored post-exercise to recovery.

Modified Borg Scale:

The modified Borg Scale was used to assess dyspnea and leg effort before the

start of loadless pedaling, every 2 minutes during exercise tests, and at the end of all

exercise.

Cardiac Output:

Cardiac output was measured using the rate of nitrous oxide uptake. The rate of

uptake is limited by the rate of blood flow through the gas exchanging lung. Nitrous

oxide was inhaled from a closed rebreathing system. The decline in the concentration of

nitrous oxide was referenced to the concentration of an inert gas (sulphur hexafluoride).

The concentrations of all gases were measured by photoacoustic spectroscopy. The

wash-out rate is proportional to the cardiac output. The validity of nitrous oxide

rebreathing is documented (Peyton & Thompson, 2004). This system has been validated

for measurements of cardiac output during exercise (Agostoni et al., 2005). This was

further validated in our laboratory during exercise in normal subjects (see Appendix 3).

This system's advantage relative to other methods of cardiac output measurement is its

ease of use.

Statistical Analysis:

All statistical analyses described below were performed utilizing the computer

software program *Statistica* (StatSoft Inc., Tulsa, Oklahoma, USA).

The data were analyzed using repeated measures ANOVA for the effects of

treatment (Tiotropium Bromide vs. Placebo) on the outcome variables cardiac output and

efficiency of gas exchange.

A further subgroup analysis was planned and also performed assessing the effects

of tiotropium bromide in responders and non-responders. Variability in outcomes from

subject to subject was expected based on the previous large scale randomized prospective

control trials. Because of the limited number of subjects (n=20) significant effects of

tiotropium bromide on gas exchange efficiency and cardiac output might not be achieved

based on the sample size. However, some subjects would be expected to improve their

exercise endurance time (exercise tolerance) while others would not. If the efficiency of

gas exchange or cardiac output were contributing to improvement in exercise tolerance,

significance would be expected in those that showed improved exercise tolerance and not

expected in those who failed to show improved exercise tolerance. Subjects were

classified as responders or non-responders based on whether they showed any improvement in exercise endurance time (exercise tolerance), measured at visits 4 and 7, following tiotropium bromide relative to placebo. Differences between responders and non-responders were again assessed for all outcome variables using repeated measures ANOVA with a covariant based on responder or non-responder classification.

All analyses compared the effects at rest, 1/3 MPO, and 2/3 MPO of tiotropium bromide at the beginning (visit 2 or visit 5, depending on randomization) and end of treatment (visit 3 or visit 6, depending on randomization) vs. placebo at the beginning (visit 2 or visit 5, depending on randomization) and end of treatment (visit 3 or visit 6, depending on randomization). As well, the effects of tiotropium bromide (visit 4 or visit 7, depending on randomization) during endurance exercise (4/5 MPO) were compared to placebo (visit 4 or visit 7, depending on randomization) during endurance exercise (4/5 MPO) (Figure 3.1).

Results:

Tiotropium Bromide vs. Placebo:

Placebo treated exercise endurance time was 331±344.5 seconds and did not significantly (p=0.66) improve with tiotropium bromide (344.1±264.8 seconds) (mean±SD).

As expected, due to low resting tidal volume (high V_D/V_T), the efficiency of gas exchange for oxygen and carbon dioxide were lowest at rest compared to exercise (p < 0.01), at the beginning (placebo V_E/VO_2: 47.1, placebo V_E/VCO_2: 59.3, tiotropium bromide V_E/VO_2: 46.8, tiotropium bromide V_E/VCO_2: 60.5) and end (placebo V_E/VO_2: 50.2, placebo V_E/VCO_2: 64.8, tiotropium bromide V_E/VO_2: 47.5, tiotropium bromide V_E/VCO_2: 62.8) of the treatment period, and improved (V_E/VO_2 and V_E/VCO_2 decreased) during exercise (Figures 3.3 & 3.4). However, there was no significant improvement in the efficiency of gas exchange for oxygen (p=0.72) or carbon dioxide with tiotropium bromide over placebo at rest or during exercise (Figures 3.3 and 3.4). The efficiency of gas exchange for carbon dioxide was significantly (p=0.005) decreased during exercise with tiotropium bromide (Figure 3.4). Tiotropium bromide did not increase the efficiency of gas exchange.

As expected, cardiac output increased as the metabolic demand (VO_2) associated with exercise increased (see *Chapter 1: Introduction: Cardiac Output;* Figure 3.5). However, there was no significant difference (slopes and intercepts within one standard deviation) in cardiac output with tiotropium bromide vs. placebo at all levels of metabolic demand (Figure 3.5). Tiotropium bromide did not improve cardiac output.

Additional variables, not directly related to the hypothesis, are presented in Chapter 4.

Responders vs. Non-Responders:

The responder population was comprised of subjects who showed any improvement in exercise endurance time, collected at visits 4 and 7, with tiotropium bromide compared to placebo. The non-responder population comprised subjects who showed no improvement or a decrease in exercise endurance time with tiotropium bromide compared to placebo. This resulted in a responder n=11 and a non-responder n=9. Responders had an FEV_1 (mean±SD) of 1.27±0.54L, while non-responders had an FEV_1 of 1.47±0.42L (Tables 3.3 & 3.4). The responders had a placebo-treated exercise endurance time (mean±SD) of 239±130sec, which was not significantly different (p=0.24) from the tiotropium bromide-treated exercise endurance time of 318±175sec. Non-responders had a placebo-treated exercise endurance time (mean±SD) of 443±485sec, which was not significantly different (p=0.74) from the tiotropium bromide-treated exercise endurance time of 376±355sec.

As expected, due to low resting tidal volume (high V_D/V_T), the efficiency of gas exchange for oxygen and carbon dioxide were lowest at rest ($p < 0.05$), at the beginning and end of the treatment period, for non-responders (placebo V_E/VO_2: 51.7 & 53.2, tiotropium bromide V_E/VO_2: 47.8 & 48.5, placebo V_E/VCO_2: 62.7 & 69.6, tiotropium bromide V_E/VCO_2: 60.4 & 63.2), and improved (V_E/VO_2 and V_E/VCO_2 decreased) during exercise (Figures 3.6 & 3.7). The same was true ($p < 0.05$) for responders (placebo V_E/VO_2: 42.5 & 47.2, tiotropium bromide V_E/VO_2: 45.8 & 46.4, placebo V_E/VCO_2: 55.8

& 60.1, tiotropium bromide V_E/VCO_2: 60.6 & 62.4) (Figures 3.6 & 3.7). However, there was no significant improvement in the efficiency of gas exchange for oxygen (p=0.65) or carbon dioxide (p=0.19) with tiotropium bromide over placebo, at rest or during exercise, in either the responders or the non-responders (Figures 3.6 & 3.7). Tiotropium bromide did not increase the efficiency of gas exchange in responders nor non-responders.

As expected, cardiac output increased as the metabolic demand associated with exercise increased, in both responders and non-responders (see *Chapter 1: Introduction: Cardiac Output;* Figure 3.8). However, there was no significant improvement (slopes and intercepts within one standard deviation) in cardiac output with tiotropium bromide vs. placebo at all levels of metabolic demand, in either the responders or the non-responders (Figure 3.8). Tiotropium bromide did not improve cardiac output in responders nor non-responders.

Additional variables, not directly related to the hypothesis, are presented in Chapter 4.

Discussion:

Tiotropium Bromide vs. Placebo:

This study explored the effects of tiotropium bromide on gas exchange efficiency and cardiac output during exercise in COPD subjects. Gas exchange efficiency was defined as the ventilation required for oxygen uptake (V_E/VO_2) and the ventilation required for carbon dioxide output (V_E/VCO_2). This simple relationship expresses the ventilation necessary for the exchange of one unit of oxygen or carbon dioxide; the greater the required ventilation, the lower the efficiency of gas exchange. The hypothesis was that tiotropium bromide improves gas exchange efficiency. The purported mechanism was opening of previously occluded small airways thereby increasing alveolar surface area for gas exchange yielding an increase in the amount of gas exchange for a given volume of ventilation. This improvement in gas exchange efficiency would manifest as a reduction in V_E/VO_2 and V_E/VCO_2, and in due course less respiratory muscle work and dyspnea. As expected due to low resting tidal volume, gas exchange efficiency for oxygen and carbon dioxide were both lowest at rest, and improved during exercise compared to resting levels (Figures 3.3 & 3.4). Comparison of tiotropium bromide-treated to placebo-treated measurements of V_E/VO_2 and V_E/VCO_2, at rest and during exercise, illustrated no significant improvements in gas exchange efficiency for either oxygen (p=0.72) or carbon dioxide (Figures 3.3 & 3.4). Analysis of the efficiency of gas exchange for carbon dioxide actually showed a significant decrease (p=0.005) during exercise with tiotropium bromide (Figure 3.4). The efficiency of gas exchange for oxygen showed a similar pattern during exercise with tiotropium bromide treatment

(Figure 3.3), but it was not statistically significant (p=0.72). While it is likely that bronchodilation with tiotropium bromide actually opened non-communicating airways, resulting in a decrease in airways resistance, alveolar ventilation might be expected to increase with a reduction in the P_aCO_2, and thus fractional alveolar CO_2 ($F_A CO_2$), explaining the increased ventilation required to exchange a unit of carbon dioxide (decreased efficiency of gas exchange for carbon dioxide) (el-Manshawi, Killian, Summers, & Jones, 1986). Given progressive increases in inspiratory resistance are accompanied by progressive decreases in alveolar ventilation (el-Manshawi et al., 1986), the inverse is also logical.

The second hypothesis was that tiotropium bromide improves cardiac output during exercise in COPD subjects. We proposed that improving alveolar ventilation and opening previously closed alveoli would reduce hypoxic and hypercapnic vasoconstriction, reducing the resistance to pulmonary blood flow and thereby increasing total pulmonary blood flow. Given that all the cardiac output must traverse the lung, improvements in pulmonary blood flow could translate into improvements in overall cardiac output. Cardiac output was assessed relative to metabolic demand. The normal resting cardiac output in the recumbent position ranges from 2.5-3.5 L/m^2 body surface area and in the upright position this drops by 20% (Shock & Norris, 1971). As the metabolic demands of exercise increase, the cardiac output rises by 5 L/min for every liter increase in oxygen uptake (Shock & Norris, 1971). The cardiac output was compared at the same oxygen uptake with and without tiotropium bromide in the same subjects. There was no significant improvement (slopes and intercepts within one

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standard deviation) with tiotropium bromide (Figure 3.5). Without an improvement in gas exchange efficiency, significant opening of previously occluded small airways and alveoli did not occur with tiotropium bromide treatment. Thus, subsequent reduction in hypoxic and hypercapnic vasoconstriction, reducing the resistance to pulmonary blood flow and thereby increasing total pulmonary blood flow, did not appear to occur.

Additional variables, not central to the hypothesis, were altered with tiotropium bromide and warrants discussion. This can be found in Chapter 4.

Responders vs. Non-Responders:

Because the beneficial effects of tiotropium bromide were not seen systematically in all subjects, a subgroup analysis was expected and planned. In the many other studies of tiotropium bromide, large numbers of subjects were required to illustrate significant effects (Barr et al., 2006; Casaburi et al., 2002; O'Donnell et al., 2004). Variability in outcomes from subject to subject was expected based on these previous large scale randomized prospective control trials. Because of the limited number of subjects (n=20) in our study, significance might not have been achieved based on the sample size. However, some subjects were expected to improve their exercise endurance time (exercise tolerance) while others would not. If the efficiency of gas exchange or cardiac output were contributing to improvement in exercise tolerance, significance would be expected in those that improved and not expected in those who failed to improve.

The responder population was more impaired by their COPD, compared to the non-responders. Responders had an FEV_1 of $1.27\pm0.54L$ and a placebo-treated exercise

endurance time of 239±130sec, while non-responders had an FEV_1 of 1.47±0.42L and a placebo-treated exercise endurance time of 443±485sec (mean±SD).

Gas exchange efficiency for oxygen and carbon dioxide were both lowest at rest, as expected due to low resting tidal volume, and improved during exercise (Figures 3.6 & 3.7). Comparison of tiotropium bromide-treated to placebo-treated measurements of V_E/VO_2 and V_E/VCO_2, at rest and during exercise, in our responders and non-responders illustrated no significant improvements in gas exchange efficiency for either oxygen (p=0.65) or carbon dioxide (p=0.19) in either group (Figures 3.6 & 3.7).

While not statistically significant, the non-responders showed a noticeable increase in both V_E/VO_2 and V_E/VCO_2 at the end of treatment for the 2/3MPO exercise level, with tiotropium bromide compared to placebo (Figures 3.6 & 3.7). While it is likely that bronchodilation with tiotropium bromide actually opened non-communicating airways, resulting in a decrease in airways resistance, alveolar ventilation might be expected to increase with a reduction in the P_aCO_2, and thus $F_A CO_2$, explaining the increased ventilation required to exchange a unit of carbon dioxide (decreased efficiency of gas exchange for carbon dioxide) (el-Manshawi et al., 1986). Given progressive increases in inspiratory resistance are accompanied by progressive decreases in alveolar ventilation (el-Manshawi et al., 1986), the inverse is also logical. This same rationale could explain the effect seen in V_E/VO_2 as well.

Assessment of cardiac output, relative to metabolic demand, by comparing tiotropium bromide-treated to placebo-treated measurements at rest and during exercise,

in our responders and non-responders, illustrated no significant improvements (slopes and intercepts within one standard deviation) in either group (Figure 3.5).

Subgroup analysis of the effects of tiotropium bromide in COPD subjects that showed improved exercise tolerance with tiotropium bromide (responders) and in those that did not show improved exercise tolerance (non-responders), illustrated no significant improvements in gas exchange efficiency or cardiac output in either group. Thus, tiotropium bromide does not yield improved exercise tolerance in COPD subjects through improvements in gas exchange efficiency or cardiac output. Lack of support for these potential mechanisms of action of tiotropium bromide suggests that the more popularly purported mechanisms, increasing ventilatory capacity by bronchodilation and deflation reducing FRC and RV, are responsible for the improvement in exercise tolerance (O'Donnell et al., 2004). Reducing hyperinflation would result in the respiratory muscles being placed in a less stretched, more efficient, position. As such, less effort would be required to drive the respiratory muscles to generate ventilation, thus reducing dyspnea. Our previous study on the factors limiting exercise in COPD showed dyspnea to be the most important symptom limiting exercise in COPD. Tiotropium bromide-mediated reduction in dyspnea, through reduction of hyperinflation, could explain the improvement in exercise tolerance seen with tiotropium bromide treatment.

Additional variables, not central to the hypothesis, were specifically altered in either the responder or non-responder group with tiotropium bromide and warrants discussion. This can be found in Chapter 4.

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Tables:

Table 3.1. *Study specific breakdown including details of each visit.*

	Run in	Treatment Period 1			Treatment Period 2		
Visit	1	2	3	4	5	6	7
Week		0	2	2	6	8	8
Day	-14	1	21(±1day)	22	50	70(±1day)	71
Informed Consent and Subject Information	X						
Demographics	X						
Medical History	X						
Smoking Status	X						
COPD Background Characteristics Questionnaire	X						
Inclusion/Exclusion Criteria	X						
Laboratory tests / Urine pregnancy test	X						
Vital Signs (seated)	X	X	X	X	X	X	X
Physical Examination	X						X
12-Lead ECG	X	X	X	X	X	X	X
Medication Washout Compliance Assessment	X	X	X	X	X	X	X
Pre-bronchodilator PFT	X						
Post-bronchodilator PFT	X						
Dispense Rescue Medication	X						
DLco	X						
Randomization		X					
Training in use of HandiHaler		X					
Dispense Study Medication		X					
Administer Study Medication		X	X	X	X	X	X
Sputum Induction		X		X	X		X
Stage 1 Exercise Test	X	X*	X*		X*	X*	
Constant Load Exercise Test				X*			X*
Collect Study Medication				X			X
Medication Accountability				X			X
Adverse Events	X	X	X	X	X	X	X

*Cardiac Output measured during these tests

Table 3.2. *Study Population Demographics.*

	n	Mean	Minimum	Maximum	Std.Dev.
pre-BD FEV$_1$ (L)	20	1.36	0.73	2.65	0.49
pre-BD FEV$_1$ % Predicted	20	44.65	19.00	68.00	14.11
post-BD FEV$_1$ (L)	20	1.64	0.83	2.61	0.47
post-BD FEV$_1$ % Predicted	20	53.85	21.00	76.00	13.50
Height (cm)	20	173.35	162.00	185.00	7.94
Weight (kg)	20	82.50	48.00	128.00	20.32
BSA (m^2)	20	1.96	1.50	2.48	0.24
Age (years)	20	64.05	37.00	78.00	9.18
Sex (% Male)	20	70			

pre-BD FEV$_1$ = pre-bronchodilator forced expired volume in one second,
post-BD FEV$_1$ = post-bronchodilator forced expired volume in one second, BSA = body
surface area

Table 3.3. *Responder Sub-Population Demographics.*

	n	Mean	Minimum	Maximum	Std.Dev.
Responders					
pre-BD FEV$_1$ (L)	11.00	1.27	0.73	2.65	0.54
pre-BD FEV$_1$ % Predicted	11.00	41.82	19.00	68.00	15.83
post-BD FEV$_1$ (L)	11.00	1.53	0.83	2.50	0.46
post-BD FEV$_1$ % Predicted	11.00	50.73	21.00	76.00	15.65
Height (cm)	11.00	172.36	163.00	185.00	8.35
Weight (kg)	11.00	89.09	48.00	128.00	22.23
BSA (m^2)	11.00	2.01	1.50	2.48	0.26
Age (years)	11.00	63.36	50.00	71.00	6.39
Sex (% Male)	11.00	64			

pre-BD FEV$_1$ = pre-bronchodilator forced expired volume in one second,
post-BD FEV$_1$ = post-bronchodilator forced expired volume in one second, BSA = body
surface area

Table 3.4. *Non-Responder Sub-Population Demographics.*

Non-Responders					
	n	Mean	Minimum	Maximum	Std.Dev.
pre-BD FEV$_1$ (L)	9.00	1.47	0.86	2.06	0.42
pre-BD FEV$_1$ % Predicted	9.00	48.11	29.00	60.00	11.62
post-BD FEV$_1$ (L)	9.00	1.77	1.25	2.61	0.47
post-BD FEV$_1$ % Predicted	9.00	57.67	40.00	70.00	9.84
Height (cm)	9.00	174.56	162.00	183.00	7.73
Weight (kg)	9.00	74.44	54.00	98.00	15.17
BSA (m^2)	9.00	1.89	1.58	2.20	0.21
Age (years)	9.00	64.89	37.00	78.00	12.16
Sex (% Male)	9.00	78			

pre-BD FEV$_1$ = pre-bronchodilator forced expired volume in one second,
post-BD FEV$_1$ = post-bronchodilator forced expired volume in one second, BSA = body surface area

