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The impact of e-cigarette smoking on pulmonary responses to maximal exercise

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Disclaimer: *Due to the COVID-19 pandemic, this project immediately ceased data collection. Therefore the manuscript was based on data collected in the initial visit.*

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Abstract

Electronic cigarettes (e-cigarettes) have emerged as popular alternatives to smoking conventional tobacco, particularly in a younger demographic. However, there is emerging and conflicting evidence on the magnitude of airway damage with e-cigarette usage. While evaluating airway health can be challenging, using a stimulus such as exercise may be used to elucidate the effects of e-cigarettes on the pulmonary system. **Purpose:** To determine the impact of an acute maximal exercise on changes in pulmonary function (i.e. bronchodilation and bronchoconstriction) in young adult exclusive e-cigarette smokers (S) compared to nonsmokers (NS). We hypothesized that S will have lower post-exercise bronchodilation after an acute maximal exercise compared to NS. **Methods:** 10 NS (3 male; 7 female; 19.8 ± 4.3 years; 67.1 ± 3.0 in; 70.7 ± 12.6 kg) and 7 S (4 male; 3 female; 21.0 ± 2.8 years; 66.3 ± 2.3 in; 65.9 ± 10.1 kg) completed an incremental test to exhaustion to determine peak oxygen consumption (VO_{2peak}) on a cycle ergometer. Subjects performed standard pulmonary function tests to assess forced vital capacity (FVC), forced expiratory volume in 1-second (FEV_1), FEV_1/FVC, forced expiratory flow between 25-75% of FVC ($FEF_{25-75\%}$) and peak expiratory flow (PEF) before the exercise test and immediately post-exercise. Post-exercise bronchodilation and bronchoconstriction were quantified as a percent change from pre-exercise values. **Results:** The NS and S group were similar for age, height, weight, body composition, VO_{2peak}, peak power, and peak heart rate (all *p's* > 0.05). There was a trend towards significance for FEV_1/FVC (0.8 ± 9.0% versus -6.3 ± 7.5%, $p = 0.081$, Cohen's $d = 0.89$) and $FEF_{25-75\%}$ (3.7 ± 17.8% versus -11.8 ± 18.9%, $p = 0.082$, Cohen's $d = 0.86$) from pre- to post-exercise for the NS and S group respectively. **Conclusion**: E-cigarette usage may

be impacting the airways despite normal resting pulmonary function in S, however decreases in pulmonary function after maximal exercise in S compared to NS.

Chapter I

Impact of Varying Physical Activity Levels on Airway Inflammation and Airway Hyperresponsiveness in Young Adult Electronic Cigarette Users versus Nonsmokers

Introduction: Tobacco smoking to electronic cigarette use

Tobacco smoking has been an implicated risk factor for respiratory disease and lung cancer.[5,46,59] Although there has been a decline in cigarette use from the early 1960s, tobacco products have changed over the years encompassing a variety of noncombustible, combustible, and electronic commodities.[61,64] Electronic cigarettes (e-cigarette) in particular have become a very popular commodity and are promoted as a safe and effective way to quit smoking.[10,11,51] The use of e-cigarettes among adolescents and young adults is drastically rising, as evidence by a 78% increase in 2017-2018 alone.[17,62] Unlike conventional cigarettes, e-cigarettes do not contain tobacco, but may contain nicotine, flavorings, and other chemicals that are heated to create an aerosol the user inhales into the lungs.[12] Although e-cigarette use is growing, the safety of e-cigarettes on physiological outcomes has yet to be elucidated.[27,33]

The impact of tobacco smoke on pulmonary function

Exclusive cigarette smoking results in a decline in lung function, a greater degree of airway inflammation (AI) and an increase in airway hyperresponsiveness (AHR) compared to nonsmokers. These changes in respiratory outcomes have been investigated over chronic and acute exposure to tobacco smoke. Lange and colleagues assessed pulmonary function of smokers and nonsmokers at two different examinations, five years apart. Nine groups were analyzed by smoking status over the study period – never-smokers, ex-smokers, light smokers, heavy smokers, light smokers who quit smoking at

least a year before the second examination, heavy smokers who quit smoking at least a year before the second examination, heavy smokers who reduced smoking to <15 cigs/day, light smokers who increased smoking to >15 cigs/day, and never-smokers/ex-smokers who started/resumed smoking after the first examination. The heavy smoking and resumption/beginning of smoking groups had greater declines in forced expiratory volume in one second (FEV_1) than any other group. The decline in FEV_1 was also more prominent in men and older adults (>55 years old) compared to women and younger subjects.[36] These findings are similar in some, but not all, pulmonary outcomes to those from Gold and colleagues who tracked lung function annually in adolescents over a 15-year period. Adolescents who smoked more than 15 cigarettes a day (heavy) had a 3-4% decline in forced expiratory flow between 25 and 75 percent of forced vital capacity ($FEF_{25-75\%}$ of FVC) over the 15-year time period. Interestingly, there was no significant change in FEV_1 between non-smokers that had never used tobacco and heavy smokers.[27] Moreover, Kiter *et al* investigated many pulmonary function outcomes of water-pipe smokers, cigarette smokers, and nonsmokers in 379 adult Turkish males. Cigarette smokers had significantly lower FEV_1, FVC, FEV_1/FVC, peak expiratory flow rate (PEF), $FEF_{25-75\%}$ of FVC, and maximal mid-expiratory flow (MMF) compared to water-pipe smokers and nonsmokers. Additionally, water-pipe smokers had lower FEV_1, FVC, FEV_1/FVC, PEF, $FEF_{25-75\%}$, and MMF than nonsmokers.[34] Therefore most evidence suggests there is an inverse relationship between tobacco smoking and pulmonary function.

