Examining the Effect of Salbutamol Use in Ozone Air Pollution by People with Asthma and/or Exercise Induced Bronchoconstriction

by

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Abstract

Introduction: Ground level ozone is a respiratory irritant component of air pollution. Exercise is a key part of a healthy lifestyle; however, when ambient air pollution is high, increased ventilation during exercise increases the inhaled dose of ozone which can be problematic for people with asthma and/or exercise-induced bronchoconstriction (EIB). Salbutamol is a medication taken by people with asthma and EIB before exercise. Rodent studies have indicated that salbutamol may exacerbate ozone-related lung inflammation.

Aim: To examine if salbutamol use before exercising in realistic ozone air pollution exacerbates ozone-related airway inflammation in individuals with asthma and/or EIB.

Methods: Participants with EIB, confirmed through an EVH test, exercised at 60% of their VO_{2max} for 30 minutes in 4 different conditions: room air + placebo, 170 ppb ozone + placebo, room air + salbutamol, and 170 ppb ozone + salbutamol. Pulmonary function was measured by spirometry. Airway inflammation was measured by FeNO. Blood pressure and symptoms were also measured. Measurements were taken before exercise, immediately after, 30 minutes after, and 1 hour after exercise.

Results: Pulmonary function, assessed through spirometry measures, was significantly better in the salbutamol condition as compared to the placebo condition. There was a marginal increase in inflammation in all conditions except the room air + placebo condition. There were no notable differences in symptoms and blood pressure between the conditions.

Conclusion: Salbutamol improved pulmonary function in ozone, however did not exacerbate ozone-related increases in airway inflammation, as indicated by FeNO. This is opposing to what has been previously found in rodent studies.

Lay Summary

Ozone gas is a component of air pollution at ground level and is generated when UV light reacts with components of typical sources of air pollution such as fossil fuel burning. At high enough concentrations, ozone gas is a respiratory irritant that causes symptoms such as cough, sore throat, and chest pain. In addition, ozone has been associated with increased mortality particularly for individuals with respiratory conditions including asthma and COPD. Salbutamol is a common inhaler medication used by people with respiratory conditions. People with exercise-induced asthma normally use salbutamol before exercise. However, some rodent studies have indicated that asthma medication such as salbutamol is associated with increased lung damage and lung inflammation caused by ozone. The purpose of this study was to examine if these findings were transferable to humans. The results of this study will be used to help inform clinicians and patients about best practices for using salbutamol in high ozone conditions.

Preface

For this project, I contributed to the design of the research protocol and experimental set up which involved converting an altitude exposure chamber into an ozone exposure chamber that could be used to control the ozone concentration of inhaled air during exercise in addition to monitoring respiratory variables. I also completed data collection and analysis. This project was approved by the UBC Clinical Research Ethics Board (H21-01080). At the time of submission of this thesis no part of this thesis has been published.

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List of Abbreviations

ANOVA: Analysis of Variance
ATS: American Thoracic Society
BP: Blood Pressure
CFCs: Chlorofluorocarbons
CI: Confidence Interval
CO_2: Carbon Dioxide
COPD: Chronic Obstructive Pulmonary Disease
EELV: End Expiratory Lung Volume
EIB: Exercise Induced Bronchoconstriction
EILV: End Inspiratory Lung Volume
EPA: Environmental Protection Agency
ERV: Expiratory Reserve Volume
EVH: Eucapnic Voluntary Hyperpnea
FEF_{25-75}: Forced Expiratory Flow between 25% and 75% of FVC
FeNO: Fraction of Exhaled Nitric Oxide
FEV_1: Forced Expiratory Volume in 1 second
FEV_1/FVC: Ratio of FEV_1 to FVC
FVC: Forced Vital Capacity
FVL: Flow-Volume Loops
IC: Inspiratory Capacity
IgA: Immunoglobin A
IQR: Inter Quartile Range
IRV: Inspiratory Reserve Volume
km: kilometers
L: Liters
L/min: Liters per minute
L/s: Liters per second
LABAs: Long Acting Beta Agonists
MDI: Metered Dose Inhaler
MEFV curves: Maximal Expiratory Flow-Volume curves
mL: milliliters
mmHg: millimeters of mercury
NAAQS: National Ambient Air Quality Standards
NH_3: Ammonia
nm: nanometers
NO: Nitric Oxide
NO_x: NO_2, NO
O_2: Dioxygen
O_3: Ozone
PAR-Q+: Physical Activity Readiness Questionnaire
PM: Particulate Matter
PM_{10}: PM with a diameter smaller than or equal to 10 µm
$PM_{2.5}$: PM with a diameter smaller than or equal to 2.5 µm

ppb: parts per billion
ppm: parts per million
rpm: revolutions per minute
RV: Residual Volume
SABAs: Short Acting Beta Agonists
SD: Standard Deviation
SO_2: Sulphur Dioxide
UV: Ultra Violet radiation
UV-B: UV radiation with a wave length between 280 nm and 315 nm
V: Volts
VE: Ventilation in L/min
VO_{2max}: Maximal Rate of Oxygen Consumption
VOCs: Volatile Organic Compounds
V_T: Tidal Volume
WHO: World Health Organization
µg: micrograms
$\mu g/m^3$: micrograms per cubic meter

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Chapter 1: Introduction

1.1 Physical Activity & Exercise

Physical activity has been recognized as an important component of a healthy lifestyle. This is especially true in high income regions of the world where sedentary behaviour has been associated with health conditions such as type 2 diabetes, hypertension, mood disorders, and certain types of cancer (Fornias Machado de Rezende et al., 2014). Regular endurance exercise has been shown to have positive effects on the cardiovascular system, musculoskeletal system, body composition, metabolic efficiency, and mental health. It has been reported that both men and women who have higher measures of physical fitness have a reduced relative risk of death of 20-30% (Vina et al., 2012). In addition to improving overall health, exercise has become an emerging treatment for health conditions including obesity, type 2 diabetes, hypertension, and depression (Febbraio, 2017; Ranjbar et al., 2015; Vina et al., 2012). Industrialization and urbanization have led to a larger number of people living in areas with increased ambient air pollution (Barbera et al., 2010). This presents an issue when it comes to exercising in poor air quality because increased ventilation during exercise results in a proportionate increase in the amount of inhaled air pollution (Giles & Koehle, 2013). As a result, air quality can act as a barrier for people to access the health benefits of exercise.

1.2 Air Pollution and Ozone Pollution

Air pollution is the world's fourth leading cause of death and disease. The World Health Organization (WHO) estimated that 99% of the world's population reside in places where air pollution exceeds WHO guidelines (*Ambient (Outdoor) Air Pollution*, n.d.). Air pollution is a complex mixture that varies in content and concentration based on the time of day, location, and

the weather. Air pollution can contain various mixtures of particulate matter (PM), primary gases, and secondary gases (Rajagopalan & Landrigan, 2021). Two PM sizes of particular interest are PM with a diameter smaller than or equal to 10 µm (PM_{10}) and particulate matter with a diameter smaller than 2.5 µm ($PM_{2.5}$). Common components of PM_{10} include dust, pollen, and mould. Common components of $PM_{2.5}$ include combustion particles such as black carbon found in smoke and other emissions (Giles & Koehle, 2013). Primary gaseous pollutants are gases that are directly emitted into the atmosphere from a source, such as nitrogen oxides (NO_x), sulphur dioxide, carbon monoxide, and volatile and semi volatile organic compounds (VOCs) (e.g., benzene, toluene, xylene, 1,3-butadine, and poly aromatic hydrocarbons) (Rajagopalan & Landrigan, 2021). Secondary gaseous pollutants, such as ozone, are gases that are formed in the atmosphere (Giles & Koehle, 2013). Ozone is an inorganic molecule with the chemical formula O_3. At room temperature, ozone is colourless and can have a chlorine- or metallic-like odour at a high enough concentration. O_3 is a powerful oxidant with a standard redox potential of +2.07 V (Mustafa, 1990). It is for this reason that O_3 is used commercially to treat drinking water, clean swimming pools, and disinfect rooms (Gray, 2013).

About 90% of ozone in the atmosphere is contained in the stratosphere (20-40 km above sea level) where O_3 concentrations range from 2-8 ppm (Chipperfield et al., 2017). The use of chemicals, such as chlorofluorocarbons (CFCs), led to thinning of the ozone layer, which increased UV-B exposure in some geographical regions including Australia (de Gruijl et al., 2003). Fortunately, since regulations were put on CFC use after the Montreal Protocol, there has been a steady recovery of the ozone layer (de Gruijl et al., 2003). Unlike stratospheric ozone, which is considered ‘good’ ozone, tropospheric ozone is considered ‘bad’ ozone because it is a component of air

pollution and has adverse health effects. The most common source of tropospheric ozone is photochemical smog (Jacob, 2000). Since UV radiation from sunlight is needed for photochemical smog, ozone pollution has a seasonal effect where it is worse in the summer months. Ozone pollution also has a time effect where ozone levels tend to be highest in the early afternoon when there is more exposure to UV radiation (Fiore et al., 1998).

Both VOCs and NO_x gases are primary pollutants. Sources of NO_x gases include fossil fuel combustion, biomass burning, soils, lightning, NH_3 oxidation, and aircrafts with over 50% coming from burning fossil fuels alone (Delmas et al., 1997). VOCs come from natural and human sources. Vegetation releases a variety of hydrocarbons into the atmosphere like isoprene, a by-product of photosynthesis. Human activities that release VOCs include fuel combustion, fuel evaporation, solvent use, and chemical manufacturing (Kansal, 2009). Depending on the atmospheric gas composition, the production of ozone either follows a 'VOC limited regime' or a 'NO_x limited regime'. In large urban areas, ozone production tends to follow a VOC limited regime while rural regions tend to follow more of a NO_x limited regime (Fiore et al., 1998). This means the components of air pollution that contain ozone and strategies used to control ozone pollution vary by region.

The WHO categorizes ozone as a respiratory irritant that can cause respiratory effects, such as difficulty breathing and inflammation of the airways, in the general population (Ambient (Outdoor) Air Pollution, 2021). These effects can aggravate lung diseases, such as asthma, emphysema, and chronic bronchitis (Nuvolone et al., 2017). Long-term exposure to ozone is one likely cause of asthma development and has been linked to increased premature death due to

respiratory illness (Koman & Mancuso, 2017; Nuvolone et al., 2017). Due to the health threats that ozone poses, a number of regulatory measures have been put in place. The WHO recommends a maximal 8-hour average ozone concentration of less than 100 μg/m^3 or 51 ppb and a daily average of 8-hour averages during the peak season of 60 μg/m^3 or 30.6 ppb (Ambient (Outdoor) Air Pollution, 2021). The National Ambient Air Quality Standards (NAAQS) set by the Environmental Protection Agency in the US set the limit for maximal 8-hour mean ozone concentrations at 70 ppb (*NAAQS Table | US EPA*, 2021). For Canada, the standard maximal 8-hour average ozone concentration is 62 ppb (Air Quality, 2021). In British Columbia, average 1-hour ozone concentrations are broken into 7 categories of increasing severity: 0-15 ppb, 15-25 ppb, 25-35 ppb, 35-45 ppb, 45-62 ppb, 62-82 ppb, and 82 ppb and above. Average 8-hour ozone concentrations in BC are broken into three levels of severity: 0-31 ppb, 31-63 ppb, and 63 ppb and above (Air Quality Health Index - Latest Air Monitoring Data Map - BC Air Quality - Province of British Columbia, n.d.).

Many places around the world experience ozone pollution. The population-weighted seasonal 8-hour daily maximal ozone average exposure across the globe in 2019 varied from 12.2 ppb to 67.2 ppb. The top 10 countries for average 8-hour ozone exposure in 2019 included Qatar, Nepal, India, Bangladesh, Bahrain, Pakistan, Kuwait, Iraq, Republic of Korea, and Saudi Arabia. Based on the same population-weighted seasonal 8-hour daily maximal ozone average, global exposure to ozone increased from 47.3 ppb in 2010 to 49.5 ppb in 2019 (Health Effects Institute, 2020). Many regions around the world also experience high one-hour average ozone concentrations during peak seasons. The Los Angeles basin regularly reaches hourly ozone levels of 110 ppb during the summer (Lu et al., 2018). In the summer of 2020, during a heat wave, Los Angeles experienced

many high one-hour ozone average events, with some reaching over 185 ppb (Barboza, 2020). Previous field observation studies have noted hourly maximal ozone concentrations in China that frequently exceed 150 ppb (Lu et al., 2018). Locally, here in Vancouver, British Columbia, the frequency and severity of ozone pollution has been reduced since the 1980's due to lower emission levels of ozone precursors. However, extreme temperatures during the summer of 2021 created high levels of ozone pollution not seen since the 1980's, with the highest recorded 1-hour average value of 151.1 ppb at the Maple Ridge air quality monitoring station (Pablo, 2021). Climate change is making ozone pollution more of a relevant issue. Since the 1990's, roughly every degree increase in global temperature has been associated with a 1.2 ppb increase in ground level ozone (Bloomer et al., 2009). Climate change also creates conditions where high levels of ozone pollution can be generated in peak season. Three effects of climate change that create these conditions are degradation of removal processes (dispersion, precipitation), higher temperatures, and UV exposure amplifying atmospheric chemistry (Orru et al., 2017).

1.3 The Lungs

The main role of the lungs in the body is facilitating the movement of oxygen (O_2) into the blood and the removal of carbon dioxide (CO_2) out of the blood. Other functions also include metabolizing some compounds, filtering unwanted material from circulation, and acting as a reservoir for blood. However, gas exchange is the most crucial role (West, 2012). In the lungs, O_2 and CO_2 move between the air and blood by simple diffusion. Venous blood, which contains a high partial pressure of CO_2 and a low partial pressure of O_2, is exposed to inhaled air that has a low partial pressure of CO_2 and a high partial pressure of O_2. Fick's Law of Diffusion describes how the rate of diffusion across a sheet of tissue is proportional to the difference in partial pressures

across the sheet and the area of the sheet, but is inversely proportional to the thickness of the sheet. This principle is optimized in the lungs by providing a blood-gas interface that has an area between 50 and 100 square metres while having a thickness between 600 nm and 2 μm (West, 2012).