Figures:
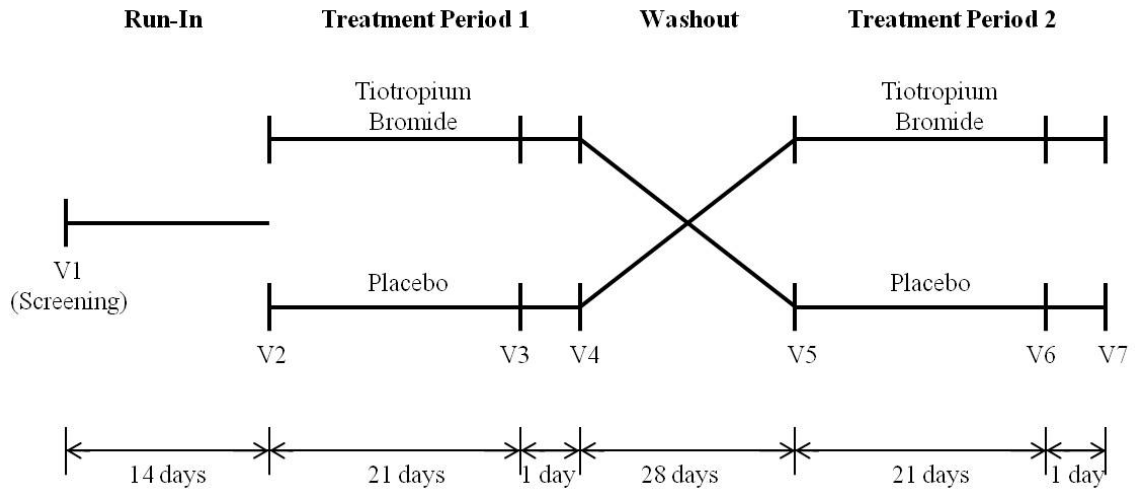
Figure 3.1. *Study design with visits 1 through 7 indicated (V1-V7).*
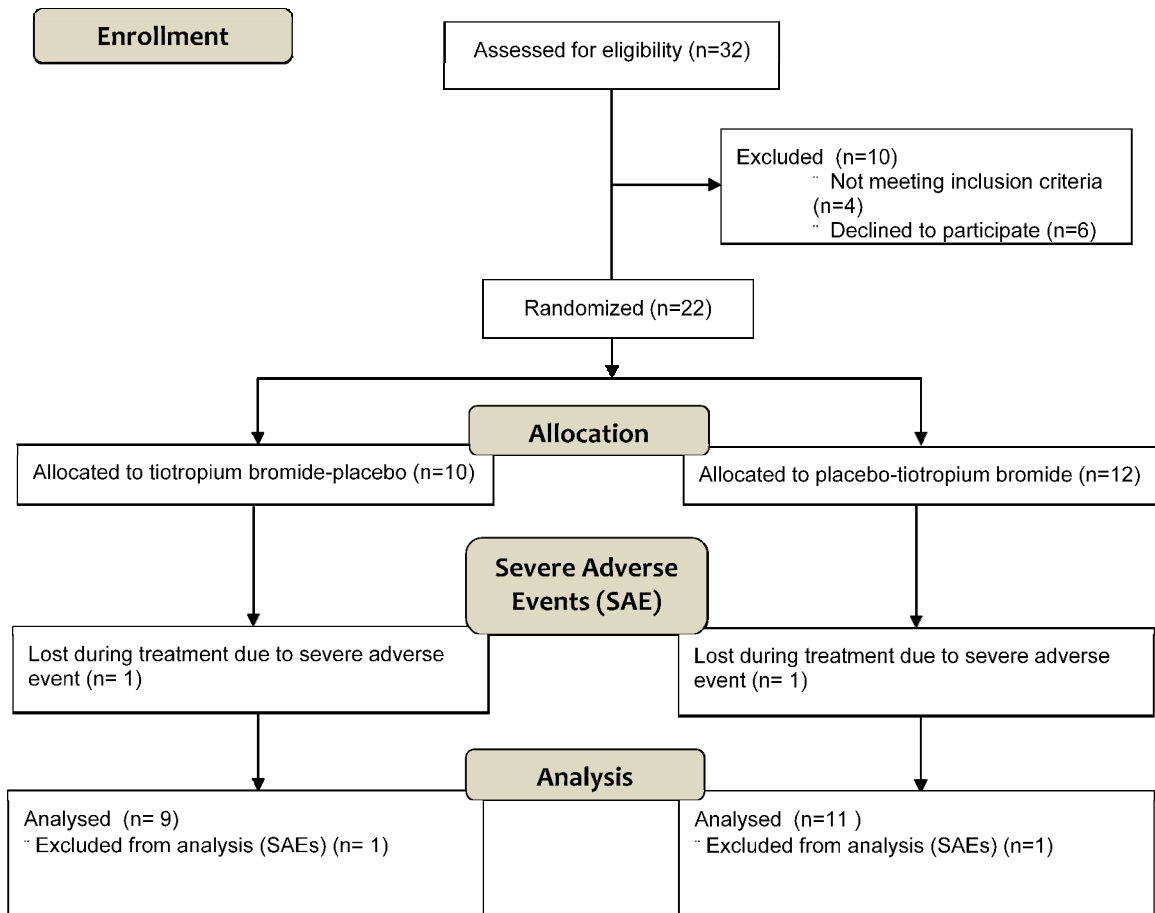
Figure 3.2. *Flow chart of study enrolment, randomization, and final status.*
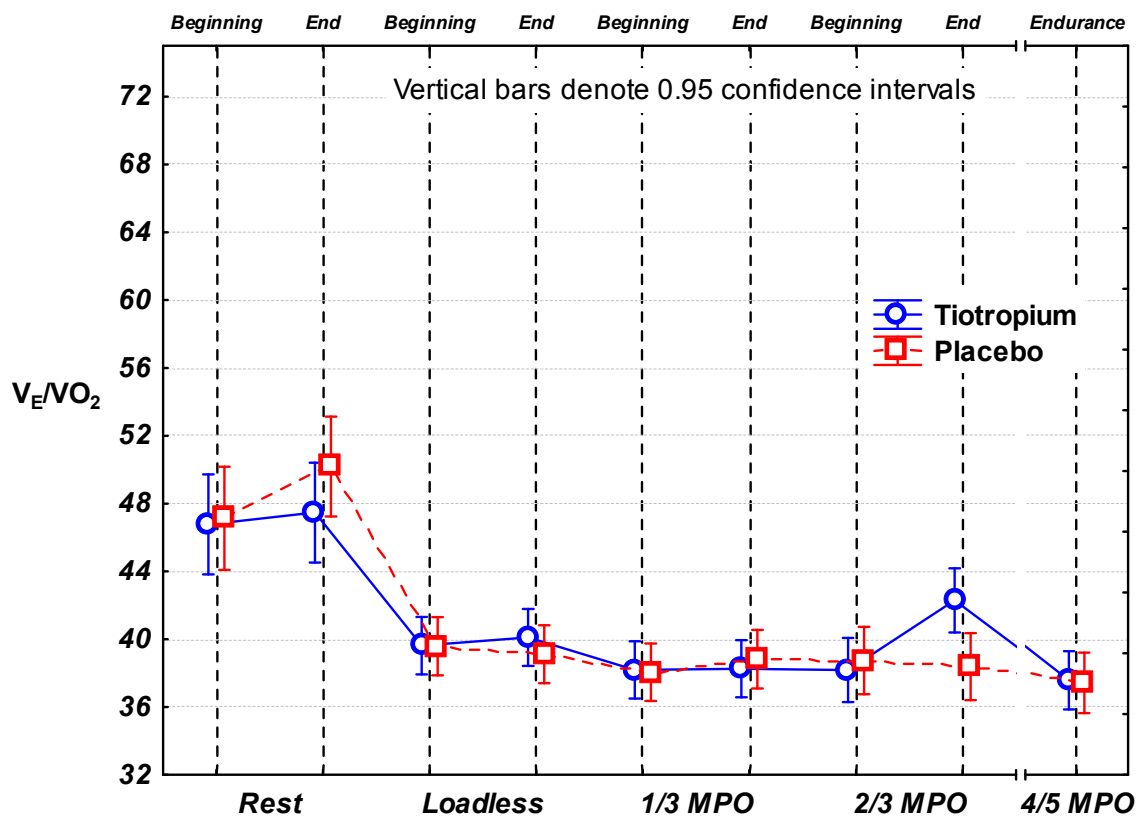
Figure 3.3. *Effects of tiotropium bromide on gas exchange efficiency of oxygen (V_E/VO_2).* Comparison of V_E/VO_2 measured with tiotropium bromide (blue) vs. Placebo (red) at rest and during cycle ergometer exercise at the start (Beginning) and completion (End) of treatment. Rest and exercise at loadless, 1/3 MPO, and 2/3 MPO performed during single exercise test performed at visits 2, 3, 5, and 6. Exercise at 4/5 MPO was performed at the end of each treatment period (visit 4 and 7) during a separate constant load endurance exercise test at 80% MPO (Endurance).
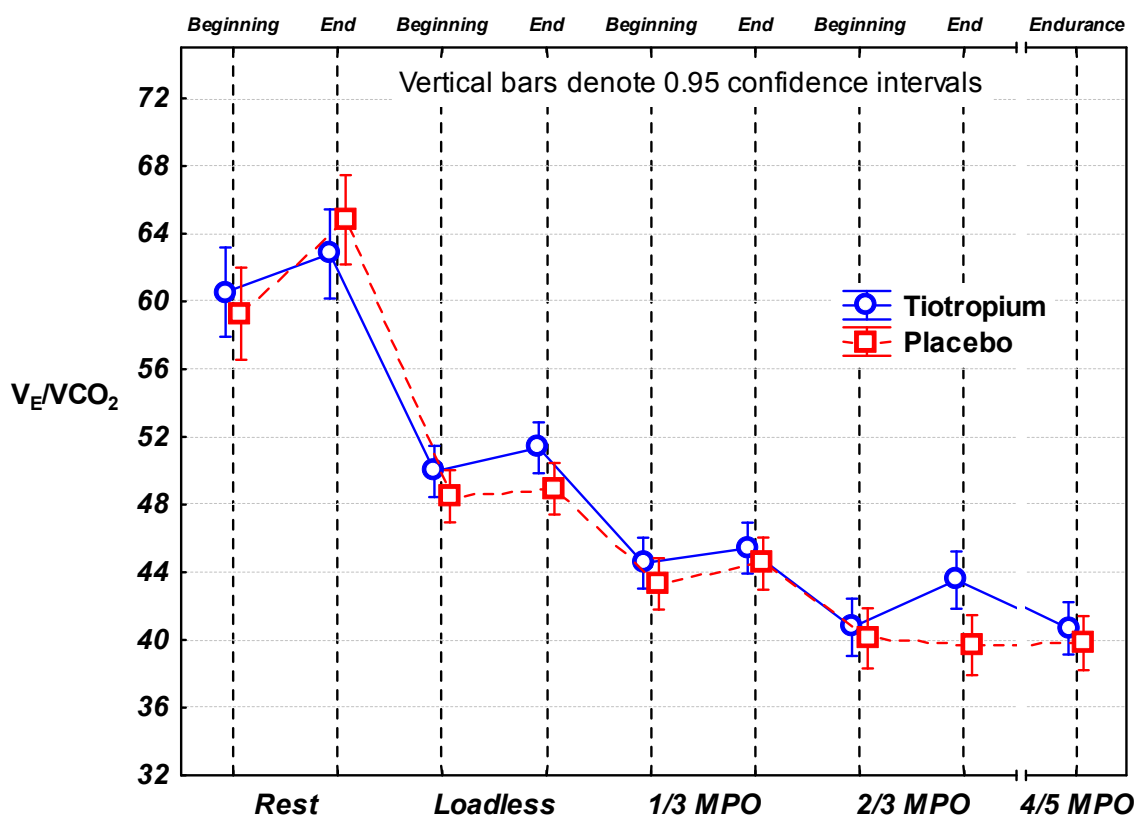
Figure 3.4. *Effects of tiotropium bromide on gas exchange efficiency of carbon dioxide (V_E/VCO_2).* Comparison of V_E/VCO_2 measured with tiotropium bromide (blue) vs. Placebo (red) at rest and during cycle ergometer exercise at the start (Beginning) and completion (End) of treatment. Rest and exercise at loadless, 1/3 MPO, and 2/3 MPO performed during single exercise test performed at visits 2, 3, 5, and 6. Exercise at 4/5 MPO was performed at the end of each treatment period (visit 4 and 7) during a separate constant load endurance exercise test at 80% MPO (Endurance).
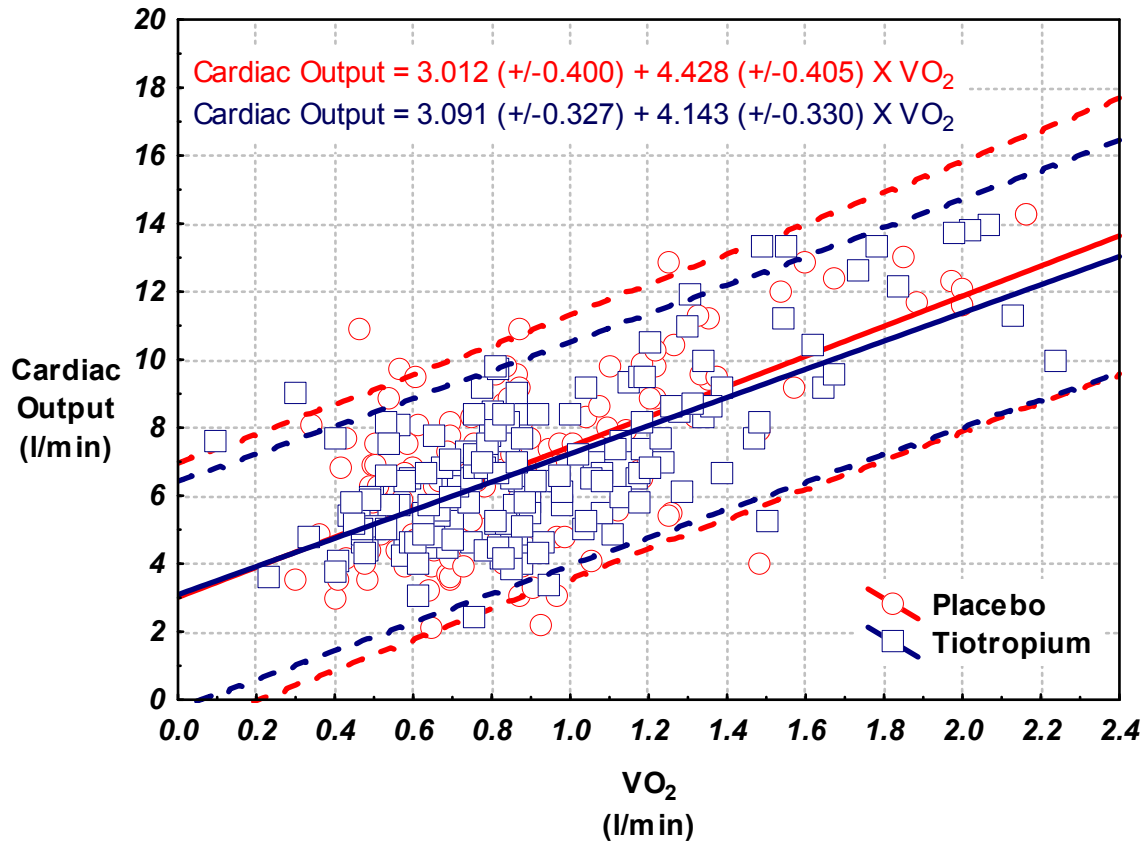
Figure 3.5. *Effects of tiotropium bromide on Cardiac Output.*
Cardiac output measured relative to oxygen uptake (VO_2) with tiotropium bromide (blue) vs. Placebo (red) at rest and during cycle ergometer exercise (all measurements from all tests). Intercepts and slopes (± standard error).
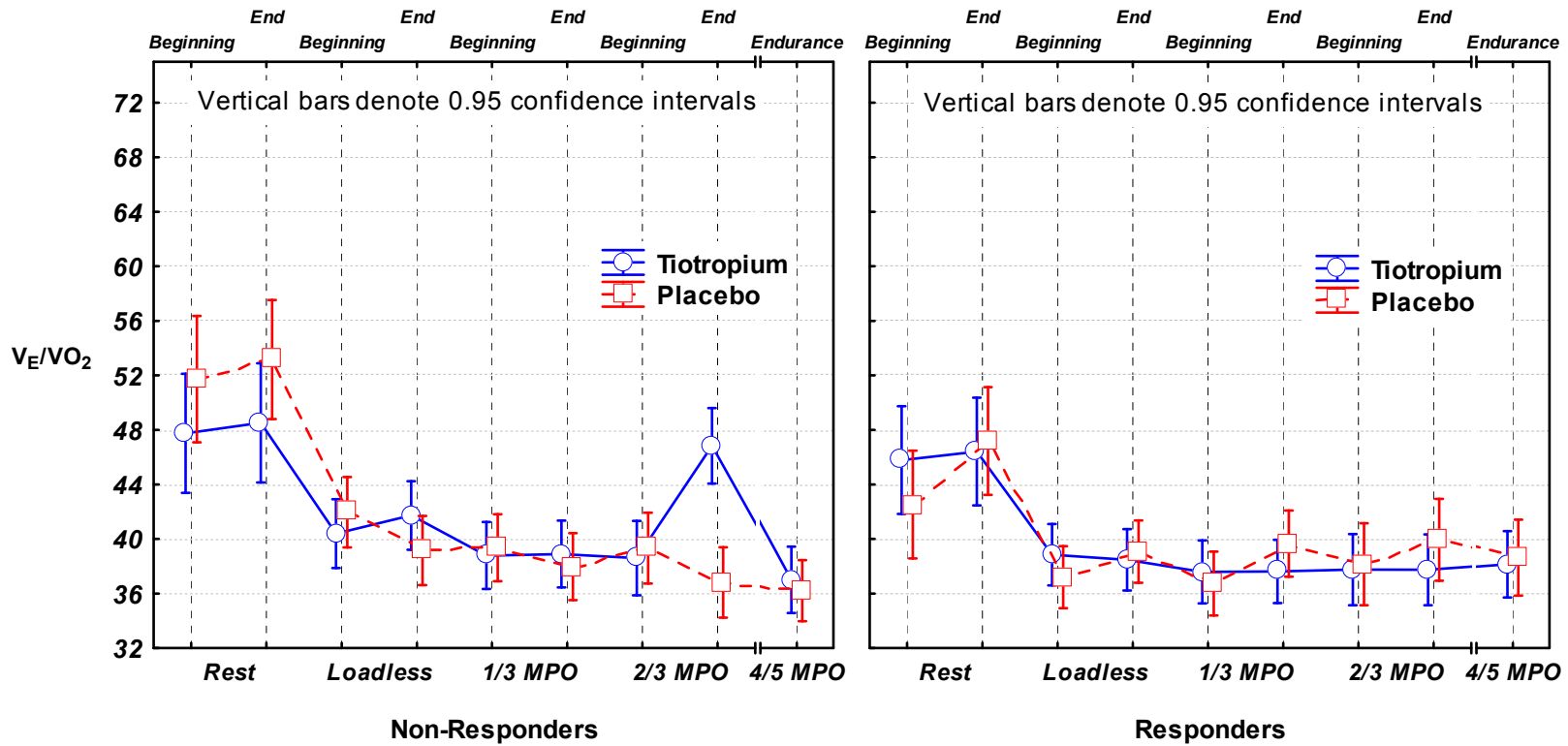
Figure 3.6. *Effects of tiotropium bromide on gas exchange efficiency of oxygen (V_E/VO_2) in Responders vs. Non-Responders.* Comparison of V_E/VO_2 measured with tiotropium bromide (blue) vs. Placebo (red) in Responders and Non-Responders at rest and during cycle ergometer exercise at the start (Beginning) and completion (End) of treatment. Rest and exercise at loadless, 1/3 MPO, and 2/3 MPO performed during single exercise test performed at visits 2, 3, 5, and 6. Exercise at 4/5 MPO was performed at the end of each treatment period (visit 4 and 7) during a separate constant load endurance exercise test at 80% MPO (Endurance).
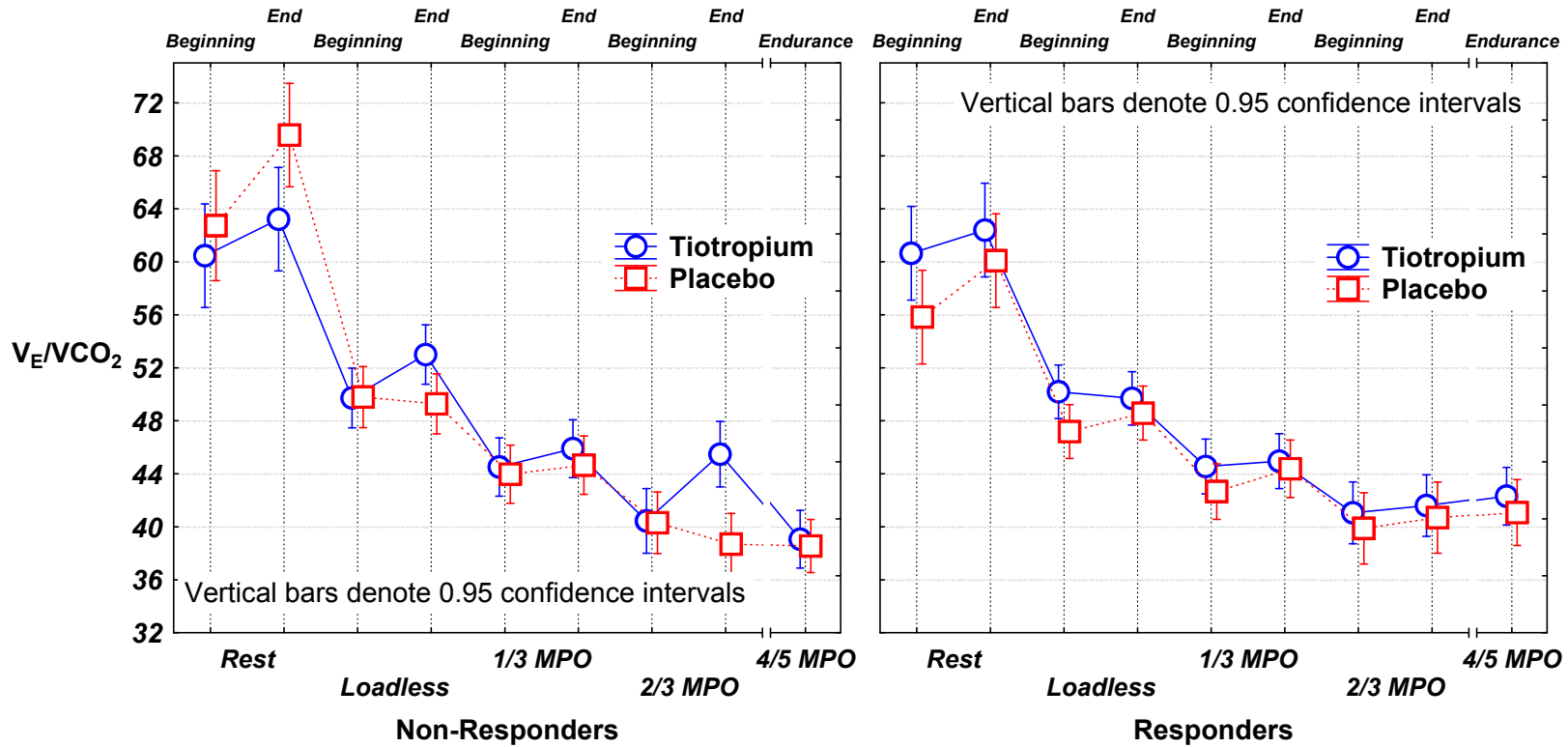
Figure 3.7. *Effects of tiotropium bromide on gas exchange efficiency of carbon dioxide (V_E/VCO$_2$) in Responders vs. Non-Responders.* Comparison of V_E/VCO$_2$ measured with tiotropium bromide (blue) vs. Placebo (red) in Responders and Non-Responders at rest and during cycle ergometer exercise at the start (Beginning) and completion (End) of treatment. Rest and exercise at loadless, 1/3 MPO, and 2/3 MPO performed during single exercise test performed at visits 2, 3, 5, and 6. Exercise at 4/5 MPO was performed at the end of each treatment period (visit 4 and 7) during a separate constant load endurance exercise test at 80% MPO (Endurance).
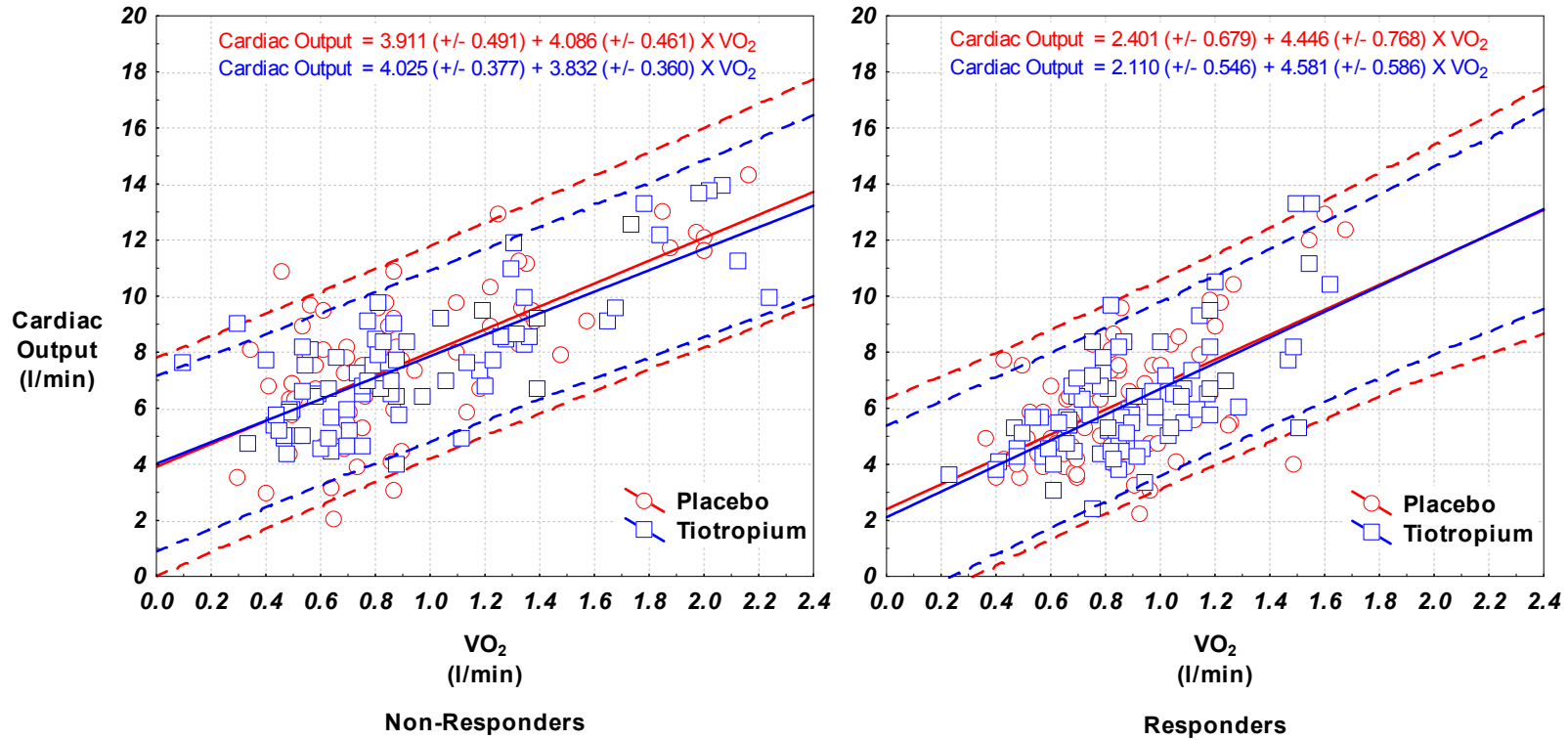
Figure 3.8. *Effects of tiotropium bromide on Cardiac Output in Responders vs. Non-Responders.* Cardiac output measured relative to oxygen uptake (VO_2) with tiotropium bromide (blue) vs. Placebo (red) at rest and during cycle ergometer exercise (all measurements from all tests) in Responders and Non-Responders. Intercepts and slopes (± standard error).

Chapter 4 - Effects of Tiotropium Bromide on Ventilatory Efficiency and Cardiac Output During Exercise in COPD: Additional Variables Considered

Introduction:

Additional variables, not central to the hypothesis, were collected during the double-blind, placebo-controlled, crossover study of tiotropium bromide and included: oxygen uptake (VO_2), carbon dioxide production (VCO_2), minute ventilation (V_E), alveolar ventilation (V_A), tidal volume (V_T), anatomical deadspace (Fowler Deadspace, V_D), respiratory exchange ratio (RER), fractional end-tidal oxygen ($F_{et}O_2$), fractional end-tidal carbon dioxide ($F_{et}CO_2$), arterial oxygen saturation (SpO_2), heart rate (HR), oxygen pulse (VO_2/HR), oxygen uptake per kilogram body weight (VO_2/kg), respiratory frequency (Resp. Freq.), dyspnea, and leg effort.

Tidal volume, alveolar ventilation, respiratory frequency, deadspace volume (Fowler), fractional end-tidal carbon dioxide, arterial oxygen saturation, respiratory exchange ratio, and dyspnea were significantly altered following treatment with tiotropium bromide. Subgroup analysis showed respiratory frequency, fractional end-tidal carbon dioxide, arterial oxygen saturation, respiratory exchange ratio, heart rate, and dyspnea were significantly altered with tiotropium bromide treatment in either the Responders or Non-Responders, exclusively. The effects of tiotropium bromide on these additional variables, in general and in Responders vs. Non-Responders, is presented and discussed.

Methods:

Arterial Oxygen Saturation & Heart Rate:

Arterial oxygen saturation and heart rate were collected continuously via pulse oximetry at rest, during all exercise intensities, and during recovery for all exercise tests.

Respiratory Exchange Ratio:

The respiratory exchange ratio was calculated as VCO_2/VO_2 from values of VCO_2 and VO_2 collected breath-by-breath at rest, during all exercise intensities, and during recovery for all exercise tests.

Anatomical Deadspace Volume:

The anatomical deadspace volume is the volume of the upper airways, trachea, and bronchi (Fowler Deadspace) and is presented as liters at body temperature and pressure, saturated with water vapor (BTPS). V_D was calculated breath-by-breath as follows: a regression line was determined for phase III of the CO_2 curve (portion of the CO_2 curve where a slower and nearly linear increase in CO_2 concentration occurs, which represents delayed alveolar concentration), and the deadspace was defined as the expired volume where two areas are equal. One area is bounded by the horizontal zero line (CO_2 at its minimum level), the vertical line at the deadspace, and the CO_2 curve. The second area is bounded by the vertical line at the dead space, the alveolar regression line (phase

III regression line), and the CO_2 curve. The instrument dead space is then subtracted from this volume, yielding V_D.

Alveolar Ventilation:

The alveolar ventilation is the volume of air exhaled from the alveoli per minute (L/min at BTPS). The alveolar ventilation was defined by the following equation:

$V_A = V_E$ – Respiratory Frequency * (V_D + Instrument Deadspace).

Fractional End-Tidal Carbon Dioxide & Oxygen:

The fractional end-tidal carbon dioxide is the carbon dioxide concentration in the expired gas at the end of the exhalation (expressed as a percentage). The fractional end-tidal oxygen is the oxygen concentration in the expired gas at the end of the exhalation (expressed as a percentage).

Modified Borg Scale:

The modified Borg Scale was used to assess dyspnea and leg effort at rest, every two minutes during all exercise intensities, and during recovery for all exercise tests.

Statistical Analysis:

The data were analyzed using repeated measures ANOVA for the effects of treatment (Tiotropium Bromide vs. Placebo) on oxygen uptake, carbon dioxide

production, minute ventilation, alveolar ventilation, tidal volume, anatomical deadspace (Fowler Deadspace), respiratory exchange ratio, fractional end-tidal oxygen, fractional end-tidal carbon dioxide, arterial oxygen saturation, heart rate, oxygen pulse, oxygen uptake per kilogram body weight, respiratory frequency, dyspnea, and leg effort.

A further subgroup analysis was planned and also performed. Variability in outcomes from subject to subject was expected based on the previous large scale randomized prospective control trials included in the metanalysis by Barr (Barr, Bourbeau, Camargo, & Ram, 2006). Because of the limited number of subjects (n=20) significance might not be achieved based on the sample size. However, some subjects would be expected to improve their exercise endurance time (exercise tolerance) while others would not. If any of the variables listed above were contributing to improvement in exercise tolerance, significance would be expected in those that improved and not expected in those who failed to improve. Subjects were classified as Responders or Non-Responders based on whether they showed any improvement in exercise endurance time (exercise tolerance), measured at visits 4 and 7, following tiotropium bromide relative to placebo. Differences between Responders and Non-Responders were again assessed for all variables using repeated measures ANOVA with a covariant based on Responder or Non-Responder classification.

Results:

Tiotropium Bromide vs. Placebo:

Tidal volume and alveolar ventilation were lowest at rest and increased during exercise, as expected (Figures 4.1 & 4.2). There was also a significant increase in tidal volume ($p<0.0001$) and alveolar ventilation ($p=0.005$) with tiotropium bromide over placebo during exercise (Figures 4.1 & 4.2). Tiotropium bromide increased tidal volume and alveolar ventilation during exercise.

The respiratory frequency was lowest at rest, and increased during exercise, as expected (Figure 4.3). The respiratory frequency was significantly ($p=0.001$) reduced with tiotropium bromide over placebo at rest and during exercise (Figure 4.3). Tiotropium bromide reduced respiratory frequency at rest and during exercise.

Deadspace volume was lowest at rest and increased during exercise, as expected (Figure 4.4). There was also a significant ($p=0.001$) increase in deadspace volume with tiotropium bromide over placebo during exercise (Figure 4.4). Tiotropium bromide increased deadspace volume during exercise.

The fractional end-tidal carbon dioxide was lowest at rest and increased during exercise, as expected (Figure 4.5). The $F_{et}CO_2$ was significantly ($p<0.0001$) reduced with tiotropium bromide over placebo during exercise (Figure 4.5). Tiotropium bromide reduced $F_{et}CO_2$ during exercise.

Arterial oxygen saturation at rest remained unchanged during all but the highest intensity exercise (4/5MPO) where it decreased (Figure 4.6). SpO_2 was significantly

(p=0.001) increased with tiotropium bromide over placebo during exercise (Figure 4.6).
Tiotropium bromide improved SpO_2 during exercise.

As expected, the respiratory exchange ratio was lowest at rest and increased during exercise (Figure 4.7). There was also a significant (p<0.0001) decrease in RER with tiotropium bromide over placebo at rest and during exercise (Figure 4.7). Tiotropium bromide reduced RER at rest and during exercise.

As expected, dyspnea was lowest at rest and increased during exercise (Figure 4.8). Dyspnea was significantly (p=0.008) reduced with tiotropium bromide over placebo during exercise (Figure 4.8). Tiotropium bromide reduced dyspnea during exercise.

Responders vs. Non-Responders:

The respiratory frequency was lowest at rest, and increased during exercise in both Responders and Non-Responders, as expected (Figure 4.9). The respiratory frequency was significantly (p=0.03) reduced with tiotropium bromide over placebo at rest and during exercise in the Non-Responders (Figure 4.9). Tiotropium bromide reduced respiratory frequency at rest and during exercise in Non-Responders.

The fractional end-tidal carbon dioxide was lowest at rest and increased during exercise in both Responders and Non-Responders, as expected (Figure 4.10). The $F_{et}CO_2$ was significantly (p=0.001) reduced with tiotropium bromide over placebo during exercise in the Responders (Figure 4.10). Tiotropium bromide reduced $F_{et}CO_2$ during exercise in Responders.

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Arterial oxygen saturation at rest remained unchanged during all but the highest intensity exercise (4/5MPO), where it decreased, in the Non-Responders (Figure 4.11). Arterial oxygen saturation at rest remained unchanged during all exercise in the Responders (Figure 4.11). SpO_2 was significantly (p=0.04) increased with tiotropium bromide over placebo during exercise in the Non-Responders (Figure 4.11). Tiotropium bromide improved SpO_2 during exercise in Non-Responders.

As expected, the respiratory exchange ratio was lowest at rest and increased during exercise in both Responders and Non-Responders (Figure 4.12). There was a significant (p=0.0002) decrease in RER with tiotropium bromide over placebo at rest and during exercise in the Responders (Figure 4.12). Tiotropium bromide reduced RER at rest and during exercise in Responders.

Heart rate was lowest at rest, and increased during exercise in both Responders and Non-Responders, as expected (Figure 4.13). Heart rate during exercise was significantly (p=0.003) reduced with tiotropium bromide, compared to placebo, in the Non-Responders (Figure 4.13). Tiotropium bromide reduced heart rate during exercise in Non-Responders.

As expected, dyspnea was lowest at rest and increased during exercise in both Responders and Non-Responders (Figure 4.14). Dyspnea was significantly (p=0.04) reduced with tiotropium bromide over placebo during exercise in the Responders (Figure 4.14). Tiotropium bromide reduced dyspnea during exercise in Responders.

Discussion:

Tiotropium Bromide vs. Placebo:

Arguably several measured variables, not central to the hypothesis, were more informative and interesting than the central hypothesis.

Proprioceptive and chemoreceptive factors interact in the generation of the ventilatory response to the metabolic demands associated with exercise. There was no change in the metabolic demand (i.e. VO_2) following treatment with tiotropium bromide (p=0.34). Tidal volume and alveolar ventilation increased with tiotropium bromide during exercise (p<0.0001 and p=0.005) (Figures 4.1 & 4.2). With the expected reduction in airway resistance, V_T and V_A would be expected to increase. In the initial study (retrospective database analysis) using incremental exercise in 4,424 patients with COPD, tidal volume increased with the power output reaching a maximum value of 53.8±11.3% (mean±SD) of the vital capacity. The vital capacity increased to an even greater extent than the FEV_1 in the large randomized prospective control trials comparing tiotropium bromide to placebo treatment, included in the metanalysis by Barr (Barr et al., 2006). Hence, it is not surprising and perhaps expected that the tidal volume during exercise would increase. The increase in alveolar ventilation (Total Ventilation – Fowler Deadspace Ventilation) with tiotropium bromide would also be anticipated. Increases in airway resistance reduce the capacity to generate ventilation. Reduced ventilation, at any given power output, might be expected. Progressive increases in inspiratory resistance are accompanied by progressive decreases in alveolar ventilation (el-Manshawi, Killian, Summers, & Jones, 1986). Hence, it is reasonable to assume that the converse (i.e.

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reducing airway resistance) would increase alveolar ventilation; which was seen. As a result, beneficial effects were observed.

There is no doubt that active treatment with tiotropium bromide is accompanied by changes in ventilation and its pattern. Consequently, as expected, respiratory frequency was reduced (p=0.0007) with tiotropium bromide (Figure 4.3), resulting from the permitted increase in tidal volume associated with the demonstrated increase in vital capacity (Barr et al., 2006; Casaburi et al., 2002). With tidal volume elevated, fewer breathes were necessary to achieve the same ventilation.

Alveolar ventilation was improved (p=0.005) with tiotropium bromide (Figure 4.2) while total ventilation at any given power was unchanged (p=0.05). The Fowler deadspace was increased (p=0.001) with tiotropium bromide (Figure 4.4). At the simplest level, large airways dilated. The total deadspace is the sum of the anatomical dead space and the physiological deadspace due to alveolar units with a high V/Q. Total deadspace can be measured using the Bohr technique, modified by substituting the $P_{et}CO_2$ with the P_aCO_2. Unfortunately this was not possible because arterial blood sampling was not performed. Nonetheless, the physiological dead space had to decrease because alveolar ventilation and Fowler deadspace increased while total ventilation remained unchanged.