The impact of tobacco smoking on airway inflammation

In addition to pulmonary function, airway inflammation has been investigated in adult tobacco smokers. Exhaled nitric oxide (eNO) is commonly used as a non-invasive way to assess airway inflammation, which has been reported to be lower in cigarette smokers. It is speculated that the decline in eNO is the result of constitutive nitric oxide synthase being downregulated by increased interlukin-8s, eosinophilia, and neutrophilia.[6,30,51,53] Yates *et al* assessed eNO of 15 nonsmokers who were exposed to tobacco smoke for an hour (passive smoking), normal room air (sham) and smoked 7 cigarettes (active smoking), with each condition lasting one hour. There was a significant decline in eNO of the active smoking group compared to the sham group.[67] These findings are in agreement with Kougias *et al,* who demonstrated that eNO decreased significantly in 50 young adults smokers after smoking one cigarette from 11.70 ± 5.37 parts per billion (ppb) to 9.85 ± 4.34 ppb.[35] Similarly, Malinovschi *et al* assessed eNO in 221 adults, reporting that ex-smokers and current smokers had lower eNO values compared to nonsmokers (17.7 ppb, 14 ppb, and 22.8 ppb, respectively).[36] From the aforementioned literature, eNO levels are inversely related to smoking and demonstrate the presence of AI.

Tobacco smoking and airway hyperresponsiveness

Airway hyperresponsiveness (AHR) is characterized by airway narrowing caused by an irritant such as exercise, cold weather, methacholine, or hypertonic saline.[54] Smokers typically express a greater degree of AHR compared to nonsmokers. Stimulation of the C-fiber afferents that innervate the smooth muscles of the airways can elicit bronchoconstriction.[14] Smoking, specifically, has been observed to increase the sensitivity of this response in human and animal models.[30,66] Much of the literature has

observed an increase in AHR in smokers compared to nonsmokers.[7,33,37,56] In these instances, AHR increases with chronic smoking. As previously mentioned, there is a decline in pulmonary function with smoking; however it is unclear whether changes in the structural network of the airways occur prior to this decline. Taylor *et al* observed an association between AHR and declining FEV_1 in smokers annually over 9 years. Smokers' had greater AHR compared to nonsmokers as well as significantly lower FEV_1 values compared to baseline FEV_1 each year.[58] This demonstrates a possible change in the airway structures before seeing a decline in pulmonary function with smoking.

Comparisons and differences in pulmonary function outcomes between e-cigarette smokers, tobacco smokers, and nonsmokers

The impact of e-cigarette smoking on pulmonary function outcomes and eNO has been investigated, but the literature is scarce in comparison to the existing literature investigating the physiological effects from tobacco smoking. Also, there are no studies, to our knowledge, to determine whether e-cigarette smoking impacts AHR. E-cigarette usage has been reported to cause declines in FEV_1/FVC, FEV_1, FVC[19] and PEF, maximal expiratory flow at 50% and 75% of vital capacity ($MEF_{50-75\%}$ of FVC).[59] Also, there is a greater decline in eNO in e-cigarette smokers compared to nonsmokers.[39,59] E-cigarette smoking degrades lung function and leads to a decline in eNO just as tobacco smoking does. It has been speculated that main reason for the decline in eNO from e-cigarette use is due to propylene glycol which functions to produce the vapor in e-cigarette.[43] Repeated exposure to this chemical compound has been associated with cough, dry throat, and airway obstruction.[65]

The degree that e-cigarettes impact pulmonary function and eNO appear to be lower when compared to tobacco smokers, however there is conflicting evidence and more research is needed to understand the impact of e-cigarettes on critical respiratory outcomes. Some literature has reported that e-cigarettes cause the same amount of decline in pulmonary function outcomes just as tobacco smoking[17,60], while some report the degradation of pulmonary function to be greater in tobacco smoking compared to e-cigarette use.[22,49] The presence of airway inflammation has been observed in both e-cigarette users and tobacco smokers, with evidence of a greater decline in eNO found with e-cigarette use compared to tobacco smoking. Marini *et al* observed 25 smokers who's eNO declined significantly from smoking tobacco cigarettes and e-cigarettes compared to not smoking by 2.8 and 3.2 parts per billion, respectively.[39] However, some literature reports no significant differences in the decline in eNO between tobacco smoking and e-cigarette use.[9]

Physical activity and pulmonary function

While smoking has adverse outcomes on pulmonary health, chronic physical activity level may impact and modify the magnitude of the deleterious respiratory outcomes. Specifically, smokers who self-reported that they engaged in moderate to vigorous intensity physical activity (MVPA) had improved FEV_1 and FVC compared to non-active counterparts.[25,26] Additionally, male smokers specifically, had increased FEV_1 and FVC with 2-3 days per week of physical activity compared to their sedentary counterparts.[31] Moreover, chronic physical activity level may impact smokers' AHR. Healthy individuals who self-reported that they partake in MVPA had decreased AHR.[54] It is speculated that the decrease in AHR may be a result of a disruption in the cross-

bridge cycles between actin and myosin as well as the remodeling of cytoskeleton in the airway smooth muscles from the increased ventilation from performing PA.[23,28] This literature demonstrates exercise's protective effect on lung function and airway health.