To reach the blood-gas interface located at the alveoli, inhaled air first travels through the oral and nasal cavities, through the pharynx, and into the larynx. Throughout this journey it is filtered, warmed, and humidified. The nose contains nasal hairs and mucous that help to filter out PM. Mucous is an aqueous secretion produced by goblet cells in the epithelium, the cells lining internal and external surfaces of the body, or by mucous and seromucous glands, pockets of epithelial cells that produce mucous along with other useful secretions. Some other components of mucous include antimicrobial enzymes and antibodies like IgA (Gartner, 2017). The fluid lining on top of the epithelium is known as the epithelial lining fluid. Evaporation of water from the epithelial lining fluid along the airway humidifies inhaled air (Warren et al., 2010). The nasal cavity has long curved shelves of bone that proceed into the nasal cavity called conchae. These bony processes increase the surface area of the nasal cavity and cause air to swirl, enhancing the warming, humidifying, and filtering function of the nose (Gartner, 2017). Alternatively, air can bypass the nose by going through the mouth. While breathing normally occurs through the nose at rest, exercise induces an increase in ventilation. As such, to match demands, there is a switch during exercise to primarily breathing through the mouth (Niinimaa et al., 1980). Air then passes the pharynx and enters the larynx, where the vocal cords are located. Aside from the vocal folds, the inferior part of the larynx contains respiratory epithelium. This epithelium contains ciliated cells in addition to some goblet cells and serous glands (Gartner, 2017). The hair-like cilia of these cells beat upwards to help move mucous-containing trapped PM, pathogens, and other debris up to the

pharynx to be swallowed or coughed out; this mechanism is termed the mucociliary escalator (West, 2012). Parts of the nasal cavity also contain respiratory epithelium (Gartner, 2017). After the larynx, the air then enters the conducting zone of the airway, starting at the trachea which divides into bronchi, which divide into lobar bronchi that supply the different lobes of the lung (two lobes on the side of the heart and three on the opposite site). From here, lobar bronchi divide into segmental bronchi, which further divide into bronchioles, which continue dividing until the terminal bronchioles at the end of the conducting zone. Until this point, the airway has divided approximately 16 times and has a volume of approximately 150 mL (West, 2012). The trachea contains respiratory epithelium and seromucous glands. Below that is connective tissue and rigid C-shaped hyaline cartilage that keep the airway patent. Either end of the C-shaped rings are connected by smooth muscle which contract or relax to help regulate airflow (Gartner, 2017). The histology (microscopic anatomy) of the bronchi is similar to the trachea, with minor differences including circumferential plates of cartilage and smooth muscle. As the airway branches and becomes narrower, the number of mucous glands, goblet cells, and amount of cartilage decreases (Gartner, 2017). Once air reaches the bronchioles, there are more substantial changes to the airway histology. Bronchioles still contain respiratory epithelium, but there are no mucous glands, few goblet cells, and the presence of Clara cells, whose secretions have lubricating and anti-inflammatory effects. While bronchioles are still surrounded by smooth muscle, they do not have cartilage (Gartner, 2017). As such, the diameter of the bronchioles is very susceptible to smooth muscle relaxation and contraction. Smooth muscle contraction leads to the narrowing of the bronchioles and contributes to bronchoconstriction. Smooth muscle relaxation leads to the dilation of the airway and contributes to bronchodilation. Bronchoconstriction and bronchodilation can be controlled by local factors released from immune and respiratory cells in the airway, in addition

to the autonomic (non-voluntary) nervous system. The epithelium and lamina propria of the lung are innervated by sensory afferent fibers that send information to respiratory centres in the central nervous system, such as the nucleus tractus solitarii (West, 2012). Examples of afferent fibers include bronchial C-fibers, which detect chemical irritants, and A-fibers, which are mechanoreceptors that respond to expansion and compression of the lung. After reaching the central nervous system, feedback from afferent fibers can result in activation or inhibition of sympathetic and parasympathetic efferent fibers (West, 2012). Parasympathetic fibers, when stimulated, release acetylcholine, which binds to muscarinic receptors on smooth muscle in the lung to induce bronchoconstriction. The release of acetylcholine from these neurons also causes increased mucous production (Richardson, 1979). Stimulation of the sympathetic nervous system results in the release of norepinephrine from sympathetic fibers innervating the smooth muscle and epithelium of the lungs. Stimulation of the sympathetic nervous system also results in the release of catecholamines (i.e., norepinephrine and epinephrine) from the adrenal gland, which bind to beta-2 adrenergic receptors on smooth muscle to promote relaxation and bronchodilation (Richardson, 1979). Bronchodilation can also occur through the withdrawal of parasympathetic tone. For example, during exercise, the airways dilate via the withdrawal of parasympathetic activity. This is also known as exercise-induced bronchodilation (McKenzie, 2012). The release of catecholamines also affects the lungs by increasing cilia beat frequency and inhibiting mast cell degranulation. Mast cells are immune cells that release histamine, which is part of the immune response that causes inflammation of tissues. In the lung, histamine can also bind to H1 receptors on smooth muscles to stimulate contraction (Sly, 1982). The degree of bronchoconstriction or dilation can also be influenced by the release of cytokines (cellular signaling molecules like interleukins) from local immune and reparatory cells (Papi et al., 2018). The terminal bronchioles

mark the end of the conducting zone of the airway. This region is also known as the anatomical dead space because no gas exchange occurs here (West, 2012).

After passing the conducting zone, air enters the transitional and respiratory zones that divide 7 more times, starting with respiratory bronchioles, which divide into alveolar ducts, and end in alveolar sacs (West, 2012). In total the lung contains roughly 300-500 million alveoli (West, 2012). The epithelium of the alveoli is very thin and is made of type I and type II pneumocytes. Type I pneumocytes are very thin simple squamous epithelium, while type II pneumocytes are thicker and secrete surfactant into the epithelial lining fluid to prevent the alveoli from collapsing due to surface tension (Gartner, 2017). The alveoli also contain macrophages, a type of white blood cell, that patrol the alveoli to clean up debris and pathogens. Here, gas exchange occurs at the blood-gas interface that is made of type 1 pneumocytes, connective tissue, and the epithelial cells of pulmonary capillaries (Gartner, 2017).

1.3.1 Ventilation and Lung Volumes

During inspiration, respiratory muscles contract to generate negative pressures along the airway that lead to an increase in lung volume from inhaled air. The major inspiratory muscle is the diaphragm, but other accessory muscles, such as the sternocleidomastoid, external intercostal muscles, and scalenes, can all assist with inspiration. Expiration at rest is a passive manoeuvre that uses elastic energy stored in the lung connective tissue (West, 2012). During forced expiration, accessory expiratory muscles, such as the internal intercostal muscles and the abdominal muscles, contract to facilitate increased expiratory flow. The average total lung volume, or the maximal amount of air the lungs can hold, is about six litres, with a large amount of variation between

individuals. Total lung volume is the sum of forced vital capacity (FVC) and residual volume (RV) (West, 2012). FVC is the amount of air that can be expired from the lungs after a maximal inhalation. RV is the leftover volume in the lungs that cannot be expired. The RV prevents alveoli from completely emptying and collapsing (West, 2012). Tidal volume (V_T) is the volume of air expired or inspired per breath. Breathing frequency is the number of breaths taken per minute. Minute ventilation (VE), the volume of air expired per breath, is therefore the product of V_T and breathing frequency (West, 2012). The lung volume after a normal inspiration is called the end-inspiratory lung volume (EILV) and the lung volume after a normal expiration is the end-expiratory lung volume (EELV). These define the operating lung volumes that are optimized for respiratory muscles (Guenette et al., 2013a). After inspiration, there is an inspiratory reserve volume (IRV) that is not used. This is also true for expiration, since after expiration, the lungs do not empty to RV; therefore, there is an expiratory reserve volume (ERV). The volume of air that can be inhaled after expiration is known as inspiratory capacity (IC) (Guenette et al., 2013a). During exercise, as intensity increases, ventilation also increases. This is achieved by increasing both V_T and breathing frequency.

Initially, as intensity increases, V_T increases more than breathing frequency. This is because increased breathing frequency increases dead space ventilation or the amount of air that flows past the anatomic dead space, mentioned above, while increasing tidal volume exposes more of the lung where gas exchange can occur with inhaled air (West, 2012).

Pulmonary function variables relevant to this study include FVC, forced expiratory volume in one second (FEV_1), and forced expiratory flow between 25% and 75% of FVC (FEF_{25-75}). FEV_1 is the

volume of air that is expired during the first second of the FVC manoeuvre (West, 2012). A ratio of FEV_1/FVC less than 0.8 indicates obstructive lung diseases, such as asthma and emphysema (West, 2012). In obstructive lung disease, it becomes difficult to expire air from the lung due to narrowing of the airways. This leads to air being trapped in the lungs and dynamic hyperinflation where the lungs compensate for air trapped in the lungs by operating at higher lung volumes (i.e., increased EILV and EELV) (Lougheed et al., 2006). In the setting of a decreased FVC, a FEV_1/FVC ratio higher than 0.8 indicates restrictive lung diseases such as sarcoidosis and pulmonary fibrosis.

1.4 Asthma and Exercise Induced Bronchoconstriction (EIB)

In discussing EIB, it is important to consider the basic mechanisms of asthma because the two conditions are similar and are often present at the same time, with some studies reporting that 80-90% of people with asthma also have EIB (Gotshall, 2002). Estimates suggest that over 300 million people worldwide are affected by asthma (Fanta, 2009). Asthma is a chronic condition characterized by attacks whereby the bronchioles of the lung become narrowed by one or more of the following mechanisms: bronchoconstriction, inflammation, and increased mucous production (Yeh & Schwartzstein, 2010). Depending on the type of asthma, attacks can be induced by different stimuli. For example, atopic asthma, otherwise known as allergic asthma, can be triggered by allergens like pollen and dust (Yeh & Schwartzstein, 2010). Narrowing of the airway results in the characteristic symptoms of asthma including cough, shortness of breath, chest tightness, and wheezing (Fanta, 2009). Both abnormal function of the autonomic nervous system (Kistemaker & Prakash, 2019) and immune system (Papi et al., 2018) have been associated with asthma-related airway narrowing. For people without asthma or EIB, bronchoconstriction occurs in a regulated

manner designed to protect the airways and assist with matching ventilation to lung perfusion (Pelaia et al., 2008). Individuals with EIB and most people with asthma have airway hyperresponsiveness, which is an exaggerated bronchoconstrictive response to specific stimuli, like histamine, and non-specific stimuli like cold dry air (Kistemaker & Prakash, 2019).

Neural-related mechanisms related to airway hyperresponsiveness include increased plasticity of lung afferent nerves, the central nervous system, and lung efferent nerves. For example, C-fibers in people with asthma have increased excitability and enhanced transmission (Kistemaker & Prakash, 2019). Immune-related causes of airway hyperresponsiveness include the increased presence and oversensitivity of immune cells such as mast cells, dendritic cells, eosinophils, neutrophils, and type 2 helper T cells (Papi et al., 2018). The oversensitivity of these cells results in the increased release of non-neural-related bronchoconstrictive mediators, like histamine, which bind to histamine receptors on smooth muscle, leading to contraction (Pelaia et al., 2008). Lung inflammation is mediated by the immune system. Once immune cells (e.g., dendritic cells) are triggered, they release inflammatory cytokines such as interleukins. This starts a cascade of events that lead to the classic immune response, whereby adjacent blood vessels become leaky, resulting in fluid release into the tissue, in addition to other immune cells and proteins (Yeh & Schwartzstein, 2010). Similar to bronchoconstriction, the increased mucous secretion can be stimulated by acetylcholine released from efferent parasympathetic nerves (Coulson & Fryer, 2003) and the release of cytokines such as interleukin-9 from immune cells (Temann et al., 2007). The resultant narrowing of the airways from bronchoconstriction, inflammation, and mucous production makes it difficult to expire air, thus increasing EELV. To compensate, EILV also increases, causing higher working lung volumes. This phenomenon is known as dynamic

hyperinflation, which creates a positive end-expiratory pressure and neuromechanical dissociation of the inspiratory muscles (Lougheed et al., 2006). Dynamic hyperinflation and sensory afferent feedback have been associated with the characteristic signs and symptoms of an asthma attack (Lougheed et al., 2006).

While EIB is more commonly known as exercise-induced asthma, EIB is used in the literature because it is the most accurate description of the phenomenon. EIB results in asthma-like symptoms that occur after exercise (Gotshall, 2002). As mentioned previously, many people with asthma will also have EIB. However, some individuals will have EIB without having asthma. This is relatively common amongst high-level endurance athletes, particularly cross-country skiers who frequently exercise in cold dry air (Zeiger & Weiler, 2020). It is believed that chronic hyperpnea, particularly with cold air, causes minor airway inflammation and remodeling resulting in the development of EIB (Zeiger & Weiler, 2020). Both heat loss and water loss/osmotic theories related to hyperpnea during exercise have been used to explain this phenomenon (Gotshall, 2002). Increased ventilation causes more evaporation from the epithelial lining fluid, resulting in an increased osmolarity due to reductions in the volume of epithelial lining fluid volume. This leads to mast cell-mediated release of prostaglandins, leukotrienes, histamine, and tryptase (Aggarwal et al., 2018a).

The heat loss theory proposes that the rewarming of the airways after cooling from conductive heat loss is the stimulus for EIB (Gotshall, 2002). Studies that replicate EIB symptoms after inhalation of a hyperosmotic saline solution support the osmotic theory (Anderson, 1996). However, airway temperature likely also plays a role in EIB. This is evident by studies that show

decreased lung function in individuals with EIB after drinking cold water and increased symptoms severity when breathing cold air (Huang et al., 2000). This could be related to the fact that the maximum humidity of air decreases as temperature decreases leading to more airway evaporation (Gotshall, 2002).

Treatment options for EIB generally depend on the presence of asthma and can be divided into pharmacological and non-pharmacological options (Aggarwal et al., 2018). Non-pharmacological treatment options include a proper warm up, wearing a face covering, and diet modifications (Aggarwal et al., 2018a; Gotshall, 2002). A warm-up consisting of short high-intensity intervals can induce a small amount of bronchoconstriction. The goal is to take advantage of the refractory period, where vigorous exercise after warm-up results in significantly less bronchoconstriction for the next 1-4 hours. While the cause of this phenomenon is not fully understood, one leading theory is once mast cells have been degranulated, it takes time for them to 'recharge' before they can be degranulated again (Stickland et al., 2012). A face covering is used to pre-humidify inhaled air and is particularly effective for sports in cold environments, like cross-country skiing. Wearing a face mask may also decrease the amount of inhaled particulate air pollution that can exacerbate asthma symptoms (Aggarwal et al., 2018a). From a dietary perspective, some evidence points to a positive effect of caffeine before exercise and a low salt diet. Caffeine enhances the amount of catecholamines from the adrenal medulla, which stimulates bronchoconstriction by binding to beta-2 receptors on smooth muscle (Gotshall, 2002). A low salt diet minimizes the osmolarity of the epithelial lining fluid, which diminishes the primary osmotic stimulus (Gotshall, 2002). Four common pharmacological treatment options include short-acting beta agonists (SABA), inhaled corticosteroids, long-acting beta agonists (LABA), and leukotriene receptor antagonists (Aggarwal

et al., 2018a). Inhaled SABAs, like salbutamol, work by binding to beta-2 receptors on airway smooth muscle causing relaxation and bronchodilation. For EIB, SABAs are commonly used in a preventative manner by taking 100-200 µg (one or two puffs) 15 minutes before exercise. This effect starts approximately 15 minutes after administration and lasts for 4-6 hours (Gotshall, 2002). SABAs can also be used as a rescue medication in asthma and EIB once bronchoconstriction has already occurred (Aggarwal et al., 2018a; Gotshall, 2002). LABAs, such as salmeterol, work the same way as SABAs except they take longer to have an effect, 30-60 minutes, and this effect lasts for about 12 hours (Gotshall, 2002).

1.5 Ozone, the Lungs, and Asthma/EIB

Air pollution is a common trigger for asthma attacks. Due to the heterogenous nature of air pollution, there can be multiple components attributed to asthma-related increases in airway responsiveness, including SO_2, PM, and ozone (Koenig, 1999). Many biological systems are negatively influenced by ozone, but the major causes of increased morbidity and mortality are related to cardiovascular and respiratory events (Taylor-Clark, 2020). Ozone causes respiratory symptoms, decrements in lung function, and airway inflammation (Nuvolone et al., 2018; Scannell et al., 2012). Respiratory symptoms include cough, throat irritation, pain with inhalation, chest tightness, wheezing, and shortness of breath. Acute ozone exposure during moderate-to-vigorous physical activity is associated with adverse effects on pulmonary function and symptoms (Hung et al., 2022). Furthermore, evidence suggests that individuals with asthma may be particularly susceptible to the effects of ozone during physical activity (McCreanor et al., 2007). For instance, in one study, short-term ozone exposure (400 ppb for 2 hours) was associated with decreases in FVC, FEV_1, and FEF_{25-75} following exercise in both healthy individuals and people with asthma.

However, the decrements in FEV_1 and FEF_{25-75} were significantly greater in participants with asthma (Kreit et al., 1989). Multi-hour ozone exposures above 80 ppb consistently produce statistically significant decreases in lung function in healthy adults (Holm & Balmes, 2022). For example, a 6.6-hour exposure to 60 ppb ozone when compared to clean air was associated with a 1.71% decrease in FEV_1 and a 2.32% decrease in FVC in healthy young adults (Kim et al., 2012). There are also negative effects related to long-term ozone exposure. Observational studies indicate that higher ozone concentrations are associated with increased asthma attacks, increased hospital admission, and increased mortality (U.S. Environmental Protection Agency, 2020). In addition, long-term ozone exposure is related to asthma development likely due to airway remodeling in response to chronic inflammation (Zu et al., 2018).