As expected with the increase in alveolar ventilation during exercise, fractional end-tidal carbon dioxide declined (p<0.0001) with tiotropium bromide (Figure 4.5). Progressive increases in inspiratory resistance result in progressive increases in $F_{et}CO_2$

(Jones, Killian, Summers, & Jones, 1985). Reduced airway resistance resulting from bronchodilation would logically lead to increased alveolar ventilation.

Arterial oxygen saturation increased (p=0.001) with tiotropium bromide (Figure 4.6). Progressive increases in inspiratory resistance result in progressive decreases in alveolar ventilation and arterial oxygen desaturation (el-Manshawi et al., 1986; Jones et al., 1985). The increase in SpO_2 is likely a product of the reduced airway resistance, resulting from bronchodilation, leading to increased alveolar ventilation. However, improving SpO_2 could conceivably imply a reduction in alveolar units with a low ventilation/perfusion (V/Q) ratio.

The respiratory exchange ratio was reduced (p<0.0001) with tiotropium bromide (Figure 4.7). The RER is a complex but frequently misunderstood measurement. RER is generally low at rest, reflecting the predominant oxidation of fat. The RER increases with meals after carbohydrate ingestion. RER systematically increases during exercise (Figure 4.15). In muscle, the rate at which fat can be oxidized is substantially lower than the rate at which carbohydrate can be oxidized. As exercise intensity increases, the oxidation of fat cannot keep pace with the energy required; leading to progressively greater amounts of carbohydrates being oxidized as power output increases. Mixed muscle contains both fast (predominantly anaerobic and glycolytic) and slow (predominantly aerobic) twitch fibers. Lactate is increasingly generated as the intensity of exercise increases, in both fiber types. Excess lactate produced by fast twitch fibers, which cannot be accommodated by the citric acid cycle, moves out of these muscle fibers and into slow twitch fibers, where it is oxidized. As power output intensifies, pyruvate

production outpaces its oxidation by either fiber type leading to lactate production and accumulation. This lactate is released into the circulation where it displaces bicarbonate which is excreted at the mouth. The increase in ventilation is commonly attributed to a metabolic acidosis (i.e. lactate accumulation). If lactate moves into the circulation from the muscle, alveolar ventilation increases causing increased CO_2 output exceeding the rate of its production. An RER >1 reflects either lactate appearing in the circulation or alveolar hyperventilation; both are seen. As cardiac output decreases relative to metabolic demand, alveolar ventilation increases augmenting CO_2 removal from muscle faced with limited perfusion. This might be considered a behavioral response. A premature increase in RER has long been recognized as having a negative effect on exercise capacity. Furthermore, in COPD RER is increased at any given power output as exercise capacity (maximum power output) decreases (Figure 4.15). This has been solely attributed to lactate accumulation, but this is an oversimplified explanation. As the RER increases, stress responses closely follow generally accompanied by further increases in heart rate. While these various factors could not be individually discriminated, the presence of a lower RER with tiotropium bromide was noted suggesting reduced stress in broad general terms.

From the prospective of the patient, which is arguably the most important, dyspnea was reduced (p=0.008) with tiotropium bromide (Figure 4.8). Popularly purported mechanisms of action for tiotropium bromide focus on increasing ventilatory capacity by bronchodilation and reducing functional residual capacity and residual volume (O'Donnell et al., 2004). Reduction in hyperinflation results in the respiratory

muscles being placed in a less stretched, more efficient, length. As such, less effort would be required to drive the respiratory muscles to generate ventilation, thus reducing dyspnea.

Responders vs. Non-Responders:

A subgroup analysis was planned because the beneficial effects of tiotropium bromide are not seen systematically in all subjects. Responder and Non-Responder groups were identified based the presence or absence of an improvement in exercise capacity with tiotropium bromide treatment.

Respiratory frequency was reduced (p=0.03) at rest and during exercise in the Non-Responders with tiotropium bromide, compared to placebo (Figure 4.9). A detailed discussion of the respiratory frequency has already been presented and can be found in the *Tiotropium Bromide vs. Placebo* section of this discussion.

Fractional end-tidal carbon dioxide was reduced (p=0.001) during exercise with tiotropium bromide, compared to placebo, in the Responders (Figure 4.10). A detailed discussion of the Fractional end-tidal carbon dioxide has already been presented and can be found in the *Tiotropium Bromide vs. Placebo* section of this discussion.

Arterial oxygen saturation was increased (p=0.04) during exercise with tiotropium bromide, compared to placebo, in the Non-Responders (Figure 4.11). A detailed discussion of the arterial oxygen saturation has already been presented and can be found in the *Tiotropium Bromide vs. Placebo* section of this discussion.

The respiratory exchange ratio was reduced (p=0.0002) with tiotropium bromide, compared to placebo, at rest and during exercise in the Responders (Figure 4.12). A detailed discussion of the RER has already been presented and can be found in the *Tiotropium Bromide vs. Placebo* section of this discussion.

Heart rate was reduced (p=0.003) during exercise with tiotropium bromide, compared to placebo, in the Non-Responders (Figure 4.13). Heart rate is determined by the intrinsic heart rate which is a function of the various ion channels determining depolarization and repolarization of the sinus node. The slope of phase four is arguably the most important. As exercise progresses, parasympathetic activity recedes and sympathetic activity increases. While the autonomic responses have both feed-forward (anticipation) and feed-back mechanisms, the feed-back responses are dominant. Assuming that the blood pressure responses are maintained (i.e. no hypotension or hypertension), feedback is largely from the exercising muscles (legs), respiratory muscles, and cardiac contraction. Reducing the stress on the respiratory or leg muscles would provide a source of reduced feed-back activity, ultimately reducing sympathetic activity and thus heart rate. The improved oxygenation (increased SpO_2) of the blood with tiotropium bromide, also seen in the Non-Responders (p=0.04), likely results in improved support of the exercising muscle through delivery of blood with a higher oxygen content. Oxygen therapy has been shown to increase SpO_2 and reduce heart rate in COPD patients, resulting in no net change in arterial oxygen flow (Mannix, Manfredi, Palange, Dowdeswell, & Farber, 1992; Palange et al., 1995). Given that tiotropium bromide increases SpO_2, blood flow would not need to be maintained at the same level in

order to deliver the same arterial oxygen flow to the working muscle. As such, heart rate would be reduced as the demand for blood flow decreases. Lastly, any expected increases in heart rate due to atropine-like effects of tiotropium bromide were not seen.

Dyspnea was also reduced (p=0.04) during exercise with tiotropium bromide, compared to placebo, in the Responders (Figure 4.14). A detailed discussion of dyspnea has already been presented and can be found in the *Tiotropium Bromide vs. Placebo* section of this discussion.

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Palange, P., Galassetti, P., Mannix, E. T., Farber, M. O., Manfredi, F., Serra, P., & Carlone, S. (1995). Oxygen effect on O2 deficit and VO2 kinetics during exercise in obstructive pulmonary disease. *Journal of Applied Physiology, 78*(6), 2228-2234.

Figures:
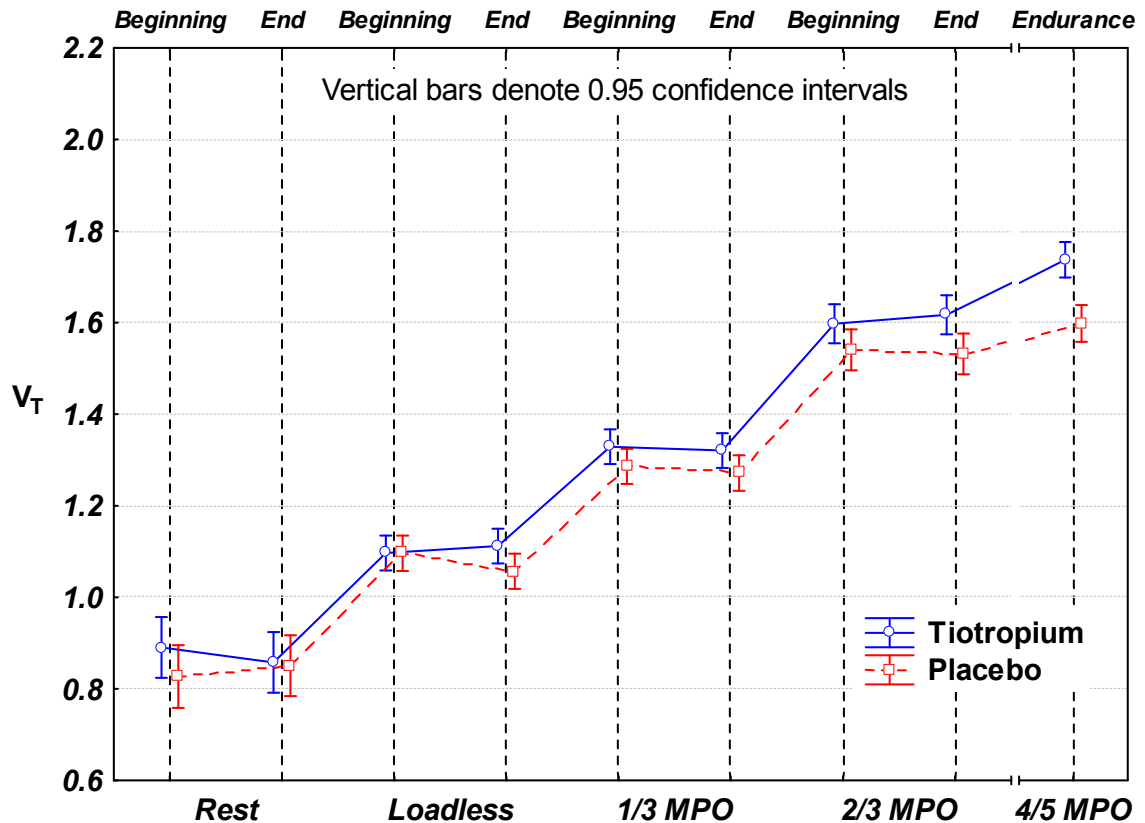
Figure 4.1. *Effects of tiotropium bromide on tidal volume (V_T).*
Comparison of tidal volume measured for tiotropium bromide (blue) and Placebo (red) at
rest and during cycle ergometer exercise at the start (Beginning) and completion (End) of
treatment. Rest and exercise at loadless, 1/3 MPO, and 2/3 MPO performed during single
exercise test performed at visits 2, 3, 5, and 6. Exercise at 4/5 MPO was performed at the
end of each treatment period (visit 4 and 7) during a separate constant load endurance
exercise test at 80% MPO (Endurance).

Figure 4.2. *Effects of tiotropium bromide on alveolar ventilation (V_A).*
Comparison of alveolar ventilation measured for tiotropium bromide (blue) and Placebo (red) at rest and during cycle ergometer exercise at the start (Beginning) and completion (End) of treatment. Rest and exercise at loadless, 1/3 MPO, and 2/3 MPO performed during single exercise test performed at visits 2, 3, 5, and 6. Exercise at 4/5 MPO was performed at the end of each treatment period (visit 4 and 7) during a separate constant load endurance exercise test at 80% MPO (Endurance).

Figure 4.3. *Effects of tiotropium bromide on respiratory frequency (Resp. Freq.).*
Comparison of respiratory frequency measured for tiotropium bromide (blue) and
Placebo (red) at rest and during cycle ergometer exercise at the start (Beginning) and
completion (End) of treatment. Rest and exercise at loadless, 1/3 MPO, and 2/3 MPO
performed during single exercise test performed at visits 2, 3, 5, and 6. Exercise at 4/5
MPO was performed at the end of each treatment period (visit 4 and 7) during a separate
constant load endurance exercise test at 80% MPO (Endurance).

Figure 4.4. *Effects of tiotropium bromide on anatomical deadspace (Fowler Deadspace, V_D).*
Comparison of anatomical deadspace measured for tiotropium bromide (blue) and Placebo (red) at rest and during cycle ergometer exercise at the start (Beginning) and completion (End) of treatment. Rest and exercise at loadless, 1/3 MPO, and 2/3 MPO performed during single exercise test performed at visits 2, 3, 5, and 6. Exercise at 4/5 MPO was performed at the end of each treatment period (visit 4 and 7) during a separate constant load endurance exercise test at 80% MPO (Endurance).

Figure 4.5. *Effects of tiotropium bromide on fractional end-tidal carbon dioxide ($F_{et}CO_2$).*
Comparison of fractional end-tidal carbon dioxide measured for tiotropium bromide (blue) and Placebo (red) at rest and during cycle ergometer exercise at the start (Beginning) and completion (End) of treatment. Rest and exercise at loadless, 1/3 MPO, and 2/3 MPO performed during single exercise test performed at visits 2, 3, 5, and 6. Exercise at 4/5 MPO was performed at the end of each treatment period (visit 4 and 7) during a separate constant load endurance exercise test at 80% MPO (Endurance).

Figure 4.6. *Effects of tiotropium bromide on arterial oxygen saturation (SpO₂).*
Comparison of arterial oxygen saturation measured for tiotropium bromide (blue) and
Placebo (red) at rest and during cycle ergometer exercise at the start (Beginning) and
completion (End) of treatment. Rest and exercise at loadless, 1/3 MPO, and 2/3 MPO
performed during single exercise test performed at visits 2, 3, 5, and 6. Exercise at 4/5
MPO was performed at the end of each treatment period (visit 4 and 7) during a separate
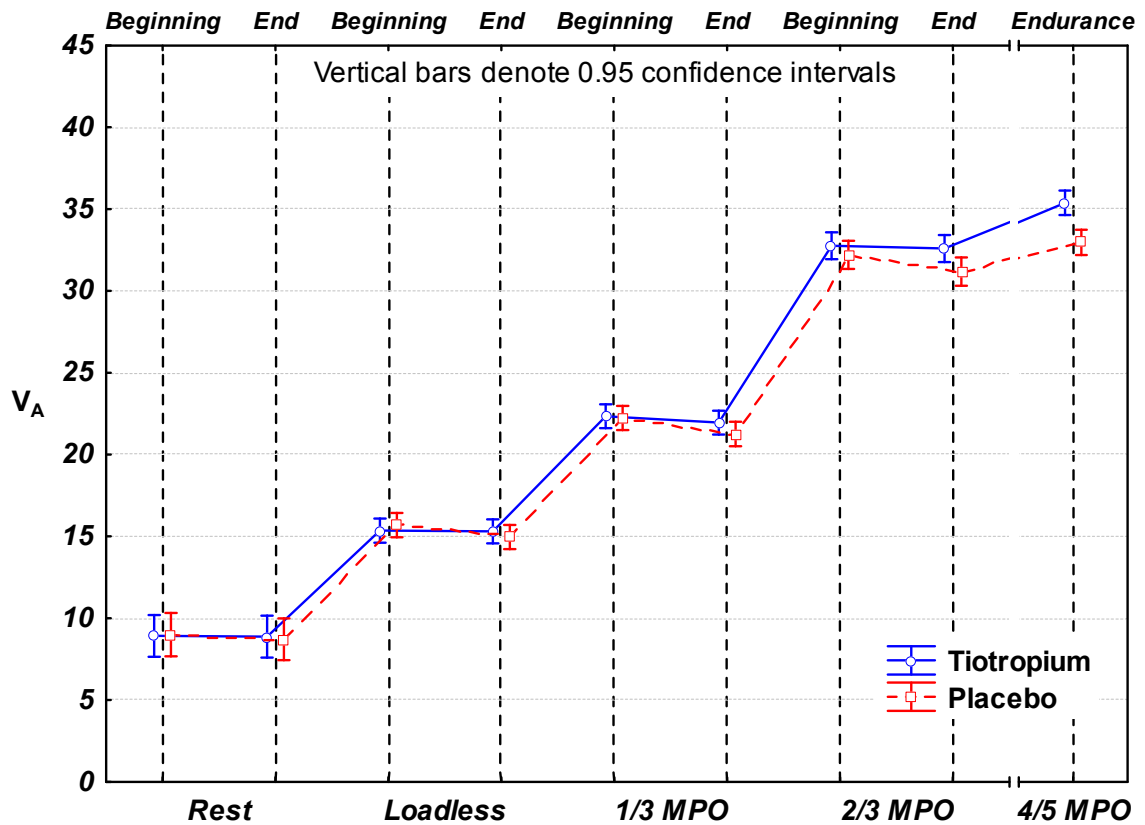constant load endurance exercise test at 80% MPO (Endurance).

Figure 4.7. *Effects of tiotropium bromide on respiratory exchange ratio (RER).*
Comparison of respiratory exchange ratio measured for tiotropium bromide (blue) and
Placebo (red) at rest and during cycle ergometer exercise at the start (Beginning) and
completion (End) of treatment. Rest and exercise at loadless, 1/3 MPO, and 2/3 MPO
performed during single exercise test performed at visits 2, 3, 5, and 6. Exercise at 4/5
MPO was performed at the end of each treatment period (visit 4 and 7) during a separate
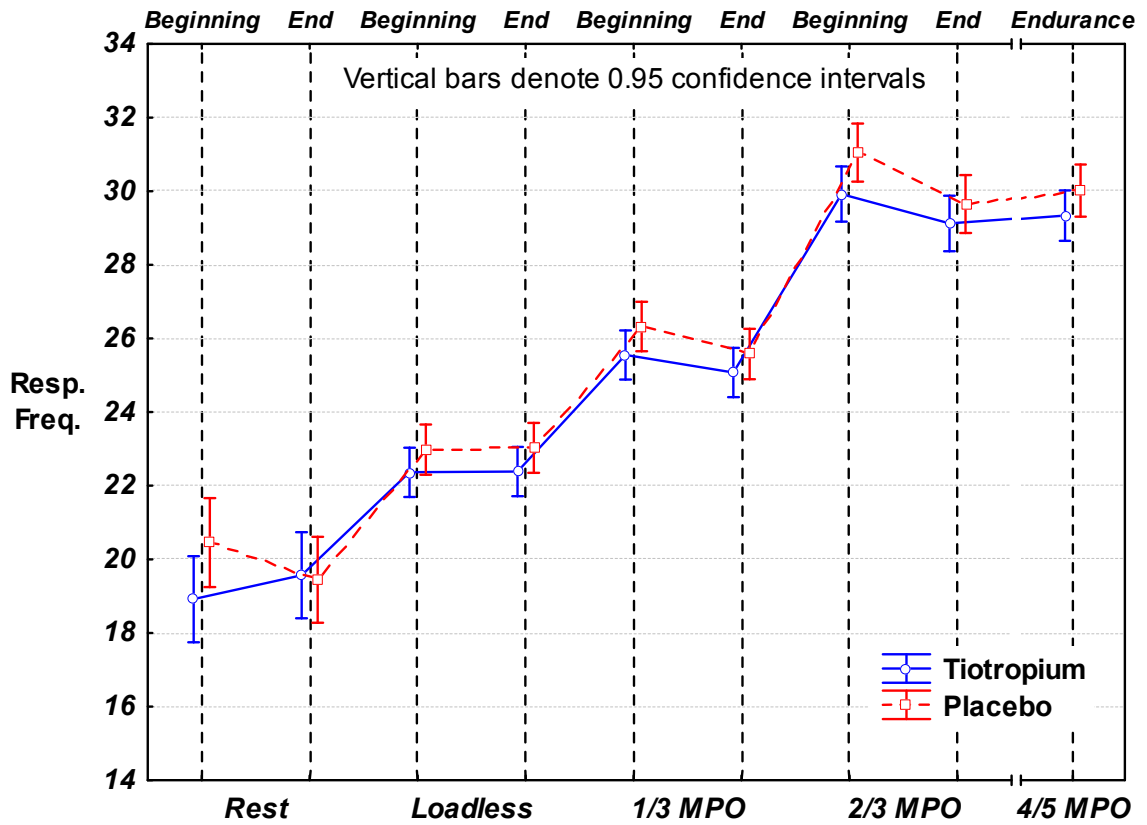constant load endurance exercise test at 80% MPO (Endurance).

