PULMONARY AND CARDIOVASCULAR RESPONSES TO A REPEATED EXPOSURE EXERCISE PROTOCOL IN OZONE AIR POLLUTION IN ADULTS WITH EXERCISE-INDUCED BRONCHOCONSTRICTION

by

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B.Sc., Universidade Federal de Minas Gerais, 2017

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

The Faculty of Graduate and Postdoctoral Studies
(Kinesiology)

THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)

November 2022

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Pulmonary and Cardiovascular Responses to a Repeated Exposure Exercise Protocol in Ozone Air Pollution in Adults with Exercise-Induced Bronchoconstriction

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the degree of Master of Science

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Abstract

Introduction: Individuals with exercise-induced bronchoconstriction (EIB) are at greater risk when exposed to air pollution, but whether impairments in pulmonary or cardiovascular functions are mitigated with repeated exposures to ozone (O_3) has yet not been investigated. This study aimed to examine whether repeated exposures to a controlled level of O_3 can induce adaptation to the impairments on pulmonary and cardiovascular functions in individuals with EIB. **Methods:** A double-blinded, cross-over, randomized trial of 10 study visits was performed. Subjects went through an EIB provocative test and a graded maximal exercise test for exercise intensity prescription. Participants cycled for 30 min at 60% of their maximal power output. They were randomized to start the first five study exposure visits on either room air (RA) or 170 ppb O_3. Spirometry and pulse wave velocity (PWV) measurements were performed at baseline and after exercise while blood pressure and dyspnea were assessed at end-exercise. A linear mixed effects model was used for differences across study visits, and t-tests for post-pre.

Results: Thirteen individuals with mild to moderate EIB completed ten study visits, 53.8% were women, 61.5% had asthma. The decrease in mean forced expiratory volume in one second (FEV_1) in the provocative test was 17.2%. On Day 1 of O_3, FEV_1 decreased 0.2L compared to baseline, p= 0.03. FEV_1 on Day 1 of O_3 was lower than on Day 4 of RA, p= 0.04, and on Day 2 of O_3 it was lower than Day 5 of RA, p= 0.03. Forced expiratory flow in the middle portion of a maximal expiratory effort (FEF_{25-75}) was also lower on Day 2 of O_3 than Day 4 of RA, p= 0.02. FEF_{25-75} was lower on Day 2 of O_3 than Days 4 and 5 of RA, p= 0.02 and p=0.04. **Conclusion:** Pulmonary function was significantly impaired on Days 1 and 2 of O_3 exposure but not on days 3,4 and 5. On Day 2, cardiovascular function showed a trend towards being impaired, which was not statistically significant.

Lay summary

People with asthma can be triggered by many factors, including air pollutants like ozone. Ozone is an air pollutant that is mostly found on sunny and hot days in its gaseous form. This study aimed to investigate whether the effects of inspired ozone while exercising could be diminished by repeated exposures. Thirteen participants with exercise-induced asthma were asked to cycle at a moderate intensity in low or high ozone. We examined how their lung and heart function changed. We found that lung function is reduced when exposed to ozone, particularly on the second day of exposure. In subsequent ozone exposures, lung function seems to recover, but is still negatively affected. The heart seems to be minimally affected on the second day of exposure. We conclude that lung function can be mitigated to repeated exposures to ozone in some people with exercise-induced asthma.

Preface

At the time of this submission, nor the entirety or part of this thesis had been published or presented. This study was designed and performed by the main author, Patric Emerson Oliveira Gonçalves, in consultation with the original idea from Dr. Michael Koehle and Dr. André Casanova. The ozone chamber was adapted from an altitude chamber by the main author of this thesis and my colleague, Ben Stothers. Data analysis was performed with the unmatched help of my friend, Dr. Rodríguez-Arelis. The writing of this thesis was conducted entirely by the main author.

The principal investigator, Dr. Koehle, provided supervision and revised all aspects of this study and thesis document. The committee members, Dr. Sheel and Dr. Borduas-Dedekind had also participated in this study design, protocol and the chamber set up.

This study was approved by the UBC Research Ethics Board under the title ‘Adaptation to ozone in individuals with asthma/exercise-induced bronchoconstriction’ and ID H21-01183.

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Abbreviations

BMI = body mass index

bpm = beats per minute OR breaths per minute

CONSORT = Consolidated Standards of Reporting Trials

DBP = diastolic blood pressure

EIB= exercise-induced bronchoconstriction

EVH= eucapnic voluntary hyperpnoea test

*f*B = breathing frequency

FEF_{25-75} = forced expiratory flow during the middle half of the FVC

FeNO = fraction of exhaled nitric oxide

FEV_1= forced expiratory volume in the first second

FVC= forced vital capacity

h = hours

HR = heart rate

L= liters

min = minutes

NO = nitric oxide

O_3= ozone

PAR-Q+ = Physical Activity Readiness Questionnaire

ppb= parts per billion

PWV = pulse wave velocity

RA = room air

rpm = revolutions per minute

SBP = systolic blood pressure

SD= standard deviation

VE = minute ventilation

VO_2max = maximal oxygen consumption

VT = tidal volume

W = watts

Acknowledgments

First, I want to thank Dr. Koehle for being so supportive throughout this journey. Starting the first half of this masters online was not easy, and Dr. Koehle was genuinely caring about the steps on my way to being physically in the Environmental Physiology Lab. His kindness and encouragement have been life changing in different aspects of this ride, from the moment he waved from outside the quarantine apartment's window to being sincerely thoughtful about my future in Canada. Despite all the hard work I put in the previous five years to get into this program, it would not have been possible without the kind recognition of that work by people like Ming-Chang Tsai.

Thank you to my committee, Dr. Borduas-Dedekind and Dr. Sheel for all the valuable support in putting this project and the ozone chamber together.

I also want to acknowledge the importance of Prof. Ligia in my academic and professional career, and for introducing me in research methods. Her support has given me the tools and courage to succeed in life goals of my own. I wish to one day impact as many people as she did to her patients and students, as she did to my own journey.

My special thanks to Tessa van de Kerkhof for the effort and hard work she put into making data collection less painful and for sharing pleasurable moments. Also, to my lab mates, Andy and Ben, who supported in unvaluable ways as did Jem and Assaf. Their help was not limited to, but went from putting together study groups in between my ICU night shifts, to offering help when I was unable to move to Vancouver due to the pandemic.

I am also grateful for all the fellow graduate lab mates and colleagues I had the pleasure to work with during this journey that supported and contributed to the completion of this thesis, with the study set up, data collection or with a coffee or pub break in the middle of the long data collection weeks: Alejandro, Owen, Josh Taylor, Josh Bouvard, Johnson and Kristina.

Dedication

To my parents, Elenice and Emerson. To my grandmoms, Mª do Carmo and Rita for their sacrifice.

To my sisters, Cynthia and Polly and to my family, who are the most supportive of my journeys.

To my husband and Brazilian friends, who keep encouraging me and celebrating every accomplishment together.

I am grateful for the love and for having you to share this life.

Eu amo vocês.

Obrigado.

Chapter 1: Introduction

Increased atmospheric temperatures and increasingly frequent and longer bouts of poor air quality resulting from climate change and events such as wildfires in western North America are concerning not only for the ecosystems but also for human health. Poor air quality jeopardizes the pulmonary and cardiovascular health and puts in check the safety of remaining physically active during these periods (Giles & Koehle, 2014). Although the health-related responses to many air pollutants such as particulate matter and diesel exhaust have been extensively studied, the same is not true about the effects on the pulmonary and cardiovascular systems when exercising while breathing ozone (O_3) air pollution.

1.1 Ozone air pollution

Ground level O_3 is alarming since it can impair human and animals cells and organs (Uysal & Schapira, 2003). O_3 is a potent gaseous pollutant formed from photochemical reactions involving volatile compounds, oxides of nitrogen, and oxygen in the presence of light, especially during sunny days when ultraviolet rays interact with nitrogen dioxide and hydrocarbons (Zhang et al., 2019). Climate change plays an important role in the O_3 formation since increased atmospheric temperatures and occurrence of prolonged warmer seasons favour O_3-forming reactions (Zhang et al., 2019). Changes in humidity and wind conditions can make the volatile compounds and nitrogen oxides stay stagnant for longer in the atmosphere, extending the time for ozone-forming reactions (Zhang et al., 2019). Interestingly, decreased levels of particulate matter and reduced concentrations of carbonaceous aerosols from combustion of diesel and coal, as we transition to cleaner fuel sources, can increase the levels of O_3 due to better air quality and increased exposure to sunlight (Zhang et al., 2019).

British Columbia is constantly concerned about wildfires during warm and dry seasons. In 2021, much of a town, Lytton, was erased by a wildfire, which is not only a burden for the city but also its surroundings. O_3 peaks downwind of the fire, with its concentration increased even in locations that are distant from the wildfire (Jaffe & Wigder, 2012). O_3 levels can be even worse in cases where the wildfire is close to urban centres with mixing of both traffic and wildfire O_3 emissions, which causes a greater pollutant peak in those regions (Jaffe & Wigder, 2012).

High O_3 levels are concerning because in humans they induce health effects, such as airway inflammation and hyperresponsiveness, oxidative stress, and can exacerbate or even cause respiratory symptoms especially in those with respiratory diseases, such as asthmatics (Guarnieri & Balmes, 2014).

1.2 Asthma and exercise-induced bronchoconstriction

Individuals with asthma and exercise-induced bronchoconstriction (EIB) are particularly at greater risk of experiencing respiratory symptoms when in environments with higher O_3 concentration (Parsons et al., 2013). The term exercise-induced asthma could be used interchangeably, but it might suggest that those with EIB must have underlying asthma when in fact not everyone, but up to 90% of people with asthma have EIB (Parsons & Mastronarde, 2005; Rundell & Jenkinson, 2002). The prevalence of EIB in the non-asthmatic general population is up to 20% while in athletes who perform their sports on ice, up to 50% might be diagnosed with EIB (Giles & Koehle, 2014; Rundell & Jenkinson, 2002; Uysal & Schapira, 2003).

1.2.1 Pathophysiology and diagnose

Asthma is an umbrella term to define a heterogeneous disease for which the diagnosis is based on the presence of episodic respiratory symptoms and airflow obstruction that could be

reversible (Busse, 2011; Jayasinghe et al., 2015). Asthma attacks can differ from EIB since the former has many triggers as compared to the single cause (exercise) of the latter. Asthmatics can have symptoms related to exposures to pollen, cigarette smoke and air pollution for example (Jayasinghe et al., 2015). The diagnosis of asthma is performed based on symptoms, history, pulmonary function and bronchodilator challenge tests, whereas EIB can be diagnosed observing pulmonary function changes in tests that will elicit increased lung ventilation (Jayasinghe, Kopsaftis, and Carson 2015; Anderson and Kippelen 2012).

EIB is defined as the transient narrowing of the airways induced by higher pulmonary ventilation during exercise with consequent airway hyperresponsiveness and inflammatory response (Jayasinghe et al., 2015; Parsons et al., 2013). The two main hypotheses to explain symptoms that occur as a response to physical activity are related to changes in osmolarity and thermal response of lower airways (Del Giacco et al., 2015). Both acknowledge that the initiating mechanism is the higher volume of air inhaled and mouth breathing during exercise that reduces the ability of upper airways to warm and humidify air before it reaches the lower respiratory tract, which can be potentially exacerbated by inhaling cold and dry air during winter season (Parsons & Mastronarde, 2005).

The ‘osmolar’ hypothesis suggests that the increased ventilation during exercise causes extracellular water loss from the airway surface increasing intracellular ion concentrations (Del Giacco et al., 2015; Parsons & Mastronarde, 2005; Rundell & Jenkinson, 2002). In those with EIB predisposition, when exercise ceases and airway ventilation is reduced, the restoration of the fluid lining the airway surface causes the release of inflammatory mediators from mast cells, such as histamine, a potent bronchoconstrictor which leads to bronchial smooth muscle constriction, mucus production, and symptoms (Parsons & Mastronarde, 2005; Rundell & Jenkinson, 2002).

These mediators alone are also thought to be responsible for a transitory oedema of the airways, which would enhance the bronchoconstriction effect. The 'vascular hypothesis' states that when the exercise ceases, a hyperemic reaction followed by a rebound vasodilation and oedema will occur to compensate the lost temperature in the airway during heavy breathing (Del Giacco et al., 2015). Increased hydrostatic volume in the peribronchial capillary bed can cause airway narrowing, which can be aggravated in those with a predisposition to increased airway resistance at rest and during exercise, such as asthmatics (Anderson & Kippelen, 2005; Carlsen & Carlsen, 2002; Del Giacco et al., 2015; Parsons & Mastronarde, 2005). EIB can be suspected when respiratory symptoms are present after exercise, such as shortness of breath, chest tightness, wheezing, and coughing; however, changes in pulmonary function will confirm the clinical history which can be done through spirometry tests (Del Giacco et al., 2015).

An eucapnic voluntary hyperpnoea (EVH) test can be performed in a laboratory setting to diagnose EIB and it is a reliable surrogate for exercise-induced changes in pulmonary function and symptoms (Anderson & Kippelen, 2012). Pulmonary function tests measured with spirometry are performed before and after the EVH test to track for changes in pulmonary function. The main pulmonary function measurement in those tests used to diagnose EIB is the forced expiratory volume in the first second (FEV_1). FEV_1 is the volume of air exhaled in the first second of a maximal and forceful expiratory maneuver from a maximal inspiration point. This is an objective measurement important not only to stratify asthma severity, but also to guide therapy, and classify the risk of asthma attacks over 3 years (Kitch et al., 2004). It has also been shown that FEV_1 is a predictor of all-cause survival rates and ischemic heart diseases mortality (Schu¨nemann et al., 2000).

A drop of 10% or greater in FEV_1, but lower than 25% after the EVH test is the main finding in a pulmonary function test to diagnose mild EIB (Anderson & Kippelen, 2012). An individual that shows an FEV_1 drop of 25% or greater is diagnosed as having a moderate EIB, whereas a decrease of 50% or more is considered a severe condition (Anderson & Kippelen, 2012). In obstructive diseases such as chronic obstructive pulmonary disease and asthma, FEV_1 is chronically reduced whereas people with EIB might have normal baseline FEV_1 until they are triggered by exercise (Del Giacco et al., 2015).

1.3 Effects of exercise in ozone

Exercise is key in increasing endurance and the ability to cope with strain in people with asthma or other chronic respiratory conditions; however, in environments with higher levels of O_3, those individuals might be at a greater risk of developing symptoms due to lung irritation (Del Giacco et al., 2015). As exercise intensity progresses, it is naturally expected that one will transition from nose breathing to mouth breathing, which occurs concomitantly with increased lung ventilation, all of which to accommodate for the higher volume of air needed for gas exchange demands in the lung tissues. However, breathing through the mouth not only can effect changes in the lung tissue level, as previously mentioned on the osmolar and vascular mechanisms, but also surpasses the protective barrier the upper airways offers to the lungs (Parsons & Mastronarde, 2005). Thus, in a polluted environment, when exercising at moderate intensities, which will likely elicit mouth breathing patterns, the increased dose of inhaled pollutants could theoretically lead to impairments in pulmonary function (Jayasinghe et al., 2015).

1.3.1 Pulmonary function

In healthy adults, studies involving exercise protocols in controlled O_3 environments showed increased lung inflammatory responses and impaired pulmonary function to exposure

(Folinsbee & Hazucha, 2000; Kim et al., 2011; Messineo & Adams, 1990). Pulmonary function is frequently and mainly measured by FEV_1, but also through forced vital capacity (FVC) and forced expiratory flow during the middle half of the FVC (FEF_{25-75}). Reduced FEV_1 is associated with obstructive lung diseases, and these spirometric values were also shown to be impaired after protocols with different durations and O_3 concentrations (Folinsbee & Hazucha, 2000; Kim et al., 2011). A study with shorter, 30-min bouts, but high concentrations of O_3, 350 parts per billion (ppb), found that acute exposure to impaired pulmonary function for up to 18 h post exposure (Folinsbee & Hazucha, 2000). Longer exposure protocols also showed that a 6.6 h intermittent exercise protocol on 120 ppb O_3 induced a small but significant decrease in pulmonary function (Horstman et al., 1990).

There are fewer studies in asthmatic individuals than in healthy subjects and none in people with EIB. An early inconclusive study with an intermittent cycling exercise protocol found a significantly greater airway obstruction in asthmatic individuals compared to non-asthmatics at 400 ppb O_3 along with increased specific airway resistance (Kreit et al., 1989). However, they concluded that despite the airway obstruction, individuals with asthma did not show greater change in lung volume, measured by FVC, nor significantly experienced more symptoms than those without asthma diagnose.