The physiologic response to ozone depends on the distribution of ozone in the upper and lower airway, breathing patterns, and the anatomy of the different parts of the airway (Ultman et al., 2004). The efficiency of ozone uptake is negatively related to breathing frequency, which results in decreased time for ozone uptake, but positively associated with V_T, which drives ozone deeper into the lung where there is less mucous in the epithelial lining fluid so ozone can more easily interact with airway epithelium (Ultman et al., 2004). Ozone has very limited water solubility which means that the upper airway is not as effective at scrubbing ozone from inhaled air compared to other pollutants like SO_2 (*Health Effects of Ozone in the General Population | US EPA*, n.d.). As a result, more ozone can reach the lower parts of the airway where it can react with biomolecules in the epithelial lining fluid. Ozone is particularly reactive with biomolecules that contain thiol groups, amine groups, or unsaturated carbon-carbon bonds (*Health Effects of Ozone in the General Population | US EPA*, n.d.). The by-products of these reactions, free radicals and

other oxidant species, in addition to ozone itself, cause oxidative damage to epithelial cells leading to the release of cellular contents. This cellular damage leads to the release of inflammatory mediators such as cytokines, prostaglandins, and leukotrienes, resulting in neutrophilic inflammation of the airway (U.S. Environmental Protection Agency, 2020). The epithelial lining fluid contains antioxidant molecules to protect the lung from antioxidant stress. It has been shown that supplementation with antioxidants like vitamin C and E may limit ozone-based lung injury (Gomes et al., 2011). The inflammatory response to oxidative damage facilitates the neural response to ozone through the release of prostaglandin E2 from injured epithelial cells which sensitizes C-fibers (Devlin et al., 2008). Neurologically, ozone, and other air pollutants, stimulate C-fibers to evoke vagally-mediated defensive cardio-pulmonary reflexes, such as cough, decreased breathing effort, hypotension, and bradycardia (Taylor-Clark, 2020). C-fibers, like other nociceptive fibers, have branched endings. It has been proposed that when an action potential generated on one branch reaches a junction with the other branches, the action potential is sent in both directions. The action potential that returns towards the end of another C-fiber branch results in the release of substance P, or other tachykinins, that bind to neurokinin receptors. Ultimately, this results in bronchoconstriction and inflammation and contributes to increased airway obstruction. This mechanism is known as the local axon reflex response (U.S. Environmental Protection Agency, 2020). Exposure to air pollutants like ozone also activates the neuroendocrine stress axis, releasing stress hormones such as epinephrine and cortisol which are thought to play an important role in the effects of ozone exposure, including lymphopenia, decreased spleen weight, hyperglycemia, glucose intolerance, and the release of free fatty acids into circulation (Henriquez et al., 2019).

1.5.1 Ozone and Salbutamol

Salbutamol is a SABA and one of the most commonly used medications for the management of both EIB and asthma. However, there are some safety concerns related to chronic salbutamol use. It has been shown that chronic SABA and LABA use can paradoxically worsen asthma and cause asthma-related death. The mechanism of this phenomenon is not known exactly but it is believed to be related to increased sensitivity to bronchoconstrictive stimuli (Chowdhury & Dal Pan, 2010). SABA use is higher in regions with more ozone pollution with a 11.3% and 8.4% increase in SABA usage in youth and adults, respectively, with every 16.8 ppb increase in ozone (Pepper et al., 2020). As a result, it is important to learn more about the interaction between ozone pollution and salbutamol use. One issue proposed regarding the use of SABAs in air pollution is that excess bronchodilation may allow for more deposition of PM further down the alveolar tree exposing more of the lung to air pollutants (Koch et al., 2021). However, in a double-blinded placebo control trial that examined the use of salbutamol in people with EIB prior to a bout of exercise in diesel exhaust, no reductions in respiratory function or exercise ventilatory response during or after exercise were found (Koch et al., 2021). This finding suggests that even if salbutamol use were associated with an increased amount of diesel exhaust exposure, this increased dose did not result in significant changes in pulmonary function.

This finding cannot be extended to salbutamol use in environments with high ozone pollution. In one study, salbutamol use in non-asthmatics was not protective against ozone toxicity (Gong et al., 2010). More lung tissue being exposed to ozone could cause a larger amount of inflammation that contributes to airway obstruction, in addition to inducing bronchoconstriction after the effects of salbutamol and exercise-induced bronchodilation have worn off. In studies done on rats, it has

been shown that rats receiving asthma maintenance medication experienced exacerbated lung injury, inflammation, and cytokine levels in bronchoalveolar lavage fluid after bring exposed to ozone (Henriquez et al., 2019). Interestingly, adrenocompromised rats and rats that had stress hormone receptors (i.e., beta-adrenergic receptors and glucocorticoid receptors) pharmaceutically blocked were resistant to ozone-induced systemic and pulmonary effects. This finding indicates that commonly used asthma medications like LABAs, SABAs, and corticosteroids, that are agonists to these receptors, may exacerbate the effects of ozone exposure (Henriquez et al., 2018).

1.6 Research Question and Hypothesis

Research Question: Does using salbutamol before exercising in realistic ozone air pollution exacerbate ozone-related airway inflammation in individuals with asthma and/or EIB?

Research Hypothesis: Individuals with asthma and/or EIB who take salbutamol before exercising in ozone will have improved pulmonary function but higher levels of inflammation after exercise compared to placebo medication.

Chapter 2: Methods

2.1 Sample and Recruitment

The target population for this study was individuals with asthma and/or EIB living in urban environments. The sample representing this population was people with asthma and/or EIB living in the Greater Vancouver area. Recruitment was accomplished using posters on public advertising boards and online advertisements. Posters were put up around the UBC campus and at community centres around Vancouver. Online advertisements were done through the UBC School of Kinesiology and UBC Psychology websites. These advertisements outlined, in lay language, the purpose of the study, inclusion and exclusion criteria, the design of the study, the potential benefits and risks to the participants, and contact information to enroll in the study.

Table 1 List of study inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
1) Have asthma and/or EIB related airway narrowing during exercise 2) Able to perform maximal exercise 3) Between 18 and 50 years of age 4) Able to communicate sufficiently using the English language	1) Use inhaled corticosteroids 2) History of smoking 3) Chronic respiratory disease other than asthma or EIB 4) Upper respiratory tract infection within the last 4 months 5) Pregnant or potentially pregnant 6) Allergy to salbutamol

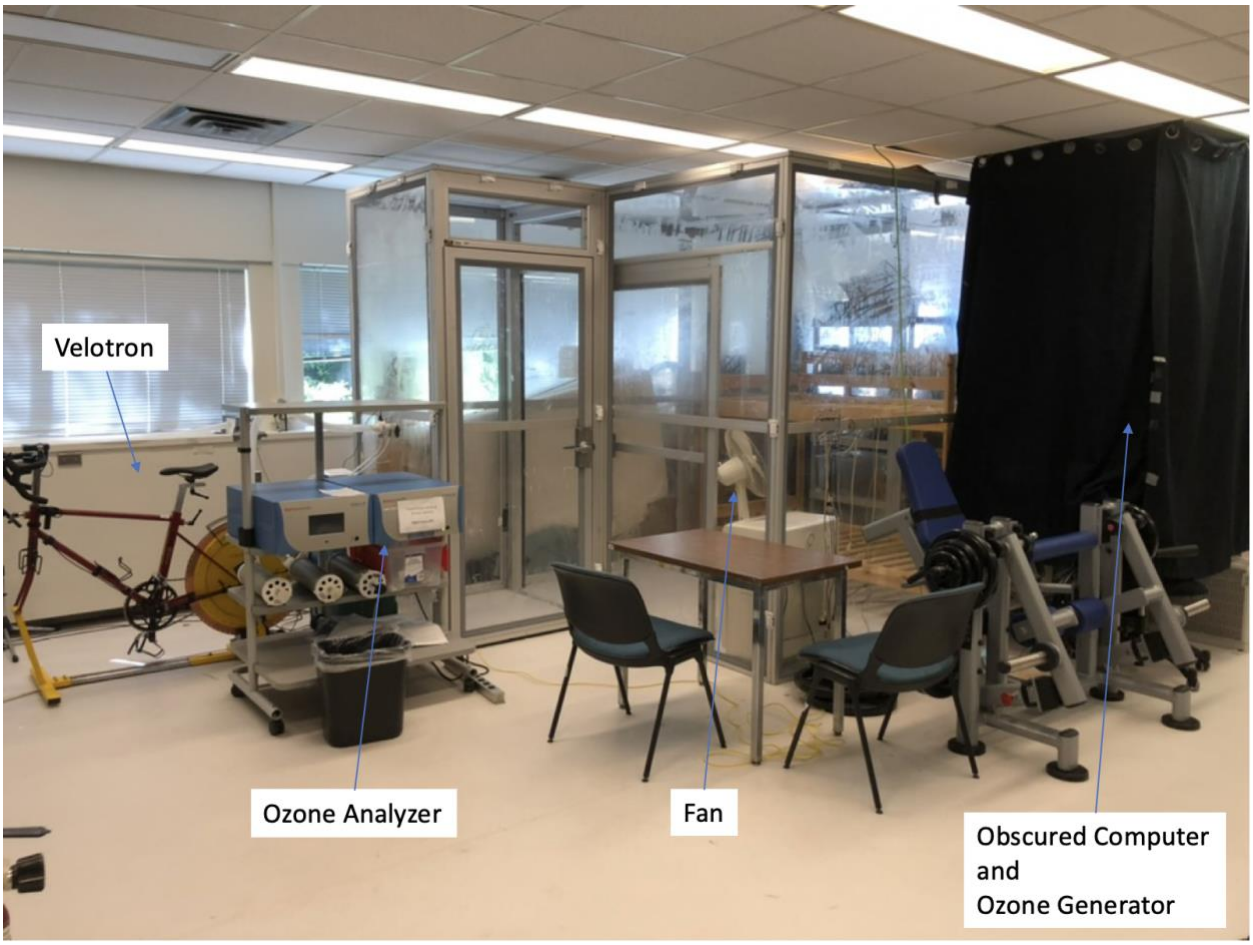
Sample size was calculated using data from previous studies that examined changes in FEV_1 in people with EIB. A study by Anderson et al., in 2001 used a similar crossover design and found a

detectable difference of 12% reduction in FEV_1 with a standard deviation of 14% (Anderson et al., 2001). Calculation for a crossover design based on analysis of variance F-tests using these values with a power of 0.8 and an alpha level of 0.05 led to an estimated sample size of 24 participants.

2.2 Experimental set up, Measurements, and Instruments

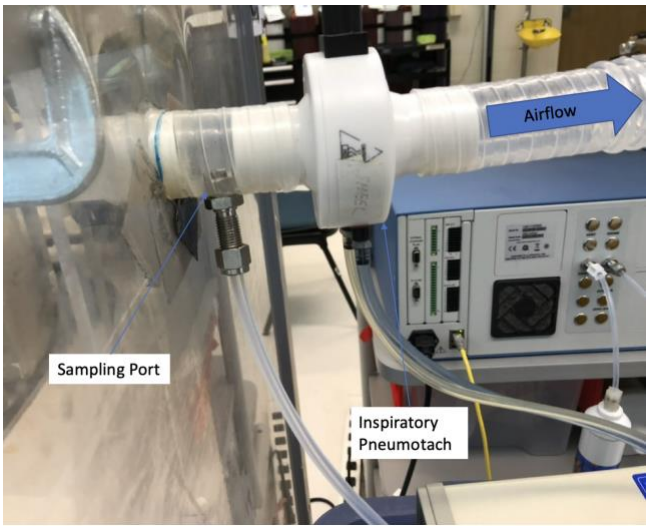
The two independent variables in this study were ozone pollution status and medication (salbutamol) status. The two levels of ozone air pollution were: 170 ppb ozone and room air. Room air in the lab where data collection was performed had low levels of ozone (typically less than 20 ppb). 170 ppb ozone was chosen for the higher ozone level because it represents the higher end of what can realistically be found in the real world (Lu et al., 2018) and half of what has been used in the past where higher (unrepresentative) concentrations were used (McKenzie et al., 1987). To create our set-up, we started with a hypoxia chamber (Colorado Altitude Training, CO, USA) that had all hypoxia equipment removed and access fenestrations closed. An ACT-500 ozone generator (Mellifiq, Hagersten, Sweden), designed to sanitize rooms, was accessible from outside the chamber while the ozone output end was inside the chamber. A standing fan was used to mix the air in the chamber. An overview of the set-up is shown in **Figure 1**.

Figure 1 Overview of experimental set up



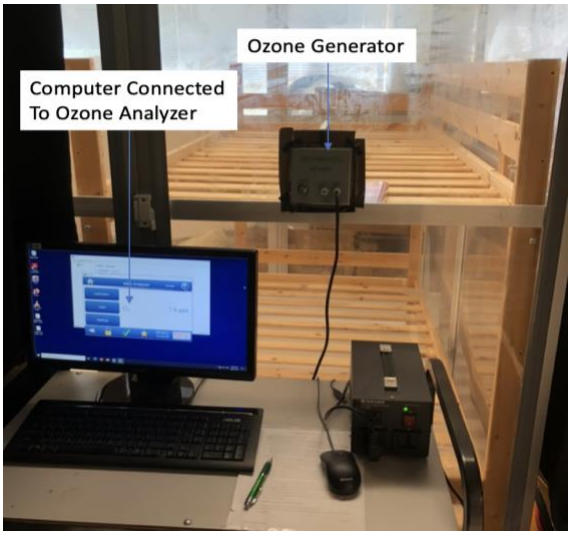
At the other end of the chamber, a bore tube connected to a R3830 adult pneumotachometer (Vacumed, California, USA), pneumotach for short, was used to measure the inspiratory breathing flow of the participant. A sampling port for a 49iQ Ozone Analyser (Thermo Scientific, Waltham, MA, USA) was installed in the bore tube that would sample air as shown in **Figure 2**.

Figure 2 Inspiratory tube connection to chamber



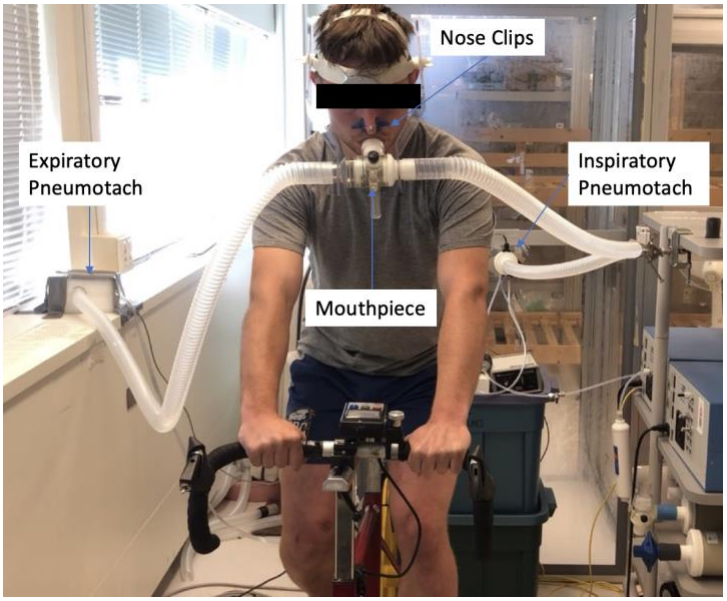
The information from the analyzer would be displayed on a computer placed near the ozone generator that would allow the operator to titrate the ozone in the chamber (**Figure 3**). This computer also recorded the ozone levels during each visit. To keep the air quality condition double-blinded, the screen of the analyzer near the bike was covered and the area that contained the computer and the generator was obscured by curtains as shown in **Figure 1**.

Figure 3 Computer and ozone generator



During testing, the participant would cycle on a Velotron stationary bike (Racemate Inc, Seattle WA, USA) while wearing nose clips and breathing from a mouthpiece with one-way non-rebreathing valves (Hans Rudolf, Kansas, USA). The mouthpiece was connected to bore tubes on the inspiratory side and expiratory side. Both bore tubes were connected to pneumotachs (**Figure 4**). The analog electrical voltage signal outputs from the pneumotachs were connected via BNC cable to a Powerlab DAQ 16/35 device (ADInstruments, Dunedin, New Zealand). The Powerlab DAQ was connected via USB to a computer with Windows XP operating system and the LabChart software (ADInstruments).