Figure 4.8. *Effects of tiotropium bromide on dyspnea (Borg scale rating).*
Comparison of dyspnea measured for tiotropium bromide (blue) and Placebo (red) at rest and during cycle ergometer exercise at the start (Beginning) and completion (End) of treatment. Rest and exercise at loadless, 1/3 MPO, and 2/3 MPO performed during single exercise test performed at visits 2, 3, 5, and 6. Exercise at 4/5 MPO was performed at the end of each treatment period (visit 4 and 7) during a separate constant load endurance exercise test at 80% MPO (Endurance).
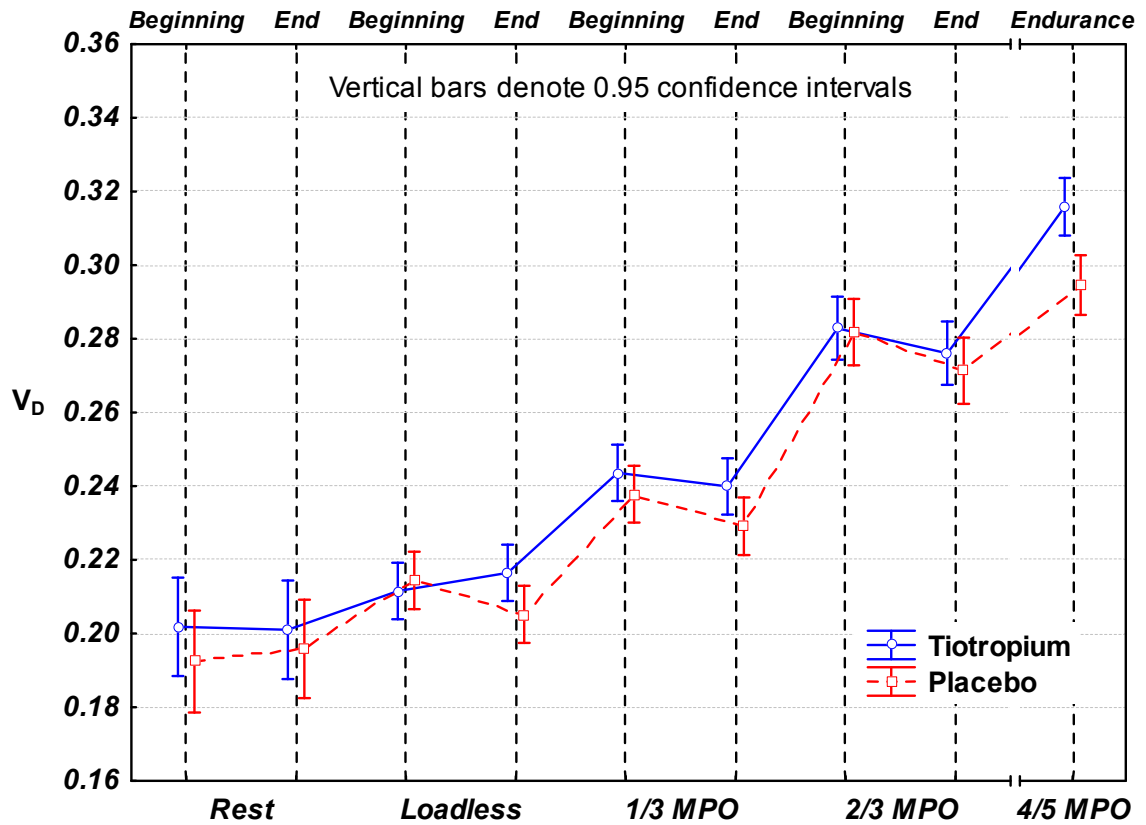
Figure 4.9. *Effects of tiotropium bromide on respiratory frequency (Resp. Freq.) in Responders vs. Non-Responders.*
Comparison of respiratory frequency measured with tiotropium bromide (blue) vs. Placebo (red) in Responders and Non-Responders at rest and during cycle ergometer exercise at the start (Beginning) and completion (End) of treatment. Rest and exercise at loadless, 1/3 MPO, and 2/3 MPO performed during single exercise test performed at visits 2, 3, 5, and 6. Exercise at 4/5 MPO was performed at the end of each treatment period (visit 4 and 7) during a separate constant load endurance exercise test at 80% MPO (Endurance).
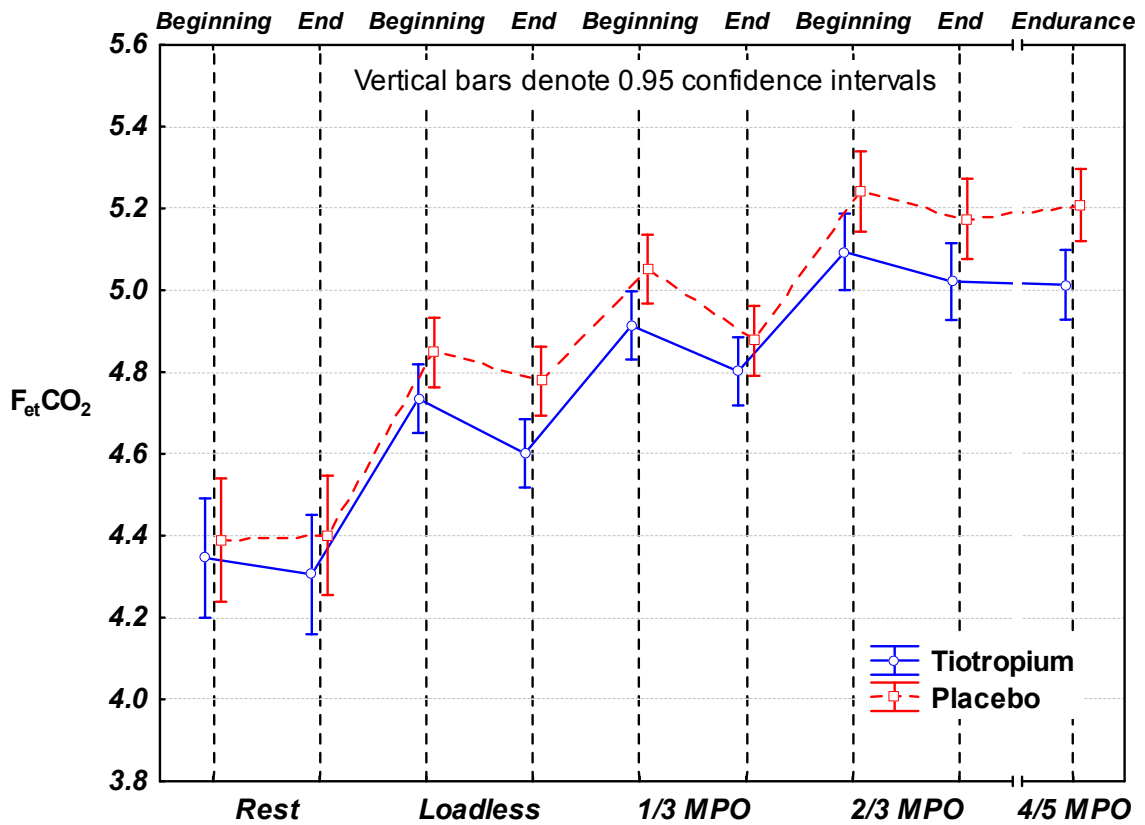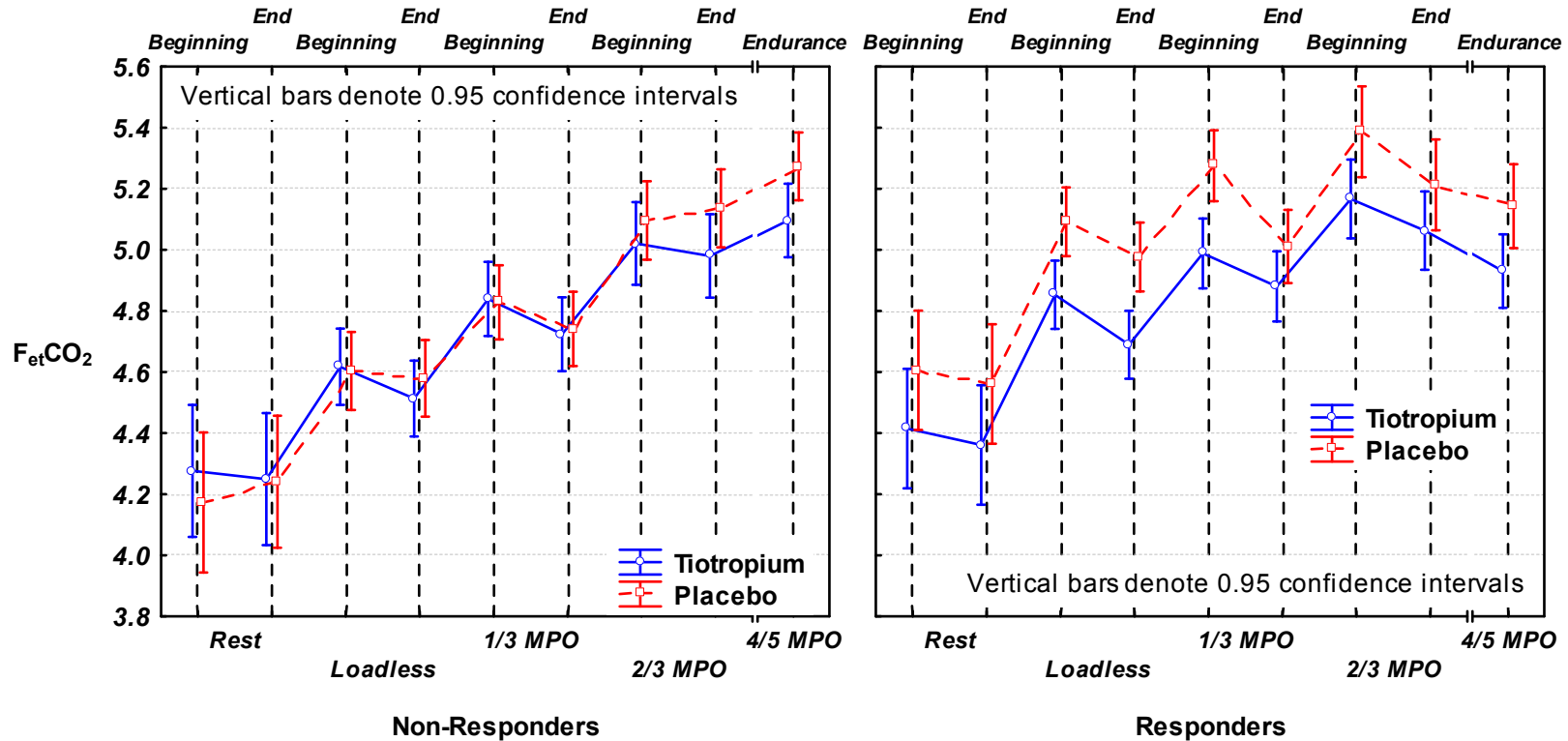
Figure 4.10. *Effects of tiotropium bromide on fractional end-tidal carbon dioxide ($F_{et}CO_2$) in Responders vs. Non-Responders.* Comparison of fractional end-tidal carbon dioxide measured with tiotropium bromide (blue) vs. Placebo (red) in Responders and Non-Responders at rest and during cycle ergometer exercise at the start (Beginning) and completion (End) of treatment. Rest and exercise at loadless, 1/3 MPO, and 2/3 MPO performed during single exercise test performed at visits 2, 3, 5, and 6. Exercise at 4/5 MPO was performed at the end of each treatment period (visit 4 and 7) during a separate constant load endurance exercise test at 80% MPO (Endurance).
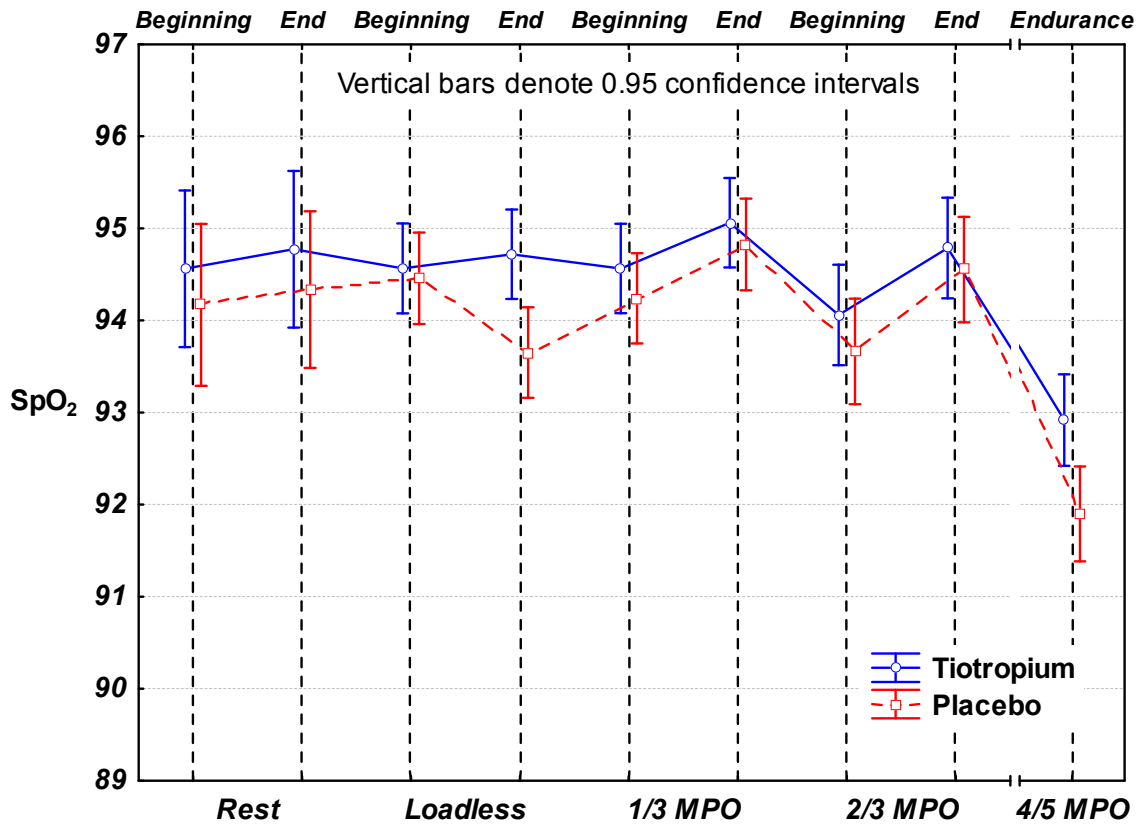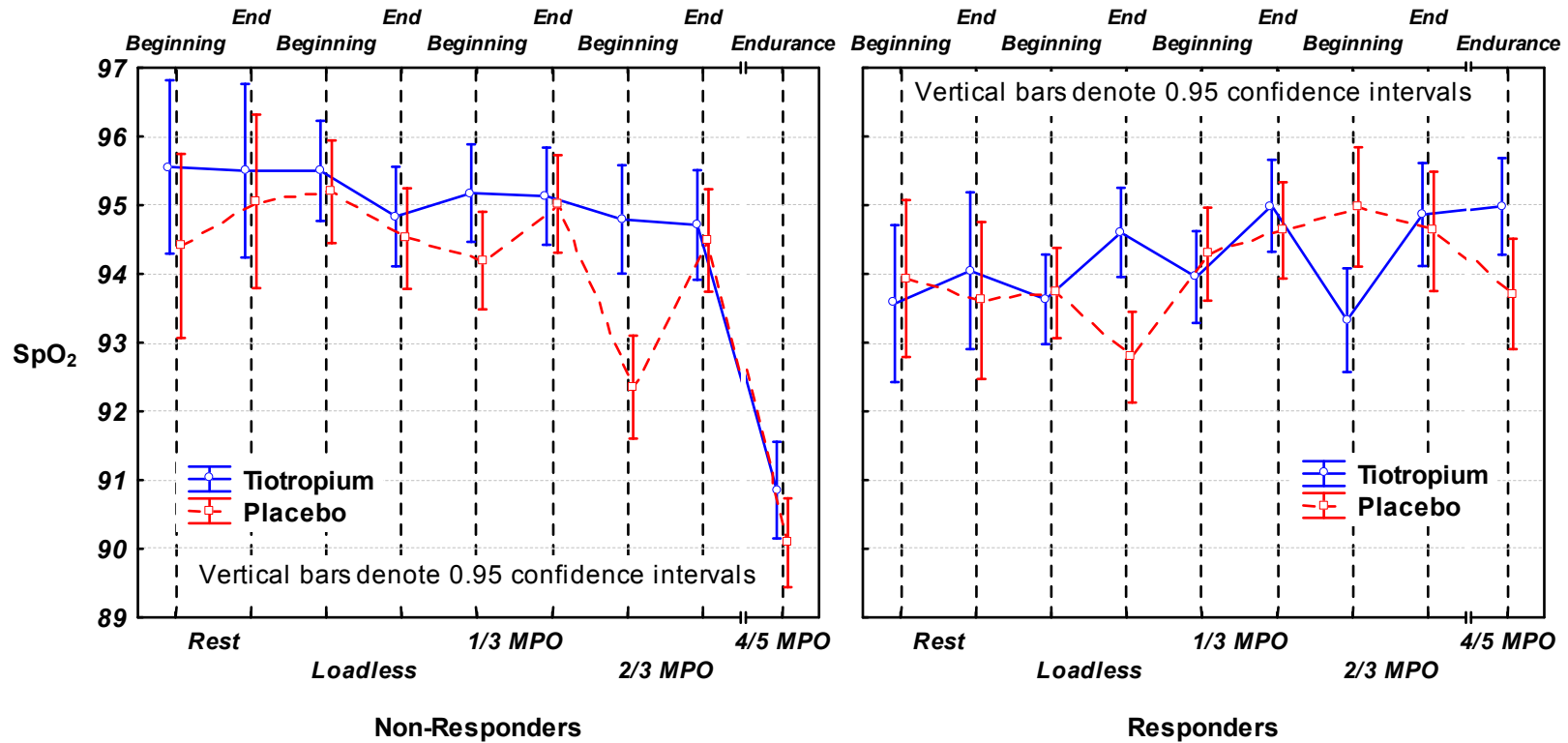
Figure 4.11. *Effects of tiotropium bromide on arterial oxygen saturation (SpO$_2$) in Responders vs. Non-Responders.* Comparison of arterial oxygen saturation measured with tiotropium bromide (blue) vs. Placebo (red) in Responders and Non-Responders at rest and during cycle ergometer exercise at the start (Beginning) and completion (End) of treatment. Rest and exercise at loadless, 1/3 MPO, and 2/3 MPO performed during single exercise test performed at visits 2, 3, 5, and 6. Exercise at 4/5 MPO was performed at the end of each treatment period (visit 4 and 7) during a separate constant load endurance exercise test at 80% MPO (Endurance).
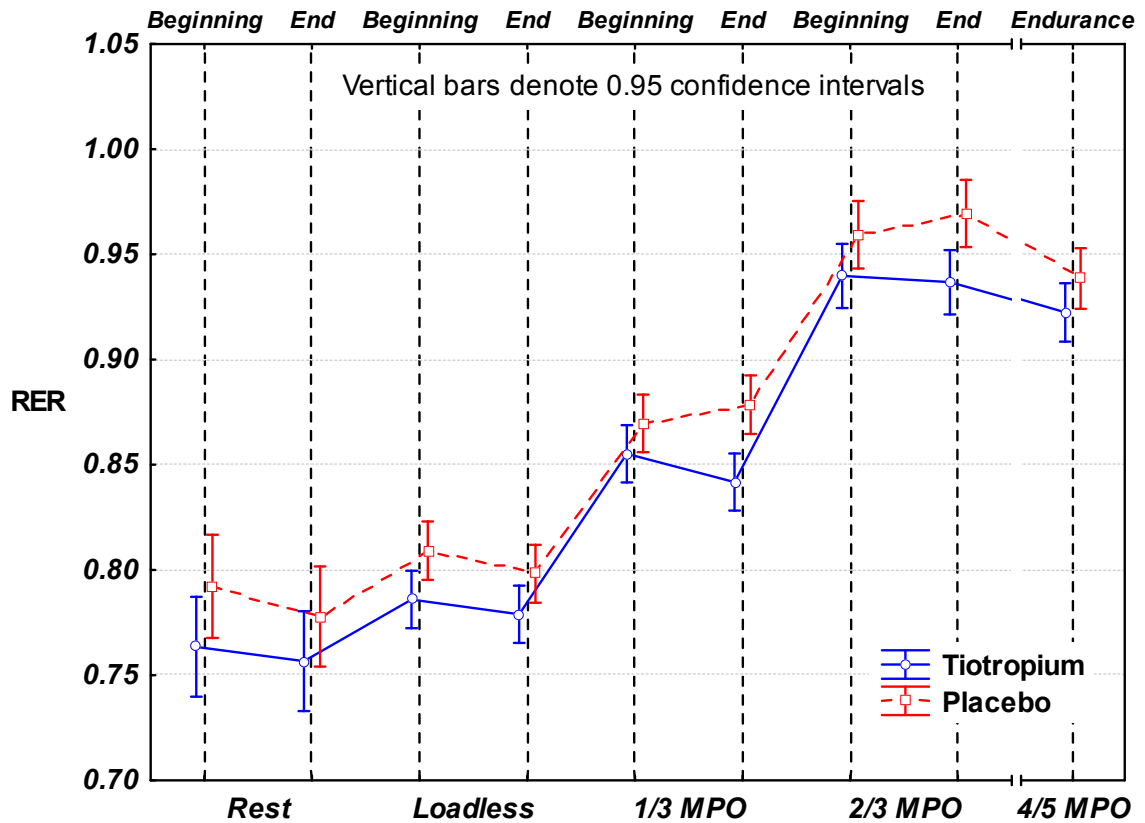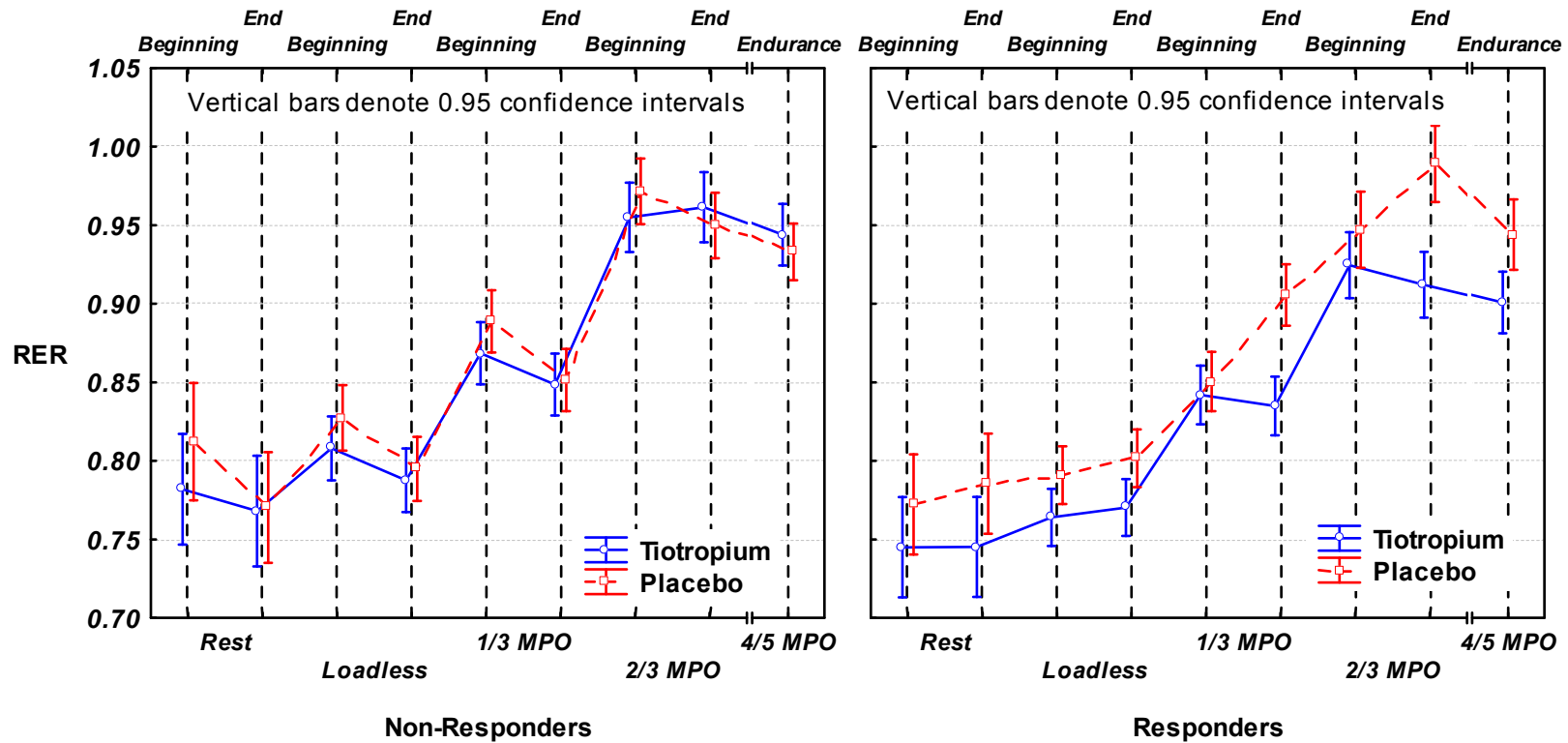
Figure 4.12. *Effects of tiotropium bromide on respiratory exchange ratio (RER) in Responders vs. Non-Responders.* Comparison of respiratory exchange ratio measured with tiotropium bromide (blue) vs. Placebo (red) in Responders and Non-Responders at rest and during cycle ergometer exercise at the start (Beginning) and completion (End) of treatment. Rest and exercise at loadless, 1/3 MPO, and 2/3 MPO performed during single exercise test performed at visits 2, 3, 5, and 6. Exercise at 4/5 MPO was performed at the end of each treatment period (visit 4 and 7) during a separate constant load endurance exercise test at 80% MPO (Endurance).
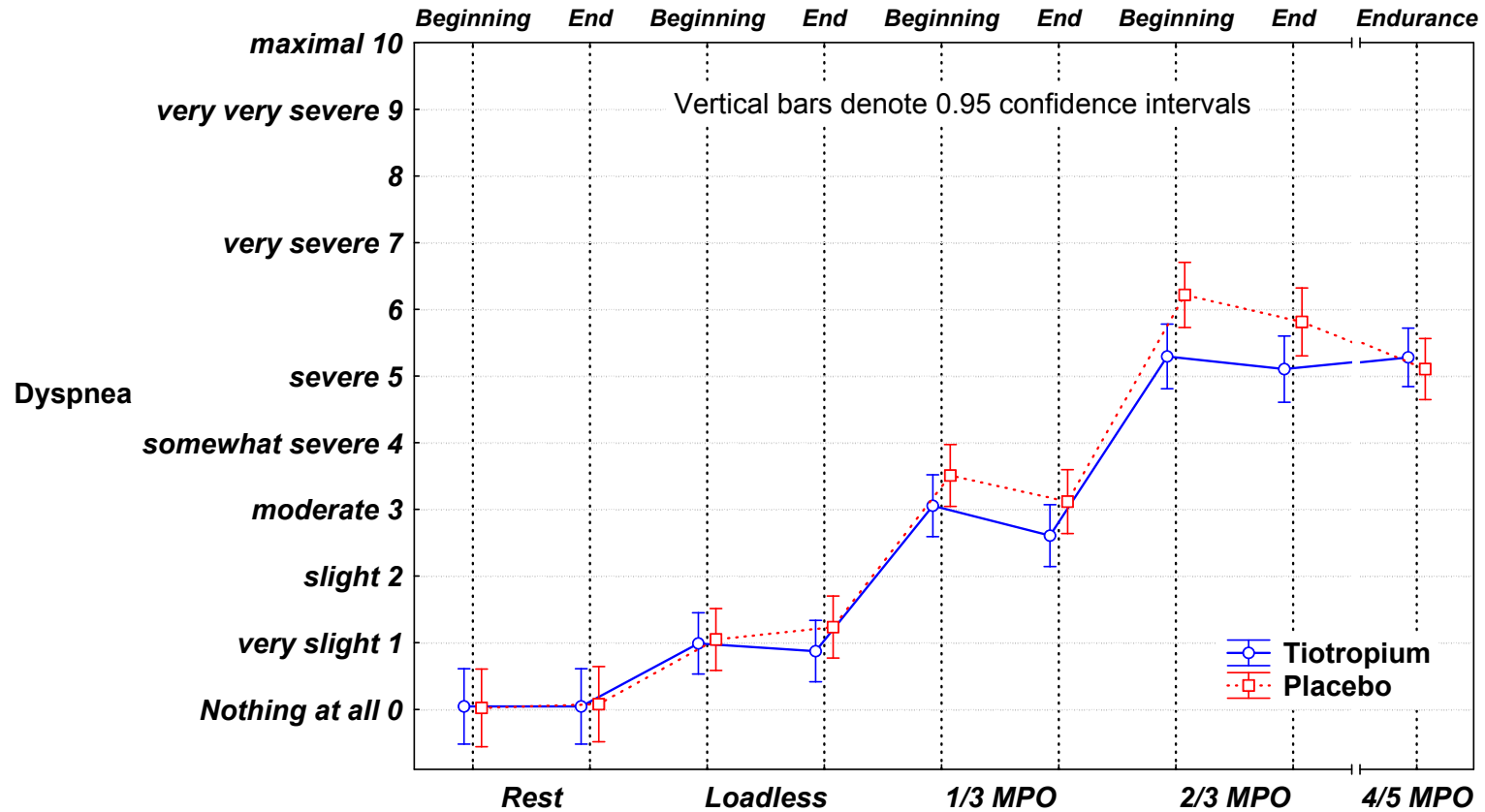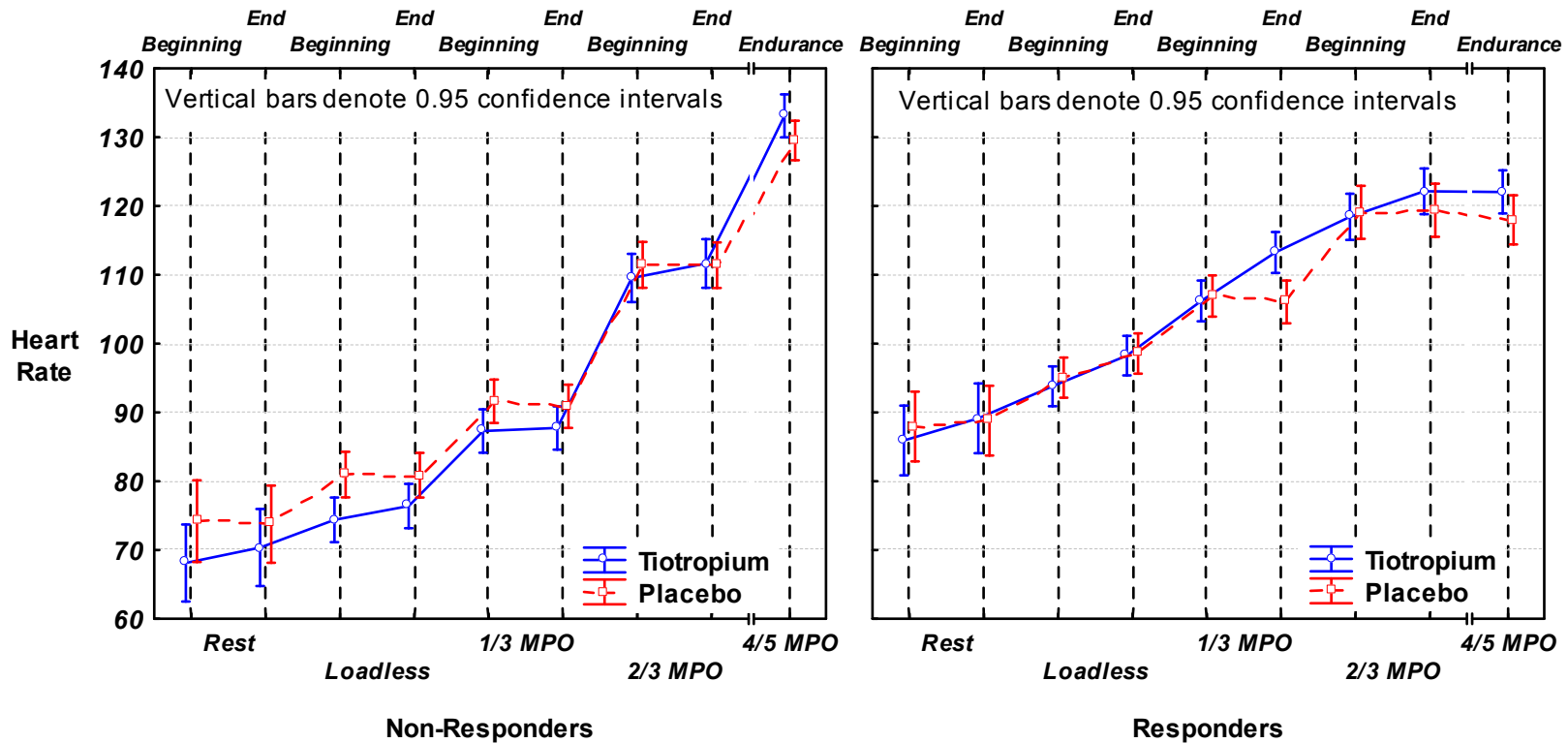
Figure 4.13. *Effects of tiotropium bromide on heart rate in Responders vs. Non-Responders.* Comparison of heart rate measured with tiotropium bromide (blue) vs. Placebo (red) in Responders and Non-Responders at rest and during cycle ergometer exercise at the start (Beginning) and completion (End) of treatment. Rest and exercise at loadless, 1/3 MPO, and 2/3 MPO performed during single exercise test performed at visits 2, 3, 5, and 6. Exercise at 4/5 MPO was performed at the end of each treatment period (visit 4 and 7) during a separate constant load endurance exercise test at 80% MPO (Endurance).
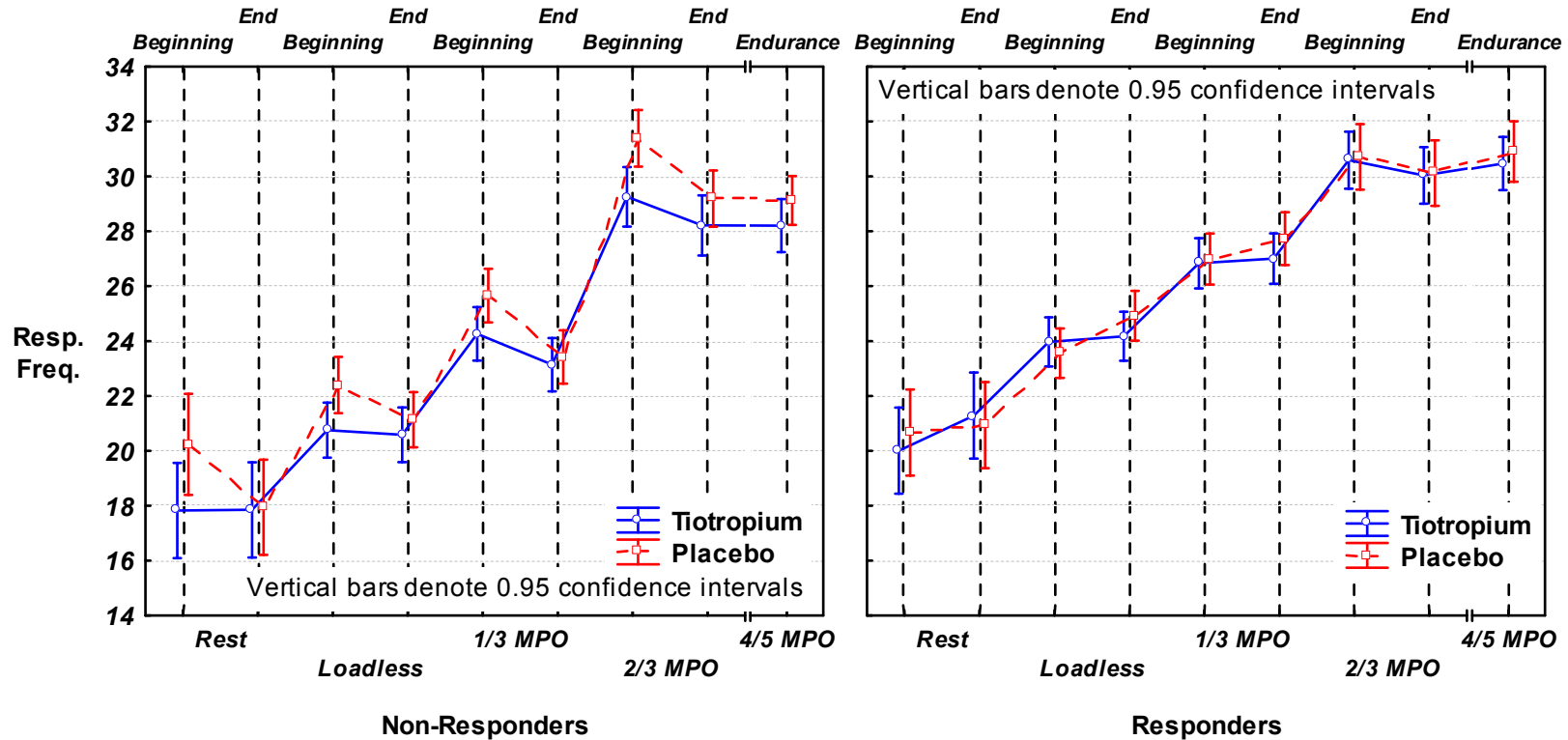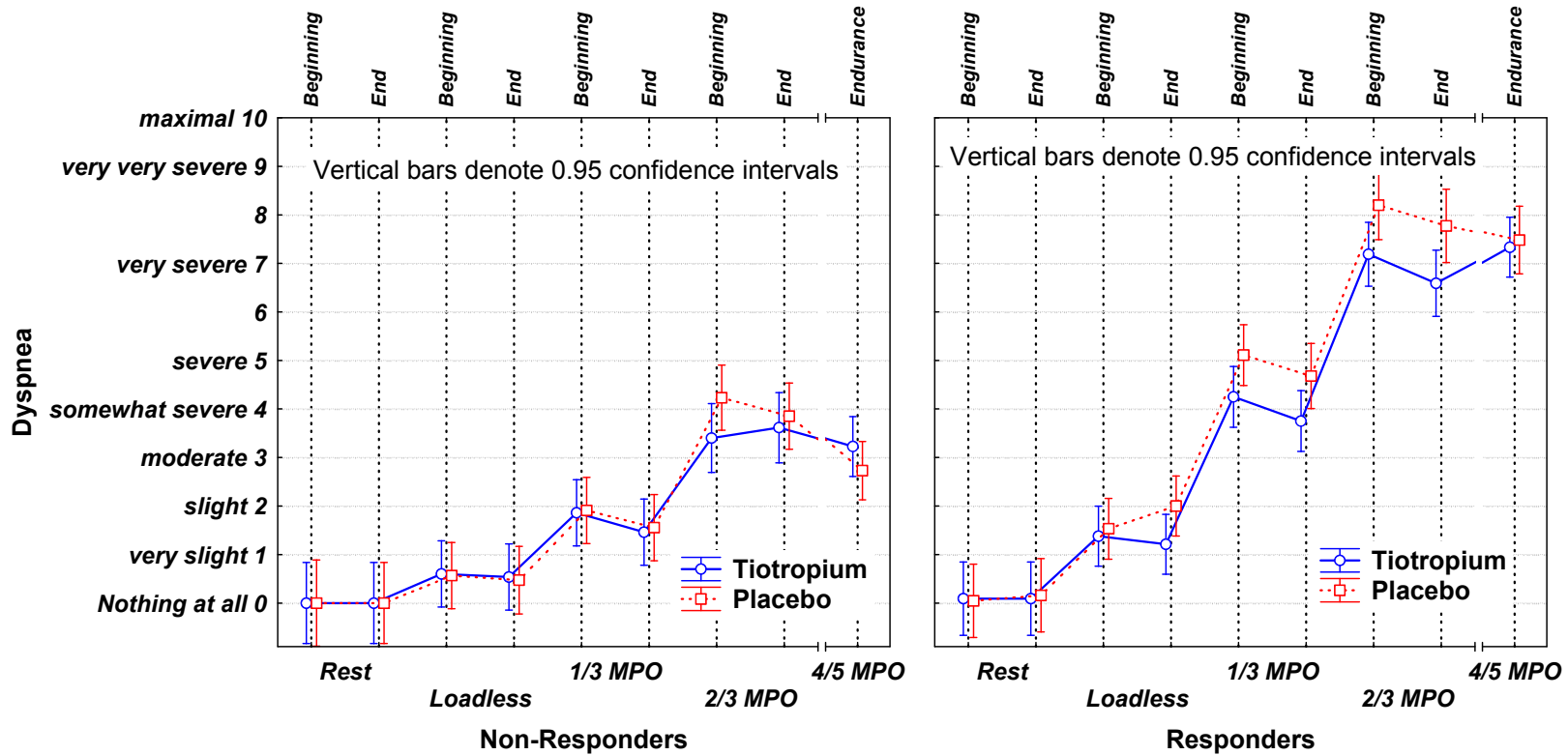
Figure 4.14. *Effects of tiotropium bromide on dyspnea (Borg scale rating) in Responders vs. Non-Responders.* Comparison of dyspnea measured with tiotropium bromide (blue) vs. Placebo (red) in Responders and Non-Responders at rest and during cycle ergometer exercise at the start (Beginning) and completion (End) of treatment. Rest and exercise at loadless, 1/3 MPO, and 2/3 MPO performed during single exercise test performed at visits 2, 3, 5, and 6. Exercise at 4/5 MPO was performed at the end of each treatment period (visit 4 and 7) during a separate constant load endurance exercise test at 80% MPO (Endurance).
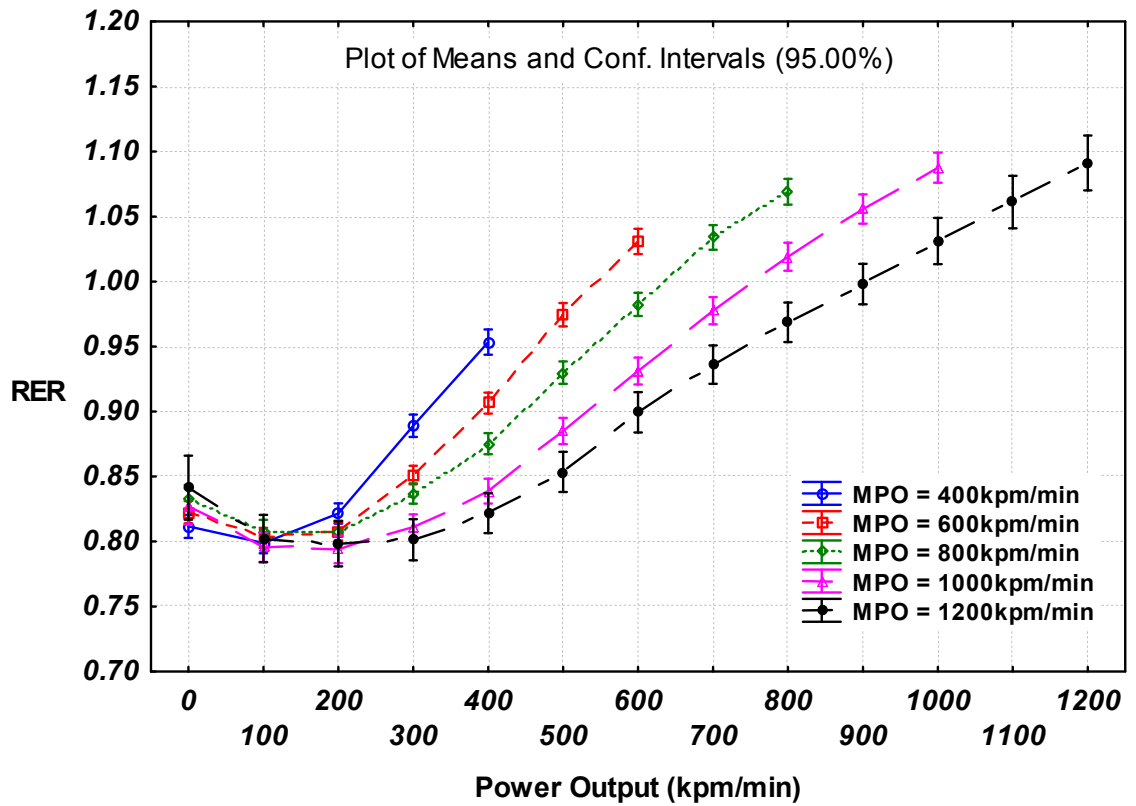
Figure 4.15. *Effect of exercise capacity (MPO) on the respiratory exchange ratio (RER) at different cycle ergometer exercise intensities (Power Output).* MPO = maximum power output.