Earlier studies in healthy population had already shown the adaptation effect of repeated exposures to O_3 following the largest drop in pulmonary function after second day of exposure (Folinsbee et al., 1980). In another study, FEV_1 and inspiratory vital capacity were significantly decreased after the first exposure while exercising in O_3 exposure (Jörres et al., 2000). These authors noted that on Day 4 of consecutive exposures, the impairment in pulmonary function was smaller than in previous days, suggesting an adaptation effect to repeated exposures. Another study

in healthy adults found that lung function impairments can accumulate over 4 to 5 days, causing further pulmonary function impairment (Barraza-Villarreal et al., 2008). Conversely, other authors did not find adaptation after Day 2 of exposure although they speculate a trend towards adaptation on Days 3 and 4 (Foxcroft & Adams, 1986).

In asthmatics, adaptation to long and repeated exposure to high O_3 levels was also found with larger reported impairment in pulmonary function than in other studies with healthy adults (Gong et al., 1997). Another study in asthmatics found that repeated exposures to 120 ppb O_3 induced an adaptation effect in pulmonary function after Day 2 at the end of a long 6.5 h exposure to intermittent exercise (Linn et al., 1994). They also found that asthmatics experienced EIB during the prolonged exposure, seen in FEV_1 and specific airway resistance measured in-between their 50 min exercise periods. Their study, however, had a potential confounder since participants most likely had been previously exposed to higher ambient levels of O_3 for long periods while living in Los Angeles.

1.3.2 Pulmonary inflammatory response

Whereas some authors mentioned that air pollution can lead to clinical asthma in those genetically predisposed, O_3 air pollution exposures only seem to aggravate that condition (Uysal & Schapira, 2003). When in contact with lower airways, ozonation and peroxidation of lipids, present in the lung surfactant, elicits proinflammatory mediators in airway epithelial cells, which can cause damage and remodelling of these cells (Uysal & Schapira, 2003). Studies that involved bronchoalveolar lavage and sputum analyses after exposure to O_3 found a greater percent of neutrophils and other markers of inflammatory response starting at concentrations of 60 ppb (Jörres et al., 2000; Kim et al., 2011; Scannell et al., 1996). In individuals with allergic asthma, O_3 exposure while exercising increased bronchial response to allergens and increased inflammation

in the airways (Scannell et al., 1996). Authors have suggested that markers of oxidative stress and antioxidant defense remained elevated after the first O_3 exposure when compared with healthy adults exercising in filtered air (Jörres et al., 2000).

In individuals with asthma, O_3 can cause shortness of breath, lung inflammation, and airway hyper-responsiveness which can result in increased symptoms and frequency of bronchodilators need (Gent et al., 2003; Guarnieri & Balmes, 2014). The mechanisms through which one might experience exacerbation or even onset of asthma is still debated (Gowers et al., 2012). However, O_3 is a potent oxidising gas, and with other pollutants, it is believed that it can deplete antioxidant defense mechanisms, causing airway inflammation and injury leading to structural remodelling (Guarnieri & Balmes, 2014). On the other hand, antioxidant supplementation has been shown to mitigate lung injury caused by O_3 (Gomes et al., 2011).

Fraction of exhaled nitric oxide (FeNO) is a marker of airway inflammation that is measured in ppb and has been found to change with air pollution exposure (Ashutosh, 2000; Lehtimäki et al., 2016). Nitric oxide (NO) is produced in the airways and is related to several diseases, including asthma (Ashutosh 2000). Normally, NO levels will be found in low concentrations, but it increases when lung cells are injured (Lehtimäki et al., 2016). Physical exercise seem to reduce FeNO, while food rich in nitrate, or respiratory infections could increase NO readings ("ATS/ERS Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide, 2005." 2005). Field studies conducted in allergic and asthmatics individuals found that low but prolonged levels of O_3 personal exposure were associated with higher FeNO (Karakatsani et al., 2017; Niu et al., 2018). A cohort study in asthmatic children found inflammatory markers to be inversely

correlated with FEV_1 with a greater effect in FeNO elicited by particulate matter and O_3 (Barraza-Villarreal et al., 2008).

After repeated exposures to O_3, oxidative stress and antioxidant defense levels were still more markedly elevated than in filtered air in a healthy cohort (Jörres et al., 2000). Their study found that the percentage of some markers of cellular injury and airway mucosal inflammation collected through bronchoalveolar lavage and biopsies was still significantly high after consecutive exposures. However, these authors concluded that airway mucosal inflammation and injury are increased or sustained, but not mitigated with repeated exposures.

1.3.3 Breathing pattern

Although still debated, changes in pulmonary function and breathing pattern with O_3 inhalation were related to sensitization of C-fibers in the lungs that caused shallow and rapid breathing and is related to bronchoconstriction and changes in O_3 uptake (Schelegle et al., 2001). They found that rapid and shallow breathing in O_3 exposure yielded greater injury to the distal airways, and its mitigation offered protection to terminal bronchioles. However, people respond differently considering the genetic variation in those mechanisms explaining asthma exacerbation to air pollution (Guarnieri & Balmes, 2014). Notwithstanding its relevance, studies involving exercise and O_3 exposure were mainly focused on pulmonary function and frequently kept changes in breathing pattern out of their reports.

At higher breathing frequencies (f_B), O_3 lung uptake is significantly reduced in the distal airways (Gerrity et al., 1988). A long exposure to low O_3 in healthy adults who went through an exercise protocol showed no significant difference in f_B, tidal volume (V_T) and minute ventilation (V_E) (Kim et al., 2011). Studies have shown a 15-50% increase in f_B and one reported a 14% drop in V_T along with increased symptoms (Folinsbee & Hazucha, 2000; Schelegle et al., 2001).

Respiratory symptoms, airway hyperresponsiveness and chest discomfort can impair performance during exercise, and reduce engagement in physical activity due to greater risks of asthma attacks (Jayasinghe et al., 2015).

1.3.2 Cardiovascular function

Changes in cardiovascular impairments in response to air pollutants have been investigated in individuals with EIB (Koch et al., 2020). They suggested that the sympathetic nervous system was activated after diesel exhaust exposure, with an increased heart rate response to exercise. Although still very debated, a population-based study using data from the Canadian Community Health Survey found a correlation between high blood pressure and asthma diagnoses (Dogra et al., 2007). They also suggest that alterations in blood pressure are related to common inflammatory pathways to the pulmonary system in asthmatics.

In turn, O_3 might not only have pulmonary effects but its potent oxidative stress also affects cardiovascular system and macrovasculature (Brook et al., 2002; Srebot et al., 2009). A randomized study exposed healthy participants to a mixture of $PM_{2,5}$ and 120 ppb O3 and found vasoconstrictor effects in the brachial artery (Brook et al., 2002). Their findings suggests that changes in arterial tone might contribute to acute cardiac events since there is a correlation between coronary and brachial arteries (Srebot et al., 2009). Inflammatory mediators in the pulmonary system, as seen in O_3 exposures, can travel through circulation to the heart and cause systemic autonomic dysfunction by stimulating the pulmonary vagal afferent reflex (Brook et al., 2002; Gold et al., 2000). The authors also suggest both vasoconstriction and impaired endothelial function in response to O_3 through inflammatory and oxidative stress.

Arterial stiffness is a way to assess cardiovascular function and can be related to decreased distensibility and elasticity capacities of the vessel and is closely linked with cerebrovascular and

heart diseases, 2nd and 3rd leading causes of death in Canada (Statistics Canada, 2022). Arterial stiffness can be measured non-invasively through pulse wave velocity (PWV), using a transducer or tonometer placed over central and peripheral arteries. PWV is a measure of how fast blood travels in the arterial system and is associated with cardiovascular risk (Mendes-Pinto et al., 2019). Living closer to busy and more polluted streets has been associated with increased arterial stiffness in children (Iannuzzi et al., 2010).

The body of literature available is still inconclusive on the effects of O_3 exposures on heart rate (HR). After a single-exposure exercise protocol in 60 ppb O_3, participants did not show differences in HR (Kim et al., 2011). However, in a repeated-exposure protocol, authors found a small but statistically significant decrease in HR between Day 1 and 4.

1.4 Adaptation to ozone

The mitigation of the health effect from O_3 exposure has been named 'resistance', 'adaptation' or 'tolerance' in early studies. The adaptation evidence in the literature however, is mainly focused on healthy individuals exposed to high doses of O_3 by combining long exposures to higher concentrations of the pollutant (Folinsbee et al., 1980, 1994; Foxcroft & Adams, 1986; Jörres et al., 2000). Such studies showed that when exposing participants to a laboratory-controlled level of O_3 for short periods on consecutive days, they became less susceptible to the adverse effects of the pollutant with a habituation effect on exercise performance, symptoms and pulmonary function. Conversely, studies on pulmonary function and inflammatory adaptation in people with asthma in long exercise protocols in O_3 were previously done, but not in those with an EIB diagnosis (Gong et al., 1997). We also did not find any studies that investigated breathing pattern in individuals with asthma or EIB to a long exposure protocol. Also, the few studies with asthmatics opted for long exposure sessions with intermittent exercise during 2– 6.6 h. Thus, it is

still unknown whether individuals with or without asthma, diagnosed with EIB can adapt to repeated and short exposures of O_3.

1.5 Objectives and Hypotheses

This study sought to determine whether repeated exposures to a controlled level of O_3 can mitigate the impairments on pulmonary and cardiovascular function in individuals with EIB.

Our first hypothesis was that the pulmonary function and airway inflammatory response impairments may be attenuated following short and consecutive bouts of continuous exercise in O_3-controlled lab environment. We also hypothesized that the pollutant would cause a cardiovascular stress response and that a repeated exposure protocol could elicit a tolerance response. This is a novel approach since no previous studies investigated the cardiovascular function response to repeated exposures of O_3.

This understanding will play a role in further uncovering the underlying mechanisms of health effects occurring during exercise in polluted environment and help plan strategies to avoid health-related risks when keeping physically active in such scenarios.

Chapter 2: Methods

2.1 Trial design

This was a crossover, double-blind study performed in individuals with EIB, in two sets of five consecutive visits, between January and August 2022. Participants were randomly exposed to each air quality, room air with low O_3 levels (RA) and 170 ppb O_3, while cycling indoors for 30 min at a moderate intensity. The exercise intensity was prescribed based on a ramp test and set to 60 % of their maximum power output.

The washout period to account for carryover effects was based on two studies, the first found inflammatory responses up to 48 h in bronchoalveolar lavage in mice that were exposed for 3 h to 800 ppb (Fakhrzadeh et al., 2002). That is almost five times the concentration and 6 times longer than what we exposed our individuals to. Also, a study performed in healthy humans that exercised for 60 min in 350 ppb of O_3 showed residual impairments in pulmonary function i.e., FEV_1, FVC and $FEF_{25-75\%}$ responses up to 18 h after exposure (Folinsbee & Hazucha, 2000). Each participant waited at least 48 h before visiting the lab for the second round of 5 consecutive visits regardless of the condition to which they were first exposed.

This protocol follows the CONSORT statement for randomised crossover trials (Dwan et al., 2019). This protocol was registered under the clinicaltrials.gov # NCT05105529. This study received an ethics certificate of full board approval in November, 2021 before the commencement of data collection. The University of British Columbia's Clinical Research Ethics Board approved this study under the # H21-01183.

2.2 Changes from protocol

An ethics amendment was needed to include a 30 min post exercise spirometry assessment halfway through data collection. Our protocol only included a spirometry immediately post exercise; however, we considered it relevant to perform another pulmonary function test before the participant left the laboratory to follow their late response to exposures. Thus, considering that they were already in the laboratory for the cardiovascular and symptoms assessments for 30 min after the exercise, adding another spirometry test was not considered as a burden for the participant. Before that change in the protocol was implemented, the amendment was approved by the ethics committee.

2.3 Participants

Our target population was physically active individuals diagnosed with asthma or EIB who were interested in participating. To be **included** they needed to be:

- able to give informed consent;
- aged ≥18 and ≤ 60 years old;
- physically active;
- non-smoker or that have not quit smoking recently;
- diagnosed with EIB (≥10% drop in FEV_1 in the EVH test)
- able to safely perform a maximal exercise test;
- able to communicate in English.

Individuals were **excluded** if they:

- had musculoskeletal injuries in the lower limb that could interfere or preclude cycling;

- were diagnosed with cardiorespiratory diseases other than asthma/EIB or vascular diseases that might affect performance;
- have had respiratory symptoms or acute conditions in the last month that could affect performance;
- could not go off of medications such as supplementation, antioxidant, vitamins E or C, antihypertensives, beta blockers or inhaled corticosteroids (e.g., beclomethasone, budesonide, fluticasone);
- were currently participating in other experimental studies (exercise, nutrition, medication);
- were pregnant or potentially pregnant.

Participants were recruited through advertisements on public advertising boards around the university common areas, the recruiting website at the School of Kinesiology, the University of British Columbia psychology graduate student council's newsletter and craigslist. Potential participants who contacted our research team that heard about the study through word-of mouth were also considered.

Potential participants that met all inclusion criteria signed the consent form and then visited the lab for an EIB diagnostic test. If individuals tested negative for EIB, they were told they were not eligible for this study. Written informed consent was obtained from all participants prior to data collection. During the consent, potential participants were informed about risks and benefits and asked whether they agreed to participate. Individuals that were still interested were invited for their first visit.

To avoid attenuation of possible lung inflammatory response when exposed to O_3, participants were asked to follow a few recommendations prior to visiting the laboratory for their exercise visits. They were asked to avoid food that contains high levels of nitrates 12 h before their visits

such as rocket, spinach, radish, beetroot, cabbage, turnips, green beans, leek, spring onion, cucumber, carrot, potato, garlic, sweet pepper, green pepper (Bjermer et al., 2014). They were also asked to refrain from food that contains antioxidants that can attenuate the effects of inhaled O_3 such as berries and some vitamins (E and C). They were also asked to refrain from exercise in the 24 h prior to each study visit. Participants were also asked to notify the researchers should they needed to take: short-acting beta 2 agonists (such as salbutamol) 8 h before each visit, leukotriene modifiers (such as montelukast) 24 h prior, long-acting beta 2 agonists 48 h, or antihistamines 72 h before each visit (Hull et al., 2016). These medications were expected to offer a protective pulmonary effect and thus to act as a confounder in this study. Data were collected at approximately the same time each day.

2.4 Settings and location

This study took place in the Environmental Physiology Laboratory, located in the Medical Sciences Block C, room 118, at the University of British Columbia, Vancouver – Point Grey campus.

2.5 Screening visit

After contacting the research team, potential participants were scheduled for their first visit in which baseline tests were done: EIB diagnosis, spirometry, and a maximal graded exercise test.

2.5.1 Eucapnic Voluntary Hyperventilation (EVH) test

The EVH test was used as a diagnostic tool for EIB, and performed with the potential participant sitting still in a comfortable position in a chair. This is a reliable tool to assess bronchoconstriction induced by exercise and has been shown to be the optimal challenge test in laboratory or field studies (Anderson et al., 2001). The protocol for this test involves the individual

hyperventilating for 6 minutes at a set target minute ventilation, 30 X FEV_1 based on the baseline spirometry while breathing 5% carbon dioxide to avoid symptoms related to carbon dioxide washout, i.e., dizziness and fainting. To be diagnosed with EIB, a decrease in FEV_1 equal to or greater than 10% after the challenge indicated the presence of EIB. After the breathing challenge, spirometry was repeated at 3, 5, 15, and 20 minutes or until lung volumes returned to baseline. If after that time the individuals were still experiencing symptoms and reduced values on the spirometry test, they were asked whether they would have had their salbutamol inhaler if they were outside of the lab setting. If they said yes, then we would recommend they would take their prescribed dose of their short-acting inhaler. Only after symptoms were resolved and spirometry had returned to baseline did we invite those with EIB to perform the next phase of the screening visit. Those without EIB were told they were not eligible for the study and did not need to perform the maximal ramp test.

After the EVH test, individuals completed the Physical Activity Readiness Questionnaire PAR-Q+ to assure safety to perform the maximal exercise test (Warburton et al., 2019). If no risks to performing physical exercise was highlighted, then they were invited to the next phase.