Purpose

Although there is emerging evidence that e-cigarettes may impact lung function, airway damage may occur prior to decrements in observed lung function. Additionally, there are benefits of exercise on lung function in conventional cigarette users, which may provide the same benefits in e-cigarette users. Therefore, the purpose of this study was to examine whether e-cigarette smoking impacts pulmonary function, eNO, and AHR in young adult exclusive e-cigarette smokers compared to nonsmokers. Furthermore, we aim to determine whether chronic physical activity level will modify the impact of e-cigarette smoking on the airways. We hypothesize that e-cigarette users will have greater AI and AHR compared to nonsmokers. Also, we hypothesize that e-cigarette smokers with the highest MVPA will have attenuated AI and AHR; and improved pulmonary function compared to their less active counterparts.

Chapter II

Methods

Study design & participants

This will be a randomized, cross-sectional study including young adults who are exclusive e-cigarette users and nonsmokers undergoing three study visits. The initial visit will include anthropometric assessment, pulmonary function tests (PFTs), assessment of exhaled nitric oxide (eNO) and an incremental exercise test to exhaustion on a cycle ergometer. The second and third visits will assess eNO, PFTs, followed by a hypertonic saline challenge only (HSC) or hypertonic saline challenge with 5 deep inspirations (HSC+DI) condition to assess AHR. Individuals will be excluded from the study if they have had past and/or present use of medications for airway and lung diseases, any evidence of lung disease or other chronic conditions that may affect pulmonary function outcomes, or if they smoked tobacco and/or used other drugs in the past 6 months. Exhaled carbon monoxide (eCO) will be assessed to determine how recently an individual has smoked, with the standard cutoff being > 4 parts per million (ppm).[15,35] Therefore, if their eCO is > 4ppm, then they will be excluded from the study. All participants will provide written informed consent as well as complete an international physical activity questionnaire (IPAQ), physical activity readiness questionnaire-plus (PARQ+), and tobacco use questionnaire before participating in the study. The study will be reviewed and approved by the James Madison University Institutional Review Board prior to data collection.

Initial Visit

IPAQ

The IPAQ (long version) is validated in adults to assess moderate to vigorous physical activity (MVPA) per week both in leisure time and work-related physical activity.[16] This will give a self-reported assessment of participants physical activity. Participants also received an accelerometer for an object assessment of MVPA per week. Subjects were asked to wear an accelerometer for one week on the hip during the day, except when going to bed and showering. Freedson-cut points will be used to quantify amount of PA per week.[24]

PARQ+

The PAR-Q+ is a validated, self-guided physical activity readiness questionnaire.[37] Participants will complete this form and if they report that they did not regularly participate in planned exercise, they must not display signs or symptoms of cardiovascular, metabolic or renal disease, and have not been diagnosed with asthma. If they have signs and symptoms or are asymptomatic but have previously been diagnosed with disease, they will be excluded from the study. Participants that do engage in regular physical activity, are asymptomatic and have never been diagnosed with renal, metabolic or cardiovascular diseases are cleared by the ACSM guidelines to undergo the incremental exercise test to exhaustion.[2]

Tobacco use questionnaire

A tobacco use questionnaire will be completed by participants to determine how much tobacco they have smoked over their lifetime as well as any use of smoking products such as e-cigs and/or drugs such as marijuana. Individuals who smoked tobacco and/or used other drugs in the past 6 months will be excluded from participating in the study.

Exhaled carbon monoxide (eCO)

Exhaled carbon monoxide will be measured to determine how recent a person had smoked a combustible tobacco product using a handheld breath CO monitor (Bedfont Scientific Ltd., Kent, UK). For this test, participants will exhale completely into a disposable mouthpiece.

Accelerometry

Participants will be given an accelerometer (Actigraph GT3X, Pensacola, FL) to wear at their hip for 7 days to determine their PA levels. Accelerometers have been validated to asses moderate to vigorous physical activity per week.[45,52] They will be asked to record non-wear time as well as when they put the accelerometer on in the morning and when they take it off at night. After one week, participants will return for their second visit where they will return the accelerometer. On the initial visit to the laboratory, subjects will be randomly assigned to either HSC or HSC+DI.