Chapter 5 – Summary and Conclusions:

Factors Contributing to Maximum Power Output in COPD:

In chapter one the hypothesis was that ventilatory capacity, gas transfer capacity, and peripheral skeletal muscle strength contributed independently to maximum power output in COPD. In chapter two it was demonstrated that the three most important factors contributing to maximum power output in COPD were: Quadriceps Strength, Maximal Breathing Capacity, and DL_{CO}. This was proven by performing forward stepwise multiple linear regression (r^2=0.703) which yielded Quadriceps Strength (β=0.373), Maximal Breathing Capacity (β=0.262), and gas transfer capacity (DL_{CO}) (β=0.246) as the top three contributing factors and generated the following linear additive equation:

$MPO = -233.3 + 5.8*$Quadriceps Strength$ + 1.9*MBC + 10.5*DL_{CO}$ (Table 2.4). To determine if the variables in our regression analysis interacted, we performed a correlation matrix analysis. This showed that all the variables interacted to varying degrees (Table 2.5). As a result, a ridge regression analysis was performed to minimize the interactive effects and to determine the major contributing factors to maximum power output in COPD. This ridge regression analysis (r^2=0.677) supported the initial findings of the forward stepwise multiple linear regression, again demonstrating that the three most important factors contributing to maximum power output were Quadriceps Strength (β=0.328), Maximal Breathing Capacity (β=0.249), and gas transfer capacity (β=0.233)

and yielded the following equation: MPO = -206.3 + 5.1*Quadriceps Strength + 1.8*MBC + 10.0*DL_{CO} (Table 2.6).

Given that the coefficients (B) for Quadriceps Strength, MBC, and DL_{CO} from the ridge regression (Table 2.6) were nearly identical to those generated from the forward stepwise multiple linear regression (Table 2.4), we concluded that Quadriceps Strength, MBC, and DL_{CO} are largely independent in their effect on MPO. Furthermore, the continued presence of variability in MPO (~32%), and the possibility that the effects of Quadriceps Strength, MBC, and DL_{CO} on MPO may be non-linear, required the pursuit of additional contributing factors and consideration beyond linear additive models.

Analysis of variance (MANOVA) was performed, using the mean values for categories (based on increasing magnitude) of Quadriceps Strength (<20, 20-40, 40-60, & >60kg), MBC (<40, 40-80, 80-120, >120l/min), and DL_{CO} (<10, 10-20, 20-30, >30mL/mmHg/min). These relationships were derived from plots of MPO vs. Quadriceps Strength (Figure 2.4), MBC (Figure 2.5), and DL_{CO} (Figure 2.6). The slope of the relationship between MPO and Quadriceps Strength was found to be 11.25. These relationships were very similar to those generated from the linear regression analyses of MPO vs. Quadriceps Strength (slope = 11.7), MBC (slope = 4.9), and DL_{CO} (slope = 28.8). Next MPO was plotted against categories of MBC stratifying the population by categories of DL_{CO} (Figure 2.13) and Quadriceps Strength (Figure 2.14) (mean and 95% confidence limits). MPO was also plotted against categories of DL_{CO}, stratifying by categories of Quadriceps Strength (Figure 2.15) (mean and 95% confidence limits). In this three-dimensional way, if the line patterns produced by the stratification had the

same slope, then the effects of the x-axis factor and the stratification factor on MPO were

independent. This yielded plots which illustrated that MBC contributed to MPO,

independent of DL_{CO} (Figure 2.13) and Quadriceps Strength (Figure 2.14); given that the

direct relationship pattern (slope of line pattern) remained no matter what the DL_{CO} or

Quadriceps Strength was. Additionally, DL_{CO} was seen to contribute to MPO,

independent of MBC (Figure 2.13) and Quadriceps Strength (Figure 2.15); also showing

a direct relationship pattern that remained unchanged with stratification. Finally, the

same plots illustrated that Quadriceps Strength was directly related to MPO and was

independent of MBC (Figure 2.14) and DL_{CO} (Figure 2.15); given there was no change in

the pattern seen. To appreciate the independent effects of Quadriceps Strength, MBC,

and DLCO on MPO, a single plot of MPO against all three independent contributors was

generated (Figure 2.16). This plot illustrated the increase in MPO that occurs with

increases in Quadriceps Strength, MBC, and DL_{CO}, respectively. It also highlighted that

at low muscle strength, there are no individuals with a high DL_{CO}. Conversely, at high

muscle strength, there were no people with low DL_{CO} or MBC. Hence we concluded that

Quadriceps Strength, Maximal Breathing Capacity, and DL_{CO} were the three most

important contributing factors to maximum power output in COPD.

Contributing Factors to the Symptoms Limiting Exercise in COPD:

Next, we hypothesized that ventilation and ventilatory capacity were major

contributors to dyspnea during exercise in COPD, but were not equal in the magnitude of

their effects. Exercising patients stop exercising when the discomfort associated with

continuing becomes intolerable. The intensity of breathing effort, leg effort, or both are

the common symptoms limiting exercise. Thus, the physiological factors contributing to

maximum power output must also logically contribute to the intensity of the effort

required to breathe or cycle. In Chapter 2, we also explored the independent factors

contributing to the effort required to breathe or cycle, in a manner similar to that utilized

to explore the factors contributing to maximum power output was used. Forward

stepwise multiple linear regression illustrated that the ventilation ($\beta = 0.75$) and the

ventilatory capacity ($\beta = -0.21$) were the two most important contributing factors to

dyspnea; with the analysis accounting for 51% of the variability in dyspnea ($r^2 = 0.512$)

(Table 2.7). Ventilation increased with exercise intensity as expected. However, at

higher powers, ventilation progressively increased as maximum power output decreased

(Figure 2.8). Maximum power output increased as maximal breathing capacity increased,

as expected (Figure 2.9). Dyspnea intensified as exercise tolerance (MPO) decreased

(Figure 2.10). Taken together, we concluded that the resulting relationship sees dyspnea

increase as ventilation increases and ventilatory capacity decreases, which supported the

findings of the forward stepwise multiple linear regression analysis.

Correlation matrix analysis illustrated interactive effects between the contributing

factors (Table 2.8), necessitating a subsequent ridge regression analysis. Ridge

regression analysis showed that the V_E ($\beta = 0.67$) and the MBC ($\beta = -0.16$) were still the

two most important contributing factors to dyspnea, with the analysis accounting for 46%

of the variability in dyspnea ($r^2 = 0.457$) (Table 2.9). Given the difference in magnitude

of the standardized β for V_E and MBC (Table 2.9), further assessment of whether improvement in ventilatory capacity would have the same magnitude of effect on improving dyspnea as a reduction in the ventilation achieved during exercise was conducted.   Dyspnea was plotted against V_E, stratifying the population based on MBC (Figure 2.11a) and against MBC, stratifying the population based on V_E (Figure 2.12a). The effect of doubling ventilation achieved during exercise, a 4.5 fold increase in dyspnea, was far greater than the effect of halving the ventilatory capacity, which yielded a 1.4 fold increase in dyspnea. Assessment based on a doubling of ventilation and a halving of ventilatory capacity illustrated the effect of an equal relative magnitude change of each on dyspnea.   If the effects of V_E and MBC were equal in magnitude in their effect on dyspnea, the standardized β scores for each would be equal (though opposite in sign (+/-)) if the variability in both factors were the same in this population. This was clearly not the case as can be seen in Tables 2.7 and 2.9. Together, this strongly illustrated the unequal effects of V_E and MBC on dyspnea, and that reductions in ventilation during exercise, by the same relative degree as improvements in ventilatory capacity, would yield a much more pronounced decrease in dyspnea.

Effects of Tiotropium Bromide on Ventilatory Efficiency and Cardiac Output During Exercise in COPD:

Tiotropium bromide vs. Placebo:

The hypothesis tested was that the efficiency of gas exchange and cardiac output increase during exercise in patients with COPD receiving tiotropium bromide. In Chapter 3 a comparison of tiotropium bromide-treated to placebo-treated measurements of V_E/VO_2 and V_E/VCO_2, at rest and during exercise, illustrated no significant improvements in gas exchange efficiency for either oxygen ($p=0.72$) or carbon dioxide (Figures 3.3 & 3.4). Analysis of the efficiency of gas exchange for carbon dioxide actually showed a significant decrease ($p=0.005$) during exercise with tiotropium bromide (Figure 3.4).

The cardiac output was compared at the same oxygen uptake with and without tiotropium bromide in the same subjects. There was no significant improvement (slopes and intercepts) with tiotropium bromide (Figure 3.5).

Responders vs. Non-responders:

Because the beneficial effects of tiotropium bromide were not seen systematically in all subjects, a subgroup analysis was planned and performed. The responder population was comprised of subjects who showed any improvement in exercise endurance time, collected at visits 4 and 7, with tiotropium bromide compared to placebo. The non-responder population comprised subjects who showed no improvement or a decrease in exercise endurance time with tiotropium bromide compared to placebo. This

resulted in a responder n=11 and a non-responder n=9. Responders had an FEV_1

(mean±SD) of 1.27±0.54L, while non-responders had an FEV_1 of 1.47±0.42L (Tables 3.3

& 3.4). The responders had a placebo-treated exercise endurance time (mean±SD) of

239±130sec and a tiotropium bromide-treated exercise endurance time of 318±175sec.

Non-responders had a placebo-treated exercise endurance time (mean±SD) of

443±485sec and a tiotropium bromide-treated exercise endurance time of 376±355sec.

We concluded that the responder population was more impaired by their COPD,

compared to the non-responders.

Comparison of tiotropium bromide-treated to placebo-treated measurements of

V_E/VO_2 and V_E/VCO_2, at rest and during exercise, in our responders and non-responders

illustrated no significant improvements in gas exchange efficiency for either oxygen

(p=0.65) or carbon dioxide (p=0.19) in either group (Figures 3.6 & 3.7). Assessment of

cardiac output, relative to metabolic demand (VO_2), by comparing tiotropium bromide-

treated to placebo-treated measurements at rest and during exercise, in our responders

and non-responders, illustrated no significant improvements (slopes and intercepts within

one standard deviation) in either group (Figure 3.5).

Subgroup analysis of the effects of tiotropium bromide in COPD subjects that

showed improved exercise tolerance with tiotropium bromide (responders) and in those

that did not show improved exercise tolerance (non-responders), illustrated no significant

improvements in gas exchange efficiency or cardiac output, with tiotropium bromide, in

either group. Thus, we conclude that tiotropium bromide does not yield improved

exercise tolerance in COPD subjects through improvements in gas exchange efficiency or

cardiac output. Lack of support for these potential mechanisms of action of tiotropium

bromide suggests that the more popularly purported mechanisms, increasing ventilatory

capacity by bronchodilation and deflation reducing FRC and RV, are responsible for the

improvement in exercise tolerance (O'Donnell et al., 2004). Reducing hyperinflation

would result in the respiratory muscles being placed in a less stretched, more efficient,

position. As such, less effort would be required to drive the respiratory muscles to

generate ventilation, thus reducing dyspnea. Our previous study (Chapter 2) on the

factors limiting exercise in COPD showed dyspnea to be the most important symptom

limiting exercise in COPD. Tiotropium bromide-mediated reduction in dyspnea, through

reduction of hyperinflation, could explain the improvement in exercise tolerance seen

with tiotropium bromide treatment.

Effects of Tiotropium Bromide on Ventilatory Efficiency and Cardiac Output
During Exercise in COPD: Additional Variables Considered:
Tiotropium bromide vs. Placebo:

Arguably several measured variables, not central to the hypothesis, were more

informative and interesting than the central hypothesis. In Chapter 4 we addressed

additional variables, not central to the hypothesis, that were collected during the double-

blind, placebo-controlled, crossover study of tiotropium bromide including: oxygen

uptake (VO_2), carbon dioxide production (VCO_2), minute ventilation (V_E), alveolar

ventilation (V_A), tidal volume (V_T), anatomical deadspace (Fowler Deadspace, V_D),

respiratory exchange ratio (RER), fractional end-tidal oxygen ($F_{et}O_2$), fractional end-tidal

carbon dioxide ($F_{et}CO_2$), arterial oxygen saturation (SpO_2), heart rate (HR), oxygen pulse (VO_2/HR), oxygen uptake per kilogram body weight (VO_2/kg), respiratory frequency (Resp. Freq.), dyspnea, and leg effort.

Tidal volume and alveolar ventilation increased with tiotropium bromide during exercise ($p<0.0001$ and $p=0.005$) (Figures 4.1 & 4.2). In our study in Chapter 2 (retrospective database analysis) using incremental exercise in 4,424 patients with COPD, tidal volume increased with the power output reaching a maximum value of $53.8\pm11.3\%$ (mean$\pm$SD) of the vital capacity. The vital capacity increased to an even greater extent than the FEV_1 in the large randomized prospective control trials comparing tiotropium bromide to placebo treatment, included in the metanalysis by Barr (Barr, Bourbeau, Camargo, & Ram, 2006). Hence, we conclude it is not surprising and perhaps expected that the tidal volume during exercise would increase.   The increase in alveolar ventilation (Total Ventilation – Fowler Deadspace Ventilation) with tiotropium bromide would also be anticipated. Increases in airway resistance reduce the capacity to generate ventilation. Reduced ventilation, at any given power output, might be expected. Progressive increases in inspiratory resistance are accompanied by progressive decreases in alveolar ventilation (el-Manshawi, Killian, Summers, & Jones, 1986). Hence, we conclude it is reasonable to assume that the converse (i.e. reducing airway resistance) would increase alveolar ventilation; which was seen.

The respiratory frequency was reduced ($p=0.0007$) with tiotropium bromide (Figure 4.3), resulting from the permitted increase in tidal volume associated with the

demonstrated increase in vital capacity. We conclude that with tidal volume elevated, fewer breathes were necessary to achieve the same ventilation.

Alveolar ventilation was improved (p=0.005) with tiotropium bromide (Figure 4.2) while total ventilation at any given power was unchanged (p=0.05). The Fowler deadspace was increased (p=0.001) with tiotropium bromide (Figure 4.4). At the simplest level, large airways dilated. The total deadspace is the sum of the anatomical dead space and the physiological deadspace due to alveolar units with a high V/Q. We conclude that the physiological dead space had to decrease because alveolar ventilation and Fowler deadspace increased while total ventilation remained unchanged.

As expected with the increase in alveolar ventilation during exercise, fractional end-tidal carbon dioxide declined (p<0.0001) with tiotropium bromide (Figure 4.5). Progressive increases in inspiratory resistance result in progressive increases in $F_{et}CO_2$ (Jones, Killian, Summers, & Jones, 1985). We conclude that reduced airway resistance resulting from bronchodilation would logically lead to increased alveolar ventilation.

Arterial oxygen saturation increased (p=0.001) with tiotropium bromide (Figure 4.6). Progressive increases in inspiratory resistance result in progressive decreases in alveolar ventilation and arterial oxygen desaturation (el-Manshawi et al., 1986; Jones et al., 1985). We conclude the increase in SpO_2 is likely a product of the reduced airway resistance, resulting from bronchodilation, leading to increased alveolar ventilation.

The respiratory exchange ratio was reduced (p<0.0001) with tiotropium bromide (Figure 4.7). We conclude the presence of a lower RER with tiotropium bromide suggests reduced stress in broad general terms.

From the prospective of the patient, which is arguably the most important, dyspnea was reduced (p=0.008) with tiotropium bromide (Figure 4.8). Popularly purported mechanisms of action for tiotropium bromide focus on increasing ventilatory capacity by bronchodilation and reducing functional residual capacity and residual volume (O'Donnell et al., 2004). We conclude a reduction in hyperinflation resulted in the respiratory muscles being placed in a less stretched, more efficient, length. As such, less effort was required to drive the respiratory muscles to generate ventilation, thus reducing dyspnea.

Responders vs. Non-responders:

Respiratory frequency was reduced (p=0.03) at rest and during exercise in the Non-Responders with tiotropium bromide, compared to placebo (Figure 4.9).

Fractional end-tidal carbon dioxide was reduced (p=0.001) during exercise with tiotropium bromide, compared to placebo in the Responders (Figure 4.10).

Arterial oxygen saturation was increased (p=0.04) during exercise with tiotropium bromide, compared to placebo, in the Non-Responders (Figure 4.11). We conclude the increase in SpO_2 is likely a product of the reduced airway resistance, resulting from bronchodilation, leading to increased alveolar ventilation.

The respiratory exchange ratio was reduced (p=0.0002) with tiotropium bromide, compared to placebo, at rest and during exercise in the Responders (Figure 4.12). We conclude the presence of a lower RER with tiotropium bromide suggests reduced stress in broad general terms.

Heart rate was reduced (p=0.003) during exercise with tiotropium bromide, compared to placebo in the Non-Responders (Figure 4.13). The improved oxygenation (increased SpO_2) of the blood with tiotropium bromide, also seen in the Non-Responders (p=0.04), likely resulted in improved support of the exercising muscle through delivery of blood with a higher oxygen content. Oxygen therapy has been shown to increase SpO_2 and reduce heart rate in COPD patients, resulting in no net change in arterial oxygen flow (Mannix, Manfredi, Palange, Dowdeswell, & Farber, 1992; Palange et al., 1995). We conclude, given that tiotropium bromide increases SpO_2, blood flow would not need to be maintained at the same level in order to deliver the same arterial oxygen flow to the working muscle. As such, heart rate would be reduced as the demand for blood flow decreases.

Dyspnea was also reduced (p=0.04) during exercise with tiotropium bromide, compared to placebo, in the Responders (Figure 4.14).). Popularly purported mechanisms of action for tiotropium bromide focus on increasing ventilatory capacity by bronchodilation and reducing functional residual capacity and residual volume (O'Donnell et al., 2004). We conclude that a reduction in hyperinflation resulted in the respiratory muscles being placed in a less stretched, more efficient, length. As such, less effort was required to drive the respiratory muscles to generate ventilation, thus reducing dyspnea.

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Appendix 1 – Additional Models

This appendix contains analyses that included the variables: mean inspiratory pressure (MIP), mean expiratory pressure (MEP), hemoglobin (Hb), and carboxyhemoglobin (HbCO), in addition to the variables included in the primary analysis presented in Chapter 2.

No differences in the results presented in Chapter 2 were obtained by performing these additional analyses.

Tables:

Table A1.1. *Forward stepwise multiple linear regression showing the independent contributors to MPO.*

Regression Summary for Dependent Variable: MPO R= .86778830 R²= .75305653 Adjusted R²= .75164273 p < 0.0000 Std. Error of estimate: 153.70						
	Beta	**Std. Err. of Beta**	**B**	**Std. Err. of B**	**t(1572)**	**p-level**
Intercept			-393.059	109.6949	-3.58320	0.000350
DL$_{CO}$ (mL/mmHg/min)	0.317696	0.020121	14.770	0.9354	15.78945	0.000000
Quad Strength (kg)	0.292078	0.020475	4.778	0.3350	14.26519	0.000000
MBC (L/min)	0.242622	0.020908	1.900	0.1638	11.60409	0.000000
Age (years)	-0.087116	0.016252	-2.284	0.4261	-5.36028	0.000000
HbCO (g/dL)	-0.044007	0.013283	-8.405	2.5369	-3.31294	0.000944
Height (cm)	0.080085	0.019211	259.237	62.1867	4.16869	0.000032
Gender (% male)	-0.059008	0.018583	-39.838	12.5459	-3.17538	0.001525
HB (g/dL)	0.037472	0.013676	8.035	2.9323	2.74004	0.006213
MIP (mmHg)	0.047338	0.017492	0.519	0.1919	2.70631	0.006877

DL$_{CO}$ = diffusing capacity for carbon monoxide, Quad Strength = strength of quadriceps muscle, MBC = maximal breathing capacity, HbCO = carboxyhemoglobin, HB = hemoglobin, MIP = maximum inspiratory pressure

Table A1.2. *Correlation matrix analysis of contributing factors to MPO.*

		Age	Gender	Height	MBC	MIP	MEP	Hb	HbCO	DL$_{CO}$	Quad Strength
Correlations											
Age (years)		1.00	0.04	-0.16*	-0.35*	-0.38*	-0.25*	-0.14*	-0.23*	-0.44*	-0.42*
Gender (% male)		0.04	1.00	0.67*	0.46*	0.33*	0.41*	0.29*	-0.02	0.42*	0.51*
Height (cm)		-0.16*	0.67*	1.00	0.58*	0.37*	0.35*	0.25*	-0.04	0.55*	0.57*
MBC (L/min)		-0.35*	0.46*	0.58*	1.00	0.63*	0.54*	0.23*	-0.07*	0.70*	0.66*
MIP (mmHg)		-0.38*	0.33*	0.37*	0.63*	1.00	0.68*	0.18*	-0.00	0.52*	0.61*
MEP (mmHg)		-0.25*	0.41*	0.35*	0.54*	0.68*	1.00	0.22*	-0.01	0.46*	0.58*
Hb (g/dL)		-0.14*	0.29*	0.25*	0.23*	0.18*	0.22*	1.00	0.04	0.33*	0.33*
HbCO (g/dL)		-0.23*	-0.02	-0.04	-0.07*	-0.00	-0.01	0.04	1.00	-0.07*	-0.02
DL$_{CO}$ (mL/mmHg/ min)		-0.44*	0.42*	0.55*	0.70*	0.52*	0.46*	0.33*	-0.07*	1.00	0.65*
Quad Strength (kg)		-0.42*	0.51*	0.57*	0.66*	0.61*	0.58*	0.33*	-0.02	0.65*	1.00

* = significant at $p < 0.05$, MBC = maximal breathing capacity, MIP = maximum inspiratory pressure, MEP = maximum expiratory pressure, Hb = hemoglobin, HbCO = carboxyhemoglobin, DL$_{CO}$ = diffusing capacity for carbon monoxide, Quad Strength = strength of quadriceps muscle

Table A1.3. *Ridge regression analysis showing the independent contributors to MPO.*

	Beta	Std. Err. of Beta	B	Std. Err. of B	t(1571)	p-level
Ridge Regression Summary for Dependent Variable: MPO R= .85378496 R²= .72894876 Adjusted R²= .72722342 p < 0.0000 Std. Error of estimate: 161.08						
Intercept			-400.300	101.6049	-3.93977	0.000085
DL$_{CO}$ (mL/mmHg/min)	0.286689	0.018366	13.328	0.8539	15.60941	0.000000
Quad Strength (kg)	0.251781	0.018938	4.119	0.3098	13.29512	0.000000
MBC (L/min)	0.224965	0.018960	1.762	0.1485	11.86550	0.000000
Age (years)	-0.102094	0.015354	-2.677	0.4026	-6.64952	0.000000
Height (cm)	0.088744	0.017679	287.268	57.2287	5.01965	0.000001
MIP (mmHg)	0.056621	0.017974	0.621	0.1971	3.15023	0.001662
HbCO (g/dL)	-0.046136	0.013080	-8.811	2.4980	-3.52737	0.000432
Hb (g/dL)	0.041770	0.013468	8.956	2.8878	3.10132	0.001961
Gender (% male)	-0.035546	0.017269	-23.998	11.6589	-2.05835	0.039721
MEP (mmHg)	0.028976	0.016916	0.223	0.1302	1.71292	0.086925

DL$_{CO}$ = diffusing capacity for carbon monoxide, Quad Strength = strength of quadriceps muscle, MBC = maximal breathing capacity, MIP = maximum inspiratory pressure, HbCO = carboxyhemoglobin, Hb = hemoglobin, MEP = maximum expiratory pressure

Table A1.4. *Forward stepwise multiple linear regression showing the independent contributors to Dyspnea.*