2.5.2 Maximal graded exercise test

Individuals were asked to perform a maximal graded exercise test using a ramp protocol in a Velotron DynaFit Pro cycle ergometer in room air. This test was used to prescribe the exercise intensity, i.e., power output, for the study visits. Expired gases were analyzed with a ParvoMedics' TrueOne® 2400 metabolic measurement system (Sandy, UT, USA). Their maximal oxygen consumption (VO_{2max}) was solely used to describe their fitness level. The maximal test was designed to start with a warm-up period at a self-selected pace and intensity if they considered necessary. After a quick warm-up, the ramp protocol started at 30 watts on the cycle ergometer

with the workload increasing by 20 watts per minute for women or 30 watts per minute for men until the participant could no longer sustain a cadence of 60 revolutions per minute (rpm) or until volitional exhaustion. For all study visits, participants were prescribed the same exercise intensity, which was 60% of their maximal power output on that maximal exercise test.

After completing the screening visit, individuals were then invited to participate on this study. They were then scheduled to start their first set of five visits.

2.6 Interventions

2.6.1 Study visits

Each study visit included the participant coming to the laboratory, having their baseline assessments, performing 30 min of cycle exercise in the assigned air quality, and before leaving, they performed their post exercise measurements. Each laboratory visit lasted for up to 1.5 h and assessments were performed in the following order:

1. Baseline symptoms questionnaire
2. Questionnaire of food ingredients consumed in the previous 12 h, medication and exercise in the last 24 – 48 h
3. Baseline pulmonary function and inflammatory status assessment
4. Baseline cardiovascular function assessment
5. 30 min exercise in the assigned weekly air quality
6. Post exercise measurements: symptoms, pulmonary function and inflammatory response, cardiovascular function

After the first five visits, participants completed a 48-h washout period and crossed over for another 5 visits in the other air quality.

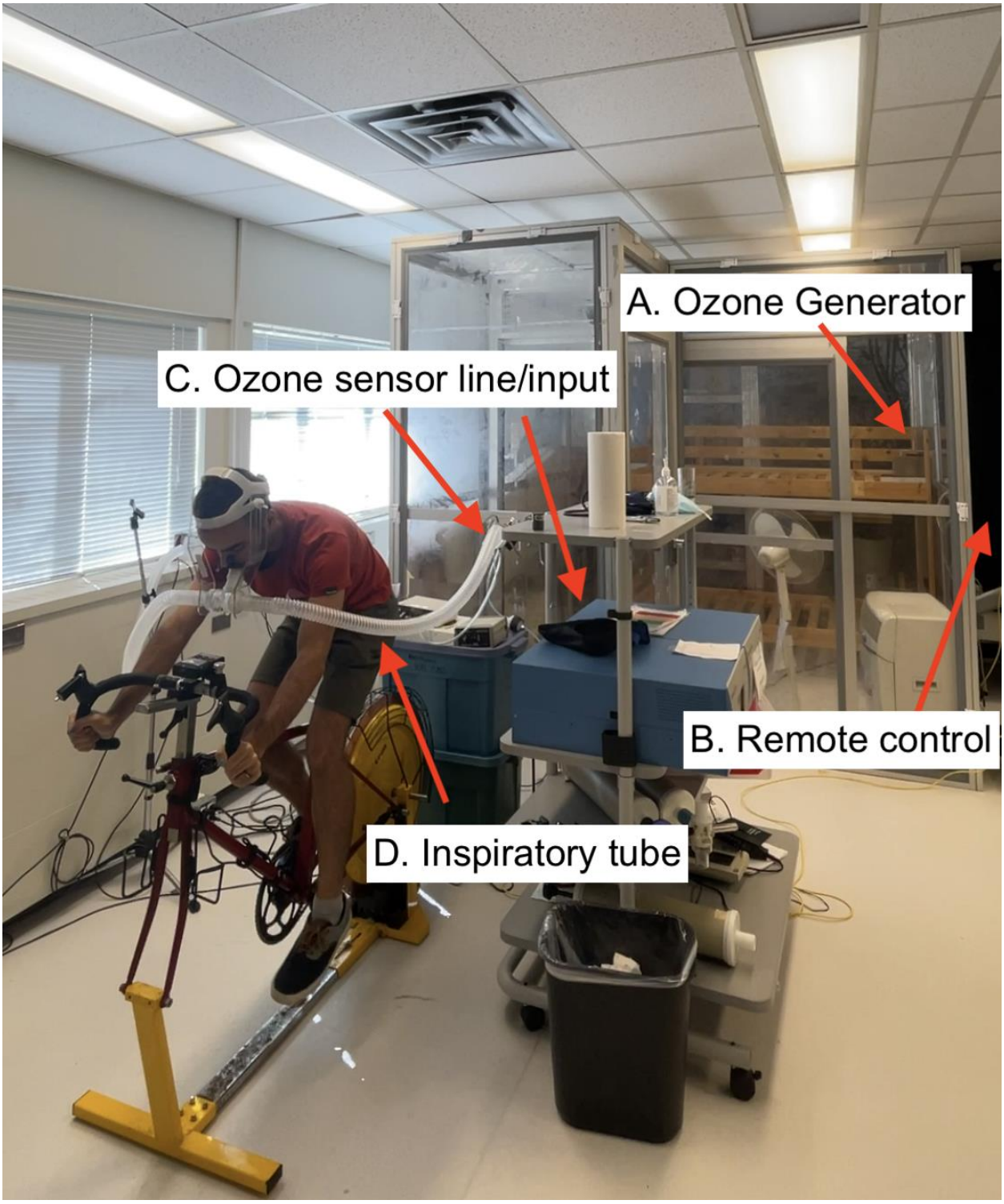
2.6.2 Ozone delivery

The O_3 at 170 ppb was fed into a 3 m x 2 m x 2 m sealed chamber that was connected to the participant's inspiratory tube outlet, Figure 1. O_3 was produced with a corona discharge generator, ACT-5000 Ozonetech (Mellifiq, Sweden) and mixed with room air in the chamber. The inspiratory breathing tube connected to the chamber was made of ethylene vinyl acetate which is not expected to react with ozone (Sisanth et al., 2017). One end of this tube was connected directly into the chamber, where the sensor was placed, and the other end was plugged into a one-way inspiratory valve on the participant's mouthpiece. No breathing masks were used in this study to avoid O_3 reaction with skin products.

The Thermo Scientific™ 49iQ Ozone Analyzer (Franklin, MA, USA) was used as an O_3 sensor and was attached to the inspiratory tube before it reached the participant mouth. This sensor was calibrated regularly according to the manufacturer using the Thermo Scientific™ 49iQ Ozone Primary Standard generator.

A trained research assistant was responsible for generating O_3 and was positioned in a blinded spot outside of the chamber where they could control the O_3 generator while watching the O_3 sensor screen remotely. This research assistant started generating O_3 a few minutes before the participant was connected to the mouthpiece with the inspiratory tube containing O_3. Before the participant was adjusted with the mouthpiece, a plug shut close the inspiratory tube so O_3 would not leak into the lab. When the participant was ready to exercise and connected to the chamber, the research assistant started recording the pollutant concentration.

Figure 1 Study setup



2.6.3 Room air delivery

Low ozone air, i.e., RA, was delivered with the same setup, through a mouthpiece plugged into the chamber. The same protocol was used for recording the pollutant concentration inside the chamber. However, when the participant was expected to exercise on RA, the O_3 generator was not turned on leaving the inhaled O_3 level to the laboratory ambient air. The O_3 concentration inside the chamber were still recorded through the exercise during the RA week. The chamber air content was cleaned with a fan every time a participant was scheduled after the other regardless of the air quality.

2.7 Primary outcomes

2.7.1 Spirometry: pulmonary function

For the main pulmonary function measures, lung volumes and flow were assessed using the ParvoMedics OUS-SPIRO on the metabolic cart (Salt Lake City, UT, USA), which was calibrated before every participant. This is the gold standard assessment of pulmonary function with a well-established protocol and substantially used in exercise-related research (Graham et al., 2019). FEV_1 and FVC were measured in liters (L), and $FEF_{25-75\%}$ in liters per second (L/s). Participants were asked to wear a nose clip and perform the maneuvers through a mouthpiece with a filter attached to a handheld pneumotach on the ParvoMedics metabolic cart. They were asked to sit comfortably in a chair, with the chest upright and the feet planted on the floor, to perform a couple of normal relaxed breaths and at the end of a normal breath out, to perform a maximal inspiration to maximal lung capacity followed by an exhalation that should be as hard and as fast as they could for 6 seconds. This was performed at baseline, when they came into the laboratory, and right after they finished the 30 min cycling.

2.7.2 Arterial stiffness: cardiovascular function

For the main cardiovascular function assessment, non-invasive carotid to femoral pulse wave velocity (PWV) measurements were performed using an automated PulsePen® system (PulsePen®, DiaTecne, Milan, Italy) and using two Pulse transducers (TN1012/ST, ADInstruments). For the pulse transducer, input signal was collected through a PowerLab data acquisition system. The PulsePen® software automatically reported PWV. For the pulse transducer, the input signal in volts acquired from LabChart was manually calculated using a peak-to-peak approach measuring the time interval between each pulse wave peak at least 20 s worth of data, which corresponded to at least 12 consecutive pulses. Distance between carotid and femoral pulses was taken with a measuring tape for both techniques. The main researcher was responsible for measurements to avoid inter-examiner variations in the technique.

Figure 2 Pulse transducer placement in carotid and femoral sites

Arterial stiffness was measured at baseline, after the lung assessments and after lying in a comfortable supine position in a quiet laboratory for at least 5 min before and after the exposures.

2.8 Secondary outcomes

2.8.1 Fractional nitric oxide in expired breath (FeNO)

This marker of airway inflammatory response was used as a secondary outcome and the NO concentration was given in ppb. We chose a more affordable tool to assess lung inflammation than previous studies, a 2nd generation NOBreathe® FeNO monitor (Bedfont® Scientific Ltd., England). The monitor's sensor was already calibrated from the company prior to the commencement of data collection. While wearing nose clips and sitting in a comfortable position, participants were asked to perform a deep inhalation away from the mouthpiece and to exhale deeply and constantly for 12 seconds into the mouthpiece of the device until told to stop by the equipment monitor. The NOBreathe® FeNO monitor screen provided constant visual and audio feedback so the participant could keep a constant flow throughout the exhalation. If the participant substantially changed flow or was not blowing out to the desired flow that was preset in the machine, the monitor would beep until they performed it correctly. This was repeated three times at each measurement timepoint, the first measure was always discarded, and the last two were averaged and recorded. The FeNO was always collected before spirometry at baseline, immediately after the participant was disconnected from the mouthpiece delivering RA or O_3.

2.8.2 Dyspnea and other symptoms

Participants were asked to report the intensity of their dyspnea by choosing a number on a 0-10 modified Borg scale in which zero represented "nothing at all" and 10 "maximal" symptom (Gaber et al., 2019). This was asked when they walked into the lab at rest, and at peak exercise.

Participants were asked to rate other symptoms using the same Borg modified scale that included: chest tightness, chest wheezing or whistling sounds, dry throat, scratchy throat, itchy nose, running nose, headache, fatigue. These symptoms have been mentioned in the literature as usual complaints in individuals with asthma and EIB and protocols that involved air pollution exposure like O_3 (Folinsbee et al., 1980; Scannell et al., 1996). These symptoms' questionnaire was completed in every session at baseline, and right after the participant finished the 30 min cycling.

2.8.3 Breathing pattern

During exercise, participants breathed through a mouthpiece that was connected to a pneumotach connected to a PowerLab signal receiver, and viewed with LabChart version 8 for Windows (PowerLab 16/35, 2014, ADInstruments Pty Ltd., Australia). Breathing pattern measures included f_B in breaths per minute (bpm), V_T in liters, VE in liters per minute (L/min).

2.8.4 Heart rate

HR was monitored with a Polar heart rate monitor (Polar Electro Oy, N2965) placed around the chest connected to the PowerLab and recorded in beats per minute (bpm).

2.8.5 Blood pressure

Systolic and diastolic blood pressure (SBP and DBP) were recorded using a manual aneroid sphygmomanometer around the left arm and expressed as mmHg.

2.9 Sample size

Sample size was calculated from previous studies that investigated pulmonary function through changes in FEV_1 after O_3 repeated exposures and changes in PWV after diesel exhaust exposure

(Folinsbee et al., 1994; Lundbäck et al., 2009). Based on those studies, a total of 16 participants were required to achieve a statistical power of 80% and significance level of 5%.

2.10 Statistical analysis

Demographic and baseline characteristics, exercise type, and training volume are summarized as means (SD). Data were tested for normality with the Shapiro-Wilk test and analyzed using R Stats Package and RStudio (2022.07.01 for macOS). A statistical significance level of 0.05 was used.

Considering a cumulative effect of the exposure on pulmonary function, changes in FEV_1, FVC, FEF_{25-75} after each exposure were expressed as a percentage change of the pre-overall exposure. Spirometry and other recorded variables were also presented as absolute values.

To test for differences between conditions at baseline, independent t tests were performed with pre-exposure measures in RA vs O_3. To test the main hypothesis of this study, whether changes in pulmonary or cardiovascular function was different between exposures, RA vs O_3, a linear mixed-effects model was performed to test for differences across each of the five study visits. Tukey's post-hoc paired tests were used to identify whether a significant difference in outcomes was still present when comparing each of the study visits. We also used paired t-tests to check the within-subject differences on each study visit (pre vs post).

2.11 Randomization

A block randomization scheme was generated prior to data collection using random.org. A sequence of eight blocks of two was generated (i.e., AB, BA, etc) in which each letter corresponded to the air condition the participant was starting their first week. This allowed a random sequence generation, a balanced sample size as well as an allocation concealment. The sequence list was

kept by the research assistant that was only responsible for operating the chamber and the allocation concealment was kept until the beginning of the participant's first visit.

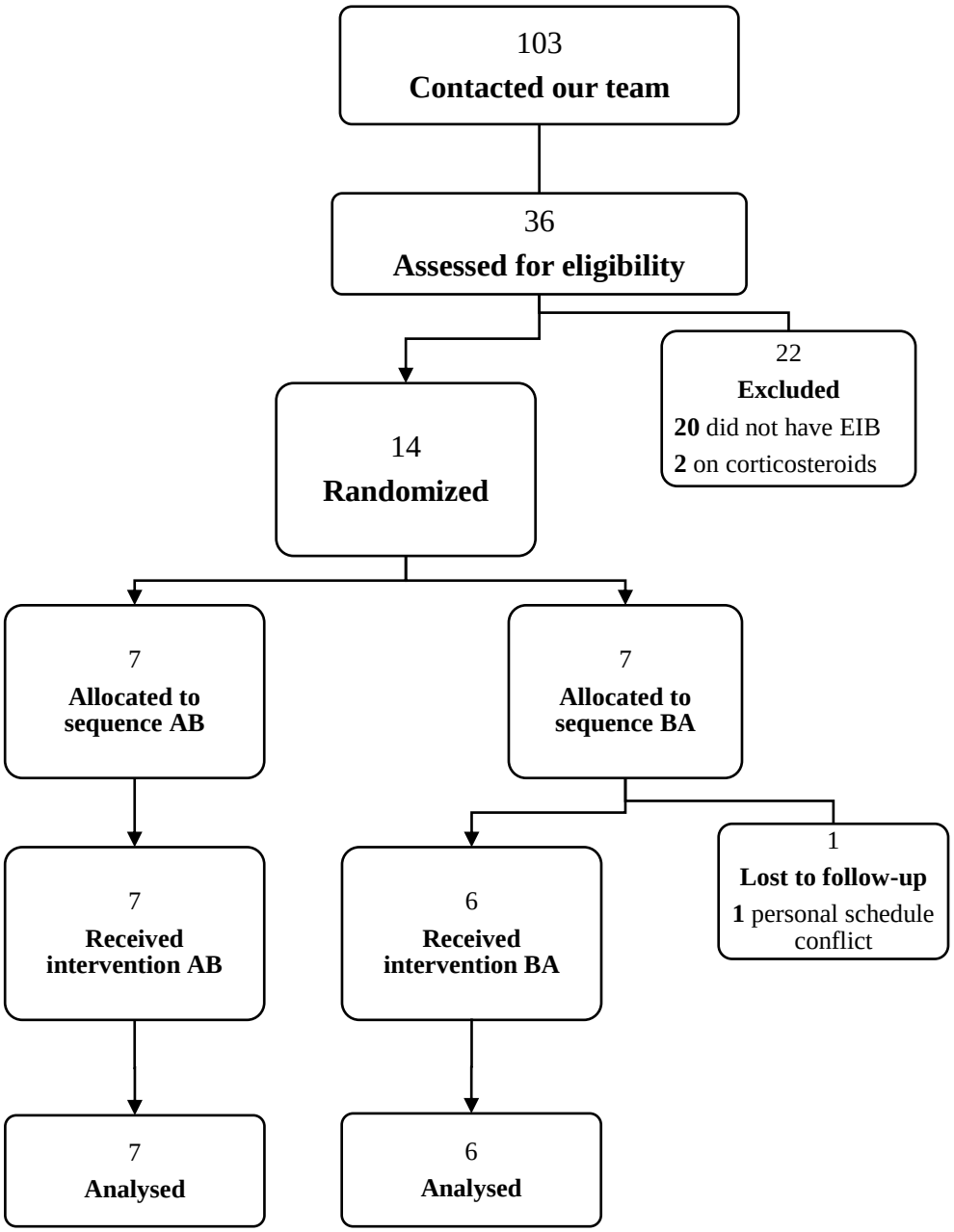
2.12 Blinding

Participants and the researcher involved with enrolling and data collection were blinded to the air quality and to the randomization scheme. Participants were not told to which exposure they were assigned until their study visits were completed. The chamber was sealed for leaks and only opened after the researcher involved with data collection left the room to avoid unblinding by the O_3 smell.