Figure 4 Participant set up on bike



For the medication condition, participants inhaled two puffs from a metered-dose inhaler (MDI) designed to deliver 100 μg of salbutamol per puff or a trainer MDI with placebo propellant. To ensure reliability of the delivered dose, participants were provided standardized instructions for using an MDI (Sanchis et al., 2013). In addition, a spacer was attached to the MDI which further

increases dose consistency and distribution in the lungs (Newman, 2004). Medication status had two levels: 200 μg of salbutamol and placebo MDI.

The main dependent variables were spirometry measures and FeNO. Spirometry measures of interest included FEV_1, FVC, and FEF_{25-75}. Spirometry was measured twice at each time point and the attempt with the highest FVC and FEV_1 values were recorded in accordance with American Thoracic Society Guidelines (Graham et al., 2019). In addition to spirometry, a series of FVC manoeuvres with graded expiratory efforts, to account for thoracic gas compression, were collected to generate maximal expiratory flow-volume (MEFV) curves before and after an exercise intervention (Guenette et al., 2010). Tidal flow-volume loops (FVL) during exercise were plotted under the MEFV curves using inspiratory capacity manoeuvres where participants took a maximal inspiration after a normal expiration. These graphs were created to assess expiratory flow limitation and operating lung volumes during exercise (Guenette et al., 2013b).

FeNO is defined as the fraction of exhaled nitric oxide in expired air which acts as a biomarker for airway inflammation (Barnes & Liew, 1995). FeNO was measured using a NObreath-v2 (Bedfont Scientific Ltd, Kent, England). When taking a FeNO measurement, participants expired at a steady rate for ten seconds into the mouthpiece (Bjermer et al., 2014). FeNO was measured before exercise, immediately after, 30 minutes after, and one hour after exercise. At each timepoint, participants performed four consecutive measurements. The first measurement was discarded and the last three were recorded and averaged. We excluded smokers and those who had a recent upper respiratory tract infection. Participants were also asked to avoid consuming foods high in nitrates (rocket, spinach, lettuce, radish, beetroot, cabbage, Chinese cabbage, turnips, green beans, leek,

spring onion, cucumber, carrot, potato, garlic, sweet pepper, green pepper) for 12 hours before each lab visit (Bjermer et al., 2014).

Rating of symptoms (dyspnea, cough, sore throat, headache, chest pain, and chest tightness) and blood pressure were also measured. The selected symptoms were chosen because they have been recognized as, or are related to, symptoms of ozone exposure (Lippmann, 2012). Dyspnea was assessed subjectively using a modified Borg scale which has been validated against other dyspnea measures (Gaber et al., 2019). The other symptoms were subjectively rated on a scale from 0-5. Blood pressure was measured using an BpTRU automated sphygmomanometer (BpTRU Medical Devices, Coquitlam, BC, Canada) at all instances except immediately after exercise (where it was measured by auscultation since the automated cuff was unable to take measurements immediately after exercise). In accordance with Hypertension Canada's guidelines on blood pressure measurement, blood pressure was measured three times and the first measure was discarded and the latter two were averaged (Nerenberg et al., 2018). For the immediate post-exercise blood pressure, only one measurement was taken since blood pressure decreased rapidly from an elevated state after exercise.

2.3 Study Design

This research design can be characterized as a placebo-controlled crossover design with pre-post measures. Participants performed 30 minutes of submaximal exercise at 60% of their VO_{2max} in all four conditions. The order of the conditions was randomized to one of four sequences representing a Latin Square crossover design, which is uniform both within periods and within sequences (Saville & Wood, 1991). FeNO, spirometry, blood pressure, and symptom measures

were obtained before exercise, immediately after, 30 minutes after, and one hour after exercise in each condition. The 30-minute and one-hour post exercise measures were added in addition to the immediately post measure because EIB-related obstruction can last approximately 30-60 minutes after exercise (Gotshall, 2002). Changes from pre- to post-measures were compared between the four conditions to examine how exercising in different combinations of ozone pollution and medication influence pulmonary function (spirometry), inflammation (FeNO), symptoms, and blood pressure.

2.4 Procedures

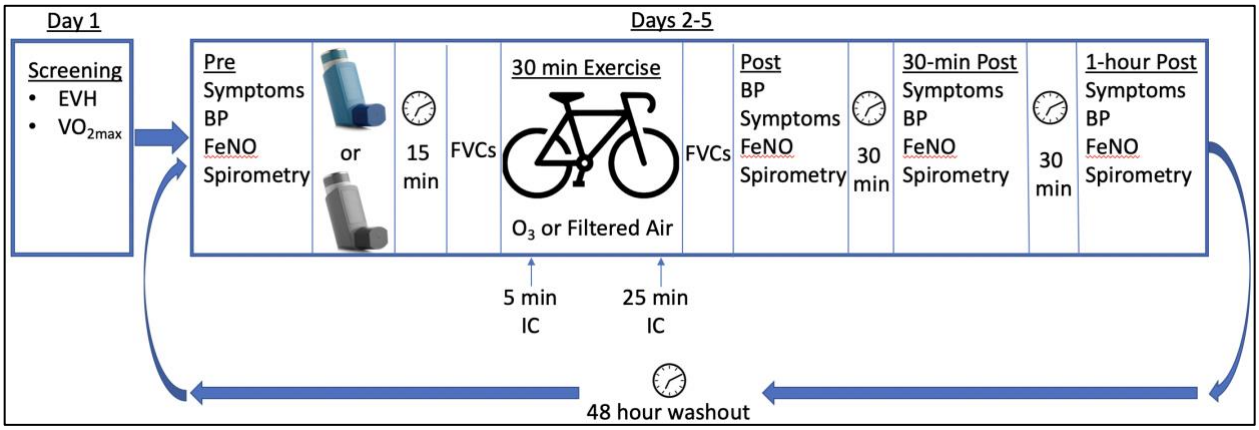
Participants visited the lab on five occasions. It was also expected that participants took no leukotriene modifier medication (Singulair or montelukast) 24 hours before each visit, no short-acting beta agonists 8 hours before each visit, no long-acting beta agonists 48 hours before each visit, and no antihistamines 72 hours before each visit. The medication and diet restrictions controlled for potential confounding effects on the eucapnic voluntary hyperpnea (EVH) test and FeNO measurement. In addition, participants were asked to avoid supplementation with vitamin C, vitamin E, and multivitamins over the course of the 5 visits. This prevented changes in the response to ozone related to vitamin supplementation (Gomes et al., 2011). Finally, participants were asked to avoid exercise 24 hours prior to each lab visit. This was to ensure no refractory period following exercise commonly seen with EIB (Stickland et al., 2012). Day one was a screening day and involved: obtaining consent, collecting height, weight, age, and sex, screening participants for EIB, conducting a VO_{2max} test, and orienting participants to FeNO and spirometry measurement. First, participants re-read and signed a consent form in addition to reviewing and signing a PAR-Q+. They then performed practice spirometry and FeNO measurements to orient

themselves to the procedure of these measures. Participants then performed an EVH test to screen for EIB. The EVH test required participants to voluntarily hyperventilate air with a 5% CO_2 concentration for 6 minutes to replicate breathing during exercise. This mimics the stimulus that leads to airway narrowing in EIB (Hull et al., 2016). Spirometry measures were obtained before and after EVH and compared. The target VE during the EVH was 30 times the pre-spirometry FEV_1 measure and during the EVH test participants were able to see their VE. A fall index, percent drop in FEV_1 from pre to post, of 10% or greater was used to confirm the presence of EIB (Hull et al., 2016). We found that it was difficult for most participants to maintain their VE close to the target. Therefore, a few participants who had a fall index greater than 9% but less than 10% who were not close to their target VE were included on the assumption that their fall index would have been at least 10% had they been able to maintain the target VE. Eligible participants then took salbutamol at the treatment dose (200 µg) and then waited 15 minutes before starting the VO_{2max} test. The VO_{2max} test followed a ramp protocol and expired gas was analyzed using a metabolic cart (True One 2400, Parvomedics Inc, UT, USA) to measure oxygen consumption and other metabolic variables. Following a self-selected warm up, participants started at 30 watts, with the workload increasing by a watt every 3 seconds for women and every 2 seconds for men until they could no longer continue or until they could no longer maintain a cadence of 60 rpm.

After the screening visit, participants returned to the lab on four separate occasions to complete the four exercise trials. Pre-exercise FeNO, blood pressure, symptoms and spirometry were measured and then participants were read a standardized set of instructions and took two puffs from one of the two inhalers. After 15 minutes, participants mounted the bike and put on a headset that included a mouthpiece connected to the chamber and the two pneumotachs as shown in **Figure**

4. The three graded FVC manoeuvres were then performed followed by a period of resting breathing and an inspiratory capacity. Once they were ready, participants started cycling at 60% of their VO_{2max} test peak wattage for 30 minutes. After 5 minutes and 25 minutes of exercise, participants repeated the inspiratory capacity manoeuvre. Immediately post exercise, blood pressure was taken followed by symptoms, FeNO, and spirometry. These measures were repeated at 30-minutes post exercise and 1-hour post exercise. All subsequent visits were separated by 48 hours to prevent carryover effects from previous exposure to ozone or salbutamol. This 48-hour duration was chosen because it is longer than five half-lives of salbutamol medication (Beregi et al., 1971). The overall procedure is summarized in **Figure 5** below.

Figure 5 Summary of experimental procedure



2.5 Statistical Analysis

Baseline characteristics were summarized descriptively for the whole sample as well as disaggregated by sex. Spirometry, FeNO, and blood pressure at baseline in the 4 different experimental conditions were compared using one-way repeated measures ANOVA to ensure there were no baseline differences between conditions. Statistical significance was defined as a p-

value less than 0.05. Mean ozone concentrations were compared between conditions to ensure consistency of ozone concentrations during the ozone and room air days.

Spirometry, FeNO, and blood pressure measures were converted into percent change from baseline values. Normality was assessed using Shapiro-Wilk tests. Mean and standard deviation percent change values were summarized and compared between conditions at each time point using one-way repeated measures ANOVA and subsequent *post hoc* paired t-tests with Bonferroni correction for multiple comparisons. Percent change values in each condition were visualized through individual and mean trajectory plots. Linear mixed effects models, adjusting for age, sex, period, and time point, were used to compare and quantify the overall effects of salbutamol and placebo as well as ozone and room air on the outcome measures.

Symptom severity scores (rated on a scale of 0-5, or 0-10 for dyspnea) were summarized across experimental conditions using median (IQR) because of the skewed severity distributions. Side-by-side boxplots were used to visualize symptom severity at the different time points (before exercise, immediately post, 30-minutes post, and 1-hour post exercise). Severity scores where then dichotomized into 0 (symptom not experienced) and 1 (symptom experienced; severity > 0) to compare differences in symptom counts across experimental conditions. Post-exercise symptom severity was compared between experimental conditions using linear mixed effects models, adjusting for age and sex as well as the pre-exercise severity score. The probability of experiencing a symptom was compared between conditions using logistic mixed effects models.

Statistical analyses were conducted in Excel and R version 4.1.2 (R Core Team 2021).

Chapter 3: Results

3.1 Participants and Pollution

A total of 15 people have been screened for this study. Of the 15, six were EVH- and therefore were excluded. Of the nine EVH+ individuals, seven have completed all five visits and two still have visits left to complete. The data analysis for this thesis was performed on the seven individuals who have completed all five visits. Recruitment is still ongoing for this project and currently we have seven people who have expressed interest but have not yet scheduled their first screening visit. The baseline characteristics of the seven completed participants are shown in **Table 2** below. Of the seven participants we had five females and two males. All included participants had a physician reported asthma diagnosis. Of the 6 excluded participants, 3 had a physician reported asthma diagnosis and 3 did not. The mean age (SD) of participants was 22.3 (2.9) years old. The mean fall index was 23.2% which falls in the moderate category for EIB (≥ 20% to ≤ 30%) (Hull et al., 2016). We had a large range of EIB severity in this sample with the largest fall index being 55.8% and the lowest being 9.2%. Another baseline characteristic of note is the baseline FeNO measures. Mean (SD) baseline FeNO was 25.4 (26.5) ppb which falls in the elevated category (20/25-50) for FeNO (Bjermer et al., 2014).

Table 2 Summary of baseline characteristics by sex[1]

	All (*n*=7)	Male (*n*=2)	Female (*n*=5)
Age (years)	22.3 (2.9)	22.5 (0.7)	22.2 (3.5)
Height (cm)	171.0 (10.3)	178.8 (4.6)	167.9 (10.6)
Weight (kg)	68.1 (9.2)	73.3 (1.7)	66.0 (10.4)
VO_{2peak} (mL/kg/min)	34.0 (7.0)	32.3 (0.1)	34.7 (8.4)
Fall index (%)	23.2 (17.0)	22.7 (13.2)	23.5 (19.7)
Asthma diagnosis	7 (100%)	2 (100%)	5 (100%)
FeNO (ppb)[2]	25.4 (26.5)	36.3 (48.0)	21.1 (19.9)
Spirometry[2]			
FVC (L)	4.41 (0.70)	5.09 (0.67)	4.14 (0.56)
FEV_1 (L)	3.43 (0.61)	3.98 (0.70)	3.20 (0.47)
FEF_{25-75} (L/min)	2.93 (0.68)	3.47 (0.85)	2.71 (0.55)
Blood pressure[2]			
Systolic (mmHg)	105.4 (7.9)	112.6 (6.1)	102.5 (6.9)
Diastolic (mmHg)	70.2 (3.3)	71.3 (0.7)	69.8 (4.0)

[1] Values are mean (SD) or *n* (%).

[2] Values are averages of all pre-exercise measurements (4 per participant).

As shown in **Table 3** there were no significant differences in baseline measurements before each exercise condition except for diastolic blood pressure. Diastolic blood pressure was higher for the room air + placebo condition. Mean (SD) ozone concentration, in ppb, for each condition were the following: 8.1 (6.6) for room air + placebo, 8.8 (7.6) for room air + salbutamol, 172.6 (5.9) for ozone + placebo, and 171.7 (5.1) for ozone + salbutamol.

Table 3 Baseline FeNO, spirometry, and blood pressure measures by treatment group[1]

	Room Air		Ozone		P-value[2]
	Placebo	Salbutamol	Placebo	Salbutamol	
FeNO (ppb)	21.6 (21.6)	23.3 (23.6)	23.4 (28.2)	26.5 (28.5)	0.202
Spirometry					
FVC (L)	4.48 (0.76)	4.51 (0.69)	4.35 (0.65)	4.32 (0.84)	0.440
FEV_1 (L)	3.43 (0.67)	3.46 (0.53)	3.46 (0.57)	3.35 (0.80)	0.837
FEF_{25-75} (L/min)	2.82 (0.73)	2.97 (0.58)	3.04 (0.67)	2.87 (0.98)	0.743
Blood Pressure					
Systolic (mmHg)	107.3 (11.6)	103.9 (7.3)	105.4 (9.8)	104.9 (6.1)	0.635
Diastolic (mmHg)	74.4 (4.6)	69.0 (3.4)	69.8 (2.3)	67.6 (6.5)	0.010*

[1] Values are mean (SD); n=7 in all treatment groups.

[2] P-values obtained from one-way repeated measures ANOVA. * indicates statistical significance ($p<0.05$).

Percent change from baseline FeNO, spirometry, and blood pressure measures were used for pre-post analysis. **Table 4** summarizes percent change values at each time point across the four conditions. Comparison between conditions was done using cross-sectional one-way repeated measures ANOVA across treatment groups at each time point. Significant differences in percent change were found for FEV_1 and FEF_{25-75} at all three post exercise time points. Further details on *post hoc* analysis of these findings are discussed in **Sections 3.2.2** and **3.2.3**.