	Beta	Std. Err. of Beta	B	Std. Err. of B	t(1575)	p-level
Regression Summary for Dependent Variable: Dyspnea R= .20720153 R²= .04293248 Adjusted R²= .03928650 p < 0.0000 Std. Error of estimate: 2.4045						
Intercept			6.620445	0.571935	11.57552	0.000000
V$_E$ (L/min)	0.254199	0.043904	0.030863	0.005330	5.78991	0.000000
MBC (L/min)	-0.172559	0.043951	-0.010750	0.002738	-3.92616	0.000090
Age (years)	-0.118478	0.030881	-0.024708	0.006440	-3.83660	0.000130
Gender (% male)	0.064031	0.029941	0.343834	0.160779	2.13855	0.032625
HbCO (g/dL)	-0.051178	0.026045	-0.077743	0.039564	-1.96497	0.049593
DL$_{CO}$ (mL/mmHg/min)	-0.063334	0.038651	-0.023420	0.014293	-1.63861	0.101495

V$_E$ = minute ventilation, MBC = maximal breathing capacity, HbCO = carboxyhemoglobin, DL$_{CO}$ = diffusing capacity for carbon monoxide

Table A1.5. *Correlation matrix analysis of contributing factors to Dyspnea.*

	Age	Gender	Height	MBC	MIP	MEP	Hb	HbCO	DL$_{CO}$	Quad Strength
					Correlations					
Age (years)	1.00	0.04	-0.16*	-0.35*	-0.38*	-0.25*	-0.14*	-0.23*	-0.44*	-0.42*
Gender (% male)	0.04	1.00	0.67*	0.46*	0.33*	0.41*	0.29*	-0.02	0.42*	0.51*
Height (cm)	-0.16*	0.67*	1.00	0.58*	0.37*	0.35*	0.25*	-0.04	0.55*	0.57*
MBC (L/min)	-0.35*	0.46*	0.58*	1.00	0.63*	0.54*	0.23*	-0.07*	0.70*	0.66*
MIP (mmHg)	-0.38*	0.33*	0.37*	0.63*	1.00	0.68*	0.18*	-0.00	0.52*	0.61*
MEP (mmHg)	-0.25*	0.41*	0.35*	0.54*	0.68*	1.00	0.22*	-0.01	0.46*	0.58*
Hb (g/dL)	-0.14*	0.29*	0.25*	0.23*	0.18*	0.22*	1.00	0.04	0.33*	0.33*
HbCO (g/dL)	-0.23*	-0.02	-0.04	-0.07*	-0.00	-0.01	0.04	1.00	-0.07*	-0.02
DL$_{CO}$ (mL/mmHg/ min)	-0.44*	0.42*	0.55*	0.70*	0.52*	0.46*	0.33*	-0.07*	1.00	0.65*
Quad Strength (kg)	-0.42*	0.51*	0.57*	0.66*	0.61*	0.58*	0.33*	-0.02	0.65*	1.00

* = significant at $p < 0.05$, MBC = maximal breathing capacity, MIP = maximum inspiratory pressure, MEP = maximum expiratory pressure, Hb = hemoglobin, HbCO = carboxyhemoglobin, DL$_{CO}$ = diffusing capacity for carbon monoxide, Quad Strength = strength of quadriceps muscle

Table A1.6. *Ridge regression analysis showing the independent contributors to Dyspnea.*

	Beta	Std. Err. of Beta	B	Std. Err. of B	t(1574)	p-level
Ridge Regression Summary for Dependent Variable: Dyspnea R= .18786264 R²= .03529237 Adjusted R²= .03100206 p < 0.0000 Std. Error of estimate: 2.4148						
Intercept			6.165346	0.535912	11.50440	0.000000
V$_E$ (L/min)	0.171786	0.037771	0.020857	0.004586	4.54808	0.000006
MBC (L/min)	-0.113521	0.037318	-0.007072	0.002325	-3.04196	0.002389
Age (years)	-0.095382	0.028910	-0.019891	0.006029	-3.29931	0.000991
Gender (% male)	0.041308	0.028812	0.221816	0.154717	1.43369	0.151860
HbCO (g/dL)	-0.039490	0.024618	-0.059987	0.037396	-1.60412	0.108888
DL$_{CO}$ (mL/mmHg/min)	-0.048046	0.034416	-0.017767	0.012726	-1.39604	0.162899
Quad Strength (kg)	0.039827	0.034355	0.005182	0.004470	1.15926	0.246525

V_E = minute ventilation, MBC = maximal breathing capacity, HbCO = carboxyhemoglobin, DL_{CO} = diffusing capacity for carbon dioxide, Quad Strength = strength of quadriceps muscle
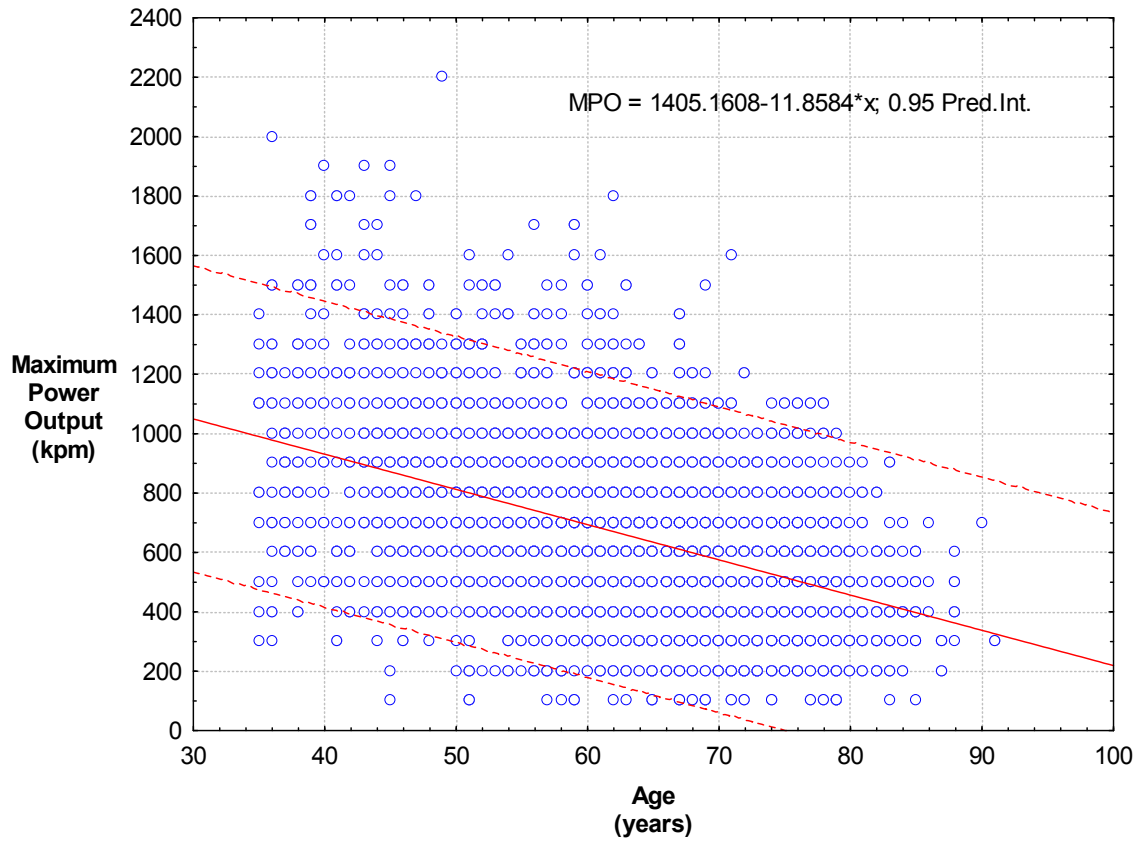
Figures:

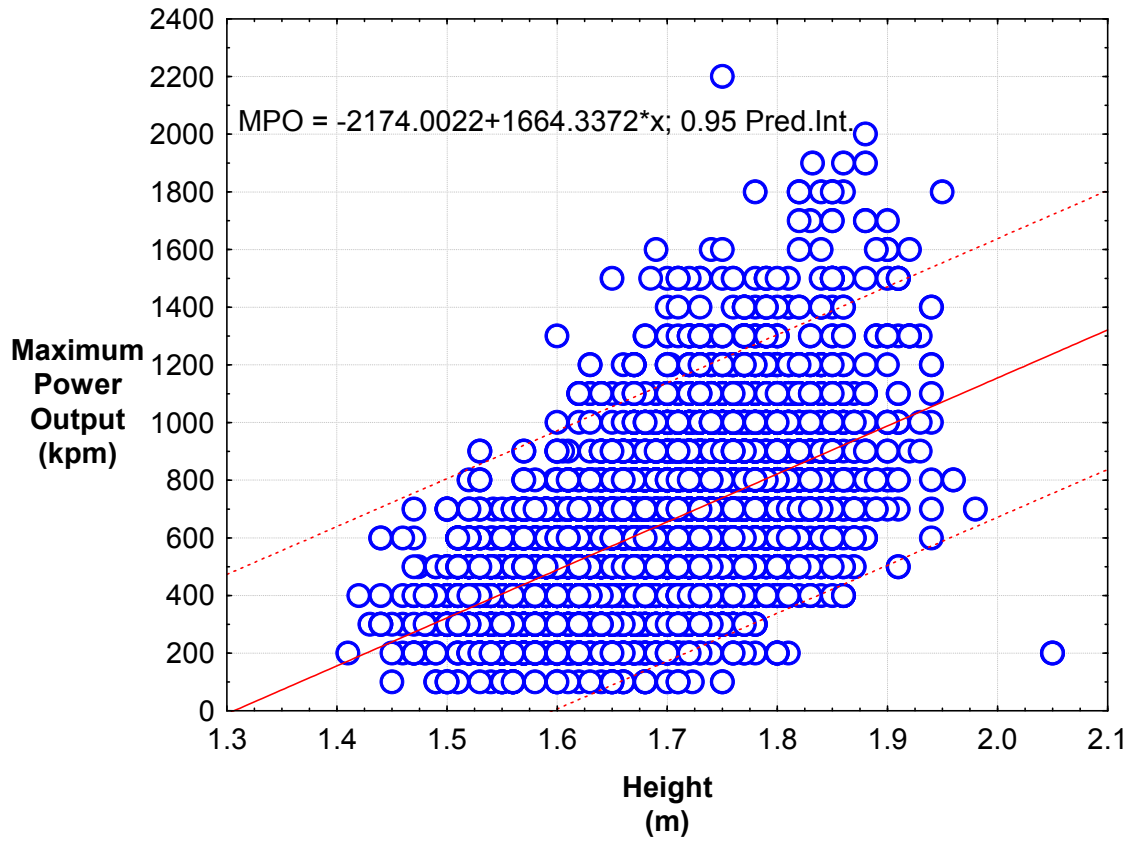Figure A1.1. *Maximum power output (MPO) achieved based on age.*

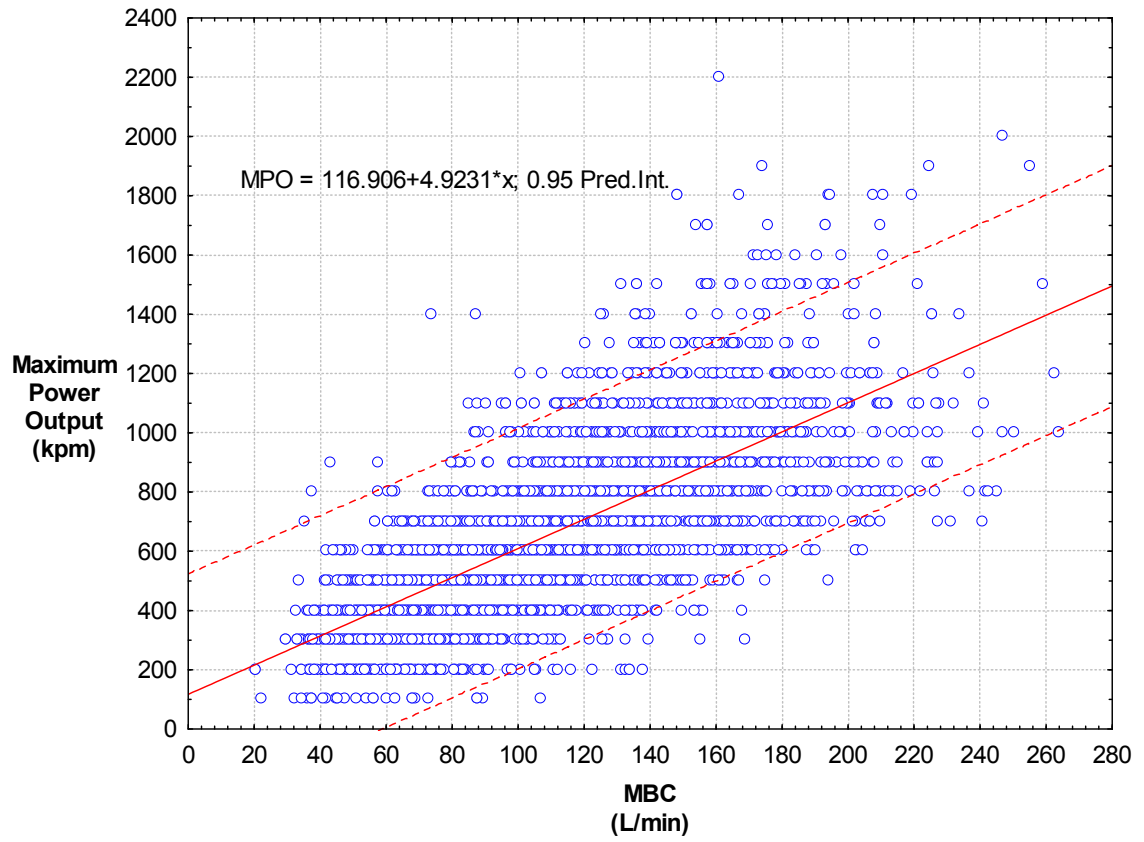Figure A1.2. *Maximum power output (MPO) achieved based on height.*

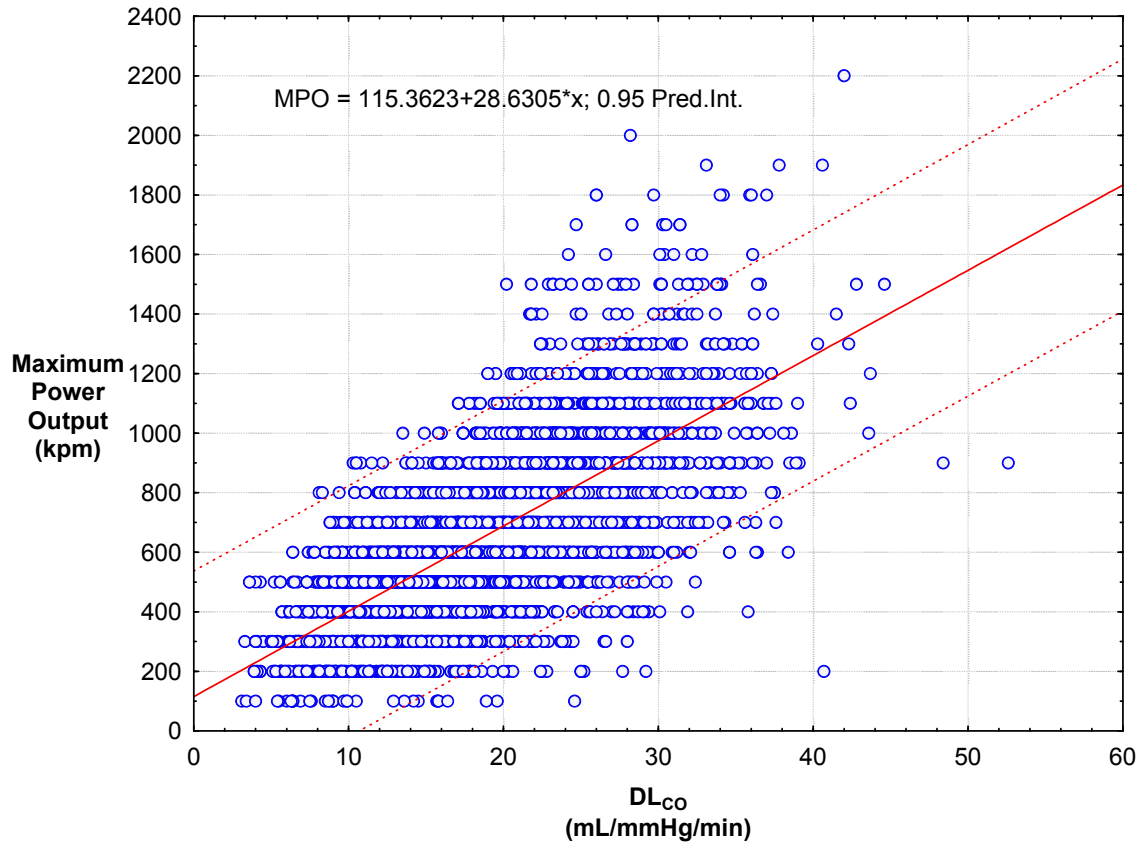Figure A1.3. *Maximum power output (MPO) achieved based on ventilatory capacity (MBC).*

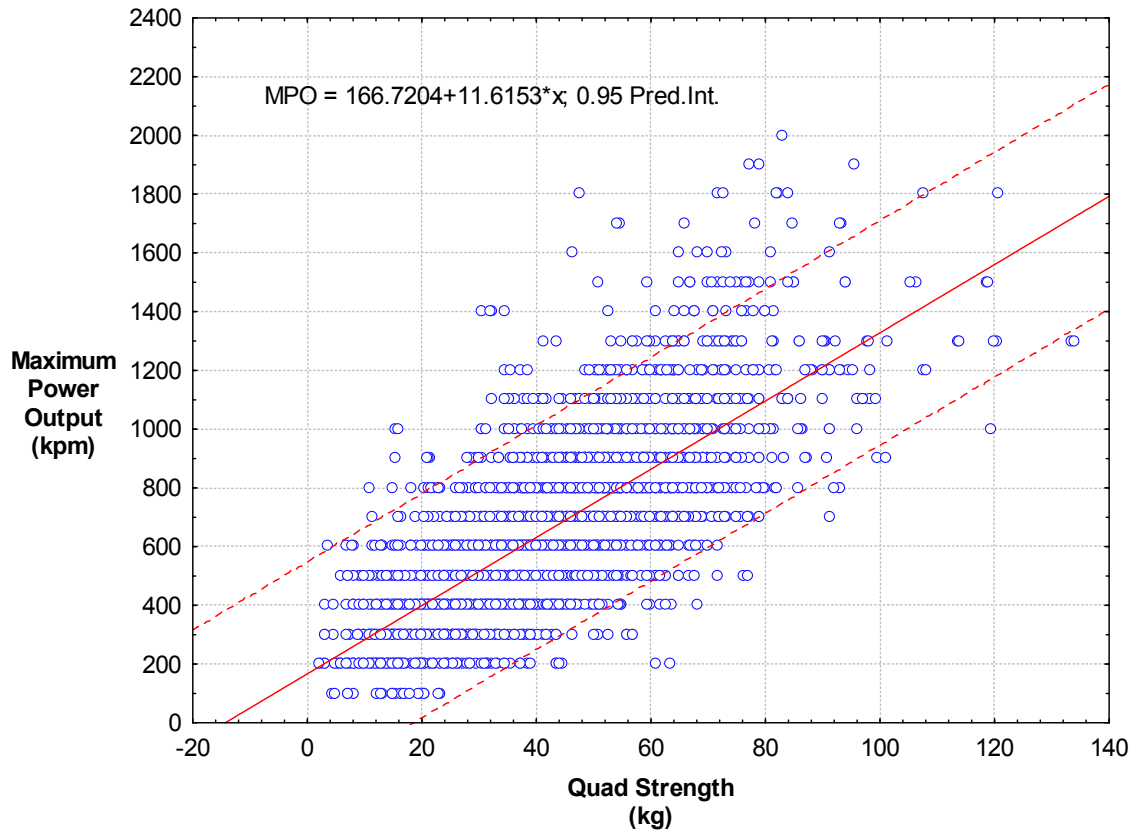Figure A1.4. *Maximum power output (MPO) achieved based on diffusion capacity (DL_CO).*

Figure A1.5. *Maximum power output (MPO) achieved based on exercising muscle strength (Quad Strength).*

Appendix 2 - Additional Pharmacological Treatments for COPD:

Xanthines:

Theophylline is a methylxanthine that has been used to treat COPD for over 70 years. Though it has fallen out of favor, theophylline is still used in developing countries due to its inexpensive cost. Though theophylline has been used for such a lengthy period of time, its modes of action in the body are still not completely clear. β agonist and anticholinergic therapy have replaced the use of xanthenes in obstructive airway disease. Theophylline is still occasionally added to these safer agents to achieve clinical improvement (ZuWallack et al., 2001). Theophylline given systemically acts on small airways reducing hyperinflation and dyspnea (Chrystyn, Mulley, & Peake, 1988).

As a bronchodilator, theophylline directly relaxes smooth muscle *in vitro* and acts as a functional antagonist, blocking the effects of bronchoconstrictor agonists (Finney, Karlsson, & Persson, 1985). Bronchodilation mediated by theophylline is believed to result largely from phosphodiesterase (PDE) inhibition, which leads to increased cyclic adenosine 3', 5'-monophosphate (cAMP) via PDE3 and PDE4, and increased guanosine 3', 5'-monophosphate (cGMP) via PDE5 (Rabe, Magnussen, & Dent, 1995). Theophylline's bronchodilatory effects in human airways is impaired by co-administration of charybdotoxin, which blocks large-conductance calcium-activated potassium channels; which suggests theophylline opens these potassium channels via increased cAMP (Miura, Belvisi, Stretton, Yacoub, & Barnes, 1992).

There is also evidence that theophylline has anti-inflammatory properties in COPD. Low doses of theophylline have been shown to reduce the total and proportion of

neutrophils in sputum, the concentration of interleukin-8 (IL8), and myeloperoxidase and neutrophil chemotactic responses (Culpitt et al., 2002). Additionally, an ability to reduce myeloperoxidase and neutrophil elastase, after 4 weeks of treatment, gives theophylline an edge over inhaled corticosteroids which fail to illicit the same effects (Culpitt et al., 1999; Keatings, Jatakanon, Worsdell, & Barnes, 1997; Kobayashi et al., 2004; Loppow et al., 2001).

At the molecular level, therapeutic concentrations of theophylline activates histone deacetylase (HDAC) activity, resulting in reduced expression of pro-inflammatory genes; which is blocked by trichostatin A, a nonselective HDAC inhibitor (Ito et al., 2002). Increased HDAC activity has also been seen in bronchial biopsies from asthmatics treated with low doses (mean plasma concentration ~ 5mg/L) of theophylline, illustrating theophylline's ability to activate HDAC *in vivo* (Ito et al., 2002). Theophylline is also able to increase, to above normal levels, the reduced HDAC activity in COPD alveolar macrophages (Cosio et al., 2004). However, the exact pathway through which theophylline activates HDACs is as yet unclear.

Adverse effects of theophylline are the major reason for its reduced usage. Adverse effects include life threatening arrhythmias and seizures, while minor side effects such as headache, nausea and vomiting, abdominal discomfort, and restlessness are difficult to tolerate (Barnes, 2005). Additionally, increased acid secretion, gastroesophageal reflux, and diuresis are common (Barnes, 2005). High doses are associated with convulsions, cardiac arrhythmias, and death (Barnes, 2005). Elimination

half lives vary widely within and between patients making these drugs very difficult to use.

β Agonists:

The early adrenergic agents stimulated the heart while relaxing airway smooth muscles. Cardiac arrhythmias were common. The usage of selective β_2 agonists in COPD is now universal. Most all β_2 agonists are similar in their selectivity for β_2 adrenoreceptors (Undem & Lichtenstein, 2001). Short acting β_2 agonists induce bronchodilation in as little as 1 to 5 minutes and have effects which last for approximately 4 hours (Rennard, 2004). Long acting β_2 agonists have a bronchodilatory effect which can last for upwards of 12 hours. Salmeterol has a slow onset of action while formoterol has a rapid onset similar to the short acting β_2 agonists (Rennard, 2004; Undem & Lichtenstein, 2001).

Selective β_2 agonists stimulate β_2 adrenoreceptors on airway smooth muscle resulting in stimulation of a G protein cascade involving the G_S protein which binds guanosine triphosphate (GTP) and activates adenylate cyclase, causing an increase in intracellular cyclic adenosine 3', 5'-monophosphate (cAMP) and subsequent activation of protein kinase A (PKA) (Johnson & Coleman, 1995). PKA activation causes myosin light chain phosphorylation reducing its affinity for calcium-calmodulin, reducing formation of active myosin light chain kinase, reduction in myosin phosphorylation, and a reduction in actin-myosin crossbridge formation; leading to bronchodilation (Johnson & Coleman, 1995). PKA stimulation also results in a reduction in intracellular calcium via

increased removal from the cell, reduced entry into the cell, and increased uptake of calcium by the smooth endoplasmic reticulum (Johnson & Coleman, 1995). Stimulation of β_2 adrenoreceptors can also increase potassium channel conduction, leading to hyperpolarization and relaxation of airway smooth muscle (Kume, Hall, Washabau, Takagi, & Kotlikoff, 1994). Activation of β_2 adrenoreceptors on inflammatory cells has been shown to increase cAMP which subsequently leads to decreased release of inflammatory mediators (leukotrienes, histamine, and cytokines) (Barnes, 1999; Hughes, Seale, & Temple, 1983). β_2 agonists have also been shown to increase mucociliary clearance, decrease microvascular permeability, and might inhibit phospholipase A_2 resulting in reduced synthesis of leukotrienes, prostiglandins, and thromboxane A_2, though the therapeutic value of these effects in COPD is not known (Montuschi, 2006; Seale, 1988).

The main side effect of selective β_2 agonists is muscle tremor, though this tends to diminish with prolonged usage (Montuschi, 2006). Higher doses can illicit tachycardia through stimulation of cardiac β_1 adrenoreceptors and also, though to a lesser extent, through stimulation of cardiac β_2 adrenoreceptors (Montuschi, 2006; Undem & Lichtenstein, 2001). Tachycardia is an important side effect to consider when treating COPD in individuals who also have ischemic heart disease or arrhythmias, as bronchodilation can result in ventilation/perfusion mismatching that generates a drop in arterial partial pressure of oxygen; though this is generally transitory and unimportant (Montuschi, 2006).

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Appendix 3- Feasibility and repeatability of non-invasive cardiac output and gas exchange efficiency measurements during exercise

Introduction:

The working hypothesis of the main study was that the improvement in exercise tolerance and reduced dyspnea in patients with COPD treated with bronchodilator medication relates to improvements in gas exchange efficiency and cardiac output. This preliminary study addressed the feasibility and repeatability of measurements of cardiac output and gas exchange efficiency (V_E/VO_2 & V_E/VCO_2) in normal subjects during exercise. The sensitivity and comparability of two non-invasive techniques for measuring cardiac output was assessed with the aim to use the second, more convenient, method during the main study. Because this methodology has not been widely used, this preliminary study was considered necessary. These measurements were tested in a normal subject population and demonstrated sufficient sensitivity for the randomized, crossover design, in patients with COPD of the main study.

Methods:

Study Design:

This was a trial to evaluate the reproducibility of cardiac output and gas exchange efficiency measurements, during exercise, in healthy subjects.

Part 1 consisted of subject screening. After informed consent, subjects attended an initial screening visit (Visit 1) for review of medical history, clinical assessment, and complete pulmonary function testing that included plethysmography and spirometry. This was followed by a symptom-limited incremental cycle exercise test, with measurement of incremental and peak oxygen uptake (VO_2), carbon dioxide output (VCO_2), minute ventilation (V_E), tidal volume (V_T), respiratory frequency, heart rate (HR), oxyhemoglobin saturation by pulse oximetry (SpO_2), and modified Borg score for leg effort and dyspnea. Only subjects with no history of smoking, an $FEV_1 > 80\%$ predicted normal and $FEV_1/FVC > 70\%$, and who were able to exercise a minimum of 50 watts during the incremental test were enrolled.

Part 2 of the study involved two incremental cycle exercise tests (Visits 2 and 3), separated by approximately 1 week. Subjects had gas exchange efficiency, cardiac output, and ventilatory measurements (incremental and peak VO_2, VCO_2, V_E, V_T, and respiratory frequency) evaluated on both occasions using an identical exercise test on each occasion. Each test consisted of 2 minutes of rest, followed by 6 minutes of pedaling at each of the following intensities: loadless, 1/3 of maximum power output (MPO) from visit 1, and 2/3 MPO from visit 1. Subjects were monitored for adequate recovery.

Study Population:

Eight healthy subjects (7 male, 1 female) were enrolled. All subjects were between 18 and 65 years of age, with no history of cigarette smoking or cardiorespiratory disease. All subjects conformed to the following spirometric criteria (measured at screening: Visit 1): FEV_1>80% predicted normal and FEV_1/FVC>70%. Subjects with significant diseases, or who had a myocardial infarction, cardiac arrhythmia, or respiratory infection in the 6 weeks prior to enrolment were excluded from the study. Specific demographics for the subject population are presented in Table A3.1.

Primary Endpoints:

The primary endpoints were the repeatability of cardiac output and gas exchange efficiency measurements during exercise.