Chapter 3: Results

A total of 103 people contacted our team and 36 responded to our e-mail and visited the lab for a screening visit, of which 14 tested positive for EIB, were invited to participate in the study, and randomized, Figure 1. One participant dropped out before their fourth visit due to a conflict in their personal work schedule and could not commit to the following study visits. Thus, the results shown in this section will be for 13 participants that completed the two sets of five consecutive visits in their assigned allocation sequence. Average O_3 level during RA study visits was 9 ppb whereas during O_3 week it was 170.5 ppb.

Figure 3 Participants flow diagram



This study included a majority of female participants, 53.8%, Table 1. Of the thirteen people, 61.5% were diagnosed with asthma previously and prescribed short-acting β-agonists, i.e. salbutamol, and two participants were prescribed long-acting β-agonists, i.e. budesonide/formoterol. However, both participants were not using this medication daily prior to

the study as they mentioned they only needed it at specific times of the year. These participants also voluntarily offered to stop taking the corticosteroids at least 48 h before their study visits to be eligible to participate. One participant stopped 48 h before and the other at least 5 days before the study started.

All participants had an EVH test performed prior to starting data collection on the screening visit, and their average drop in FEV_1 was 17.2%. The minimum drop in FEV_1 in the EVH test was 9.2%. That is due to one participant not being able to reach the VE target on the EVH test for most of its duration. The maximal drop in FEV_1 in the EVH test was 43%. Peak power output on the maximal graded exercise test on the cycle ergometer was 247 Watts. The main physical activity performed by participants in this study was cycling, and 30.8% of the total sample competed in local cycling events. Two participants (15.4%) were not regularly performing any kind of physical activity, but considered themselves active.

Table 1 Baseline demographic and clinical characteristics

	All (n= 13)
Age, years	28.8 (11.5)
Female, n (%)	7 (53.8)
BMI	23.4 (2.3)
Asthma diagnose, n (%)	8 (61.5)
Short-acting β-agonist, n (%)	8 (61.5)
Long-acting β-agonist, n (%)	2 (15.4)
FEV_1 drop in % (SD)	17.2 (9.1)
VO_{2peak}, ml/kg.min^{-1}	36 (10)
Peak power output, W	247 (92.3)
Physically active, n (%)	12 (85.7)
Cyclists, n (%)	5 (38.5)
Runners/joggers, n (%)	3 (23.1)

Legend: Data is shown in mean (SD). BMI: body mass index. FEV_1: forced expiratory volume in the first second. $VO_{2peak:}$ peak oxygen consumption in the maximal graded exercise test. W: watts.

No statistically significant difference was found in the baseline (Day 1) between exposure order for pulmonary, FeNO, and cardiovascular function measurements.

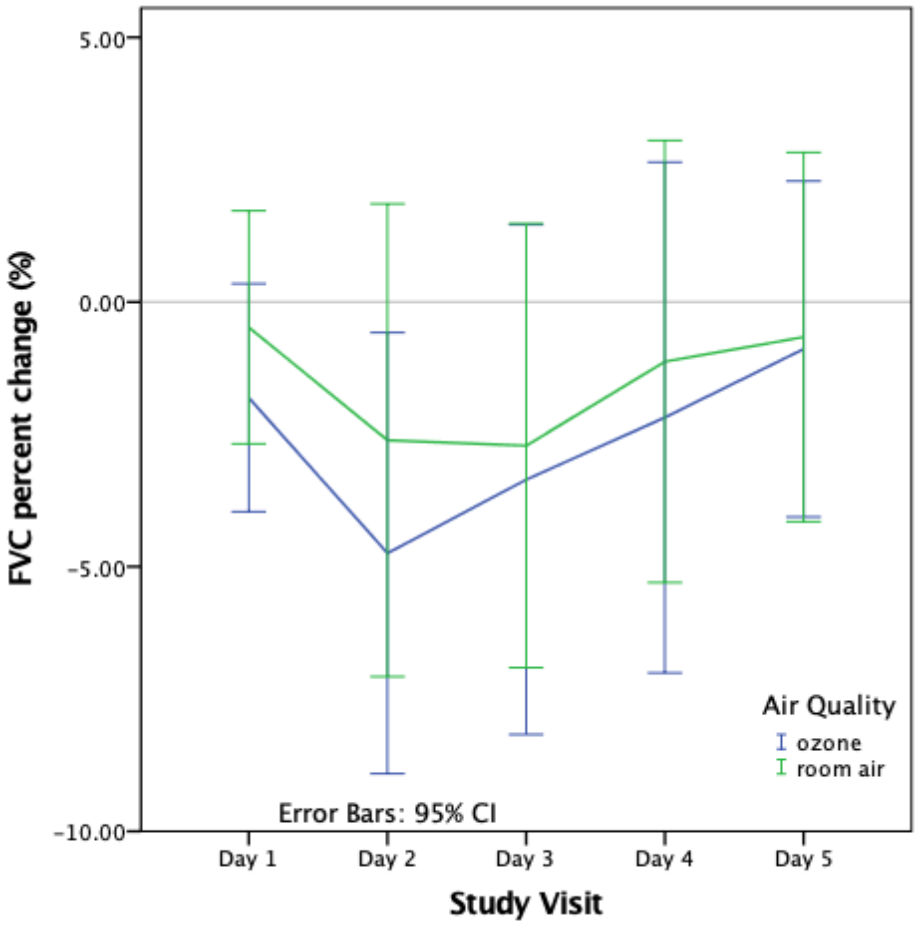
3.1 Pulmonary function

Overall, measurements of pulmonary function showed a minor decrease through Days 1-3 of O_3 exposure with a recovery towards Days 4 and 5, whereas in RA there were either an increase

post exercise or no change. In contrast to the RA exposures, absolute lung volumes dropped after all study visits in which they were exposed to O_3, Table 2 in Appendix A.

Absolute FVC values dropped after O_3 exposure on Days 1, 3, 4 and 5 and remained the same as baseline on Day 2, Table 2. The greatest percent drop in FVC from baseline, -4.6% (6.9), was on Day 2 of O_3 exposure, Figure 4. The linear mixed effects models did not show a statistical difference in absolute values between study visits. Percent changes from baseline (Day 1) were not statistically significant for FVC, Table 3 in Appendix B.

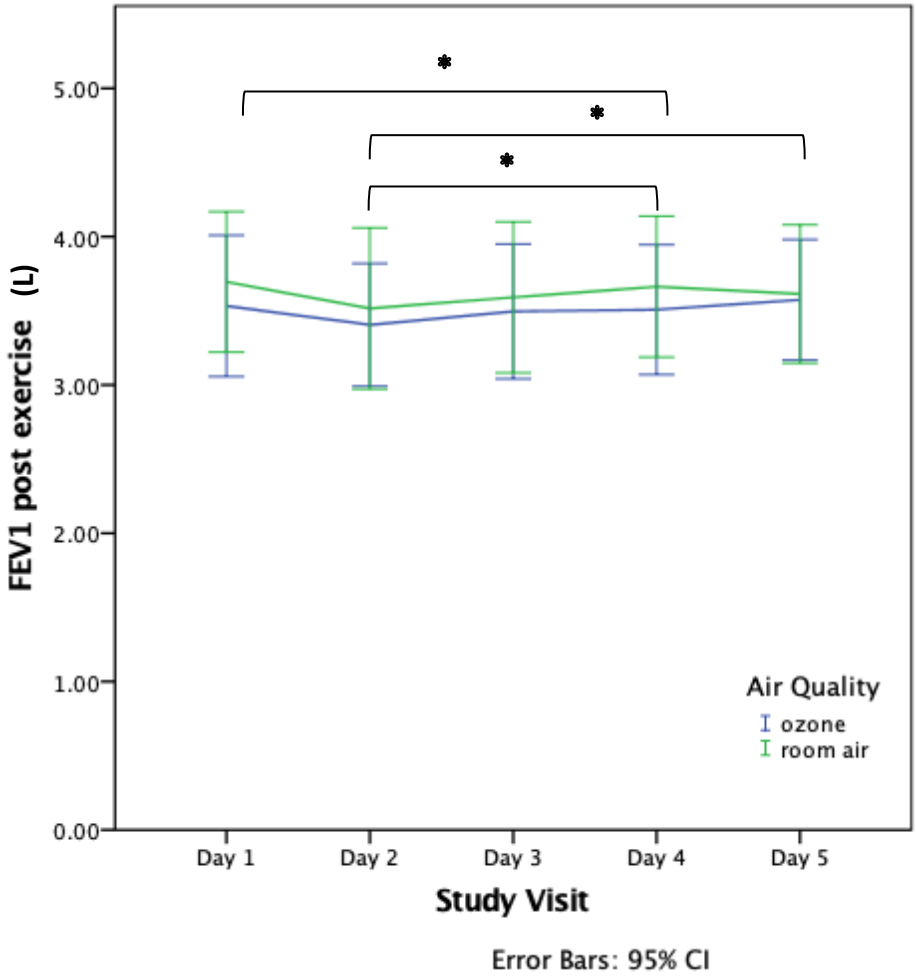
Figure 4 Percent change in forced vital capacity (FVC) from baseline on Day 1



Analysis of absolute values showed that FEV_1 after exercise was significantly lower on Day 1 of O_3 compared to Day 4 of RA (p= 0.04), on Day 2 of O_3 vs Day 5 of RA (p= 0.03), and

also on Day 2 of O_3 vs Day 4 of RA (p= 0.05), Figure 5. The decrease in FEV_1 of 0.2 L seen post O_3 exposure on Day 1 was small yet statistically significant compared to its baseline, p= 0.03 (95% CI: 0.02 – 0.23), Table 2.

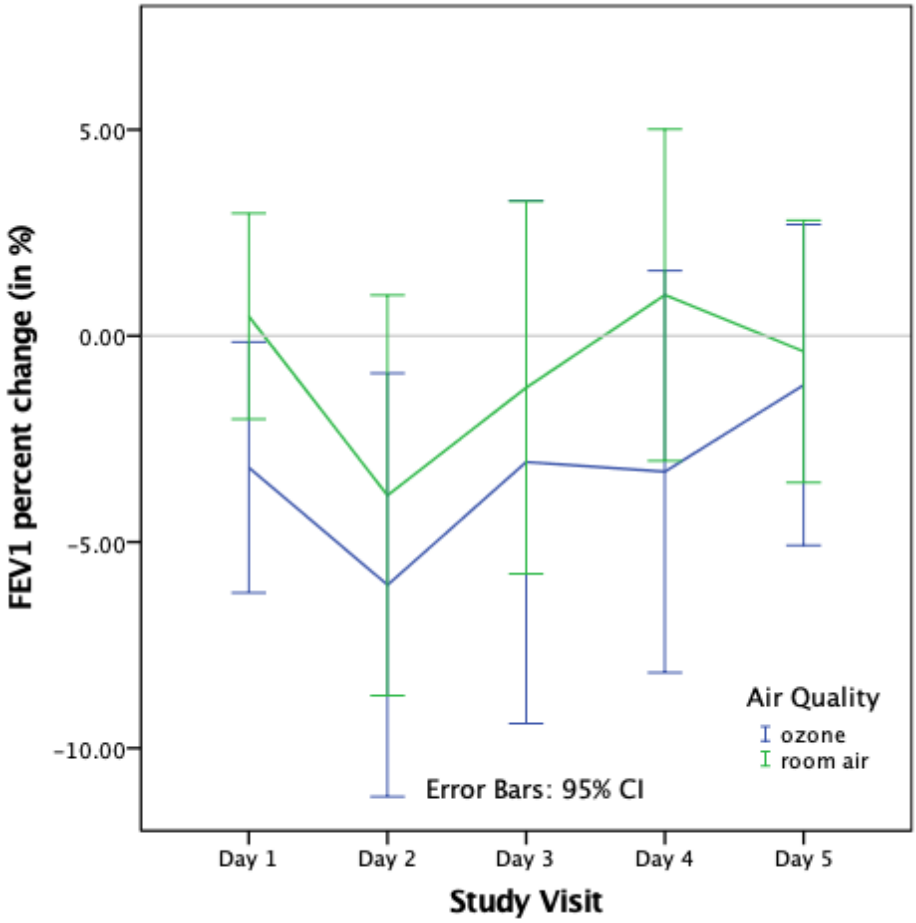
Figure 5 Forced expiratory volume in one second (FEV_1) measured post exercise



Legend: * indicates statistically significant differences between ozone and room air with p< 0.05.

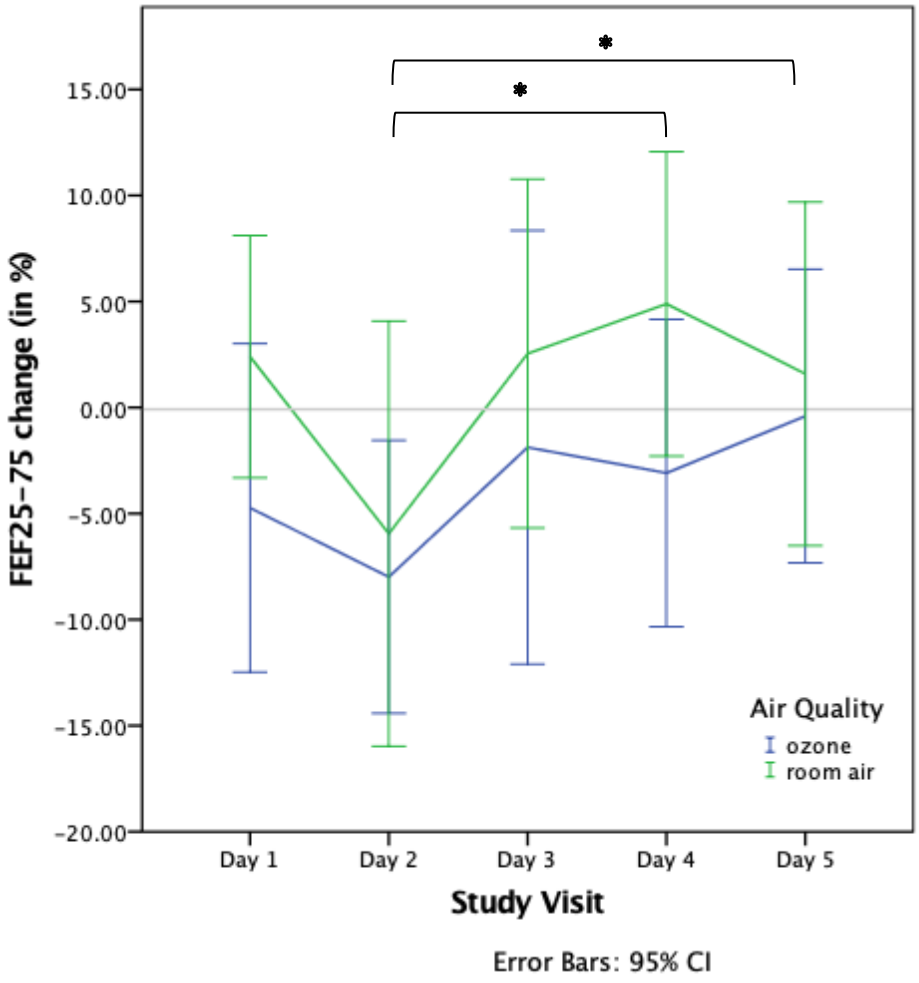
In the percent change analysis from baseline, FEV_1 reached its lowest value on Day 2 of O_3 exposure, -6.1% (8.4), Figure 6. None of the percent changes in FEV_1 were statistically significant when comparing to baseline on Day 1.