Anthropometrics

Participants height will be measured using a portable stadiometer (Invicta Height Measure; Invicta Plastics Limited, Leicester, England, UK) and weight will be taken using a standard physician's scale (Pelouze 4040; Health-o-meter, Inc., Bridgeview, Illinois, USA).Waist circumference will be measured using a Gulick measuring tape according to ACSM standards.[2]

Dual energy x-ray absorptiometry (DEXA) (GE iDXA Lunar; Fairfield, CT) will be used to assess body fat percentage lean mass, and fat mass. Participants will be advised to wear comfortable clothing and lay supine while the total body scan takes

approximately 7-10 minutes to be completed. The DEXA will be operated by one trained personnel.

PFT

Pulmonary function will be assessed by the maximum flow-volume loop (MFVL) using a Vmax Encore metabolic cart (Vyaire Medical, Mettawa, IL). PFTs will be done before and 2 minutes after the bout of exercise. All administered PFTs will follow the American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines.[3] To be considered a successful MFVL: (1) the expiratory volume in FEV_1 must be <5% of the FVC or 0.150 L, whichever is greater; (2) there must be no cough during the first second of the expiratory portion; (3) the participant does not inhale too early (test does not terminate early); (4) there is no hesitation during the maneuver which may prevent an accurate measurement of FEV_1 and FVC; (5) there is no leak or obstruction in the mouthpiece and (6) the maneuver is performed correctly without an extra breath taken. All participants will be given verbal instructions during this test as well as encouragement throughout the test by the primary investigator. Participants will maximally inhale and forcefully exhale for 6 seconds then maximally inhale into a spirometer while wearing a nose-clip. Forced expiratory volume in 1-second (FEV_1), forced vital capacity (FVC), forced expiratory flow between 25 and 75% of forced vital capacity ($FEF_{25-75\%}$) of FVC, and peak expiratory flow (PEF) will be recorded. Three measurements within 10% will be averaged and used in the analysis.

eNO

Measurements of eNO via chemiluminescence is a validated measure for airway inflammation.[1] The test will follow the ATS guidelines.[3] eNO will be assessed before and

after the bout of exercise using the Niox Vero (Niox Vero, Circassia, Morrisville, NC). Participants will be seated comfortably and instructed to sit straight with their feet flat on the ground. Participants will be instructed to maximally inhale, then exhale for approximately 6 seconds into the eNO analyzer. Testing will be performed until two values within 10% of each other are achieved. The average value will be recorded and used in the analysis.

Incremental test to exhaustion

An incremental test to exhaustion will be performed on an electronically braked cycle ergometer (Lode; Groningen, Netherlands) to determine VO_{2peak} that will last 12-15 minutes. Resting metabolic measurements will be taken for 3 minutes prior to beginning the test. Participants will then begin a warm-up for 2 minutes at a work rate of 60 watts (W), keeping a cadence above 50 revolutions per minute (rpm). After the warm-up, work rate will be increased by 10W/minute for females and 20W/minute for males. Ventilatory and metabolic data will be recorded through breath by breath analysis for the entirety of the test with a SensorMedics 229 Metabolic Cart (SensorMedics Corp., Yorba Linda, Calif., USA). Heart rate (HR) will be recorded for the entirety of the test with a PolarLink HR monitor and chest strap. Ratings of perceived exertion (RPE) using the Borg Scale will be recorded every minute of the test as well as how difficult breathing felt using a Borg CR10 Dyspnea Scale. Metabolic and ventilatory data will be recorded throughout the entire test. The test will be terminated when the participant can no longer continue exercise or maintain a cadence of 50 rpm and reaches volitional fatigue. Participants will be advised to give their best effort; however investigators will clearly state that they can end the test whenever they want if they are uncomfortable.

Second and third visit

On the second visit, participants will perform PFTs using the MFVL, eNO, and airway resistance testing before and after they perform the treatment session they were randomly assigned to (HSC or HSC+DI). At least two days later, subjects will come back to the laboratory for the other treatment, where participants will repeat the same tests in the same order.

Airway Resistance

Airway resistance will be assessed using impulse oscillometry Vmax Encore metabolic cart (Vyaire Medical, Mettawa, IL). Participants will breathe normally through a mouthpiece while the machine records resistance in the central and peripheral airways. This is done before and after the HSC session.

HSC session

Participants will breathe in a 25% hypertonic saline (salt and water) solution from an over-the-counter ultrasonic nebulizer that converts the solution from a liquid to a mist. Participants will sit comfortably and breathe normally on the nebulizer for 20 minutes while wearing a nose clip. They will be asked to not talk, laugh, or take deep breaths to prevent harm on the airways. MFVLs, eNO, airway resistance assessments will be performed before and after the HSC.

HSC+DI session

Participants will complete the same procedures as done in the HSC session with the exception of adding 5 DIs after the HSC. During the DIs, participants will be asked to inhale deeply and exhale passively.

Statistical analysis

Data will be analyzed using SPSS V.24 Statistical software analyses. AHR, AI and pulmonary function will be analyzed using an ANOVA (time [pre- and post-challenge] x group [e-cig user or nonsmoker]). A moderation analysis will be conducted to determine the impact of chronic PA level on AI, AHR and pulmonary function in JUUL users. Additionally, we will conduct exploratory analyses to determine whether other factors (body composition and fitness level) are associated with pulmonary function, AI and AHR.