Spirometry and Body Plethysmography:

Forced vital capacity over a minimum of 6 seconds (FVC), FEV_1, and a maximum inspiratory and expiratory flow volume manoeuvre were measured conforming to American Thoracic Society (ATS) minimal performance recommendations (Miller et al., 2005). The spirometer flow sensor was calibrated prior to pulmonary function testing. A 3 liter calibration syringe was attached to the flow sensor and, after a zero flow reading was captured 5-15 complete cycles of the syringe (in and out) at varying rates of speed (flow rate) were performed. This process was then repeated to verify the results and the flow and volume measurements were updated. Calibration results were

maintained in a calibration log. Lung volumes were measured with a pressure body

plethysmograph (body box), conforming to the guidelines from the American Thoracic

Society (Wanger et al., 2005). Calibration of the body plethysmograph occurred daily.

The door to the body box was closed and an automatic internal 50ml syringe pump was

cycled 16 times. This process was repeated for validation, and the volume and pressure

measurements were updated. A calibration log showing the date, calibration values,

barometric pressure, and technician initials was maintained.   A more detailed

description of the calibration process can be found in the operator's manual

(SensorMedics Corporation, 2002). At least three (with no more than 8) separate

measurements were obtained and the three acceptable measurements agreed within 5% of

the average of the three measurements.

Stage One Exercise Tests:

At Visit 1 (screening) an incremental cycle exercise test was performed to a

symptom-limited maximum (maximum power output, MPO). The initial work rate was

set at 0 watts. The subject performed 1 minute of loadless pedaling at 50-70 rpm prior to

increasing the work rate. The subject was encouraged to continue to exercise for as long

as possible at this pedaling frequency, with the work rate increased in increments of 15

watts at the end of each minute. MPO was defined as the highest work rate that was

maintained for at least 30 seconds. One week later, subjects returned for Visit 2.

At Visit 2, and one week later at Visit 3, exercise was performed on a cycle ergometer consisting of 6 minutes of loadless pedaling, followed by 6 minutes at 1/3 MPO and 6 minutes at 2/3 MPO (total 18 minutes).

Before all exercise tests, while seated comfortably on the cycle ergometer, the subject breathed for 2 minutes through the mouthpiece with a noseclip in place while metabolic measurements were taken. Measures of ventilation (VO_2, VCO_2, V_E, V_T, and respiratory frequency) and circulation (BP, HR, and SpO_2) were monitored from rest to maximum exercise. Using the modified Borg Scale, subjects rated the intensity of leg effort and dyspnea every two minutes. At the third and fifth minute of 1/3 MPO and 2/3 MPO exercise, the subjects had their cardiac output measured via CO_2 rebreathing and nitrous oxide rebreathing, respectively (see below). Subjects were monitored post-exercise for recovery.

Modified Borg Scale:

The modified Borg Scale was used to assess dyspnea and leg effort before the start of loadless pedaling, every 2 minutes during exercise tests, and at the end of all exercise.

Cardiac Output:

Measurements of cardiac output were obtained with the $V_{max}29c$ metabolic system (Sensormedics, USA) using the CO_2 rebreathe technique and with the Innocor

system (Innovision, Denmark) using the nitrous oxide rebreathe technique, at minute 3 and minute 5 of the 1/3 MPO and 2/3 MPO work rates, respectively.

The CO_2 rebreathe technique required the subject to rebreathe from a bag with a gas mixture containing carbon dioxide at levels slightly higher than that in mixed venous blood (6 – 15%). The subject continued to rebreathe until an equilibrium plateau was reached where no further carbon dioxide exchange occurred across the alveolar-capillary junction and in the bag (Auchincloss, Gilbert, Kuppinger, & Peppi, 1980; Collier, 1956; Jones, 1975). The CO_2 at the point of equilibrium was taken as equal to the mixed venous CO_2 (P_vCO_2). The arterial PCO_2 (P_aCO_2) was estimated from the alveolar PCO_2 (P_ACO_2), determined from continuous end tidal CO_2 ($P_{et}CO_2$) (Auchincloss et al., 1980; Jones, 1975). Once P_vCO_2 and P_aCO_2 had been determined, the venous-arterial CO_2 content difference ($C_{v-a}CO_2$) was calculated using Jones' equation (Jones, 1975). Cardiac output was then calculated using the Fick equation: $Q = VCO_2 / C_{v-a}CO_2$.

Nitrous oxide rebreathe cardiac output was measured using the rate of nitrous oxide uptake which is limited by the rate of blood flow through the gas exchanging lung. Nitrous oxide was inhaled from a closed rebreathing system. The decline in the concentration of nitrous oxide was referenced to the concentration of an inert gas (sulphur hexafluoride). The concentrations of all gases were measured by photoacoustic spectroscopy. The wash-out rate is proportional to the cardiac output. The validity of nitrous oxide rebreathing has been documented in pre- and post cardiac surgery patients (Peyton & Thompson, 2004). This system has been used for measurements of cardiac

output in space, in healthy astronauts, and during exercise, in chronic heart failure patients (Agostoni et al., 2005). This system's advantage is its ease of use.

Data Analysis:

The sensitivity of CO_2 and nitrous oxide rebreathing to measure cardiac output was assessed by plotting cardiac output against metabolic demand (VO_2) at 1/3 and 2/3 MPO, from both exercise tests (Visits 2 & 3). A linear fit was applied, generating slope, intercept, and correlation (r^2) for both techniques.

The comparability of cardiac output measures collected via the CO_2 and nitrous oxide rebreathe techniques was assessed by plotting nitrous oxide cardiac output measures against CO_2 cardiac output measures at 1/3 and 2/3 MPO, respectively. Paired t-tests were performed to detect differences between cardiac outputs measured by the two techniques at both 1/3 and 2/3 MPO.

The repeatability of cardiac output and gas exchange efficiency was assessed by performing paired t-tests to detect significant differences, between visits, in cardiac output, V_E/VO_2, and V_E/VCO_2. Plots were generated illustrating cardiac output, V_E/VO_2, and V_E/VCO_2 at visits 2 and 3, respectively.

Results:

Sensitivity of Cardiac Output Measurements:

The nitrous oxide technique for measuring cardiac output was strongly correlated with metabolic demand (VO_2) for both exercise tests (visits 2 and 3), with $r^2 = 0.93$ and 0.87 (Figure A3.1). The intercepts were 2.8 and 2.9 and the slopes were 5.7 and 5.8 for visits 2 and 3, respectively (Figure A3.1).

The CO_2 rebreathe technique for measuring cardiac output was strongly correlated with metabolic demand for both exercise tests (visits 2 and 3), with $r^2 = 0.90$ and 0.93 (Figure A3.2). The intercepts were 5.2 and 5.0 and the slopes were 5.7 and 5.8 for visits 2 and 3, respectively (Figure A3.2).

Both techniques are sensitive to changes in cardiac output that accompany changes in metabolic demand with exercise, showing strong correlation (r^2) and nearly identical slopes. The intercepts were lower for the nitrous oxide technique, illustrating systematically lower values obtained via nitrous oxide compared to CO_2 rebreathing (Figures A3.1 & A3.2). This has been a consistent characteristic of this technique (Krogh & Lindhard, 1912; Shock & Norris, 1971).

Comparability of Cardiac Output Measurements:

Nitrous oxide measures of cardiac output were significantly lower than measures obtained via CO_2 rebreathing at both 1/3 ($p = 0.006$) and 2/3 MPO ($p = 0.00004$) (Figures A3.3 & A3.4).

Nitrous oxide measures of cardiac output are lower than measures obtained via CO_2 rebreathing.

Repeatability of Cardiac Output Measurements:

Nitrous oxide measures of cardiac output at 1/3 MPO were significantly different (p = 0.04) between visit 2 and visit 3 (Figure A3.5). Nitrous oxide measures of cardiac output at 2/3 MPO were not significantly different (p = 0.70) between visit 2 and visit 3 (Figure A3.7).

CO_2 rebreathe measures of cardiac output at both 1/3 and 2/3 MPO were not significantly different (p = 0.55 & 0.80) between visit 2 and visit 3 (Figures A3.6 & A3.8).

Both techniques generate cardiac output measures that are repeatable over time at 2/3 MPO.

Repeatability of Gas Exchange Efficiency Measurements:

The gas exchange efficiency for oxygen at 1/3 MPO was significantly different (p = 0.03) between visit 2 and visit 3 (Figure A3.9). V_E/VO_2 at 2/3 MPO was not significantly different (p = 0.08) between visit 2 and visit 3 (Figure A3.10).

The gas exchange efficiency for carbon dioxide at both 1/3 and 2/3 MPO was not significantly different (p= 0.36 & 0.96) between visit 2 and visit 3 (Figures A3.11 & A3.12).

Both V_E/VO_2 and V_E/VCO_2 are repeatable over time at 2/3 MPO.

Discussion:

Sensitivity of Cardiac Output Measurements during exercise:

During exercise, for every liter increase in oxygen consumption, cardiac output increased by 5.7 L/min (Visit 2) and 5.8 L/min (Visit 3) with the nitrous oxide technique and by 5.7 L/min (Visit 2) and 5.8 L/min (Visit 3) with the CO_2 rebreathe technique. The sensitivities were essentially the same.

Comparability of Cardiac Output Measurements:

Direct comparison of cardiac output measurements collected via nitrous oxide and CO_2 rebreathing, at the same metabolic demand in the same subjects, illustrated significantly lower measurements of cardiac output via the nitrous oxide rebreathe technique. There was also a significantly lower intercept for the nitrous oxide technique. Historically, the cardiac output measured using the nitrous oxide uptake technique at rest was always lower than the cardiac output measured at rest using rebreathing techniques (Krogh & Lindhard, 1912; Shock & Norris, 1971). In this study, both techniques were highly correlated ($r^2 \geq 0.87$), showing the same increases in cardiac output, with metabolic demand (VO_2) despite the lower absolute values obtained with the nitrous oxide technique.

Repeatability of Cardiac Output Measurements:

Cardiac output measured using the nitrous oxide technique varied significantly from Visit 2 to Visit 3 during low intensity exercise (1/3 MPO), but did not vary

significantly during high intensity exercise (2/3 MPO). The CO_2 rebreathe technique provided measures of cardiac output that were not significantly different from Visit 2 to Visit 3 at both low and high exercise intensities. Cardiac output measured via the nitrous oxide technique is repeatable during exercise at higher intensities, while cardiac output measured via CO_2 rebreathing is repeatable during both low and high intensity exercise.

Repeatability of Gas Exchange Efficiency Measurements:

The efficiency of gas exchange for oxygen varied significantly from Visit 2 to Visit 3 during low intensity exercise (1/3 MPO). There was no significant variability from Visit 2 to Visit 3 during high intensity exercise (2/3 MPO). The efficiency of gas exchange for carbon dioxide did not vary significantly from Visit 2 to Visit 3 during low or high intensity exercise.

Conclusions:

The nitrous oxide technique provides a sensitive measure of cardiac output during exercise that is repeatable at 2/3 MPO. Measurements of gas exchange efficiency for oxygen and carbon dioxide are also both repeatable during exercise at 2/3 MPO. For the purposes of assessing cardiac output during exercise in the main study, the nitrous oxide technique is both sensitive and repeatable at high intensity exercise, while being easy to use. The efficiency of gas exchange for oxygen and carbon dioxide are also repeatable at high intensity exercise.

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Tables:

Table A3.1. *Study Population Demographics.*

	n	Mean	Minimum	Maximum	Std.Dev.
pre-BD FEV$_1$ (L)	8	4.3388	3.1400	5.7700	0.77567
pre-BD FEV$_1$ % Predicted (%)	8	100.0000	84.0000	126.0000	14.97617
post-BD FEV$_1$ (L)	8	4.6212	3.2700	6.5800	0.94980
post-BD FEV$_1$ % Predicted (%)	8	106.2500	87.0000	128.0000	15.26668
Height (cm)	8	183.5000	174.0000	200.0000	7.80110
Weight (kg)	8	83.0000	66.0000	100.0000	11.94033
BSA (m^2)	8	2.0375	1.8000	2.4000	0.19226
Age (years)	8	31.3750	20.0000	57.0000	14.09090
Sex (% male)	8	0.8750			

pre-BD FEV$_1$ = pre-bronchodilator forced expired volume in one second, post-BD FEV$_1$ = post-bronchodilator forced expired volume in one second, BSA = body surface area

Table A3.2. *Mean (±SD) values for ventilatory measurements made 1/3 & 2/3 MPO.*

Visit	Work Rate	VO_2 (l/min)	VCO_2 (l/min)	P_VCO_2 (mmHg)	V_E (l/min)	V_E/VO_2	V_E/VCO_2
2	1/3 MPO	1.52±0.17	13.76±1.58	59.91±2.93	34.92±6.34	22.77±2.45	28.65±4.58
3	1/3 MPO	1.43±0.30	14.65±1.75	60.60±1.50	39.41±3.52	28.25±4.44	27.02±1.27
2	2/3 MPO	2.54±0.29	18.95±2.08	70.92±2.82	63.35±9.51	24.84±1.57	25.83±1.38
3	2/3 MPO	2.58±0.43	19.16±2.21	71.76±2.86	67.22±10.00	26.24±2.64	25.79±1.62

MPO = maximum power output, VO_2 = oxygen uptake, VCO_2 = carbon dioxide production, P_VCO_2 = mixed venous partial pressure of carbon dioxide, V_E = minute ventilation, V_E/VO_2 = gas exchange efficiency for oxygen, V_E/VCO_2 = gas exchange efficiency for carbon dioxide
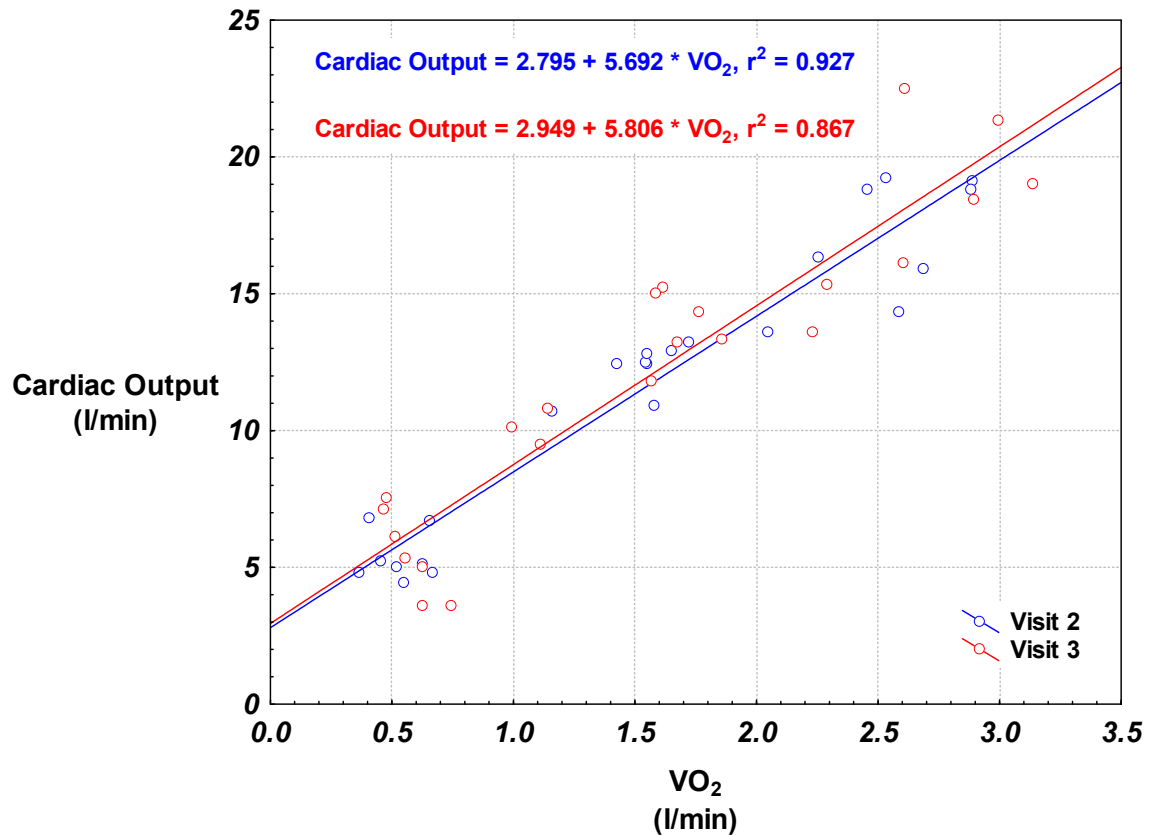
Figures:

Figure A3.1. *Sensitivity of the Inert Gas technique to detect changes in cardiac output associated with increasing metabolic demand (VO₂).* Linear equation and correlation (r^2) presented for measurements made at visit 2 (blue) and 3 (red), respectively.
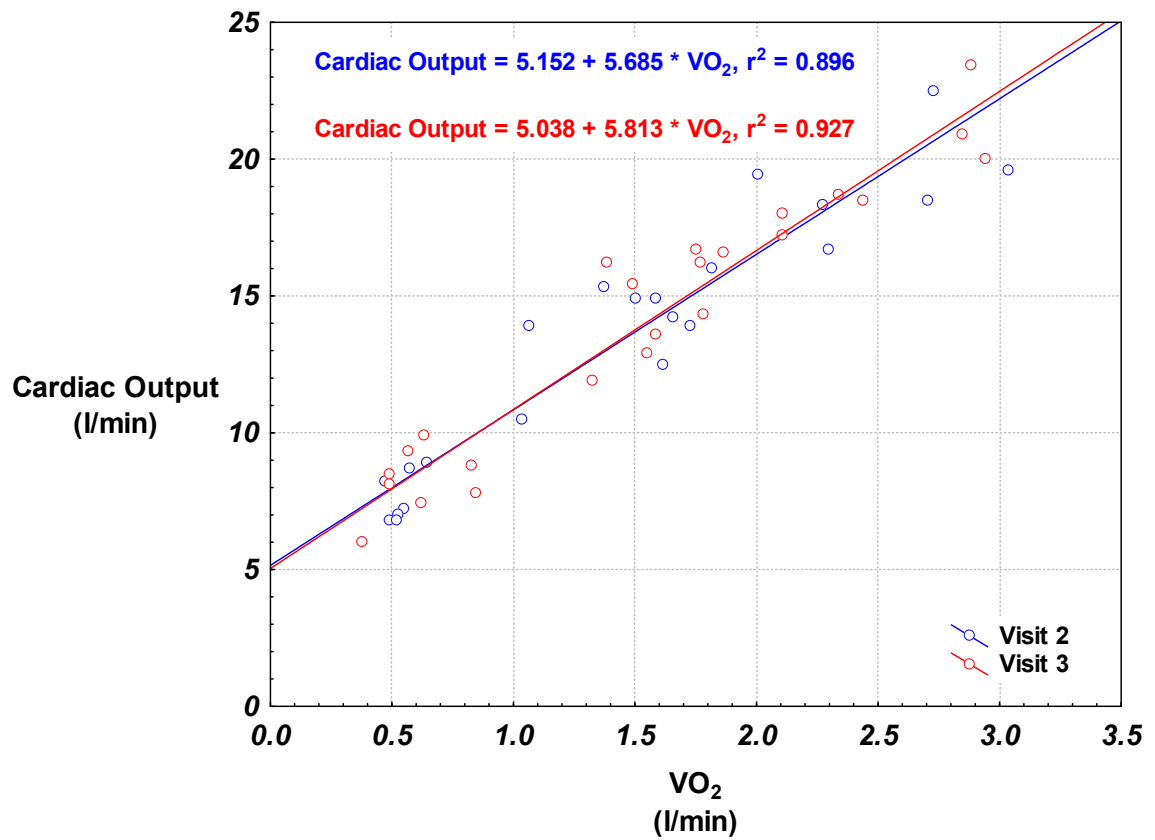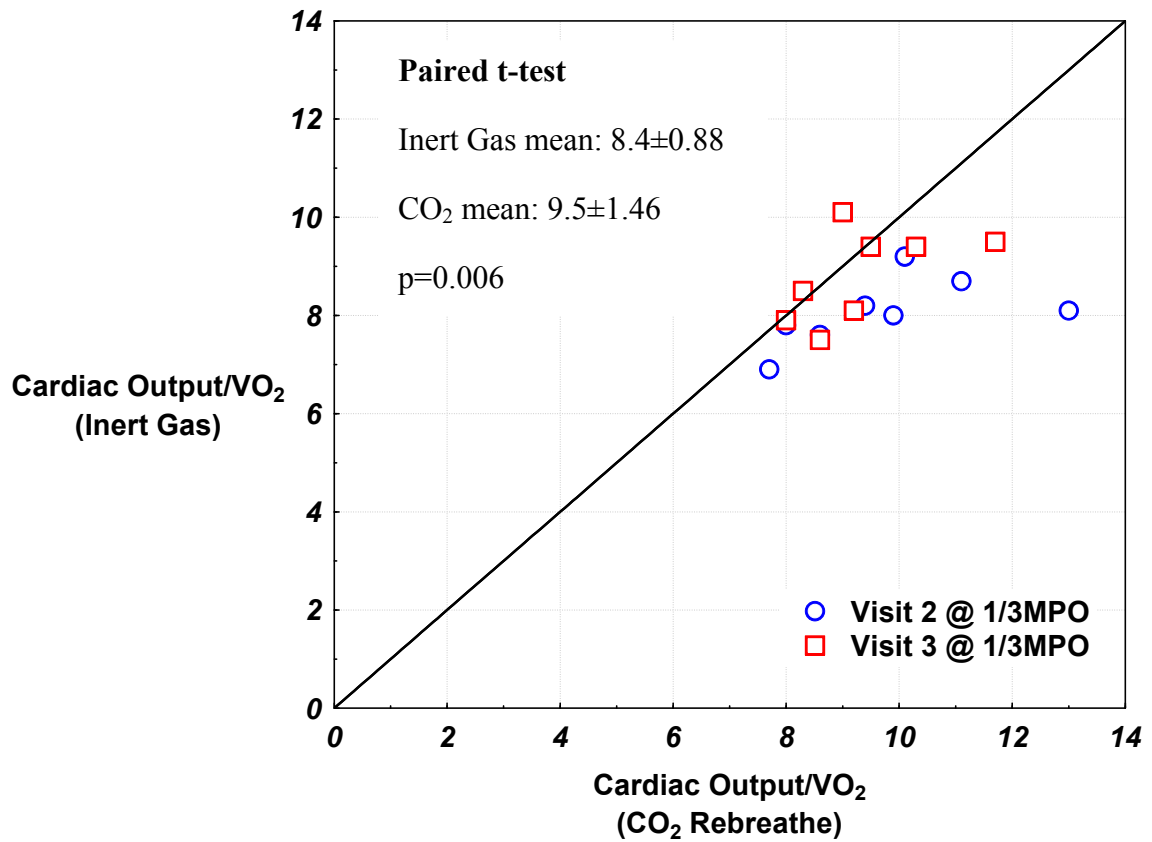
Figure A3.2. *Sensitivity of the CO$_2$ Rebreathe technique to detect changes in cardiac output associated with increasing metabolic demand (VO$_2$).* Linear equation and correlation (r^2) presented for measurements made at visit 2 (blue) and 3 (red), respectively.