Table 4 Summary of percent change from baseline FeNO, spirometry, and blood pressure measures by experimental group[1]

	Exposure	Room Air		Ozone		P-value[2]
		Placebo	Salbutamol	Placebo	Salbutamol	
FeNO	FeNO					
	Post	-18.6 (33.6)	14.0 (39.9)	17.0 (41.7)	12.3 (42.6)	0.416
	30-min post	-11.7 (16.7)	39.3 (76.0)	19.9 (39.8)	30.6 (56.3)	0.387
	60-min post	-6.12 (52.0)	6.37 (38.4)	7.89 (37.7)	36.3 (77.3)	0.436
SPIROMETRY	FVC					
	Post	-2.96 (3.58)	-2.47 (5.33)	-2.65 (4.00)	-0.02 (1.86)	0.301
	30-min post	-1.25 (3.21)	0.44 (3.95)	-1.33 (2.61)	0.44 (2.10)	0.436
	60-min post	-2.39 (2.62)	1.06 (2.01)	-0.09 (2.67)	0.93 (2.87)	0.121
	FEV_1					
	Post	-1.16 (3.69)	8.15 (4.83)	-2.79 (5.83)	11.2 (8.49)	0.002*
	30-min post	1.72 (5.39)	10.3 (4.99)	-1.41 (3.27)	11.1 (7.68)	<0.001*
	60-min post	0.34 (6.26)	11.3 (4.84)	-1.41 (4.36)	10.1 (7.60)	<0.001*
	FEF_{25-75}					
	Post	7.63 (10.8)	40.2 (7.63)	1.08 (12.8)	38.7 (28.1)	0.005*
	30-min post	11.5 (15.6)	33.0 (13.7)	2.63 (6.70)	35.4 (22.9)	<0.001*
	60-min post	8.06 (15.3)	33.2 (13.5)	-0.48 (9.07)	31.4 (25.8)	<0.001*
BLOOD PRESSURE	Systolic					
	Post	28.7 (12.6)	34.3 (11.7)	40.9 (12.9)	29.4 (8.80)	0.113
	30-min post	-3.57 (6.17)	-2.13 (3.47)	-5.38 (6.65)	-2.97 (5.44)	0.784
	60-min post	-7.25 (6.22)	-3.03 (4.41)	-4.67 (4.19)	-5.50 (5.61)	0.536
	Diastolic					
	Post	10.4 (11.1)	-1.54 (9.42)	17.4 (9.35)	8.53 (15.7)	0.081
	30-min post	-2.82 (2.81)	0.72 (7.37)	-3.15 (8.43)	-1.11 (7.42)	0.713
	60-min post	-3.77 (8.56)	1.50 (7.03)	-1.56 (4.97)	0.52 (8.28)	0.560

[1] Values are mean (SD); n=7 in all treatment groups.

[2] P-values obtained from cross-sectional one-way repeated measures ANOVA across treatment groups at each time point. * indicates statistical significance ($p<0.05$).

3.2 Spirometry

3.2.1 Forced Vital Capacity (FVC)

Individual and mean FVC trajectories are displayed in **Figure 6** and **Figure 7**, respectively. No statistically significant differences were found between the percent change in FVC at the three time points (**Table 4**). However, although the ANOVA indicated insignificant differences between treatment groups, there was a trend for larger percent change in FVC values at 30-minutes and 1-hour post exercise in the salbutamol conditions compared to the placebo conditions (**Figure 7**). Linear mixed effects models adjusting for age and sex showed a significant difference between salbutamol and placebo, with overall percent change in FVC being 1.9% higher with salbutamol use compared to placebo (95% CI: [0.8%, 3.0%]; p=0.002). There was no difference in FVC values when comparing ozone to room air conditions.

Figure 6 Individual percent change from baseline post exercise FVC measures by condition

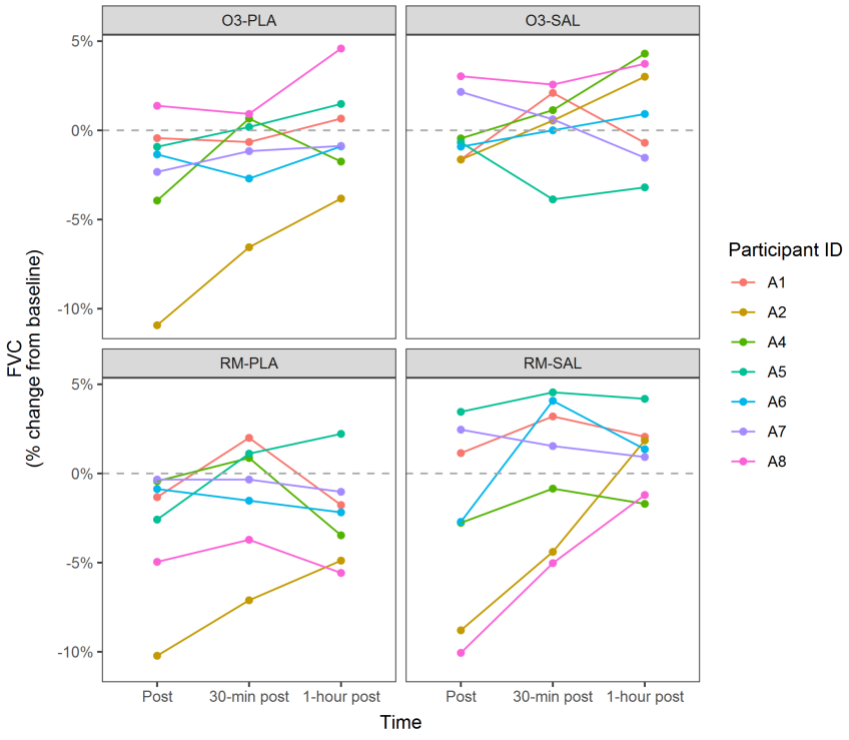
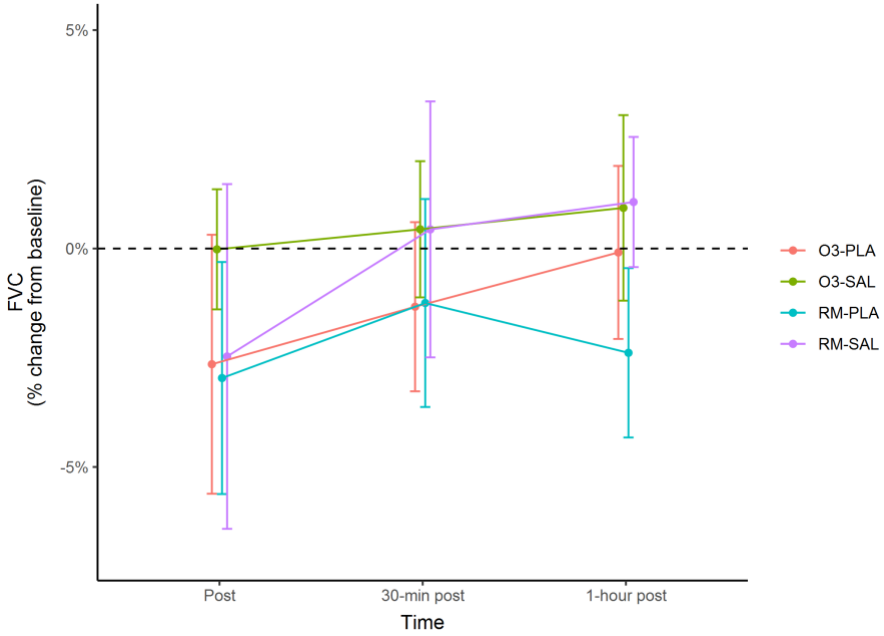


Figure 7 Mean (95% CI) percent change from baseline post exercise FVC measures by condition



3.2.2 Forced Expiratory Volume in 1 Second (FEV_1)

Individual and mean FEV_1 trajectories are displayed in **Figure 8** and **Figure 9**, respectively. For FEV_1 in both air quality conditions, the salbutamol condition showed improvement while there was a minor decrease for the placebo condition. *Post hoc* paired t-tests revealed significant differences between room air + placebo and room air + salbutamol conditions at all three time points (Bonferroni-adjusted p-value 0.014, 0.030, and 0.011 for immediately post, 30-min post, and 1-hour post, respectively). Significant differences were also observed between room air + salbutamol and ozone + placebo (adjusted p-value 0.009, 0.002, and <0.001 for immediately post, 30-min post, and 1-hour post, respectively) as well as ozone + placebo and ozone + salbutamol (adjusted p-value <0.001, 0.008, and 0.003 for immediately post, 30-min post, and 1-hour post, respectively). The comparison between room air + placebo and ozone + salbutamol was found to be marginally significant at all time points. Linear mixed effects modelling to assess the overall drug effect showed significant differences between the salbutamol and placebo conditions, with percent change in FEV_1 being 10.7% higher with salbutamol use compared to placebo (95% CI: [8.9%, 12.6%]; p<0.001). No significant differences were observed between room air and ozone among the salbutamol conditions; however, among the placebo conditions, overall percent change in FEV_1 was 2.4% higher in room air compared to ozone (95% CI: [0.1%, 4.7%]; p=0.065).

Figure 8 Individual percent change from baseline post exercise FEV_1 measures by condition

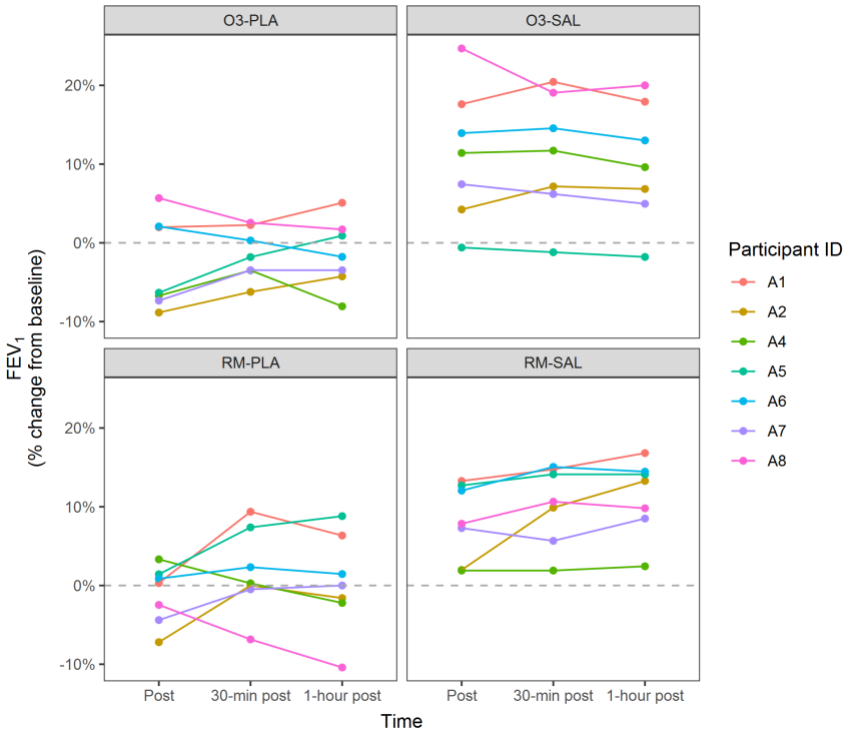
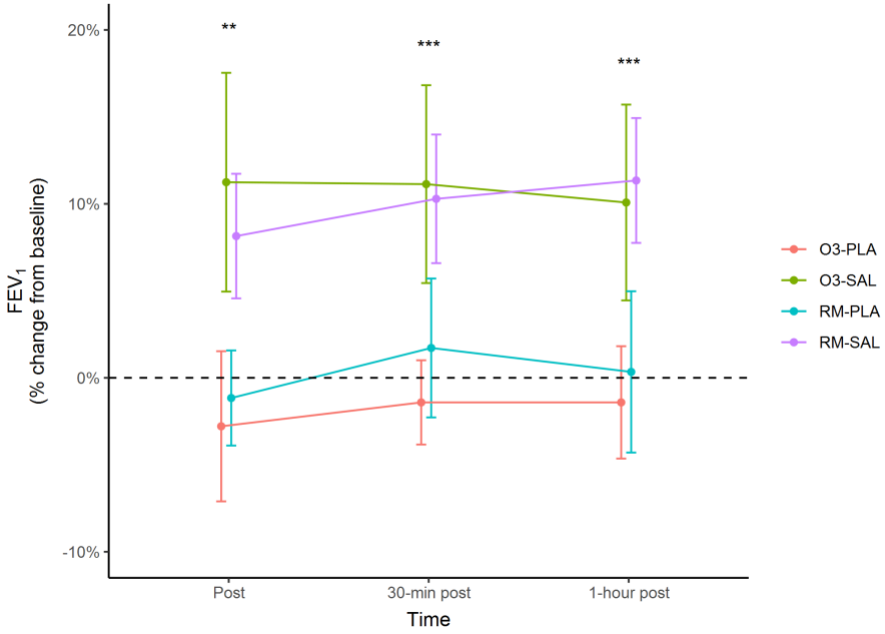


Figure 9 Mean (95% CI) percent change from baseline post exercise FEV_1 measures by condition (note: *, **, *** indicate statistical significance; p<0.05, <0.01, <0.001, respectively)



3.2.3 Forced Expiratory Flow between 25% and 75% of FVC (FEF_{25-75})

Individual and mean FEF_{25-75} trajectories are displayed in **Figure 10** and **Figure 11**, respectively. As with FEV_1, there was a large amount of variability in % change in FEF_{25-75} between participants in the ozone + salbutamol condition. *Post hoc* paired t-tests revealed significant differences between room air + salbutamol and ozone + placebo conditions across all three post exercise time points (Bonferroni-adjusted p-value <0.001). Differences were also observed between room air + placebo and room air + salbutamol (adjusted p-value <0.001, 0.097, 0.032 for immediately post, 30-min post, and 1-hour post, respectively) as well as ozone + placebo and ozone + salbutamol (adjusted p-value 0.015, 0.024, 0.062 for immediately post, 30-min post, and 1-hour post, respectively). The comparison between room air + placebo and ozone + salbutamol was found to be marginally significant. Linear mixed effects modelling adjusting for age and sex revealed significant differences between salbutamol and placebo conditions, with percent change in FEF_{25-75} being 28.8% higher with salbutamol use compared to placebo (95% CI: [23.6%, 33.9%]; p<0.001). No differences were observed between ozone and room air among the salbutamol conditions; however, among the placebo conditions overall percent change in FEF_{25-75} was 7.4% higher in room air compared to ozone (95% CI: [2.3%, 12.3%]; p=0.011).