Figure 6 Percent change in forced expiratory volume in one second (FEV_1) from baseline



The absolute value for post exercise FEF_{25-75} was significantly lower on Day 2 in O_3 compared to Day 4 in RA, p= 0.02. On Day 4 in RA exposure, FEF_{25-75} increased 0.1 L/s, and that was statistically significant compared to pre-exposure to RA on the same day, p= 0.04 (95% CI: -0.2 – -0.01). Analysis of absolute values showed that FEF_{25-75} was lower after O_3 exposure on Day 2 when compared to RA on Day 4, p= 0.02 and Day 5, p= 0.04. The percent change in FEF_{25-75} was significantly different in Day 2 of O_3 when compared to RA on Day 4, p= 0.04 and to Day 5, p= 0.05, Figure 7.

Figure 7 Percent change of FEF_{25-75} in % from baseline on Day 1



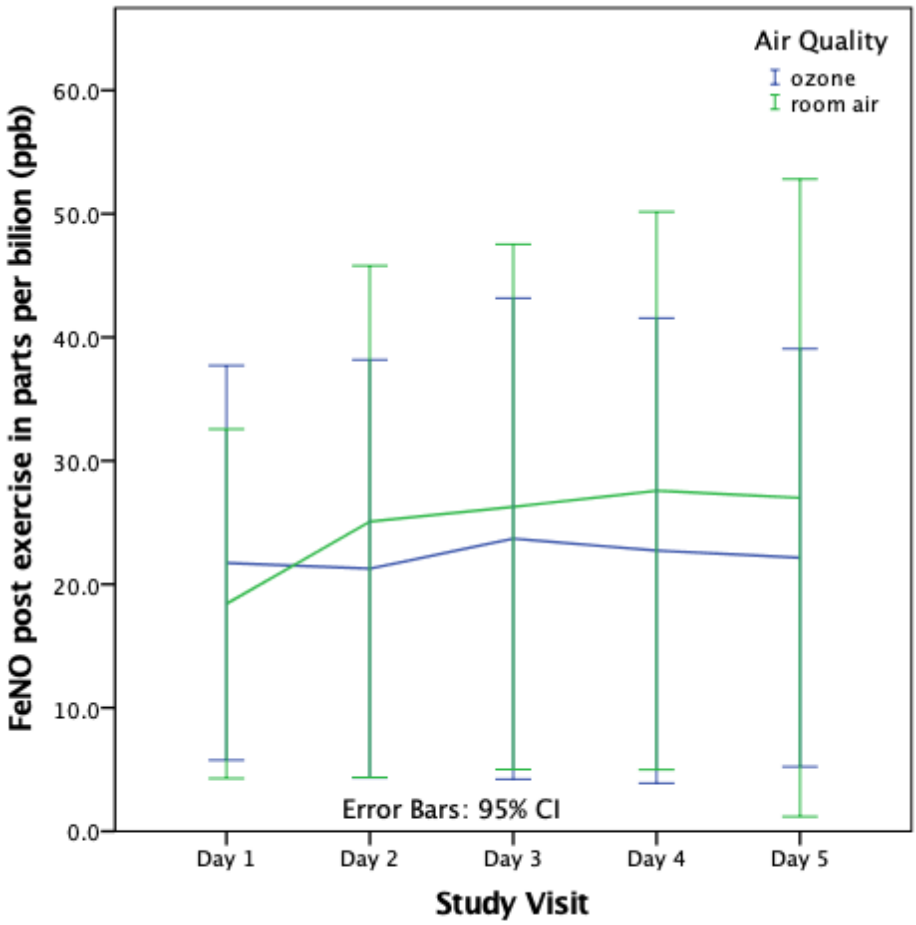
Legend: * indicates statistically significant differences between ozone and room air with $p<0.05$.

Individual response for FEV_1, FVC and FEF_{25-75} are plotted in the appendix

3.2 Pulmonary inflammatory response

FeNO was only greater than RA after O_3 exposure on Day 1 (by 3.3 ppb) with no statistically significant difference on the independent t-test for that visit. On all other visits FeNO was greater in RA than in O_3, also with no statistical significance, Figure 8.

Figure 8 Fraction of exhaled NO post exercise

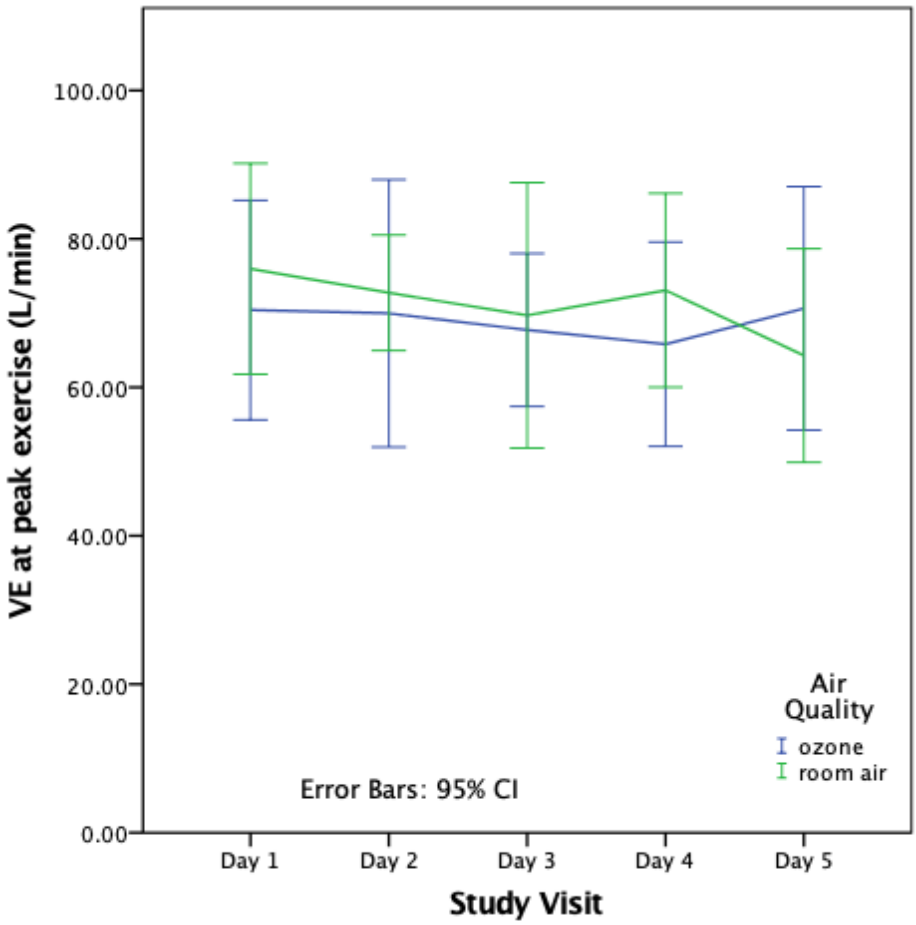


3.3 Breathing pattern

At peak exercise during the O_3 exposures, V_T was consistently lower than RA in all study visits, Table 3. The greatest difference between RA and O_3 was on Day 2, of 0.3 L. Also, f was lower on O_3 exposures on Days 1 and 4. VE was lower at peak exercise with O_3 exposure on the first 4 visits and greater than RA on the last visit, Figure 9. During the O_3 exposure, participants had a peak exercise VE 7.3 L/min lower than RA on Day 4.

The average VE at peak exercise in O_3 exposure was 68.9 L/min (18) and in RA 71.1 L/min (17.7). There were no statistically significant differences between air qualities amongst study visits for V_T, f or VE.

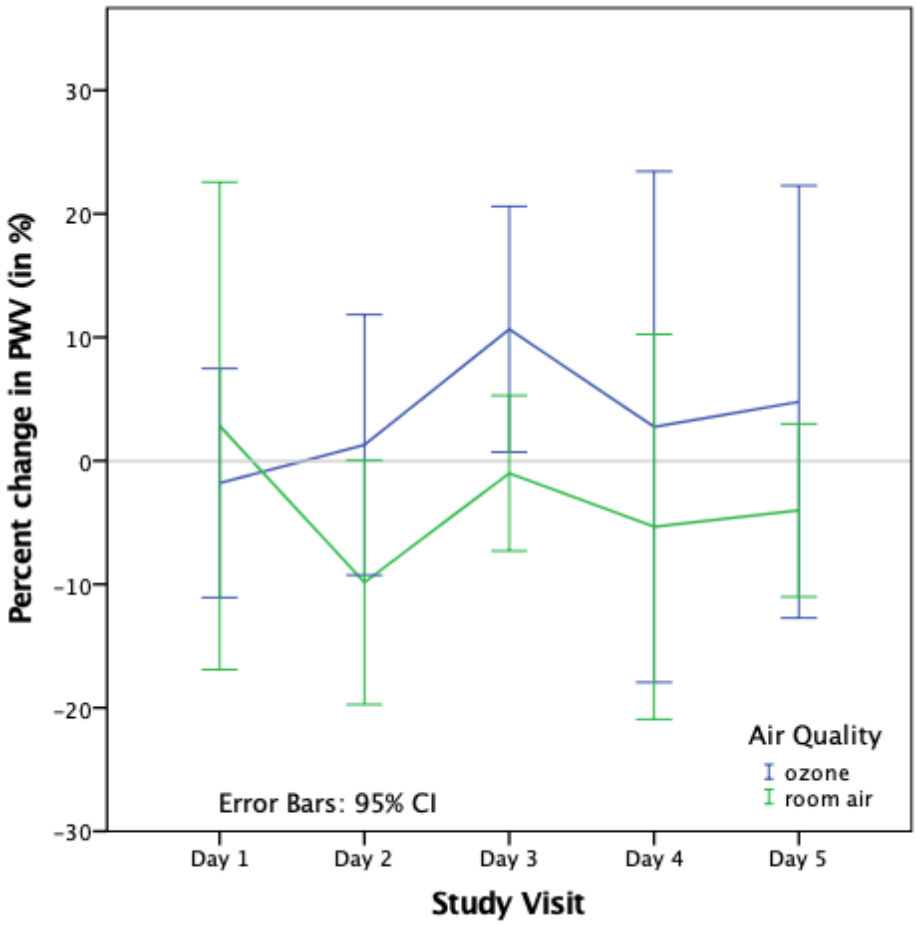
Figure 9 Minute ventilation (VE) at peak exercise



3.3 Cardiovascular function

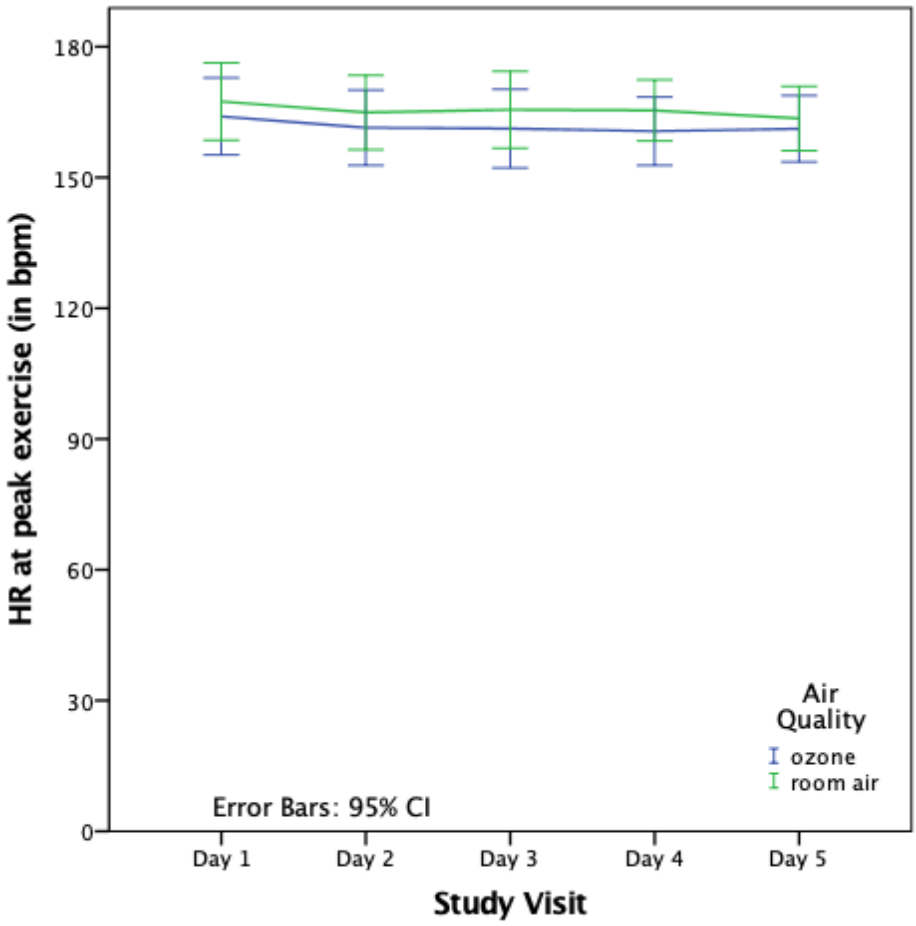
On Day 2 of O_3, PWV was higher than any of the other days in any air quality, Table 2. The percent change from each day's baseline was lower after O_3 exposures than RA on Day 1 and greater on the remainder visits, Figure 10. However, none of those differences were statistically significant in the linear mixed effects model analysis.

Figure 10 Percent change in Pulse Wave Velocity (PWV)



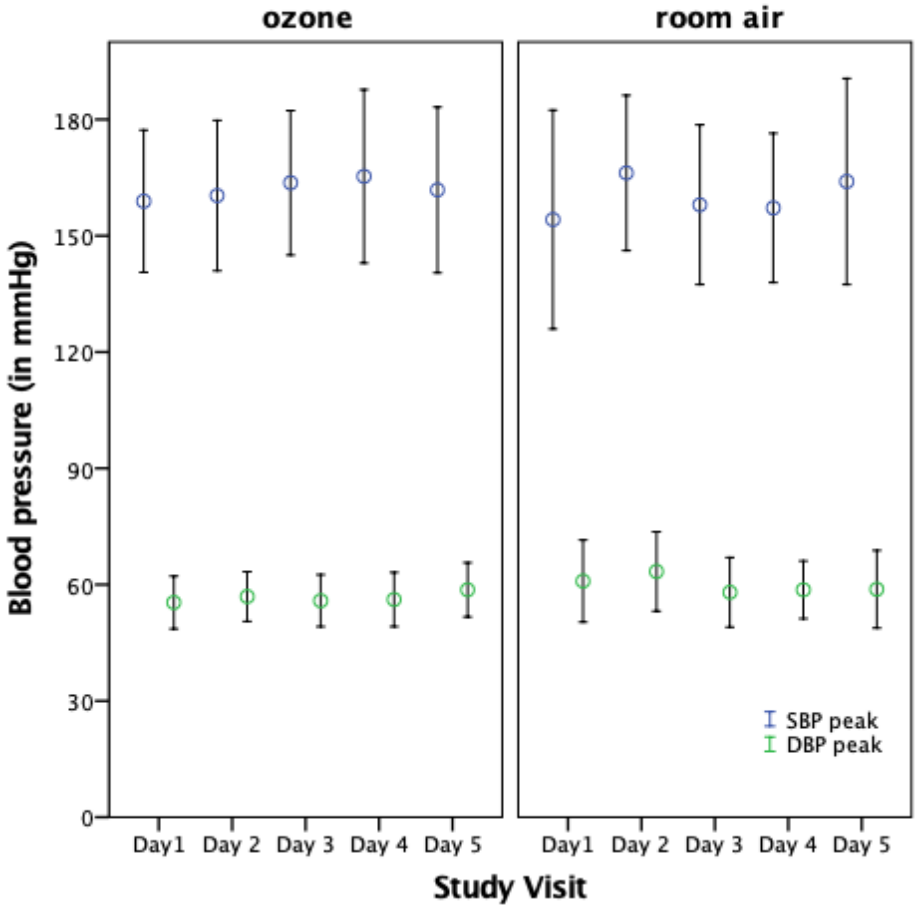
HR was lower at peak exercise on all study visits when participants were exposed to O_3; however, that small difference was not statistically significant across study visits, Figure 10. The largest difference was seen on Day 4, of 5 bpm, and was also not statistically significant.