Chapter III

Manuscript

The impact of e-cigarette smoking on pulmonary responses to maximal exercise.

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Abstract

Electronic cigarettes (e-cigarettes) have emerged as popular alternatives to smoking conventional tobacco, particularly in a younger demographic. However, there is emerging and conflicting evidence on the magnitude of airway damage with e-cigarette usage. While evaluating airway health can be challenging, using a stimulus such as exercise may be used to elucidate the effects of e-cigarettes on the pulmonary system. **Purpose:** To determine the impact of an acute maximal exercise on changes in pulmonary function (i.e. bronchodilation and bronchoconstriction) in young adult exclusive e-cigarette smokers (S) compared to nonsmokers (NS). We hypothesized that S will have lower post-exercise bronchodilation after an acute maximal exercise compared to NS. **Methods:** 10 NS (3 male; 7 female; 19.8 ± 4.3 years; 67.1 ± 3.0 in; 70.7 ± 12.6 kg) and 7 S (4 male; 3 female; 21.0 ± 2.8 years; 66.3 ± 2.3 in; 65.9 ± 10.1 kg) completed an incremental test to exhaustion to determine peak oxygen consumption (VO_{2peak}) on a cycle ergometer. Subjects performed standard pulmonary function tests to assess forced vital capacity (FVC), forced expiratory volume in 1-second (FEV_1), FEV_1/FVC, forced expiratory flow between 25-75% of FVC ($FEF_{25-75\%}$) and peak expiratory flow (PEF) before the exercise test and immediately post-exercise. Post-exercise bronchodilation and bronchoconstriction were quantified as a percent change from pre-exercise values. **Results:** The NS and S group were similar for age, height, weight, body composition, VO_{2peak}, peak power, and peak heart rate (all *p's* > 0.05). There was a trend towards significance for FEV_1/FVC (0.8 ± 9.0% versus -6.3 ± 7.5%, $p = 0.081$, Cohen's $d = 0.89$) and $FEF_{25-75\%}$ (3.7 ± 17.8% versus -11.8 ± 18.9%, $p = 0.082$, Cohen's $d = 0.86$) from pre- to post-exercise for the NS and S group respectively. **Conclusion**: E-cigarette usage may

be impacting the airways despite normal resting pulmonary function in S, however decreases in pulmonary function after maximal exercise in S compared to NS.

Introduction

The use of tobacco cigarettes has declined among adolescents and young adults, however there has been a growing emergence of electronic cigarette (e-cigarette) use.[17,61] Several types of e-cigarettes contain the same nicotine content as conventional cigarettes, but use chemicals and flavorings to create an aerosol that is inhaled.[12] The variety of flavorings as well as the fact that e-cigarettes have been marketed as a safe and effective treatment to quit smoking conventional cigarettes,[10,11,51] have likely contributed to the increased use in adolescents and young adults.[62] Still, the impact of e-cigarettes on physiological outcomes, specifically in the respiratory system (i.e. pulmonary function and airway health), has yet to be elucidated.[3,33]

While conventional cigarette use is associated with decreased pulmonary function[27,34,36] and decreases in exhaled nitric oxide (eNO) which indicates increased airway inflammation[38,67], it is equivocal whether these findings translate to e-cigarette use exclusively. Although some studies suggest that e-cigarettes have minimal effect on the lungs[20,22]and airways[22], these studies are based primarily on the maximum flow volume loop (MFVL) at rest. While the resting MFVL is the gold standard to assess pulmonary function, declines in pulmonary function are only present after long-term structural changes (i.e. airway remodeling), and therefore may not be evident until years of damage have occurred. Therefore, using a stimulus that may impact pulmonary function, such as exercise, in addition to the MFVL, may provide information as to initial lung damage with e-cigarette use.

Exercise is a stimulus which induces post-exercise bronchodilation of approximately 10% in healthy airways and may alter airway inflammation.[21] However, it

is also possible for the airways to bronchoconstrict after intense exercise, which may indicate increased airway inflammation and remodeling. eNO is used as a non-invasive marker to assess airway inflammation[47] and numerous evidence exists in healthy, nonsmoking adults that eNO increases as exercise intensity increases, then decreases rapidly after 2 or more minutes of recovery.[8,13,50,53] However, whether eNO changes in conventional cigarette users to a similar magnitude as healthy, nonsmokers from pre- to post exercise is unknown. Therefore, the purpose of this study was to examine the impact of acute maximal exercise on changes in pulmonary function in young adult e-cigarette users compared to nonsmokers. An exploratory aim was to investigate whether changes in eNO are present from pre- to post-exercise in e-cigarette users and nonsmokers, and whether changes in eNO are associated with changes in pulmonary function. We hypothesize that e-cigarette users will have less post-exercise bronchodilation after acute maximal exercise compared to nonsmokers.

Methods

Participants

Seventeen participants participated in the present study between the ages of 18 and 35 years old (20.3±3.7 years). The study was composed of a nonsmoking (NS) group (n=10, 3M /7 F) who reported never using e-cigarettes or other combustible tobacco products and an exclusive e-cigarette (S) group (n=7, 4M/7F) who had reported not using any combustible tobacco products except e-cigarettes for the past 6 months. All procedures were approved by the James Madison University Institutional Review Board and informed consent was obtained prior to data collection.