Figure 10 Individual percent change from baseline post exercise FEF_{25-75} measures by condition

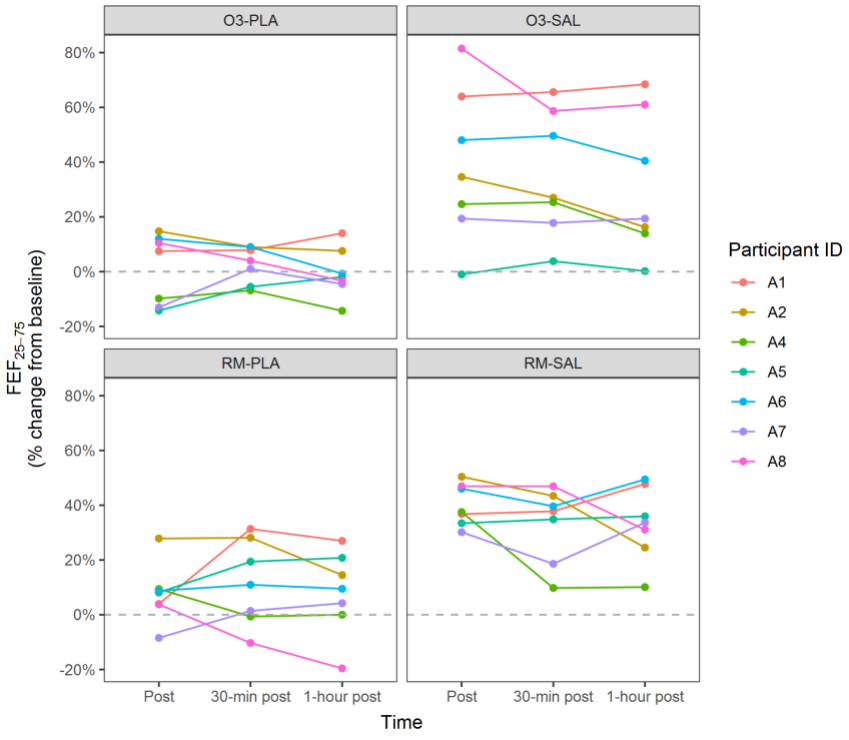
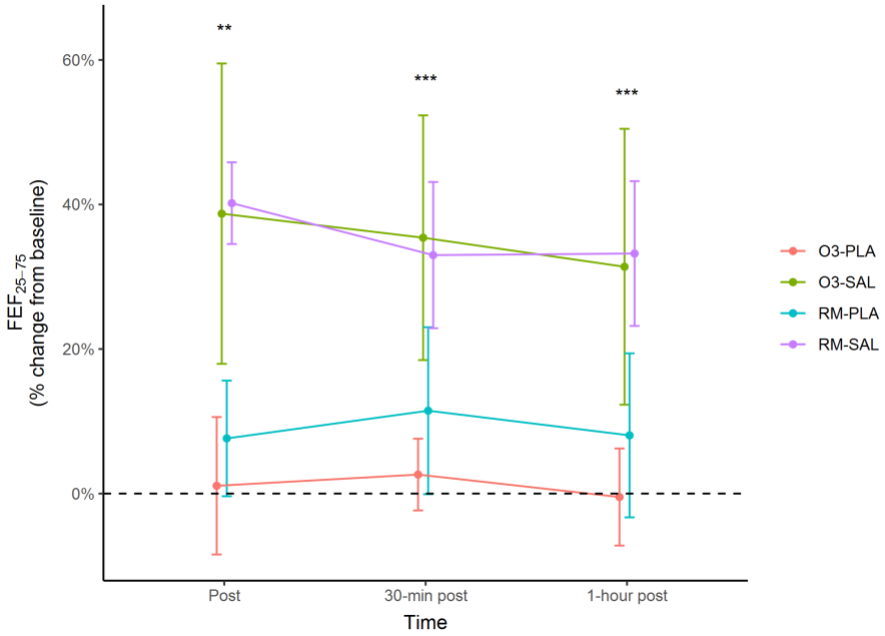


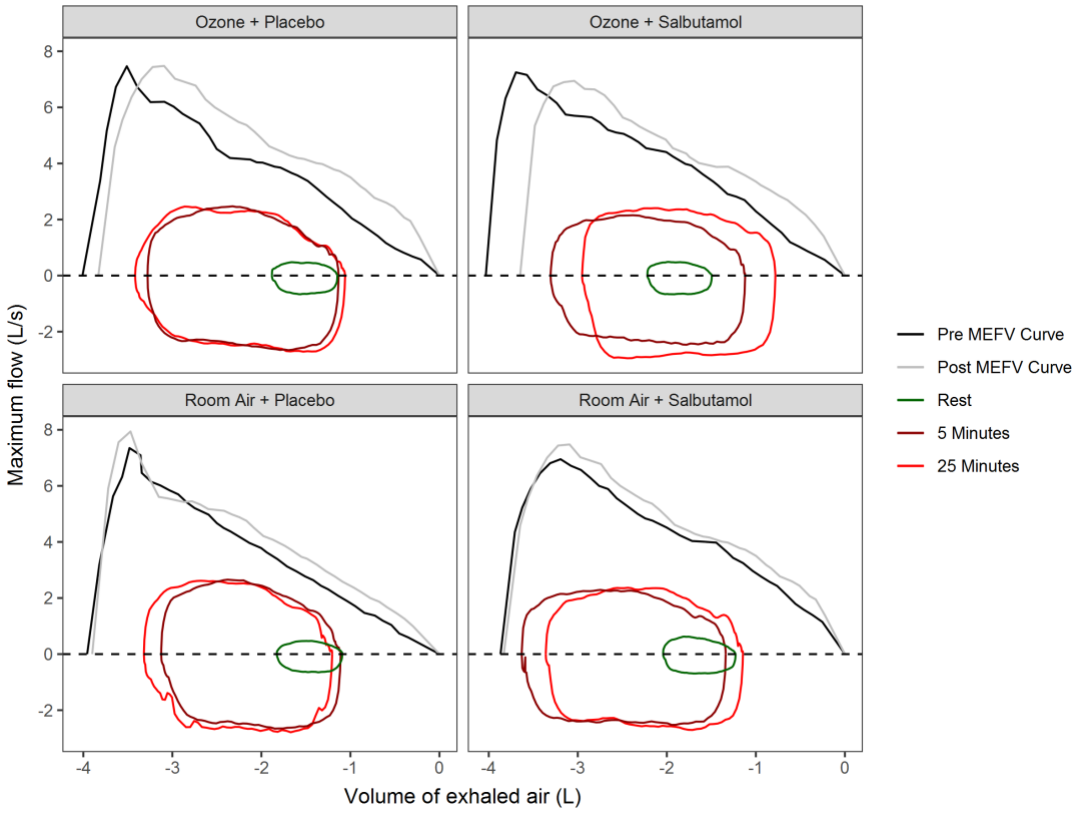
Figure 11 Mean (95% CI) percent change from baseline post exercise FEF_{25-75} measures by condition (note: *, **, *** indicate statistical significance; p<0.05, <0.01, <0.001, respectively)



3.3 Flow-Volume Loops

The flow-volume loops for only one participant have been analyzed. The chosen participant had the largest fall index from the EVH test (55.8%). Flow-volume loops for this participant are displayed in **Figure 12**. In all 4 conditions we did not find any expiratory flow limitation during exercise. In the ozone conditions there was a smaller decrease in FVC compared to the room air conditions. Operating lung volumes also did not shift consistently between 5 minutes and 25 minutes during exercise. However, in the salbutamol conditions, the 25-minute flow-volume loops appear to be shifted more to the right compared to the 5-minute flow-volume loops.

Figure 12 Maximal expiratory flow-volume (MEFV) curves pre- and post-exercise with tidal flow-volume loops at rest, 5 minutes during exercise, and 25 minutes during exercise



3.4 Fraction of Exhaled Nitric Oxide (FeNO)

Individual and mean FeNO trajectories are displayed in **Figure 13** and **Figure 14**, respectively. FeNO was found to have the most variability out of all the measured variables. Looking at the individual data it appears some participants had large changes in FeNO while others did not have much change at all. Repeated measures ANOVA testing showed no significant differences between the conditions at any time points. However, looking at the mean plot there is potentially a trend where it seems FeNO decreased at all time points for the room air + placebo condition and increased for all the other conditions. Linear mixed effects modelling adjusting for age and sex revealed a significant difference between ozone and room air among the placebo conditions, with percent change in FeNO being 31.6% higher in ozone compared to room air (95% CI: [11.9%, 51.3%]; p=0.008). This difference between ozone and room air was not observed for the salbutamol conditions. Looking at the overall drug effect, percent change in FeNO was also found to be 31.2% higher for salbutamol compared to placebo in the room air conditions (95% CI: [6.5%, 55.9%]; p=0.033); however, this significant effect was not observed in the ozone conditions.

Figure 13 Individual percent change from baseline post exercise FeNO measures by condition

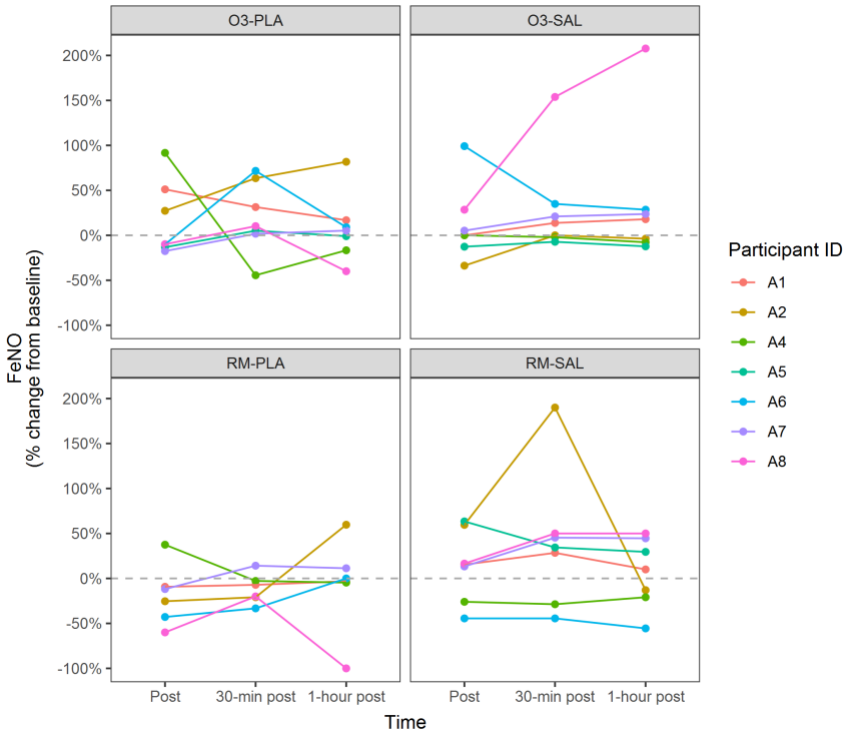
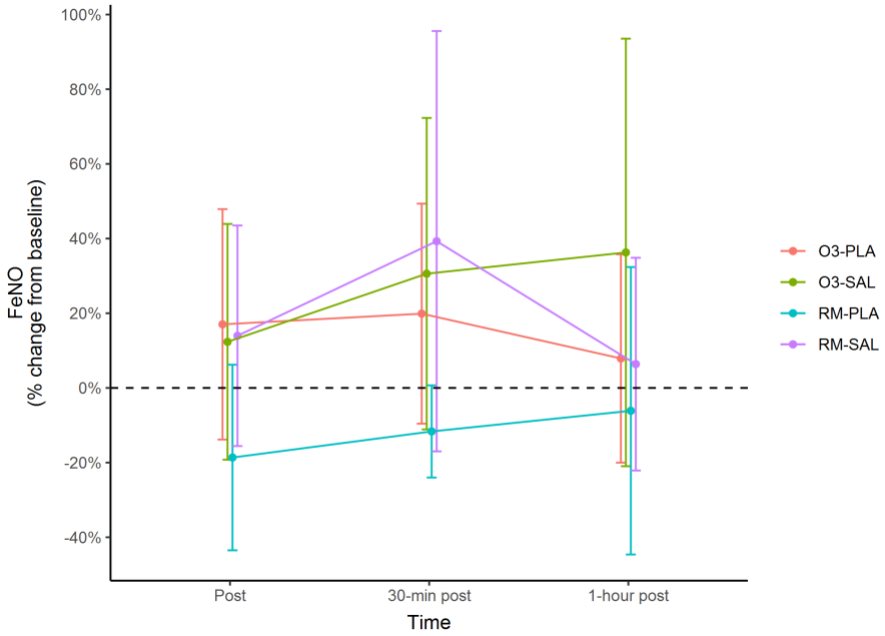


Figure 14 Mean (95% CI) percent change from baseline post exercise FeNO measures by condition



3.5 Blood Pressure

Individual and mean trajectories for systolic blood pressure are displayed in **Figure 15** and **Figure 16**. All participants had a similar response in systolic blood pressure where blood pressure was elevated post exercise and then decreased to baseline or below baseline at 30-minutes and 1-hour after exercise. The mean trajectory plots show that there were no differences in blood pressure between the four experimental conditions. Systolic blood pressure decreased by 3.84 mmHg at 30 minutes post exercise and by 5.50 mmHg 1-hour post exercise. Diastolic blood pressure decreased by 1.16 mmHg at 30 minutes post exercise and by 0.61 mmHg 1-hour post exercise.

Figure 15 Individual percent change from baseline post exercise systolic blood pressure measures by condition

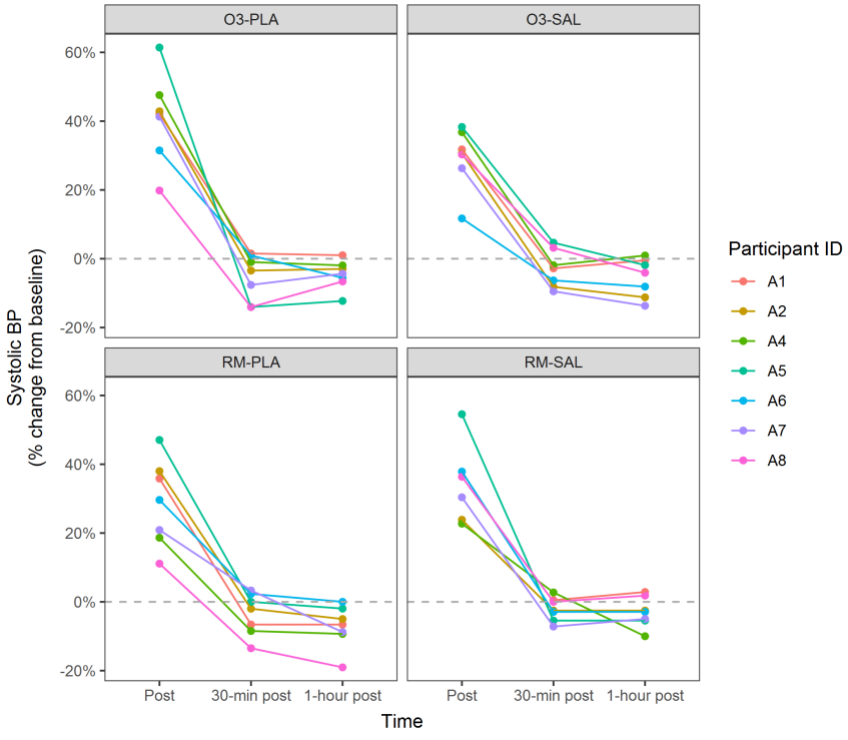
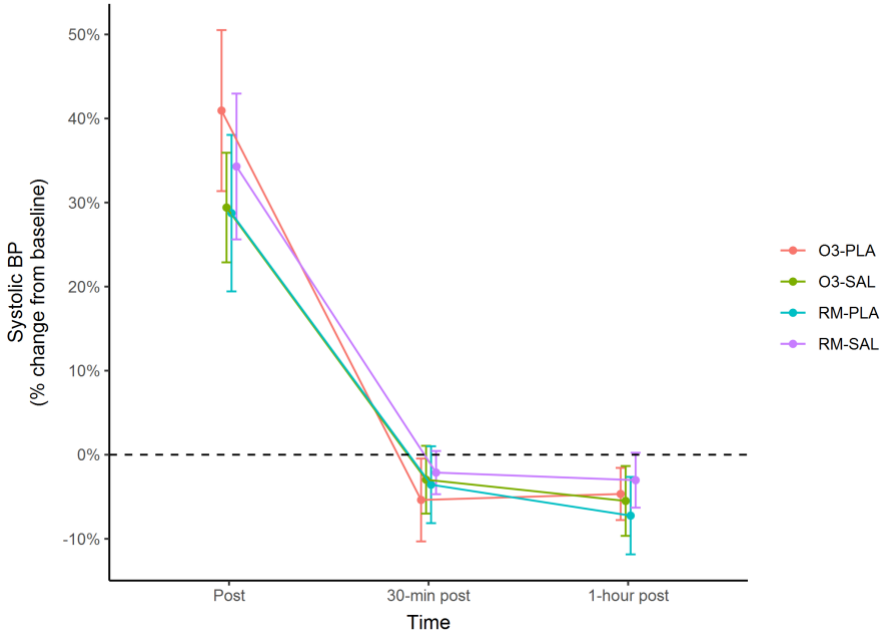


Figure 16 Mean (95% CI) percent change from baseline post exercise systolic blood pressure measures by condition



Individual and mean trajectories for diastolic blood pressure are displayed in **Figure 17** and **Figure 18**, respectively. As discussed in **Section 4.4** there were limitations that affected diastolic blood pressure measurement. This may explain why individual trajectories for diastolic blood pressure displayed in **Figure 17** do not match the individual trajectories in systolic blood pressure presented in **Figure 15**.