Figure 11 Heart rate (HR) at peak exercise in beats per minute (bpm)



When participants exercised in O_3, peak SBP was lower than in RA on Days 2, and 5, Table 3 in Appendix B. DBP at peak exercise was lower on the first 4 visits in O_3 exposure. No statistically significant differences were found in these differences in SBP or DBP at peak exercise, Figure 12.

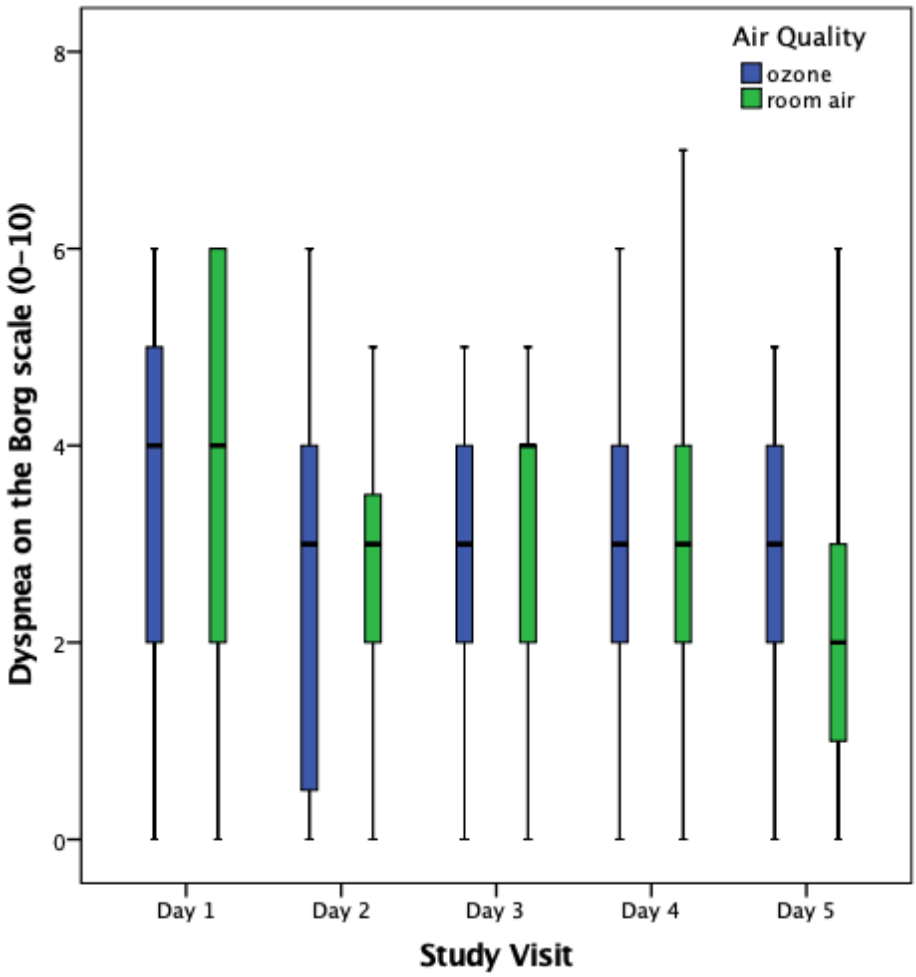
Figure 12 Systolic (SBP) and diastolic blood pressure (DBP) at peak exercise



3.4 Dyspnea and other symptoms

Participants exposed to O_3 did not show statistically significant difference to RA, Figure 13. Boxplots for other symptoms are shown in Appendix C, Figures 14-21. No statistically significant difference was found in the linear mixed effect model for any symptoms when comparing both exposures through the five days.

Figure 13 Dyspnea at peak exercise in both exposures



When questioned about which air quality they were exposed to at the end of each round of five study visits, participants were accurate 46% of the time. The main reason participants gave to why they chose whether that week was RA or O_3 was due to the frequent presence of respiratory symptoms during O_3 week compared to RA. Only one participant reported that they noticed a different taste in the air delivered through the mouthpiece during the actual O_3 week, but was unsure whether that was from the plastic components of the tubing.

Data were analysed by the original group to which participants were assigned at the commencement of the study. That is, there were no changes in the randomization sequence and allocation. No harms were reported during this study.

Chapter 4: Discussion

This study aimed to investigate the changes in pulmonary and cardiovascular function of individuals with EIB following repeated exposures to both RA and 170 ppb O_3 during exercise. We hypothesized that repeated exposures to O_3 would mitigate the acute pulmonary impairments that most likely would reach the nadir during the first days, as seen in other studies with healthy individuals, but in individuals diagnosed with EIB. The main analyses demonstrate that pulmonary function was slightly, yet significantly, impaired especially on Days 1 and 2 of O_3 exposure. We also hypothesized that the cardiovascular function could be impaired by O_3 exposure in people with EIB. We observed that arterial stiffness in response to O_3 tended to be most pronounced on the day that pulmonary function was most impaired (Day 2) but this trend was not significant.

4.1 Pulmonary function

Our main finding in this study is that pulmonary function was mostly impaired on the first two days of O_3 exposure compared to RA and that is in accordance with other repeated exposure studies (Folinsbee et al., 1980). In the post exercise spirometry on Day 1 of O_3, FEV_1 was significantly lower than its baseline (-0.2 L). This finding is in accordance with an earlier study performed in asthmatics that found a similar reduction in FEV_1 (Linn et al., 1994). Nevertheless, in their study that difference was only seen after 6.5 h of exposure to 120 ppb O_3 whereas we found the same drop in a 30-min exposure protocol. Moreover, their sample included participants with severe asthma with a range of predicted FEV_1 from 39-90%. On the other hand, in our sample, almost 40% participants did not have chronic asthma, and those who had been diagnosed only had mild to moderate EIB and were comfortable to discontinue their long acting β-agonist medication for the duration of the study. However, FEV_1 impairments are not limited to people with asthma/EIB. There are inconsistent findings for single exposure to O_3 in the literature, in which

healthy or asthmatics seem to have similar responses. A study that exposed healthy participants to 160 ppb O_3 showed a significant drop in FEV_1 also around 0.2 L (Avol et al., 1984). But, the novelty of our findings is that we looked into the adaptation to repeated exposures in people with EIB, with or without asthma.

With regard to adaptation, on day 1 of O_3 exposure in our study, absolute FEV_1 was significantly lower in O_3 than on Day 4 of RA, and on Day 2 it was lower than on Days 4 and 5 of RA. On Days 3, 4 and 5 that difference was no longer significant. These spirometry findings show that the greater airway obstruction seen on the first two exposures to O_3 was mitigated towards the last three exposures.

Another important finding of this study was that FEF_{25-75} was significantly lower on Day 2 of O_3 compared to Days 4 and 5 of RA exposures. Although we found a smaller decline (-0.1 L/s) in our participants, this was a significant change which is in accordance with another study in asthmatics that found a significant decrease of 0.9 L/s in FEF_{25-75} (Kreit et al., 1989). However, in that study participants were exposed for 2 h to 400 ppb, which can explain the lesser prominent impairment in FEF_{25-75} seen in our results for both reasons, our protocol being shorter and exposing participants to less than half the O_3 concentration of that study. Despite the low decline, the significant impairment in FEF_{25-75} implies an obstruction effect of O_3 on medium to small airways. Moreover, on Day 2 of O_3 exposure, FEF_{25-75} dropped -8.1% from baseline and remained impaired for the entirety of the study visits. However, on Day 5 it was only -0.3% below baseline levels, whereas in RA exposures, FEF_{25-75} only dropped below baseline on Day 2 sustaining scores above baseline for all other study visits.

Being exposed to O_3 played a role in impaired pulmonary function on Day 1 and 2, since obstruction kept spirometry values for FEV_1 and FEF_{25-75} significantly lower than RA.

Furthermore, the significant impairments on first days of O_3 exposure were not significant in the last three days, which could be interpreted as a mitigation of the initial pulmonary function impairment in response to repeated exposures to O_3.

Although FVC was lower in O_3 than in RA in all of our post exercise measurements, the robust statistical analysis method performed did not find a significant difference between exposures, so we cannot state that O_3 was responsible for that change. Another early study exposed healthy subjects to 200 ppb for 2 h and could not find significant changes in spirometry values after 3 days exposure (Folinsbee et al., 1980).

4.2 Breathing pattern

Studies in animal models and also in humans showed that in addition to bronchoconstriction caused by O_3, rapid and shallow breathing patterns were also found (Foxcroft & Adams, 1986; Schelegle et al., 2001). One theory for these changes relates to C-fiber stimulation by O_3 in which a defense mechanism to the air pollutant is elicited from these vagal afferent fibers present in the airways, causing increased f and reduced V_T (Schelegle et al., 2001).

In the study by Foxcroft & Adams, they found that healthy subjects increased f and reduced V_T when exposed to O_3 compared to filtered air (Foxcroft & Adams, 1986). Moreover, they noted that these changes were mitigated with repeated exposures. In our study, we found that V_T was lower at peak exercise in all study visits when participants were exposed to O_3, and f and VE were lower from Days 1-4. Although the lower V_T found across all study visits in O_3 in our study could explain the lower FEV_1, these findings were not significant. Their results could be attributed to their longer 2-h protocol in a greater concentration of O_3, 400 – 600 ppb which would elicit a greater stimulation of C-fibers as a defense mechanism, and therefore, reflecting in larger changes in breathing pattern.

4.3 Lung inflammatory response

FeNO was expected to increase after O3 exposures as found in other studies involving exposures to the same air pollutant in asthmatics (Barraza-Villarreal et al., 2008). In our study however, we found FeNO to be lower after O_3 exposure than RA, but not statistically significant. According to FeNO guidelines, many factors can contribute to altering its readings, such as eating a list of food high in nitrates. Our participants reported eating food that were not recommended, but usually 8 h before the study visit. Also, drinking water can transiently affect FeNO readings, and that was not controlled prior to each assessment and could have misled our results. Overall, in both exposures, FeNO readings at peak exercise were below or slightly above normal range.

4.4 Cardiovascular function

We expected a vasoconstrictor response to O_3 exposures, which was seen in the post exercise PWV measures in Days 2-5, but not statistically significant. The greatest PWV increase from baseline, was of 0.4 m/s on Day 3, which is 10% greater than baseline. A study in healthy subjects found increased arterial stiffness after diesel exhaust exposures. The only study we are aware that investigated the effect of O_3 in arterial stiffness, did not expose participants to O_3 alone (Brook et al., 2002).

The cardiovascular effects to O_3 exposure seem to occur as a cascade of pathophysiological events initiated in the lung. C-fiber stimulation by air pollutants in airways are thought to cause bradycardia and hypotension through an increased parasympathetic drive to the heart (Taylor-Clark, 2020). Thus, the small pulmonary impairment in our study could explain the minor reduction in HR seen in response to O_3 in our study.

4.5 Symptoms

Authors found their participants reported greater symptoms on first days which reduced on last days of 4 consecutive exposures to O_3 (Jörres et al., 2000). We expected that respiratory symptoms would increase to air pollution exposure, however changes in our study were not significant.

4.6 Strengths and limitations

Our study was the first to examine changes in pulmonary and cardiovascular functions to repeated O_3 exposures in people with EIB. Another strength of this study is its external validity considering the differences in the group of participants we recruited that included a broad age range, EIB severity and different physical activity levels. We also included people with EIB/asthma and those that had EIB, but had not been diagnosed with asthma. However, we believe that including people with asthma could have reduced the magnitude of the results seen, since samples with no underlying pulmonary condition had shown in previous studies to be significantly more impaired than our participants. We included people with asthma, which could mean our participants might have been experiencing chronic reductions in pulmonary function prior to even being recruited and tested in our study visits. This could have made our participants show a blunted effect to O_3 since their pulmonary function is chronically impaired, meaning it would require a greater concentration of O_3 or longer protocol to see an impairment. Authors have suggested that geographic location could blunt response to O_3 by a sensitization to prior long-term exposure to higher O_3, like in Los Angeles (Linn et al., 1994). However, that premise cannot be applied in this study, since ozone levels in Vancouver are considerably low throughout the year.

However, small decreases in spirometry scores are still relevant specially for those living with asthma, who have a chronic baseline obstruction and that is a strength of this study. Another

population that could likely benefit from our findings are athletes diagnosed with asthma that have sport events in cities with high O_3 levels since competing when your pulmonary function is at the lowest impairment possible could mean improved performance.

The major limitation to this study is its sample size. We believe collecting data in more participants will help increase its statistical power and reveal greater changes in the cardiovascular function. Another limitation to this study was the FeNO monitor used, which did not give consistent readings and could have been affected by many factors as listed previously. We believe that a future approach to investigate inflammatory changes in response to O_3 should consider more reliable means to assess FeNO. Also, one could consider exercise intensity was not sufficient to increase O_3 intake. However, our participants exercised at 60% of their maximal power output while other studies found significant changes even with lower intensities (e.g., 50%) (Linn et al., 1994). Also, in this study we used pulse transducers for the first time as a technique to assess PWV between carotid and femoral sites. PWV is a marker of cardiovascular morbidity and mortality risk and its utility has been validated with tonometer devices which are expensive and require extra software acquisition, increasing research burden. However, this could be a limitation to the study since the exact specificity of this technique has yet not been studied. However, on that premise we believe introducing this technique will make arterial stiffness assessment through PWV between femoral and carotid more often. Accessible means to record arterial stiffness make research in arterial stiffness more feasible as well as accessible to clinicians and healthcare professionals.

Chapter 5: Conclusion

This study sought to investigate changes in pulmonary and cardiovascular function in response to repeated exposures to 170 ppb O_3 in individuals with EIB. This was a novel approach and to our knowledge, no other study focused on the cardiovascular response to repeated exposures to O_3. We found that pulmonary function, assessed by FEV_1 and FEF_{25-75} was significantly impaired on Days 1 and 2 of O_3 exposure and improved following the next exposures, but did not surpass the baseline values from Day 1. We also found changes in the cardiovascular function that were not statistically significant. For future studies, we recommend the investigation of the response to O_3 in individuals who experience different asthma phenotypes, as well as the response from those diagnosed with EIB solely.

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Appendices

Appendix A

Table 2 Daily changes from baseline to peak exercise on both exposures

	Day 1		Day 2		Day 3		Day 4		Day 5	
	Baseline	**Post**	**Baseline**	**Post**	**Baseline**	**Post**	**Baseline**	**Post**	**Baseline**	**Post**
FVC (L)										
RA	4.7 (1.1)	4.8 (1.1)	4.7 (1.2)	4.6 (1.2)	4.6 (1.1)	4.6 (1.1)	4.7 (1.1)	4.7 (1.1)	4.6 (1.1)	4.7 (1)
O_3	4.7 (1.1)	4.6 (1.1)	4.5 (1.1)	4.5 (1)	4.6 (1.1)	4.5 (1.1)	4.6 (1.1)	4.6 (1)	4.7 (1)	4.6 (1)
FEV_1 (L)										
RA	3.6 (0.8)	3.7 (1.1)	3.5 (0.9)	3.5 (0.9)	3.6 (0.9)	3.6 (0.8)	3.6 (0.8)	3.7 (0.8)	3.5 (0.8)	3.6 (0.8)
O_3	3.7 (0.8)	3.5 (0.8)*	3.5 (0.7)	3.4 (0.7)	3.6 (0.9)	3.5 (0.8)	3.6 (0.7)	3.5 (0.7)	3.6 (0.7)	3.6 (0.7)
FEF_{25-75} (L/s)										
RA	3.1 (0.9)	3.3 (1.1)	3 (1.2)	3 (0.3)	3.1 (1.1)	3.2 (1.1)	3.2 (1.1)	3.3 (1.1)*	3.1 (1.2)	3.2 (1.2)
O_3	3.2 (1.2)	3.1 (1.2)	3 (1.1)	2.9 (0.9)	3.1 (1.1)	3.1 (1)	3.2 (1.1)	3.2 (1.3)	3.2 (1.3)	3.2 (1.2)
FeNO (ppb)										
RA	20.5 (27.4)	18.4 (23.4)	29.5 (48.4)	25 (34.3)	32.7 (48.3)	26.3 (35.2)	32.6 (52.5)	27.6 (37.4)	35.9 (58.2)	27 (40.6)
O_3	27.3 (41)	21.7 (26.5)	26.9 (40.8)	21.3 (28)	32.7 (48.3)	23.7 (32.2)	25.4 (35)	22.7 (31.2)	25.1 (38.4)	22.2 (28)
PWV (m/s)										
RA	6.2 (1.4)	6.5 (1.1)	6.9 (1.3)	6.3 (1.7)	6.7 (1.4)	6.6 (1.3)	6.7 (1.2)	6.2 (1.7)	6.7 (1.4)	6.6 (1.7)
O_3	6.2 (1.4)	6 (1.1)	7.1 (1.7)	7.3 (1.5)	6.2 (1.5)	6.6 (1.6)	6.8 (1.5)	6.8 (1.3)	6.9 (1.5)	6.9 (1.8)

Legend: Data is shown in mean (SD). * = p-value was statistically significant ($p<0.05$).
RA: room air exposure. O_3: ozone exposure. FVC: forced vital capacity. FEV_1: forced expiratory volume in the first second. FEF_{25-75}: forced expiratory flow in the midportion of the FVC maneuver in liters per second. FeNO: fraction of exhaled nitric oxide in parts per billion. PWV: carotid to femoral pulse wave velocity in meters per second.