Figure A3.3. *Comparability of Cardiac Output measurements obtained via Inert Gas and CO₂ Rebreathe techniques at 1/3 maximum power output (MPO).* Measurements are from visit 2 (blue) and 3 (red) with the line of identity (y = x) illustrated in black. Paired t-test: Inert Gas and CO₂ Rebreathe cardiac outputs (mean±SD) and statistically significant at p<0.05.
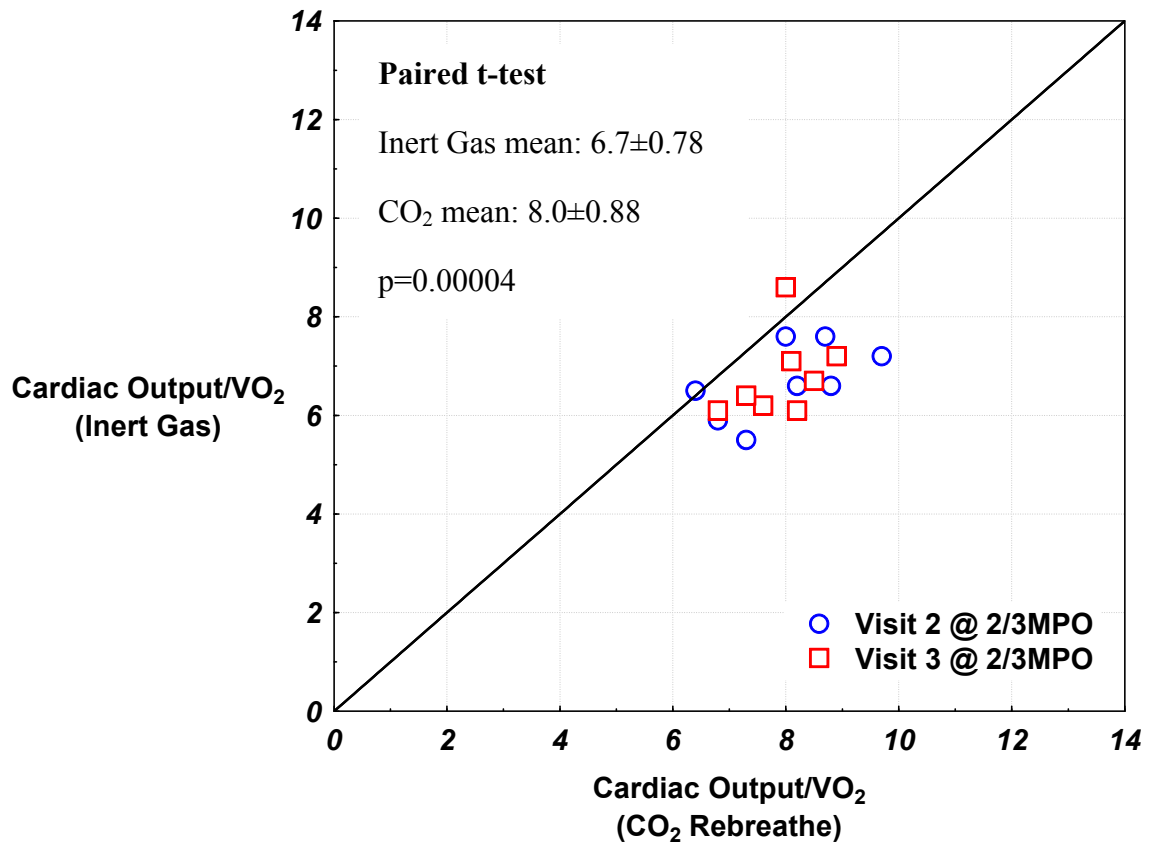
Figure A3.4. *Comparability of Cardiac Output measurements obtained via Inert Gas and CO₂ Rebreathe techniques at 2/3 maximum power output (MPO).* Measurements are from visit 2 (blue) and 3 (red) with the line of identity (y = x) illustrated in black. Paired t-test: Inert Gas and CO₂ Rebreathe cardiac outputs (mean±SD) and statistically significant at p<0.05.
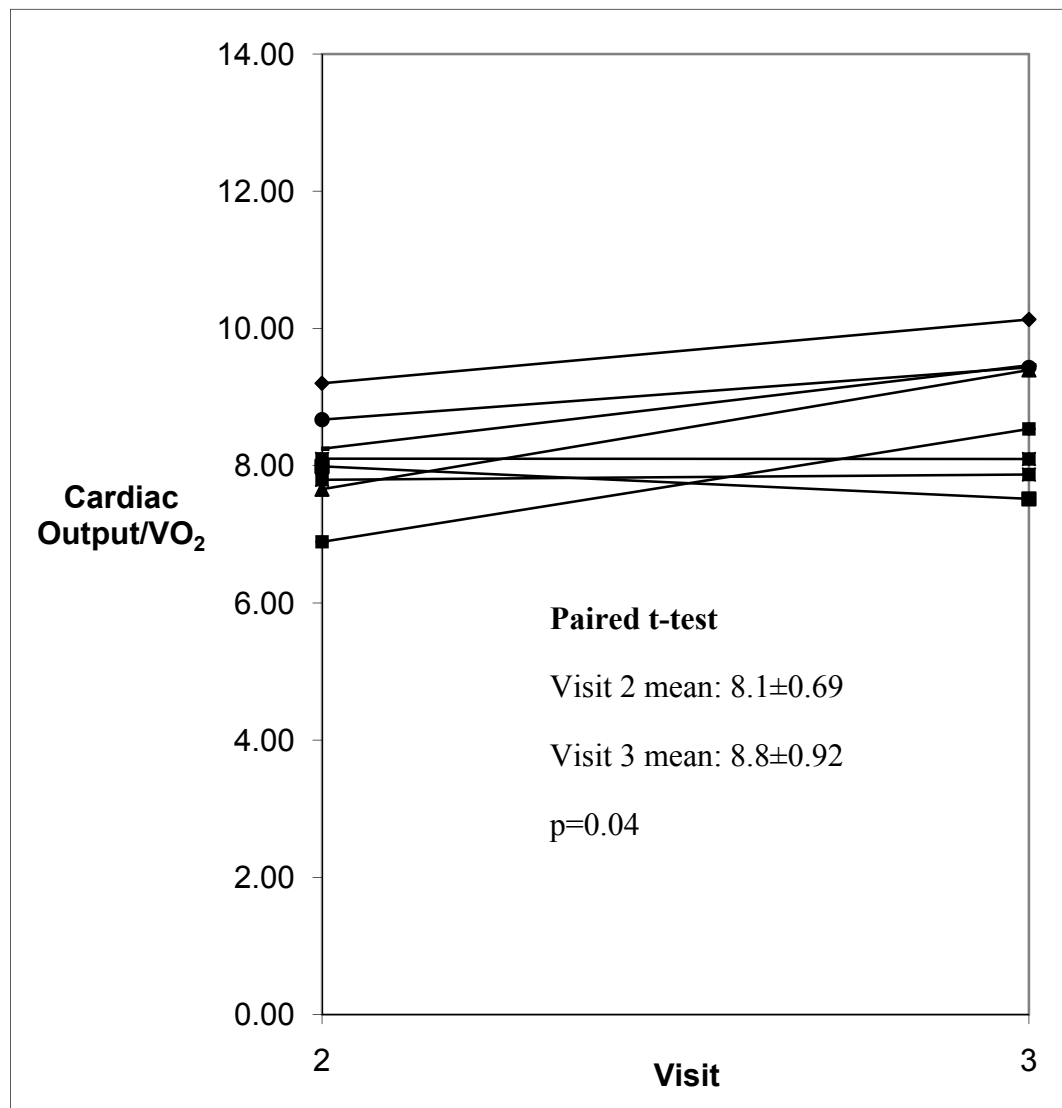
Figure A3.5. *Cardiac output relative to metabolic demand (VO_2) measured at visits 2 and 3 via the Inert Gas technique at 1/3 maximum power output (MPO)*. Paired t-test: visit 2 and visit 3 cardiac output (mean±SD) and statistically significant at p<0.05.
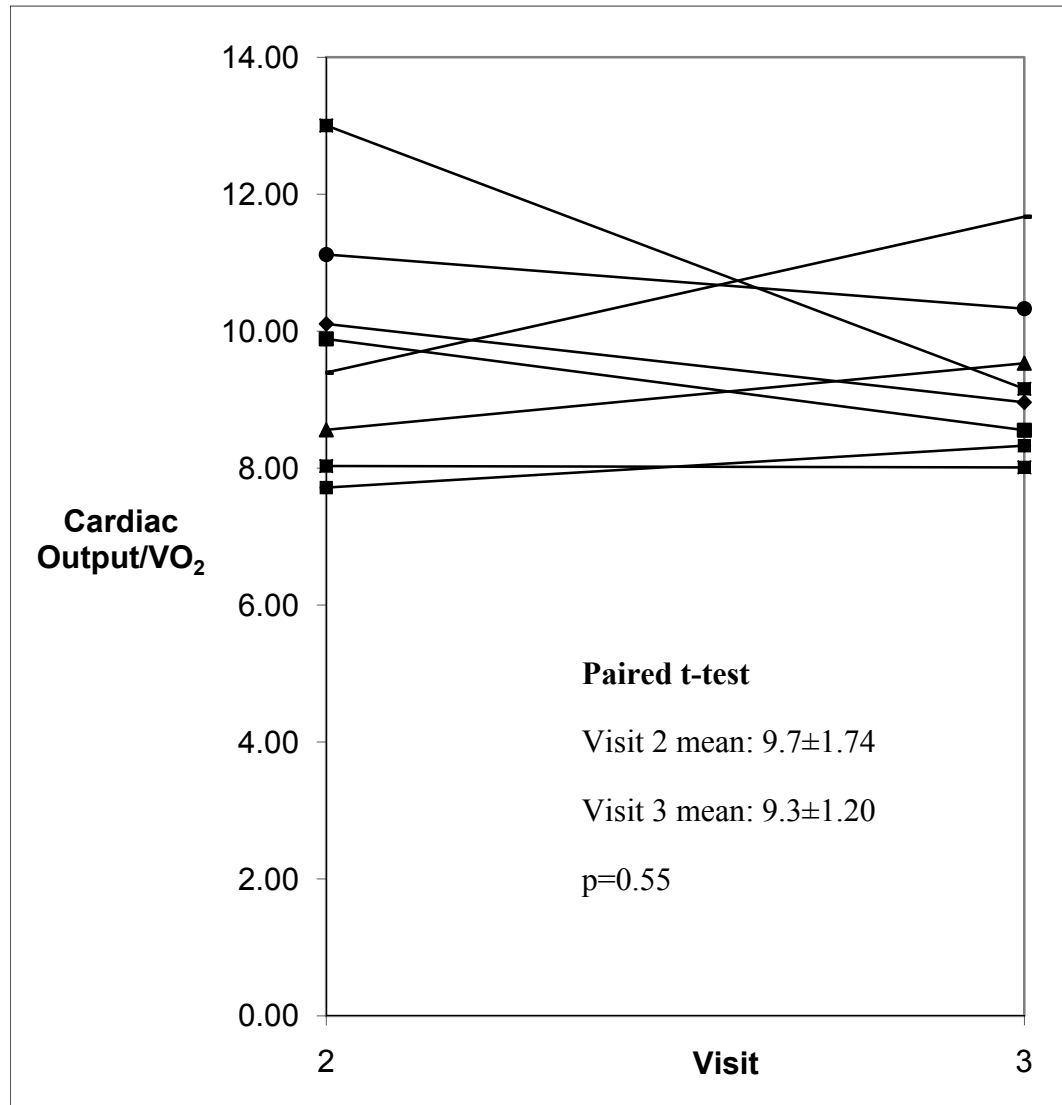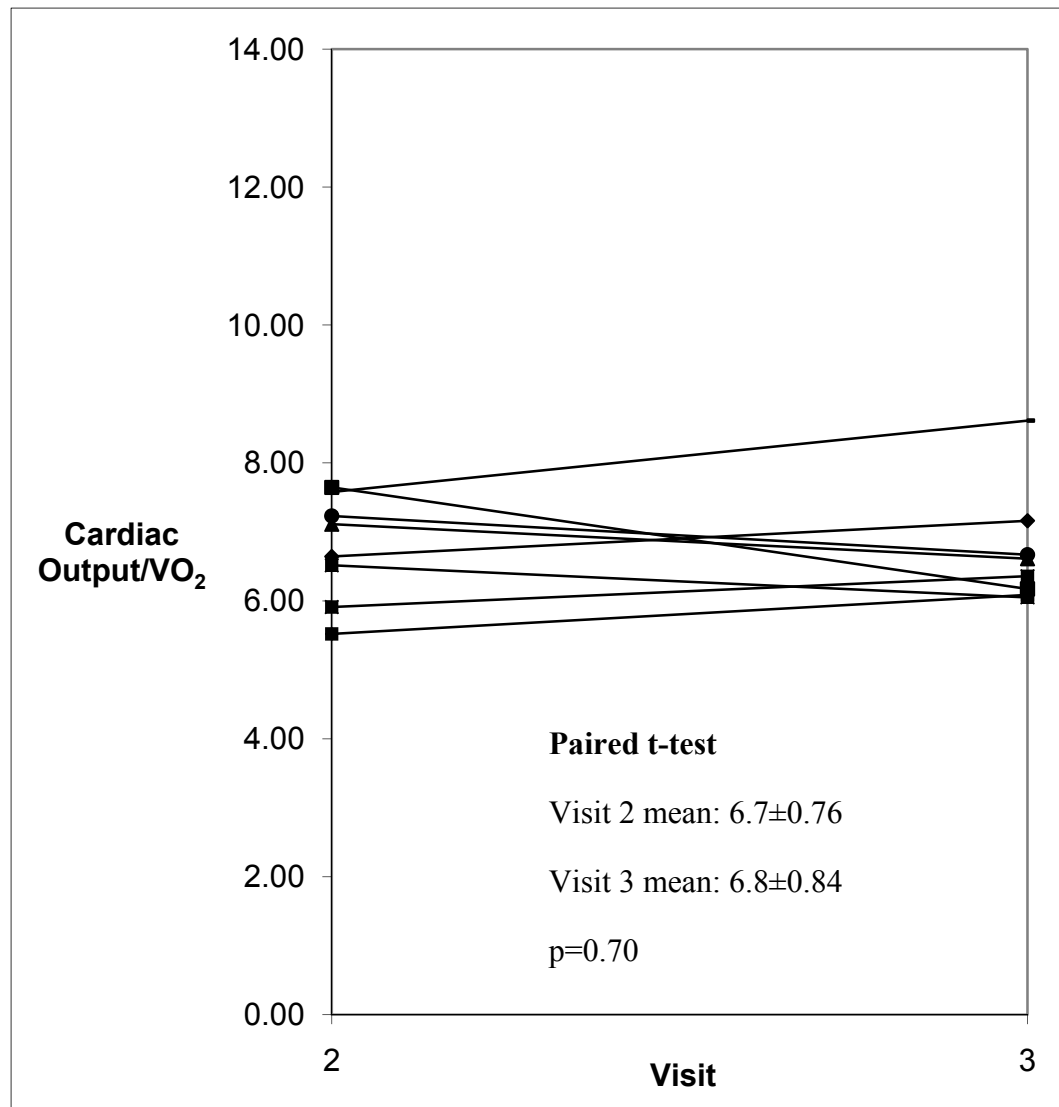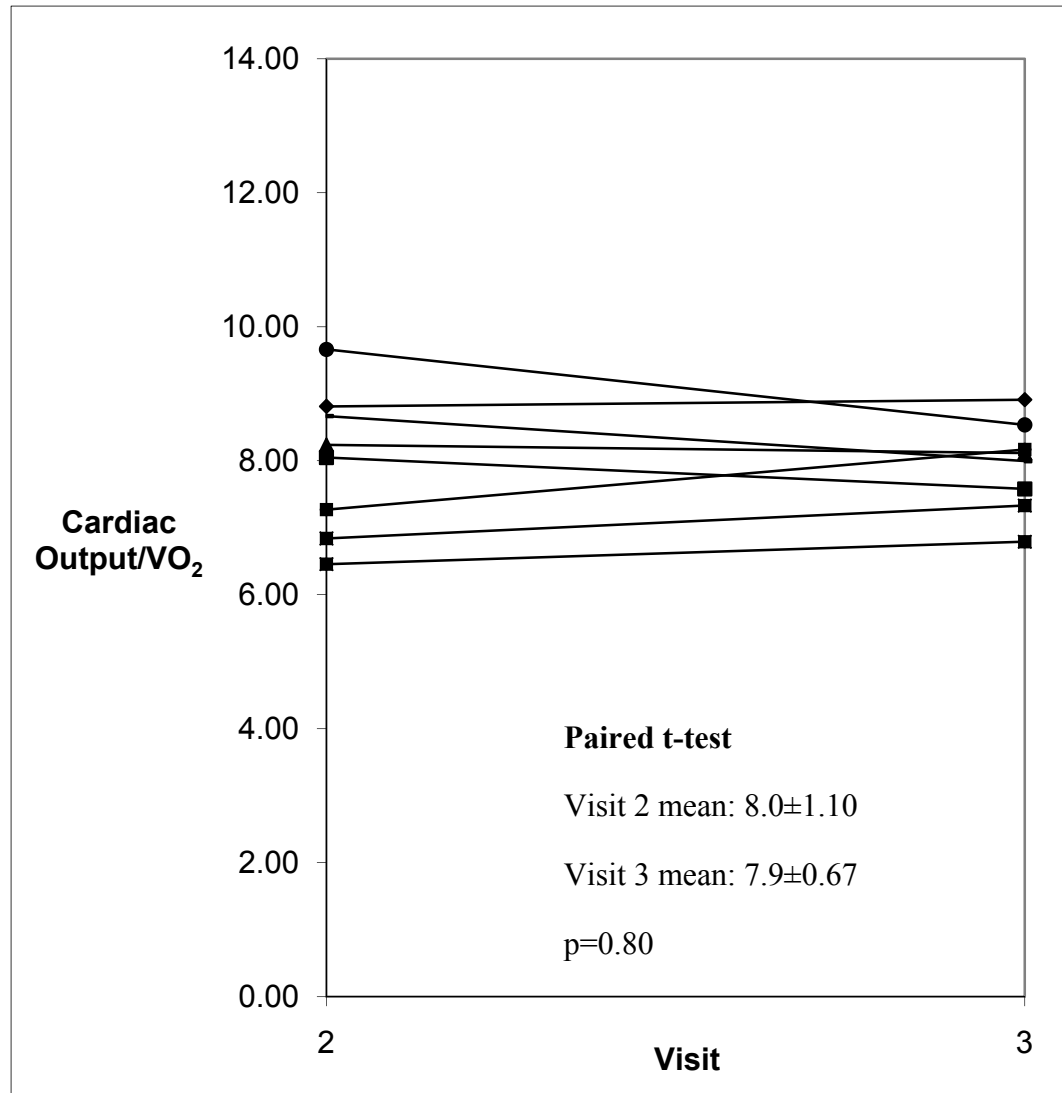
Figure A3.6. *Cardiac output relative to metabolic demand (VO_2) measured at visits 2 and 3 via the CO_2 Rebreathe technique at 1/3 maximum power output (MPO).* Paired t-test: visit 2 and visit 3 cardiac output (mean±SD) and statistically significant at p<0.05.

Figure A3.7. *Cardiac output relative to metabolic demand (VO$_2$) measured at visits 2 and 3 via the Inert Gas technique at 2/3 maximum power output (MPO).* Paired t-test: visit 2 and visit 3 cardiac output (mean±SD) and statistically significant at p<0.05.
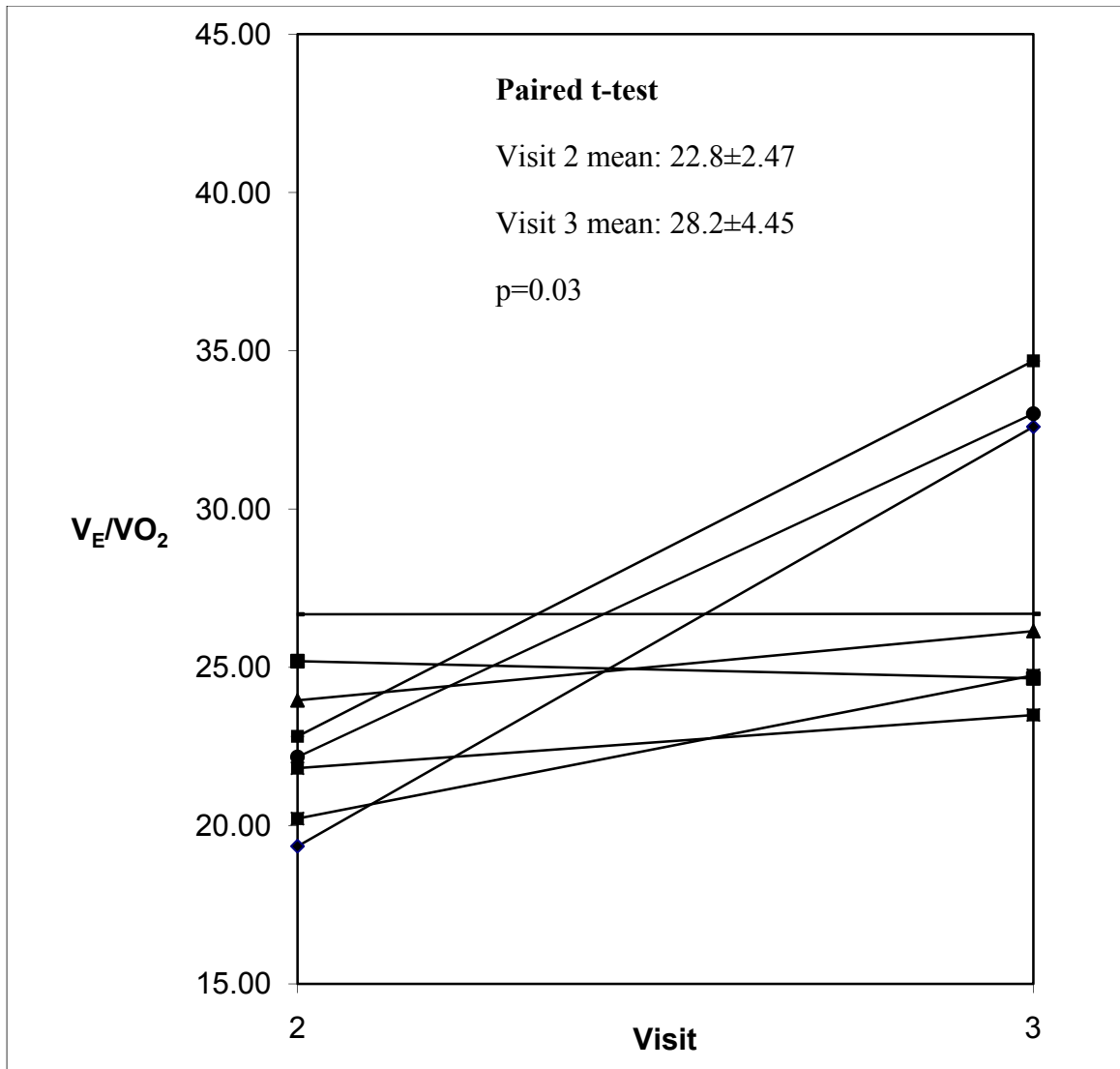
Figure A3.8. *Cardiac output relative to metabolic demand (VO₂) measured at visits 2 and 3 via the CO₂ Rebreathe technique at 2/3 maximum power output (MPO).* Paired t-test: visit 2 and visit 3 cardiac output (mean±SD) and statistically significant at p<0.05.

Paired t-test

Visit 2 mean: 22.8±2.47

Visit 3 mean: 28.2±4.45

p=0.03

Figure A3.9. *Ventilatory efficiency for oxygen (V$_E$/VO$_2$) at 1/3 maximum power output (MPO) for visits 2 and 3.* Paired t-test: visit 2 and visit 3 V$_E$/VO$_2$ (mean±SD) and statistically significant at p<0.05.

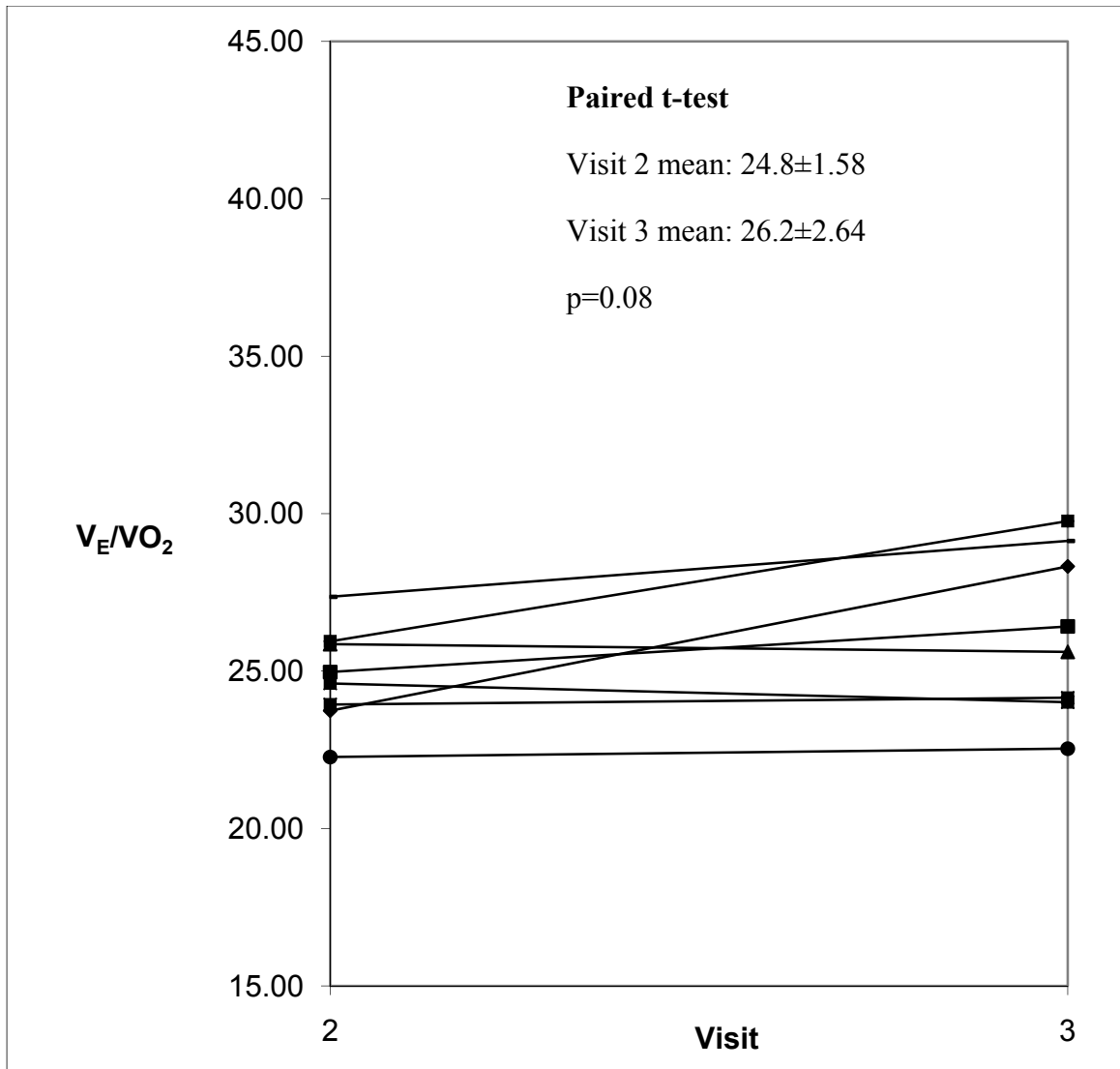Figure A3.10. *Ventilatory efficiency for oxygen (V_E/VO_2) at 2/3 maximum power output (MPO) for visits 2 and 3.* Paired t-test: visit 2 and visit 3 V_E/VO_2 (mean±SD) and statistically significant at p<0.05.
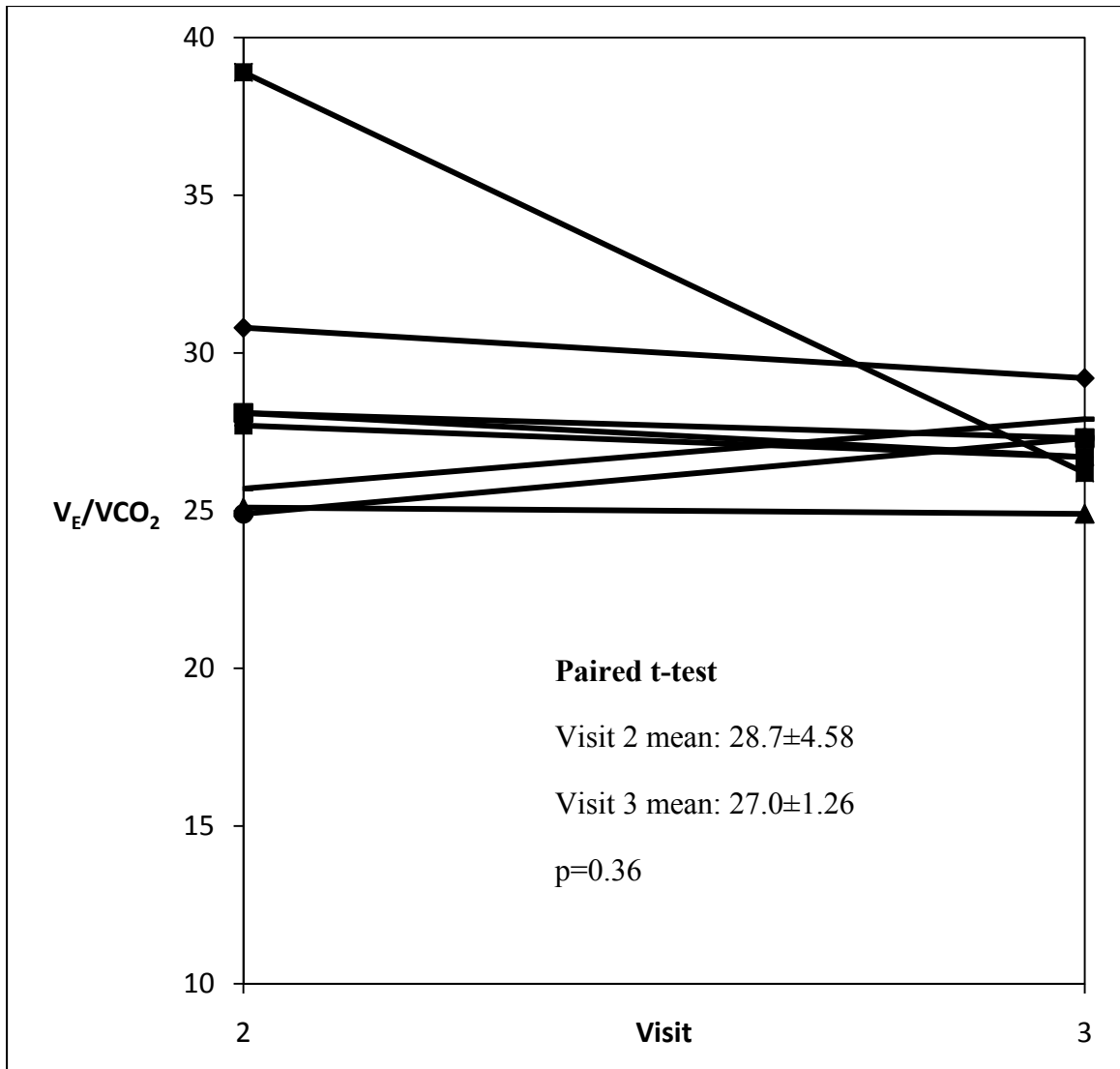
Figure A3.11. *Ventilatory efficiency for carbon dioxide (V_E/VCO_2) at 1/3 maximum power output (MPO) for visits 2 and 3.* Paired t-test: visit 2 and visit 3 V_E/VCO_2 (mean±SD) and statistically significant at $p<0.05$.
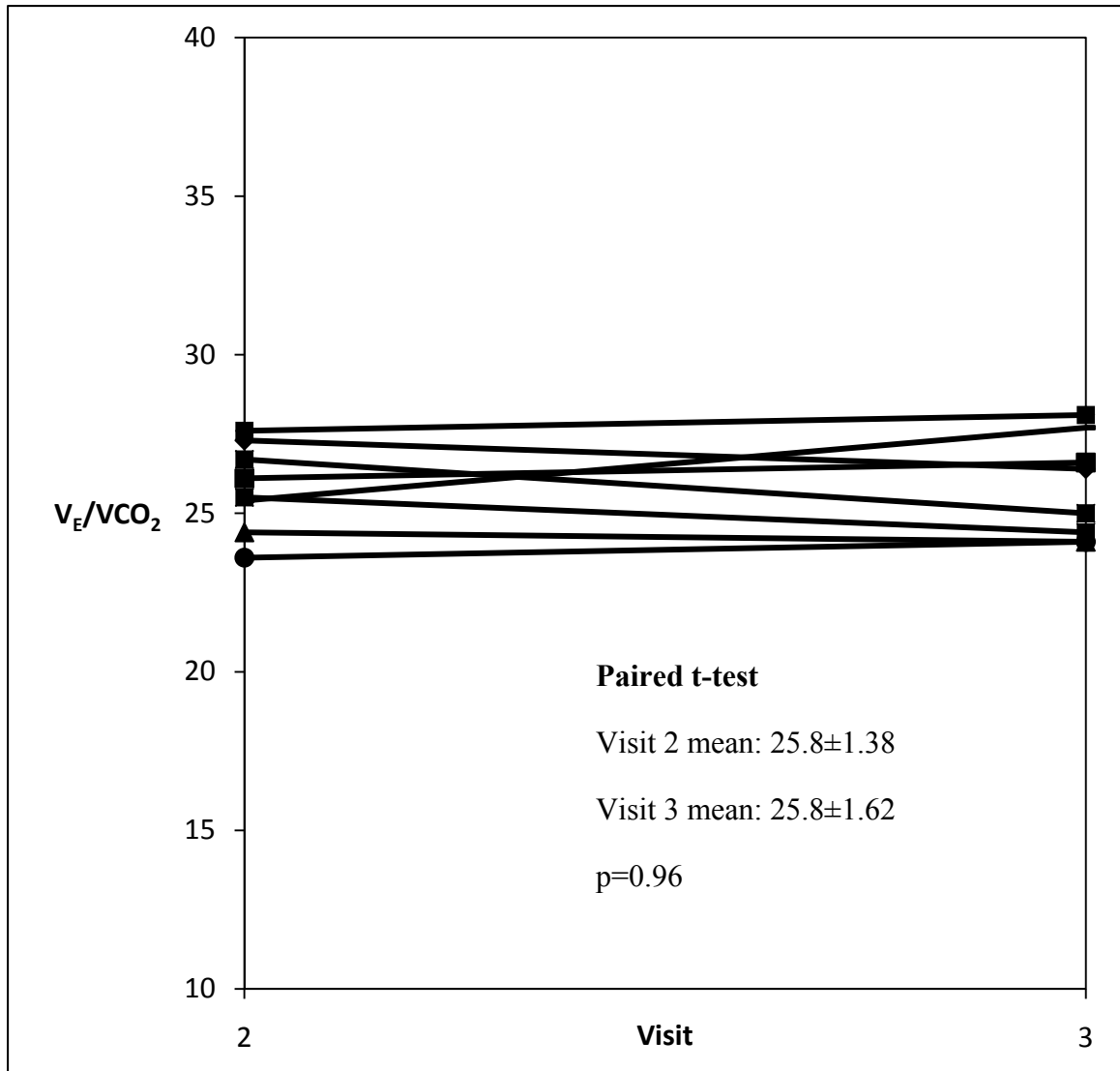
Figure A3.12. *Ventilatory efficiency for carbon dioxide (V_E/VCO_2) at 2/3 maximum power output (MPO) for visits 2 and 3.* Paired t-test: visit 2 and visit 3 V_E/VCO_2 (mean±SD) and statistically significant at $p<0.05$.