Figure 17 Individual percent change from baseline post exercise diastolic blood pressure measures by condition

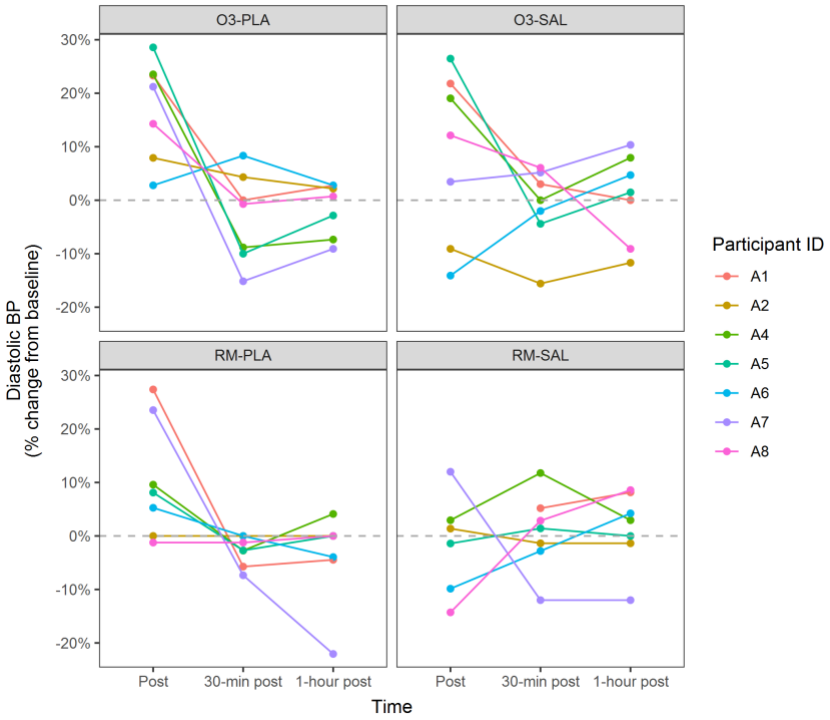
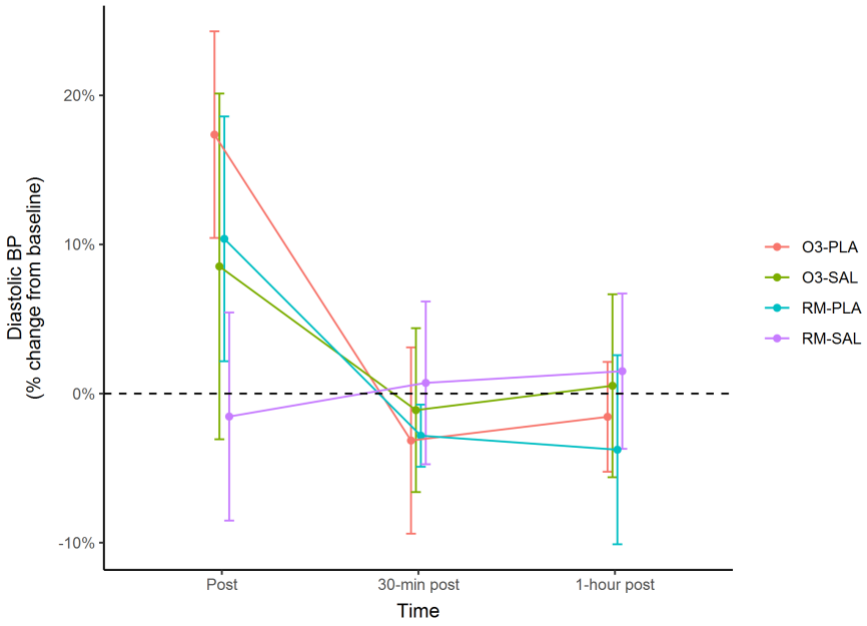


Figure 18 Mean (95% CI) percent change from baseline post exercise diastolic blood pressure measures by condition



3.6 Symptoms

Table 5 summarizes symptom severity scores and counts across experimental conditions. Side-by-side boxplots for symptom severity are shown in **Figure 19**. Symptom severity appeared to be most severe immediately post and 30 minutes post exercise, before decreasing to a level comparable to pre-exercise severity by the 1-hour time point. Overall, the most common symptoms experienced were dyspnea, chest tightness, sore throat and cough, with all participants reporting dyspnea and/or sore throat at some point during the experimental trial and all but one participant reporting chest tightness and/or cough.

Table 5 Summary of symptom severity and counts across experimental conditions at each time point[1]

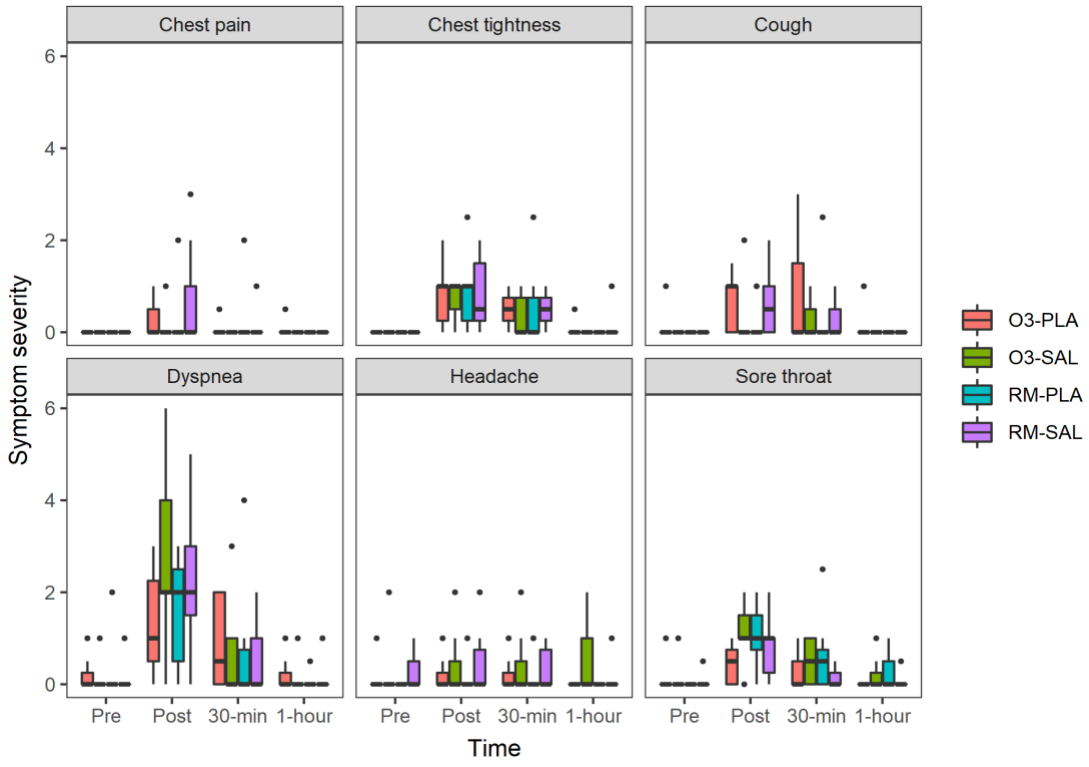
	Room Air								Ozone							
	Placebo				Salbutamol				Placebo				Salbutamol			
	Pre	Post	30 min	1 hour	Pre	Post	30 min	1 hour	Pre	Post	30 min	1 hour	Pre	Post	30 min	1 hour
Dyspnea																
Severity[2]	0 (0, 0)	2 (0.5, 2.5)	0 (0, 0.75)	0 (0, 0)	0 (0, 0)	2 (1.5, 3)	0 (0, 1)	0 (0, 0)	0 (0, 0.25)	1 (0.5, 2.25)	0.5 (0, 2)	0 (0, 0.25)	0 (0, 0)	2 (2, 4)	0 (0, 1)	0 (0, 0)
Count	1 (14.3%)	5 (71.4%)	3 (42.9%)	1 (14.3%)	1 (14.3%)	6 (85.7%)	3 (42.9%)	1 (14.3%)	2 (28.6%)	6 (85.7%)	4 (57.1%)	2 (28.6%)	1 (14.3%)	6 (85.7%)	3 (42.9%)	1 (14.2%)
Cough																
Severity[3]	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.5 (0, 1)	0 (0, 0.5)	0 (0, 0)	0 (0, 0)	1 (0, 1)	0 (0, 1.5)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0.5)	0 (0, 0)
Count	0 (0%)	1 (14.3%)	1 (14.3%)	0 (0%)	0 (0%)	4 (57.1%)	2 (28.6%)	0 (0%)	1 (14.3%)	4 (57.1%)	3 (42.9%)	1 (14.3%)	0 (0%)	1 (14.3%)	3 (42.9%)	0 (0%)
Sore throat																
Severity[3]	0 (0, 0)	1 (0.75, 1.5)	0.5 (0, 0.75)	0 (0, 0.5)	0 (0, 0)	1 (0.25, 1)	0 (0, 0.25)	0 (0, 0)	0 (0, 0)	0.5 (0, 0.75)	0 (0, 0.5)	0 (0, 0)	0 (0, 0)	1 (1, 1.5)	0.5 (0, 1)	0 (0, 0.25)
Count	0 (0%)	6 (85.7%)	4 (57.1%)	2 (28.6%)	1 (14.3%)	5 (71.4%)	3 (42.9%)	1 (14.3%)	1 (14.3%)	4 (57.1%)	3 (42.9%)	0 (0%)	1 (14.3%)	6 (85.7%)	4 (57.1%)	2 (28.6%)
Headache																
Severity[3]	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0.5)	0 (0, 0.75)	0 (0, 0.75)	0 (0, 0)	0 (0, 0)	0 (0, 0.25)	0 (0, 0.25)	0 (0, 0)	0 (0, 0)	0 (0, 0.5)	0 (0, 0.5)	0 (0, 1)
Count	0 (0%)	1 (14.3%)	1 (14.3%)	0 (0%)	2 (28.6%)	3 (42.9%)	3 (42.9%)	1 (14.3%)	1 (14.3%)	2 (28.6%)	2 (28.6%)	0 (0%)	1 (14.3%)	2 (28.6%)	2 (28.6%)	2 (28.6%)
Chest pain																
Severity[3]	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 1)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0.5)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
Count	0 (0%)	1 (14.3%)	1 (14.3%)	0 (0%)	0 (0%)	2 (28.6%)	1 (14.3%)	0 (0%)	0 (0%)	2 (28.6%)	1 (14.3%)	1 (14.3%)	0 (0%)	1 (14.3%)	0 (0%)	0 (0%)
Chest tightness																
Severity[3]	0 (0, 0)	1 (0.25, 1)	0 (0, 0.75)	0 (0, 0)	0 (0, 0)	0.5 (0.25, 1.5)	0.5 (0.25, 0.75)	0 (0, 0)	0 (0, 0)	1 (0.25, 1)	0.5 (0.25, 0.75)	0 (0, 0)	0 (0, 0)	1 (0.5, 1)	0 (0, 0.75)	0 (0, 0)
Count	0 (0%)	5 (71.4%)	3 (42.9%)	0 (0%)	0 (0%)	5 (71.4%)	5 (71.4%)	1 (14.3%)	0 (0%)	5 (71.4%)	5 (71.4%)	1 (14.3%)	0 (0%)	5 (71.4%)	3 (42.9%)	0 (0%)

[1] Severity measures are summarized using median (IQR); counts are presented as *n* (%).

[2] Severity scored on a scale from 0-10.

[3] Severity scored on a scale from 0-5.

Figure 19 Side-by-side boxplots displaying symptom severity across time points for each experimental condition (note: dyspnea rated on a scale from 0-10, other symptoms rated on a scale from 0-5)



Linear mixed effects modelling of post exercise symptom severity scores, adjusting for age, sex, and pre-exercise severity, showed significant differences in cough and sore throat severity between ozone + placebo and room air + placebo conditions (p=0.005 and p=0.002, respectively), with the ozone group having an average cough severity 0.54 (95% CI: 0.20-0.89) units higher and a sore throat severity 0.45 (95% CI: 0.19-0.71) units lower compared to the room air group. Significant differences in cough and sore throat severity were also observed between ozone + placebo and ozone + salbutamol conditions (p=0.014 and p=0.010, respectively), where cough severity was 0.47 (95% CI: 0.13-0.82) units lower with salbutamol use whereas sore throat severity was 0.36 (95% CI: 0.11-0.62) higher with salbutamol use. Cough severity was also significantly higher in

the ozone + placebo condition compared to the room air + salbutamol condition (p=0.044). Sore throat severity was found to be significantly lower in the room air + salbutamol condition compared to both the room air + placebo condition (p=0.005) as well as the ozone + salbutamol condition (p=0.027). Headache severity was found to be significantly higher in the ozone + salbutamol condition compared to both room air + placebo and ozone + placebo conditions (p=0.013), with severity being 0.29 (95% CI: 0.08-0.50) units higher compared to room air + placebo and 0.27 (95% CI: 0.07-0.46) units higher compared to ozone + placebo. Looking at the overall drug effect between salbutamol and placebo, a significant drug effect was only observed for headache where severity was 0.19 units higher with salbutamol use compared to placebo (95% CI: [0.04, 0.34]; p=0.020).

When looking at symptom prevalence, logistic mixed effects models adjusting for age and sex showed significant differences between room air + placebo and room air + salbutamol for cough and headache. Both probability of cough and probability of headache were higher with salbutamol use (p=0.047 and p=0.021, respectively). Among the ozone groups, probability of cough was marginally lower with salbutamol use compared to placebo (p=0.078). Probability of cough was found to be significantly higher in ozone + placebo compared to room air + placebo (p=0.005). Overall, the probability of cough tended to be higher in ozone than in room air (p=0.073). Probability of headache was marginally higher in room air + salbutamol compared to ozone + placebo (p=0.076). Overall, there was a trend for the probability of headache to be greater with salbutamol use compared to placebo (p=0.056). No other differences between experimental conditions were observed for the other symptoms.

Chapter 4: Discussion

We hypothesized that individuals with asthma and/or EIB who took salbutamol before exercising in ozone would have improved pulmonary function but higher levels of inflammation after exercise compared to placebo medication. We found that salbutamol did improve pulmonary function as shown by the larger, statistically significant, improvements in FEV_1 and FEF_{25-75} in both the ozone and room air conditions compared to placebo. However, salbutamol did not appear to exacerbate ozone-related airway inflammation. We did not find a significant difference in percent change in FeNO between ozone + salbutamol and ozone + placebo conditions. However, a significant difference was observed between ozone and room air amongst the placebo conditions where change in FeNO was higher in ozone. Mixed effects modelling also showed a significant difference between salbutamol and placebo in room air, where inflammation was higher with salbutamol use. Since salbutamol did not exacerbate the effects of ozone pollution this study provides evidence that it may be safe to use salbutamol in ozone air pollution in the acute setting.

4.1 Spirometry and Flow-Volume Loops

We did not see significant differences for percent change in FVC between any of the four experimental conditions. This result shows that participants had a consistent effort effect for spirometry measures pre and post exercise and there was no evidence of learning effect between conditions. Furthermore, we saw approximately 10% larger greater in FEV_1 and 30% larger increase in FEF_{25-75} with salbutamol use compared to placebo. Previous studies in healthy individuals have shown that salbutamol was not able to prevent ozone-related decreases in pulmonary function (Gong et al., 2010). Since our participants did show improvements in pulmonary function with salbutamol use, this finding confirms that our sample had EIB and that

the medication achieved the expected effect (Aggarwal et al., 2018b; Shin et al., 2006). When looking at the impact of air quality on spirometry, differences were observed between room air and ozone in the placebo conditions, with percent change in FEV_1 and FEF_{25-75} being 2.4% and 7.4% higher, respectively, in room air compared to ozone, however, these differences were not observed in the salbutamol conditions. This finding is similar to other ozone exposure studies that found larger decreases in FEV_1 and FEF_{25-75} after exposing people with asthma to ozone compared to filtered air (Kreit et al., 1989). We likely found a smaller difference than Kreit et al. because we used a lower dose of ozone and exposed participants for a shorter period of time. We also used a higher exercise intensity than the intermittent low intensity protocol used in the past (Kim et al., 2012; Kreit et al., 1989; Weinmann et al., 1995). As a result, exercise-induced bronchodilation likely blunted the potential bronchoconstricting effects of ozone in the placebo conditions. With salbutamol, there was no difference in spirometry between the two air quality conditions indicating that the effect of salbutamol likely obscured the effects of ozone.