Appendix B

Table 3 Baseline and peak exercise measures across study visits in each air quality

	Day 1		Day 2		Day 3		Day 4		Day 5	
	Baseline	**Peak**	**Baseline**	**Peak**	**Baseline**	**Peak**	**Baseline**	**Peak**	**Baseline**	**Peak**
V_{T} (L)										
RA	0.8 (0.3)	2.1 (0.7)	0.7 (0.2)	2.2 (0.6)	0.8 (0.5)	2 (0.7)	0.7 (0.2)	2.1 (0.5)	0.6 (0.2)	2 (0.6)
O_3	0.8 (0.2)	2 (0.5)	0.6 (0.1)	1.9 (0.6)	0.8 (0.5)	1.9 (0.6)	0.8 (0.6)	1.9 (0.6)	0.7 (0.3)	1.9 (0.5)
f_B (bpm)										
RA	21 (8)	41 (7)	21 (7)	37 (8)	21 (8)	38 (5)	21 (8)	39 (8)	20 (9)	36 (6)
O_3	17 (5)	40 (7)	17 (4)	38 (6)	21 (8)	38 (6)	19 (9)	37 (7)	19 (6)	41 (7)
VE (L/min)										
RA	17.5 (10.1)	75.9 (18.5)	14.7 (5.9)	72.7 (9.3)	12.8 (4.5)	69.7 (23.3)	14.3 (7.4)	73.1 (17)	11.6 (5.7)	64.2 (18.7)
O_3	13.3 (3.2)	70.4 (19.2)	9.9 (2.1)	69.9 (21.5)	12.8 (4.5)	67.7 (13.4)	14.4 (8.5)	65.8 (17.9)	15.8 (11.1)	70.6 (21.3)
HR (bpm)										
RA	68 (14)	167 (14)	61 (9)	165 (13)	61 (11)	166 (15)	66 (14)	165 (12)	61 (7)	164 (12)
O_3	62 (7)	164 (15)	63 (4)	161 (14)	62 (7)	161 (15)	65 (8)	161 (13)	66 (15)	161 (12)
SBP (mmHg)										
RA	109 (14)	158 (42)	107 (13)	170 (30)	105 (7)	158 (31)	109 (11)	162 (34)	104 (9)	164 (37)
O_3	108 (9)	159 (30)	104 (87)	161 (29)	109 (9)	164 (29)	115 (31)	165 (35)	106 (8)	162 (34)
DBP (mmHg)										
RA	70 (8)	61 (16)	67 (7)	63 (14)	67 (7)	58 (13)	70 (6)	59 (12)	66 (7)	59 (14)
O_3	68 (8)	55 (11)	71 (8)	57 (10)	69 (9)	56 (11)	69 (7)	56 (11)	70 (8)	60 (11)

Legend: Data is shown in mean (SD).
RA: room air exposure. O_3: ozone exposure. V_T: tidal volume in liters. f_B: breathing frequency in breaths per minute. VE: minute ventilation in liters per minute. HR: heart rate in beats per minute. SBP: systolic blood pressure in millimeters of mercury. DBP: diastolic blood pressure in millimeters of mercury.

Appendix C

Figure 14 Chest tightness (0-10) at peak exercise in both exposures

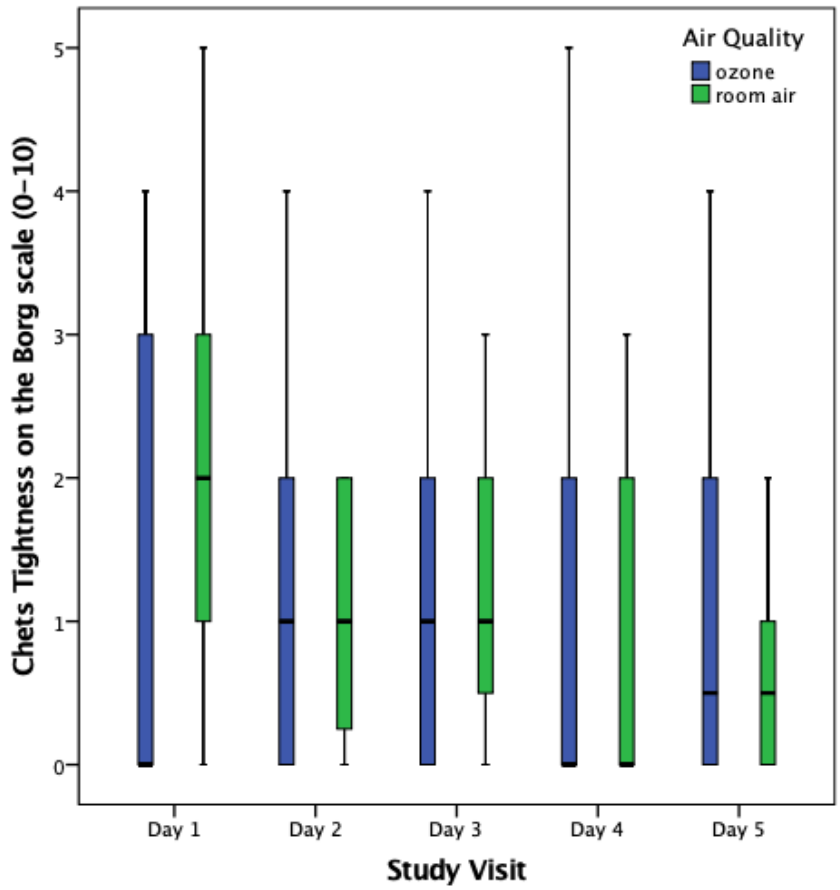

Figure 15 Chest wheezing or whistling sounds (0-10) at peak exercise in both exposures