Experimental design

Participants completed the following questionnaires before participating in this study: international physical activity questionnaire (IPAQ), physical activity readiness questionnaire-plus (PAR-Q+), and tobacco use questionnaire. Upon arrival to the lab, height, weight, waist circumference, and exhaled carbon monoxide (eCO) were measured. Participants then performed an incremental, maximal exercise test to assess peak oxygen consumption (VO_{2peak}). eNO and pulmonary function were assessed immediately before and after the VO_{2peak} test. Following completion of the exercise test, participants underwent a body composition assessment via dual-energy x-Ray absorptiometry (DEXA).

Questionnaires

A tobacco use questionnaire approved by the Food and Drug Administration (FDA) was completed by participants to determine how much tobacco they smoked over their lifetime as well as any use of smoking products such as e-cigs and/or drugs such as

marijuana. Individuals who smoked tobacco and/or used other drugs in the past 6 months were excluded from participating in the study.[2]

Exhaled carbon monoxide (eCO)

eCO was measured using a handheld breath CO monitor (Bedfont Scientific Ltd., Kent, UK) to ensure participants had not used any combustible tobacco products. For this test, participants were seated comfortably with feet flat on the ground and instructed to exhale fully into a disposable mouthpiece without a nose-clip for at least 6 seconds or until all the air had been exhaled from the lungs.[3]

Anthropometrics

Participants height were measured using a portable stadiometer (Invicta Height Measure; Invicta Plastics Limited, Leicester, England, UK) and weight was taken using a standard physician's scale (Pelouze 4040; Health-o-meter, Inc., Bridgeview, Illinois, USA).Waist circumference was measured using a Gulick measuring tape according to American College of Sports Medicine (ACSM) guidelines.[2]

Exhaled carbon monoxide (eNO)

Participants completed the eNO test using the Niox Vero nitric oxide analyzer (Circassia, Morrissville, NC, USA). This test is a validated, noninvasive assessment of airway inflammation.[1] Testing was performed following ATS guidelines.[3] eNO was assessed before and after exercise testing. Participants were instructed to sit straight with their feet flat on the ground and wear a nose clip. Participants were then instructed to maximally inhale, then exhale for approximately 6 seconds at a steady flow rate into the Niox Vero analyzer. Testing was performed until two values within 10% of each other

were achieved. No participant needed to perform the assessment more than two times. The average value was recorded and used in the analysis.

Pulmonary Function Testing

Following eNO assessment, pulmonary function was assessed by the MFVL using a Vmax Encore metabolic cart (Vyaire Medical, Mettawa, IL). The MFVL is the gold standard to assess the maximum capacity of the pulmonary system, and all testing was administered following the American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines.[3] MFVLs were done before and 2 minutes after the bout of exercise. To be considered a successful MFVL: (1) the expiratory volume in FEV_1 needed to be <5% of the FVC or 0.150 L, whichever is greater; (2) there could be no cough during the first second of the expiratory portion; (3) the participant should not have inhaled too early (test does not terminate early); (4) there was no hesitation during the maneuver which may prevent an accurate measurement of FEV_1 and FVC; (5) there was no leak or obstruction in the mouthpiece and (6) the maneuver was performed correctly without an extra breath taken. All participants were given verbal instructions during this test and were encouraged throughout the test by the primary investigator. Participants were instructed to maximally inhale and forcefully exhale for 6 seconds then maximally inhale into a spirometer while wearing a nose-clip. Forced expiratory volume in 1-second (FEV_1), forced vital capacity (FVC), forced expiratory flow between 25 and 75% of forced vital capacity ($FEF_{25-75\%}$) of FVC, and peak expiratory flow (PEF) were recorded. Three measurements within 10% were averaged and used in the analysis.

Incremental test to exhaustion

An incremental test to exhaustion was performed on an electronically braked cycle ergometer (Lode; Groningen, Netherlands) to determine VO_{2peak}. Resting metabolic measurements were taken for 3 minutes prior to beginning the test. Participants began the test with a warm-up for 2 minutes at a work rate of 60 watts (W), keeping a cadence above 50 revolutions per minute (rpm). After the warm-up, work rate was increased by 10W/minute for females and 20W/minute for males. Ventilatory and metabolic data were recorded through breath by breath analysis for the entirety of the test with a Vmax Encore metabolic cart (Vyaire Medical, Mettawa, IL). Heart rate (HR) was recorded every minute for the entirety of the test with a PolarLink HR monitor and chest strap. Ratings of perceived exertion (RPE) using the Borg Scale were recorded every minute. Perceptions of breathing were obtained using a Borg CR10 Dyspnea Scale. Metabolic and ventilatory data were recorded throughout the entire test. The test was terminated when the participant could no longer continue exercise or maintain a cadence of 50 rpm.

Body Composition

Following the exercise protocol, pulmonary assessments, and an adequate rest time, the subject underwent a DEXA (GE iDXA Lunar; Fairfield, CT) scan for body composition assessment. DEXA analysis provided subjects' body fat percentage, lean mass, and fat mass.