With the flow-volume loops, the pre and post MEFV curves were similar in all conditions with the largest difference being in the ozone + salbutamol condition where there was a minor decrease in volume in the post-MEFV curve compared to the pre-MEFV curve. However, this may have been a result of inconsistencies in data collection for the generation of these curves and increased familiarity with later visits. The tidal volume loops did not appear to encroach on the MEFV curves indicating that expiratory flow limitation did not occur. Tidal volume also did not appear to change during exercise. These findings are consistent with the flow-volume loops generated in a similar study by Koch et al. assessing the effect of salbutamol on people with EIB exposed to diesel exhaust at an equivalent exercise intensity (Koch et al., 2021). However, these findings do not

match previous studies that have shown that ozone exposure decreases tidal volume and increases breathing frequency (Folinsbee et al., 1975; Foxcroft & Adams, 1986). There are two potential reasons for this: 1) we used a lower ozone concentration and 2) we used a higher exercise intensity than what has been used in ozone studies in the past. Thus, the dose of ozone may have been too low to elicit an effect on tidal volume while the greater exercise intensity may have obscured any effects of ozone on tidal volume.

4.2 FeNO

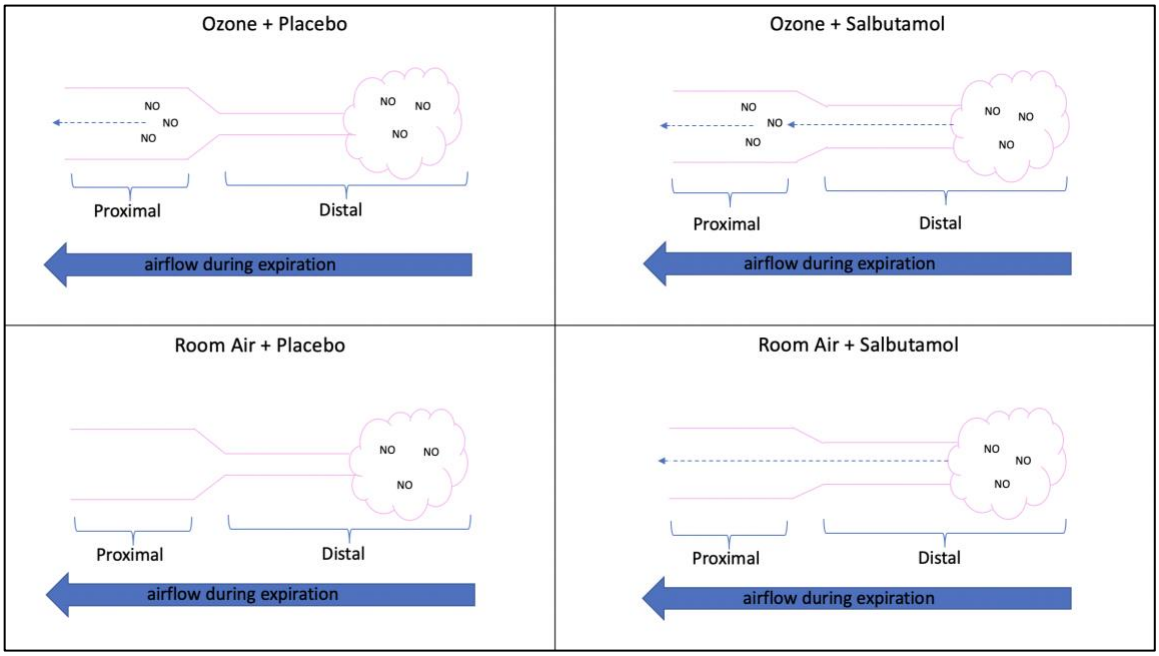
The elevated average baseline FeNO of our participants is consistent with other studies that show higher FeNO levels in people with asthma who do not take corticosteroid medication (Shin et al., 2006). Looking at **Figure 14,** differences between the four experimental conditions are not obvious. However, mixed effects models adjusting for age and sex showed a significant difference in percent change in FeNO between placebo and salbutamol in the room air conditions and a difference between ozone and room air in the placebo conditions. Inconsistencies between ANOVA and mixed effects modelling make it difficult to discern the true effect of the experimental conditions on FeNO; however, the mixed effects model may have more power, taking into consideration all the data through a longitudinal approach and adjusting for potential confounders such as age and sex, whereas the ANOVA was conducted cross-sectionally at each time point.

There are three main variables that could have affected FeNO: exercise, salbutamol, and ozone. Normally in people without asthma, FeNO decreases after exercise and then becomes elevated for at least 30 minutes after exercise. For people with EIB, FeNO decreases after exercise but then

stays below or returns to baseline levels (de Gouw et al., 2001). In people with asthma, salbutamol has been shown to increase FeNO measurement by about 10% for up to an hour after administration (Silkoff et al., 2012). It is believed that the cause of increased FeNO in people with asthma after taking salbutamol is mechanical due to the rapid onset where more nitric oxide in the distal portions of the lung can be exhaled following bronchodilation (Silkoff et al., 2012). The effect of ozone on FeNO is still unclear. One study found that healthy participants exposed to 300 ppb ozone for 75 minutes did not have elevated FeNO levels compared to room air (Barath et al., 2013a), while another study using a 3-day environmental ozone exposure, found increased FeNO following ozone exposure that was related to the methylation of the NOS2A gene and increased inducible nitric oxide synthase transcription. In the present study, the pattern where FeNO was decreased for people with asthma following exercise was only seen in the room air + placebo condition. In the other conditions, the mean post-exercise FeNO was elevated across all three timepoints after exercise. This leads one to believe that both ozone and salbutamol increase FeNO in individuals with EIB after exercise. According to ATS guidelines, a 20% change in FeNO is considered clinically significant for people with asthma (Dweik et al., 2012). Therefore the 31.6% higher FeNO change in ozone compared to room air in the placebo conditions and the 31.2% higher FeNO change for salbutamol compared to placebo in the room air conditions could be clinically significant. From our results, it appears that both salbutamol and ozone may increase FeNO in people with EIB but the combination of the two are not much different than ozone or salbutamol on their own. This could potentially be because the increases in FeNO caused by ozone occur in the proximal airway and the increases in FeNO due to salbutamol occur from increased expired NO from the distal airway. FeNO being elevated in the room air + salbutamol condition and diminished in the room air + placebo condition indicates that without the bronchodilation effect of

salbutamol more nitric oxide could have been trapped in the distal airway in the room air + placebo condition. If this were the case, then increases in FeNO for the ozone + placebo condition could have been due to increases in NO production in the proximal airway and not the distal airway because this condition had elevated FeNO but did not have the bronchodilation effect of salbutamol at the distal airway. This idea is supported by our spirometry results that show a significant increase in FEF_{25-75} in the salbutamol condition compared to the placebo condition indicating improved flow from the distal airways in the salbutamol condition. Therefore, increased flow from the distal airway could explain increases in FeNO in both salbutamol conditions. Since both the ozone + placebo condition and the ozone + salbutamol condition showed similar increases in FeNO, it is possible that the increase in FeNO from the proximal airway due to ozone is more significant than the increase in FeNO from the distal airway resulting from salbutamol use. This idea is illustrated in **Figure 20**.

Figure 20 Illustration depicting proposed effects of salbutamol and ozone on FeNO



However, one inconsistency with this explanation is that the room air + salbutamol condition also had a similar increase in FeNO compared to the ozone + placebo and ozone + salbutamol conditions. If the previous explanation were correct, we would have expected to see the highest FeNO in the ozone + salbutamol condition where both NO flow from the distal airway, due to salbutamol, and increased NO production in the proximal airway, due to ozone, should have led to the highest measured FeNO. This appears to be the case one hour after exercise, however without an adequate sample size it is difficult to discern FeNO effects when there is a large amount of variation between individuals.

Henriquez et al. found that clenbuterol, an agonist of beta-2 adrenergic receptors, and dexamethasone, an agonist of glucocorticoid receptors, exacerbated ozone-related lung inflammation in rats. Since salbutamol, similar to clenbuterol, is also an agonist of beta-2 adrenergic receptors we expected that FeNO measures would be highest in the ozone + salbutamol condition; however, this was not the case. A potential explanation for this would be that we did not use a similar method of measuring lung inflammation as Henriquez et al. so it is possible the effect of highest inflammatory measures being present in the ozone + salbutamol condition could have been present had we used bronchoalveolar lavage. It is also worth noting the difference between salbutamol and clenbuterol. Although they are both are beta-2 adrenoceptor agonists, clenbuterol has an anabolic effect that are dependent on interactions with beta-2 adrenergic while salbutamol does not have an anabolic effect (Choo et al., 1992). We also used a much lower effective dose of ozone compared to Henriquez et al. so it is possible there was not enough ozone-induced inflammation for salbutamol to have an observable effect. Furthermore, the corticosteroid medication used had more of an effect than the bronchodilator medication.

4.3 Other Measures

We suspect that the higher diastolic blood pressure for the room air + placebo condition was due to low sample size and inaccuracy in diastolic measurement. Compared to baseline, we saw elevated blood pressure directly after exercise as well as post exercise hypotension at both 30 minutes and one hour after exercise. No differences were observed between the four conditions. The fact that we did not see differences between the ozone and room air conditions indicates that the changes in blood pressure after exercise were due to the exercise itself and not ozone or salbutamol. This finding is consistent with a previous study that did not find differences in blood pressure between filtered air and 300 ppb ozone after a 3-hour exposure with intermittent exercise (Gong et al., 2012). Salbutamol can have an effect on diastolic blood pressure, systolic blood pressure, and plasma potassium at higher doses but these effects typically do not occur at 200 µg (Bennett et al., 1994). Similar to Bennett et al. we did not find a significant increase in blood pressure from 200 µg of salbutamol. Post exercise hypotension was more evident in systolic blood pressure than diastolic blood pressure likely due to measurement error for diastolic pressure (see **Section 4.4**). A review of post exercise hypotension found that, in healthy individuals, the magnitude of post exercise hypotension was 8 mmHg for systolic and 9 mmHg for diastolic (MacDonald, 2002). We also observed decreases in blood pressure, however the magnitude was not as high.

Overall, the most common symptoms experienced were dyspnea, chest tightness, sore throat and cough, with all participants reporting dyspnea and/or sore throat at some point during the experimental trial and all but one participant reporting chest tightness and/or cough. There were

no notable significant differences between the four groups for symptoms and symptom severity scores were very low overall. The observed patterns mentioned in the results need a greater sample size to improve the reliability of results generated from statistical models.

4.4 Strengths and Limitations

Using a double-blinded placebo-controlled crossover design with simulated air pollution had both strengths and limitations. The benefits of a crossover design are that participants are compared to themselves in different conditions which helps control for individual confounding variables. The double blinding of both the medication and the air quality condition helped control for potential bias caused by expectations of the researchers and participants. A weakness of this study design was that it measured only acute responses to ozone and exercise and thus is not representative of chronic exposure to air pollution that leads to adverse health outcomes in the real world (Long et al., 2022). The advantage of generating our own pollution was that it could be precisely controlled and the specific effects of ozone could be examined. However, real-world air pollution is quite different to the pollution to which we exposed our participants. There are other components of realistic air pollution, including PM, NO_X gases, VOCs, and carbon monoxide, that depend on the location, source, time of day, and weather (U.S. EPA, 2020) thus affecting the generalizability of our findings. One strength of this study regarding generalizability is that we had participants complete a bout of exercise that is representative of what people might do when training, exercising, or commuting in real life. This is unlike other pollution studies that have used a less representative 15 minutes on 15 minutes off fixed ventilation protocol (Barath et al., 2013; Kim et al., 2012).

The methodology for some of the dependent variables in this study was another potential limitation. Regarding the timing of post exercise measurements, we did not perform any measurements after the effects of salbutamol had abated. Thus, if salbutamol were to cause an increase in ozone exposure, it is possible that these effects would not have been noticeable until after the effects of salbutamol had abated. However, such a premise assumes that the effects of ozone would still be present after the salbutamol effects wore off. Choosing FeNO as the primary method of measuring lung inflammation has both advantages and disadvantages. FeNO measurement is simple and non-invasive; however, FeNO is specific to T-helper cell 2 mediated airway inflammation. This inflammation pathway is exacerbated in some types of asthma (Bjermer et al., 2014). This specific form of measuring inflammation may not have been sensitive to ozone-related airway inflammation. One study that looked at diesel exhaust (300 $\mu g/m^3$) and ozone (300 ppb) exposure during an hour of intermittent exercise on FeNO in healthy adults found an effect with diesel exhaust but not ozone (Barath et al., 2013). Previously, biomarkers of inflammation correlated to ozone exposure, like kinins, PGE2, and PGF2a, were measured in bronchoalveolar lavage fluid (Weinmann et al., 1995). Although these measures would have been more specific to ozone, they would have been significantly more invasive than FeNO which could lead to participant discomfort and make recruiting and data collection more difficult. Furthermore, FeNO can remain elevated for up to an hour after spirometry measurement (Silkoff et al., 2012). This factor could have artificially inflated our FeNO measures at 30 minutes and one hour post exercise. There were also limitations when it came to blood pressure measurement. The device we used to measure blood pressure did not work reliably during resting measurements and did not work at all for the immediately post exercise measurements. As a result, blood pressure was measured manually immediately post exercise by a researcher. Inexperience with taking manual blood

pressure measures after exercise and inconsistencies due to the nature of manual measurement likely made the diastolic measurements taken immediately post exercise less accurate.

Our set-up made it impossible to directly measure the temperature and humidity of inhaled air. However, since the exposure was performed in a climate-controlled lab the variation in humidity and temperature between conditions would have been limited. Studies on women with asthma have shown worsening of asthma symptoms from the late follicular phase (high estrogen) to the late luteal phase (declining estrogen and progesterone) (Haggerty et al., 2003). It has also been reported that FEV_1 and FVC are lowest in the follicular phase of the menstrual cycle (Bonds & Midoro-Horiuti, 2013). In addition, rodent studies have shown that estrogen enhances inflammation and airway hyperresponsiveness triggered by ozone (Fuentes et al., 2019). Due to the complex nature of the study and recruitment challenges, we were unable to control for the effects of sex hormones in the female participants.

Due to externally applied hard deadlines, this thesis represents only a partial sample; post defence, we will continue to collect data and increase sample size. It took longer than expected to start data collection, and it was difficult to get people with asthma to come in to the lab during the COVID-19 pandemic. This low sample size and large variation in some outcome measures limits the power of statistical tests conducted and the reliability of results; however, patterns were still evident from data summaries and visualizations. This limitation was particularly difficult for analyzing FeNO which is known to have a high level of variability due to it being influenced by numerous factors (Bjermer et al., 2014).

Chapter 5: Conclusion

Does using salbutamol before exercising in realistic ozone air pollution exacerbate ozone-related airway inflammation in individuals with asthma and/or EIB? This was the question this project was designed to answer. We hypothesized that individuals with asthma and/or EIB who take salbutamol before exercising in ozone will have improved pulmonary function but higher levels of inflammation after exercise compared to placebo medication. This hypothesis was only partially correct. Salbutamol did improve pulmonary function in ozone and this improvement was comparable to the improvement seen in room air as indicated by spirometry measures. However, the effect of salbutamol on inflammation, as indicated by FeNO, in ozone was different than expected. We found similar increases in FeNO in the room air + salbutamol, ozone + placebo, and ozone + salbutamol conditions indicating that salbutamol did not make ozone-related inflammation worse for our exposure of 30 minutes exercising at 60% of VO_{2max}. However, further research in this area is warranted that may address some of the limitations experienced in this study. Our research question could be repeated using more advanced measures of airway inflammation such as those used by Henriquez et al. Another related topic worth researching is the effect of corticosteroid medication use on ozone-related airway inflammation since corticosteroid medication was also used in the studies by Henriquez et al. Additionally, our study was conducted in a laboratory setting with a single pollutant (ozone) instead of a realistic mixture of air pollutants. As such, the findings need to be interpreted with caution. Nonetheless, this study indicates that using salbutamol before exercise in ozone pollution may not acutely cause adverse effects on lung function.

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