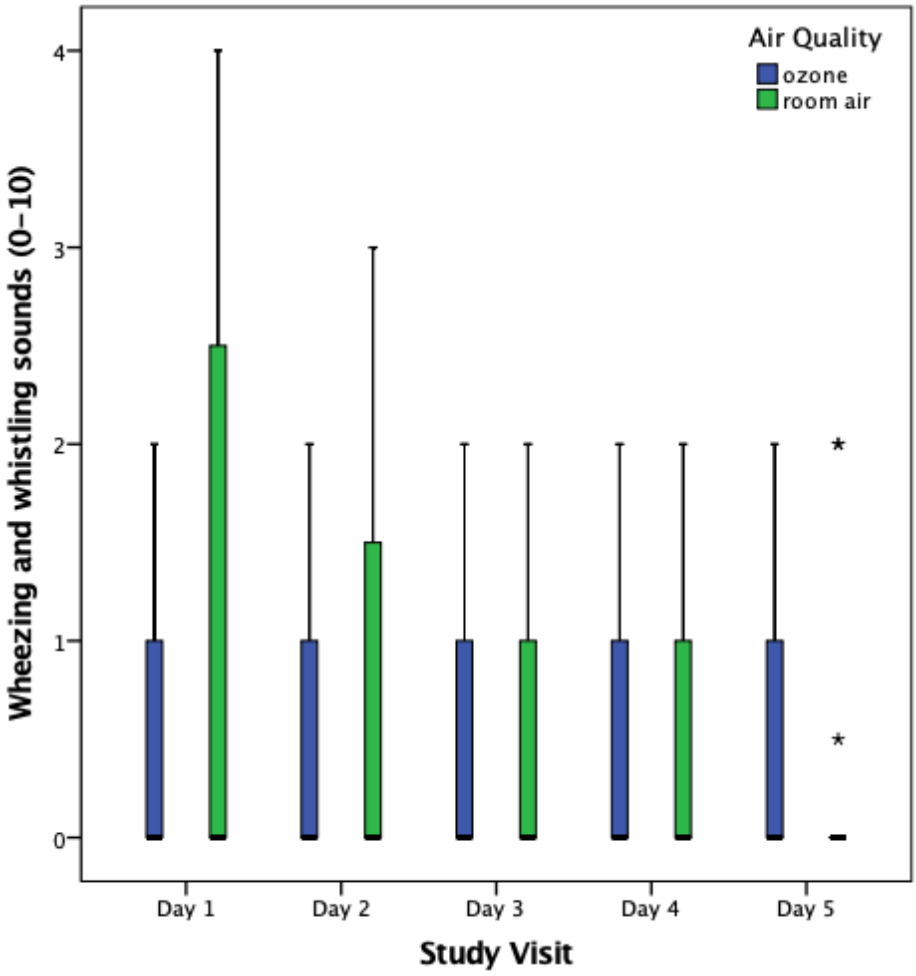

Figure 16 Dry throat (0-10) at peak exercise in both exposures

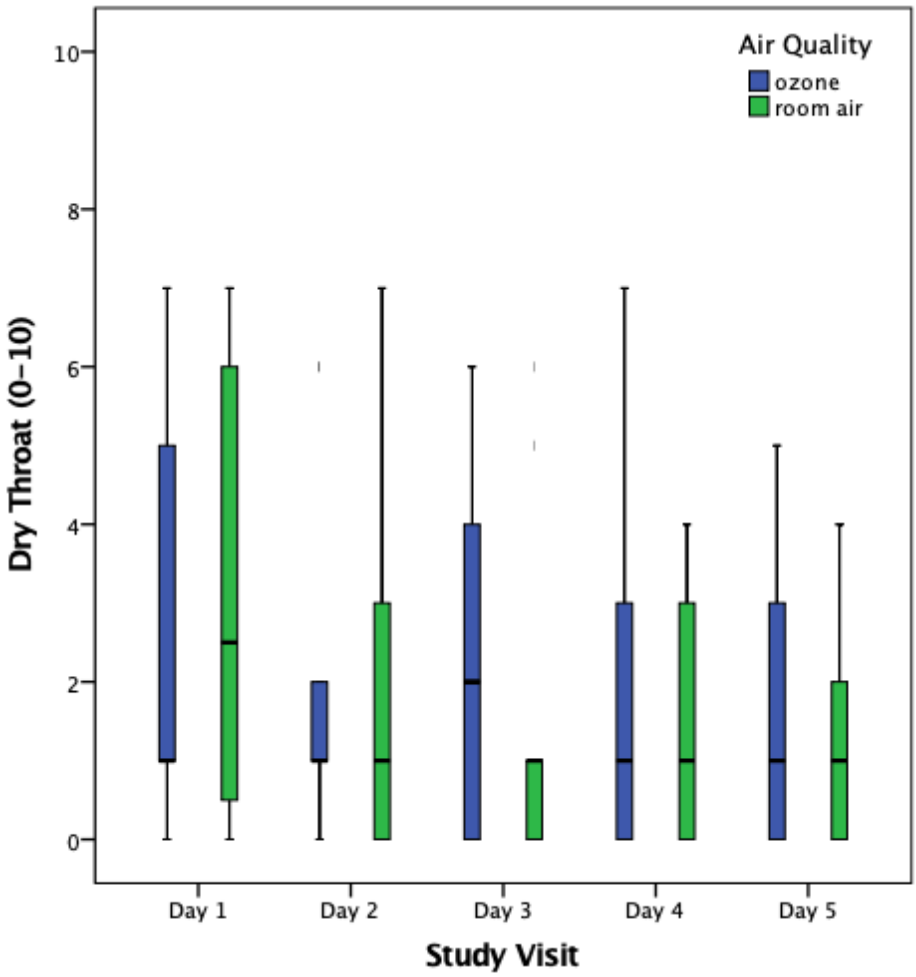

Figure 17 Scratchy throat (0-10) at peak exercise in both exposures

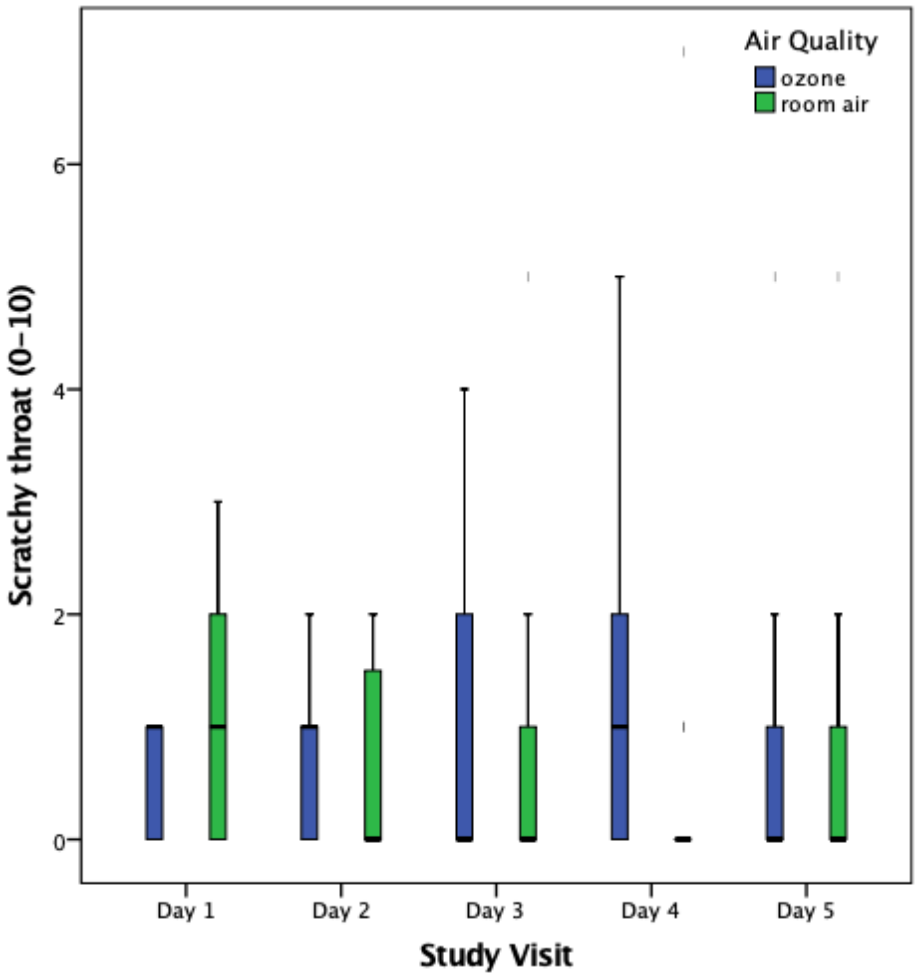

Figure 18 Itchy nose (0-10) at peak exercise in both exposures

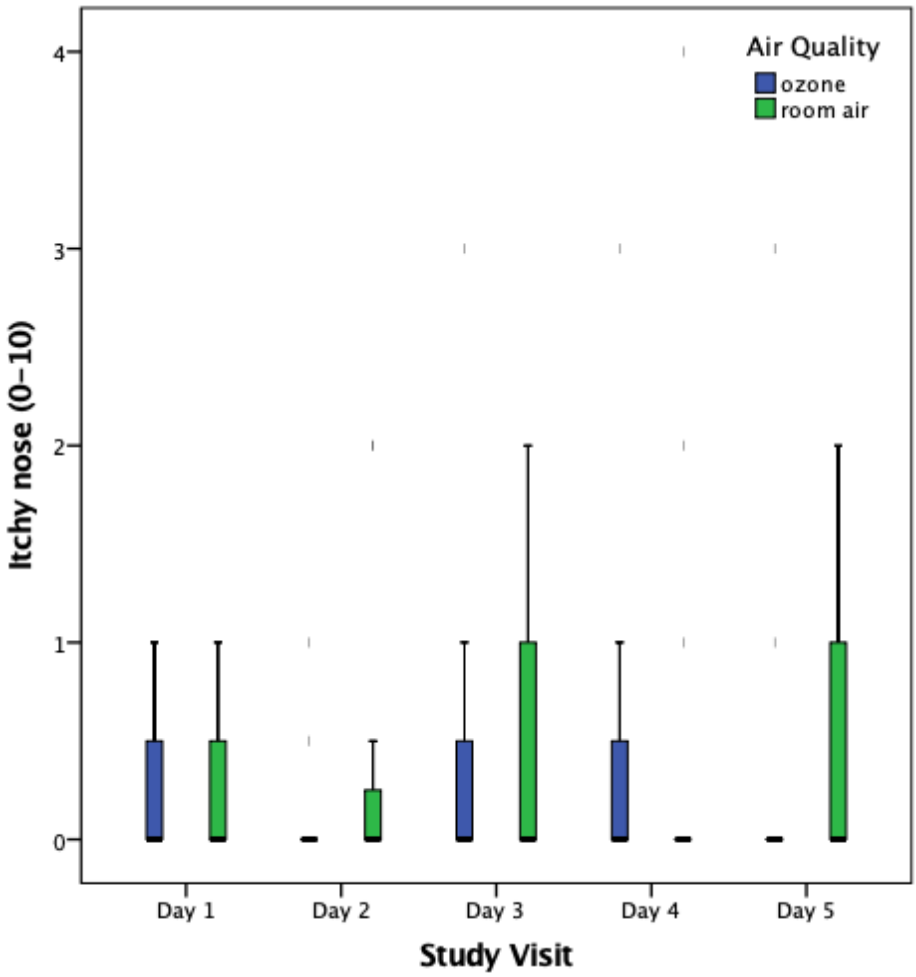

Figure 19 Running nose (0-10) at peak exercise in both exposures

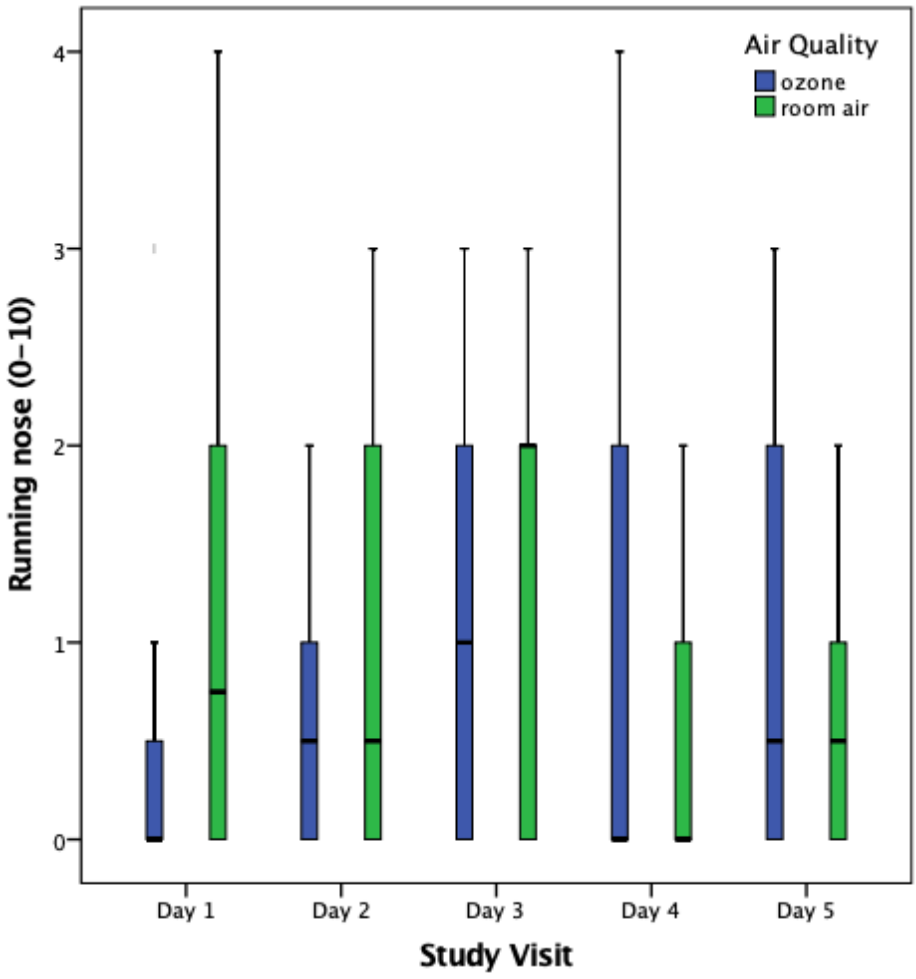

Figure 20 Headache (0-10) at peak exercise in both exposures

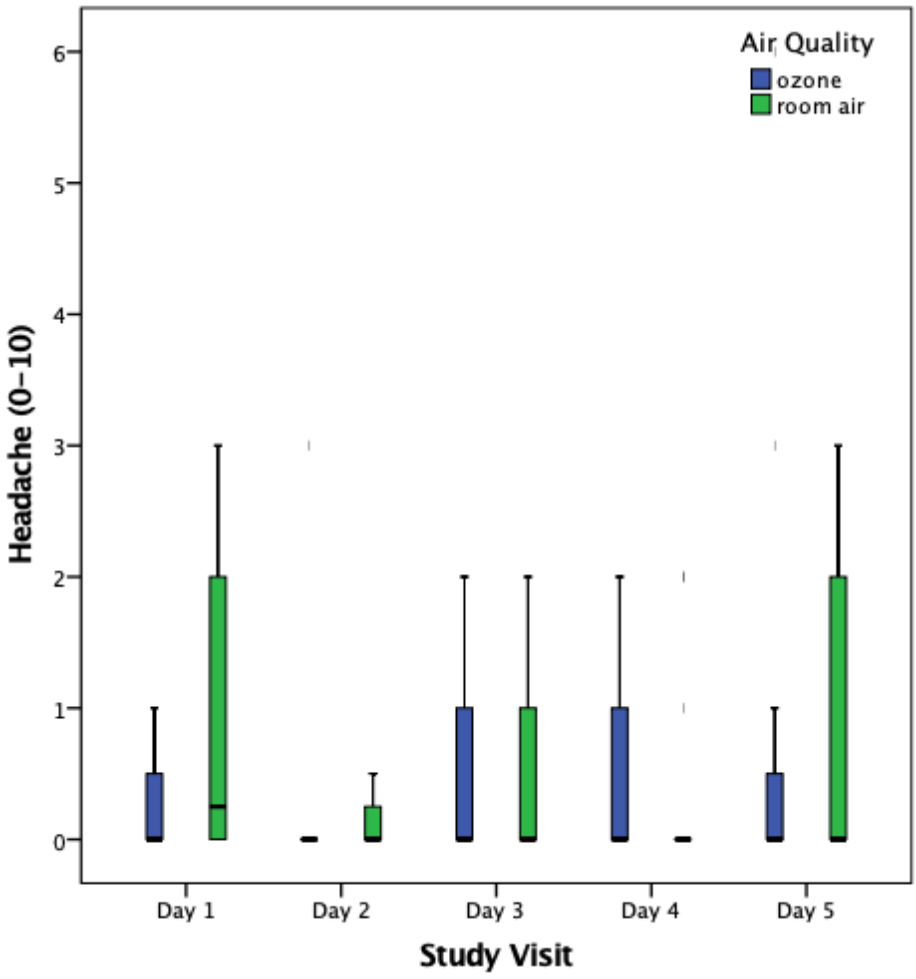

Figure 21 Fatigue (0-10) at peak exercise in both exposures

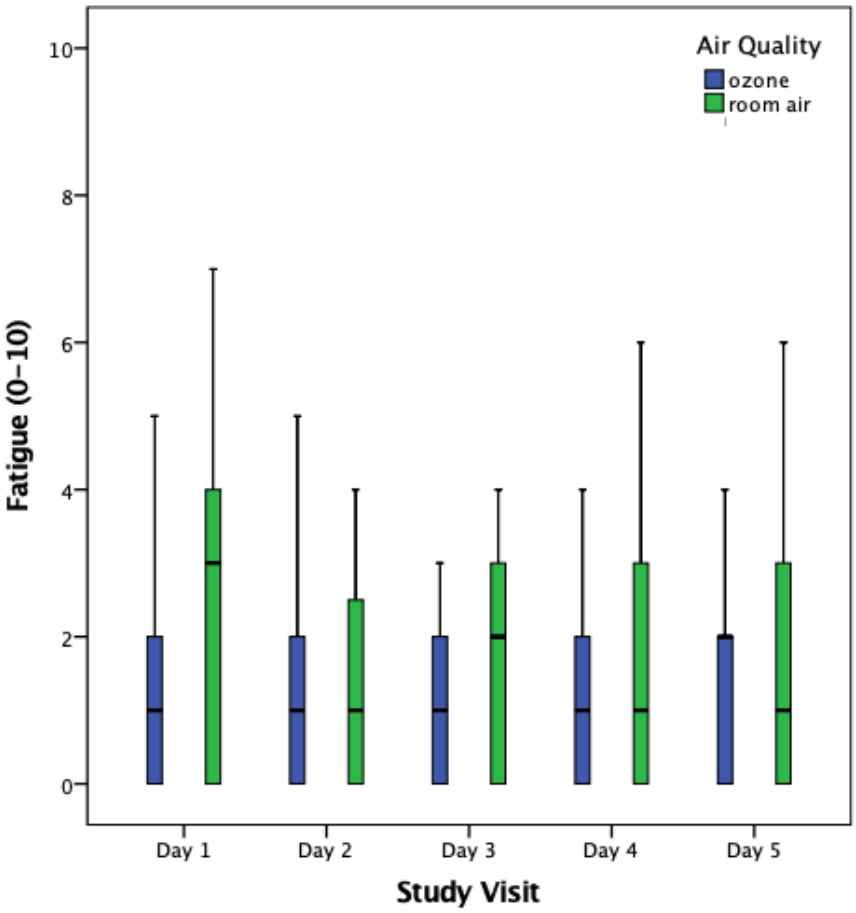

Figure 22 Individual changes in FEV_1 in both exposures

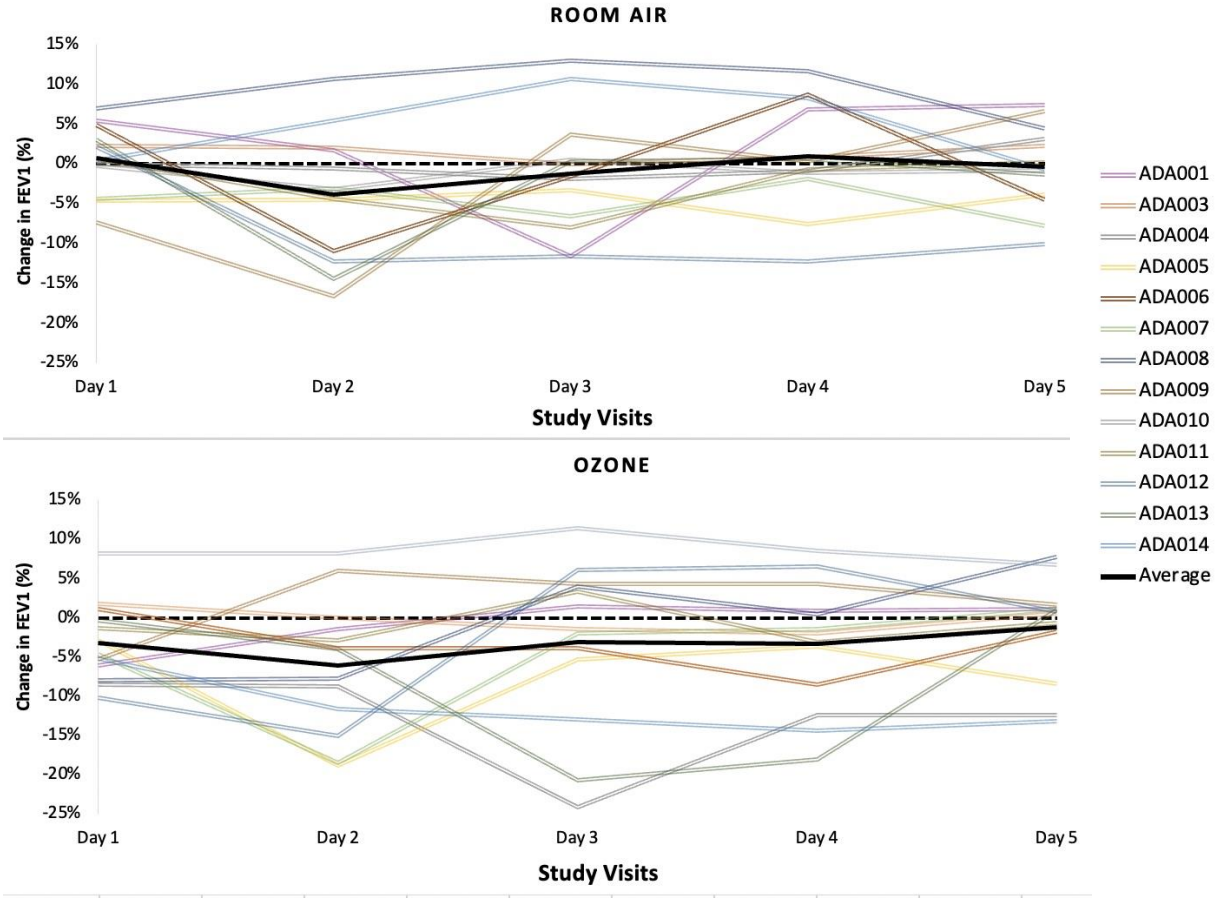

Figure 23 Individual changes in FVC in both exposures

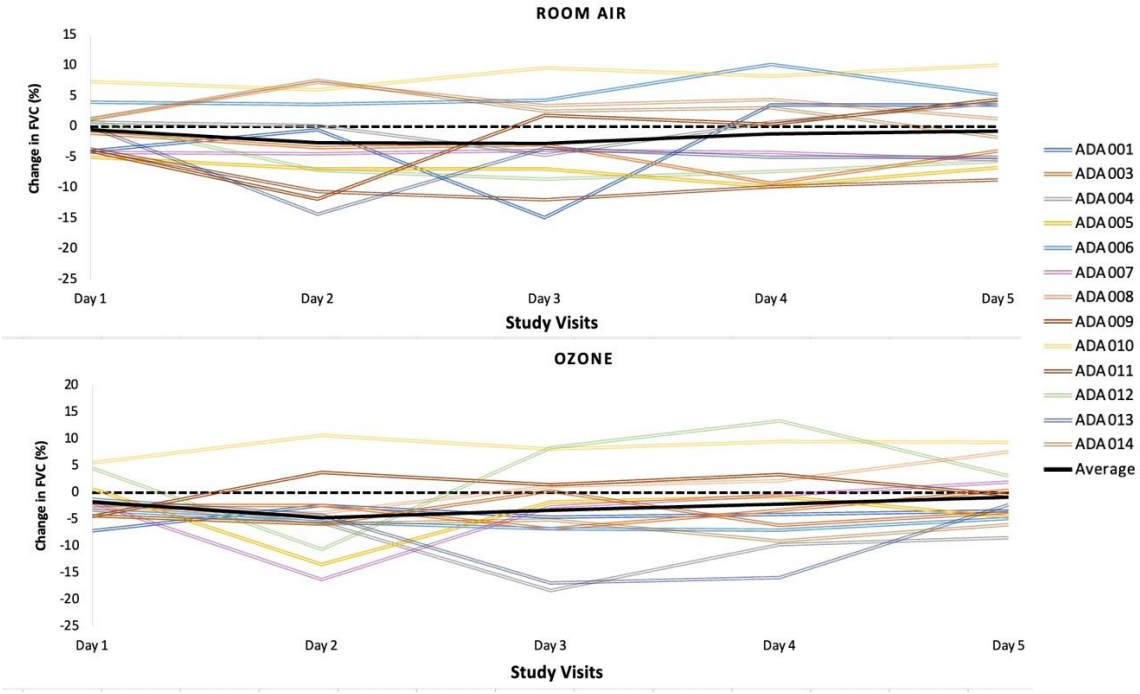

Figure 24 Individual changes in FEF_{25-75} in both exposures