Statistical Analysis

Data was analyzed using SPSS V.26 Statistical software (IBM, Armonk, NY, USA). Data are expressed as means ± SD. Prior to analyses; data were checked for normality to verify parametric assumptions were met. eNO and FVC data did not pass the Shapiro-Wilk test for normality and were log10 transformed prior to analyses. Data

passed normality testing after transformations were performed. eNO and MFVL's were analyzed using a 2-way ANOVA (time [pre- and post- exercise] x group [S or NS]). In consideration of our sample size and trends for significance, post-hoc testing was done on main outcomes to assess time * group interactions using the percent changes in eNO and PFTs from pre- to post-exercise and Cohen's *d* values were computed to provide information with regard to effect size. Additionally, an exploratory analysis was done to determine whether eNO was associated with changes in pulmonary function, and whether other factors (the number of months and number of times per day of e-cigarette use) were associated with pulmonary outcomes using Pearson product-moment correlation for parametric data and Spearman's rho for nonparametric data. Significance was set to $p < 0.05$ for all analyses.

Results

Subject Characteristics

Subject characteristics are shown in Table 1. There were no significant differences between the NS and S groups for age, height, weight, BMI, waist circumference, body fat percent, lean mass, and fat mass (*all p's* > 0.05). Maximal exercise values from the VO_{2peak} test are shown in Table 2. There were no significant differences between the groups for relative VO_2, absolute VO_2, peak heart rate, and peak power (*all p's* > 0.05.

Pulmonary function

The percent of predicted pulmonary function responses pre- and post-exercise in the NS and S groups are shown in Figure1A-E. No pulmonary function measures were different at rest in the NS compared to the S (*all p's* > 0.05) and all were within normal percent of predicted values. There was a trend towards significance in the percent of predicted FEV_1/FVC (p = 0.081, Cohen's d= 0.89), and $FEF_{25-75\%}$ (p = 0.082, Cohen's d= 0.86) from pre- to post-exercise depending on smoking status. The NS had increases in FEV_1/FVC (0.8 ± 9.0%) and $FEF_{25-75\%}$ (3.7 ± 17.8%) while the S had decreases (-6.3 ± 7.5% and 11.8 ± 18.9%, respectively). There were no significant associations in the number of months e-cigarettes users reported vaping or times per day of vaping with any changes in pulmonary function (all p's > 0.05).

eNO

Baseline eNO was not statistically different between NS (17.4±10.7 ppb) and S (21.5±12.3 ppb) groups at baseline (p = 0.480). The percent change in eNO from baseline to post-exercise for the NS and S groups are shown in Figure 2. For the NS and S groups,

eNO increased significantly from baseline by 7.5% and 5%, respectively ($p = 0.049$). There was no significant difference in eNO between the two groups ($p = 0.662$). There were no significant associations between eNO and usage in the e-cigarette smokers. Additionally, there was no association between eNO and any of the pulmonary function outcomes.

Discussion

Primary Findings

The present study investigated the impact of acute maximal exercise in NS and S on pulmonary function and exhaled nitric oxide. Our primary finding was that from pre- to post-exercise, there was a trend towards significance in $FEF_{25-75\%}$ and FEV_1/FVC in the NS group compared to the S group. Although not statistically significant, effect size calculations showed a large effect for e-cigarette smoking on $FEF_{25-75\%}$ and FEV_1/FVC from pre- to post-exercise in S compared to NS. Additionally, eNO significantly increased from pre- to post-exercise by the same amount in both groups. These pulmonary responses to exercise were not associated with e-cigarette usage.

Pulmonary function and eNO in nonsmokers versus e-cigarette users

Previous literature has reported that a clinically meaningful exercise-induced bronchodilation or bronchoconstriction (EIB) corresponds to a 10% increase or 10% decrease in FEV_1 respectively.[48] Furthermore, increases and decreases of less than 10% in FEV_1, and other pulmonary outcomes such as FEV_1/FVC and $FEF_{25-75\%}$ suggest bronchodilation and bronchoconstriction are occurring, and therefore these findings will be discussed even if they are not clinically meaningful. Zamel et al. observed increased post-exercise bronchodilation to an even greater magnitude in healthy non-smokers than our NS group, despite similarities in subject characteristics.[68] Specifically, FEV_1 increased by 20% in 6/7 non-smoking asthmatic men. However in healthy, adolescent endurance-trained and recreationally active non-smokers, Cox et al. recently reported a 5.3 and 5.8% increase in FEV_1 in untrained and trained subjects, respectively[15] which is a similar magnitude of increases in bronchodilation as the present study. Only 3/24 subjects

in their study experienced exercise-induced bronchodilation. Our NS group exhibited increased $FEF_{25-75\%}$ of 3.7 ± 17.8% and an increase in FEV_1/FVC by 0.8 ± 9.0%. Additionally, 3/10 participants in the NS group showed post-exercise bronchodilation for FEV_1, $FEF_{25-75\%}$, and 2/10 for FEV_1/FVC.

Conversely, the S group had decreased pulmonary function post-exercise suggesting bronchodilation did not occur. The average percent change in FEV_1 from pre to post-exercise was -6.3 ± 7.5%%, while it was -11.8 ± 18.9%, for $FEF_{25-75\%}$. In our subjects, 4/7 exhibited decreases >10% in FEV_1, $FEF_{25-75\%}$, and FEV_1/FVC. There was also a large effect of smoking on FEV_1 in the S compared to the NS (p=0.18, Cohen's d= 0.77) Similarly, Minov et al. observed an average of 23% decline in FEV_1 post-exercise in office cleaners positive for EIB exposed to tobacco smoke.[42] Therefore, decreasing pulmonary outcomes in the S group is more similar to tobacco smokers who experienced EIB as compared to the NS group. Although our S group did not show as large of a decrease in pulmonary outcomes compared exposed nonsmokers in Minov et al. study, it is possible that their e-cigarette usage is beginning to lead to structural changes in the airway.

There are several plausible explanations for why there may be varying pulmonary function responses in the S group compared to the NS group. In healthy, nonsmokers, the most plausible pathology for EIB is the dehydration of the airway membranes as a result of breathing in dry air causing the airways to narrow. In turn, there is an inflammatory response whereby histamine is released in the airways causing bronchoconstriction.[4] Additionally, there may be stimulation of the C-fiber afferents that innervate the smooth muscles of the airways.[14] Smoking, specifically, has been observed to increase the

sensitivity of this response in human and animal models.[30,66] E-cigarette aerosol may be causing the same sensitivity to the C-fiber afferents like tobacco smoke resulting in the bronchoconstriction seen post-exercise. A combination of dry airways and stimulated C-fiber afferents may lead to the bronchoconstriction only seen in S.

In the current study, a potential decline in pulmonary function was observed between the groups despite no differences in initial airway inflammation as assessed by pre- and post-exercise eNO. Therefore, there may be changes to airway structures before inflammation is present. The eNO increases in both groups post-exercise may be due to an inflammatory response within the pulmonary system. Kurti et al. has previously shown that ~30 minutes of submaximal exercise may upregulate various pathways that increase eNO[54], though precise mechanisms need to be understood. Nitric oxide comes from multiple sources. When an inflammatory response occurs, inducible nitric oxide synthase(iNOS) is upregulated through its release from inflammatory cells.[44] This may increase the endogenous origin of nitric oxide and lead to an increased eNO.[13] iNOs may have continued to be upregulated post-exercise which would continue to increase eNO resulting in a higher eNO observed post-exercise. However, more research is needed to understand these changes in eNO post-exercise among healthy, nonsmokers and e-cigarette users.

Limitations

The primary limitation of our study is the sample size of our S group, making us unable to draw clear conclusions from our data. Due to the COVID-19 pandemic, data collection was halted and therefore more data will be collected in the future to better understand pulmonary responses in e-cigarette users. Additionally, eCO assessment has

not been standardized for e-cigarette smoking, and it is primarily used to see whether individuals have had recent exposure to tobacco smoke. While participants were instructed to not use an e-cigarette for at least three hours prior to their initial visit, we could only verbally ensure they had not used any products through verbal questioning.

Conclusion

This is the first study, to our knowledge, that investigates changes in pulmonary outcomes (i.e. pulmonary function and eNO) in e-cigarette users after acute maximal exercise. There were trends towards bronchodilation in the NS group and bronchoconstriction in the S group. Considering eNO increased from pre- to post-exercise in both groups and was not associated with any pulmonary outcomes, it is likely that the decreases in pulmonary function present in the S group are occurring independently of eNO. However data from more e-cigarette smokers needs to be collected to fully understand the impact of e-cigarettes on airway health, and whether exercise could be used as a stimulus to enable earlier detection of deleterious pulmonary effects in e-cigarette users. Considering that there appears to large effects in the pulmonary responses after maximal exercise in e-cigarette users compared to nonsmokers, future research should elucidate the magnitude of these changes as well as mechanisms for their occurrence.

Table 1. Subject characteristics of nonsmoking controls (NS) and e-cigarette users (S).

	NS (n=10)	S (n=7)
Gender (M/F)	3/7	4/3
Age (years)	19.8±4.3	21.0±2.8
Height (in)	67.1±2.9	66.3±2.3
Weight (kg)	70.7±12.6	65.9±10.1
Body mass index (BMI) (kg/m^2)	24.2±3.9	23.3±4.3
Waist circumference (cm)	79.6±9.7	79.1±8.8
Body fat (%)	27.9±10.1	24.7±8.5
Lean mass (kg)	48.1±11.2	46.9±6.2
Fat mass (kg)	19.2±9.0	15.9±6.9

Data are expressed as the mean value with the standard deviation.

Table 2. Maximal exercise data on nonsmoking controls and e-cigarette users.

	NS	S
VO_2 (L/min)	2.71±0.69	2.55±0.22
VO_2 (mL/kg/min)	38.4±8.1	39.2±5.1
Peak heart rate (bpm)	185.5±10.6	186.6±6.2
Peak power (W)	192±47.3	192.8±17.0

Data are expressed as the mean value with the standard deviation.

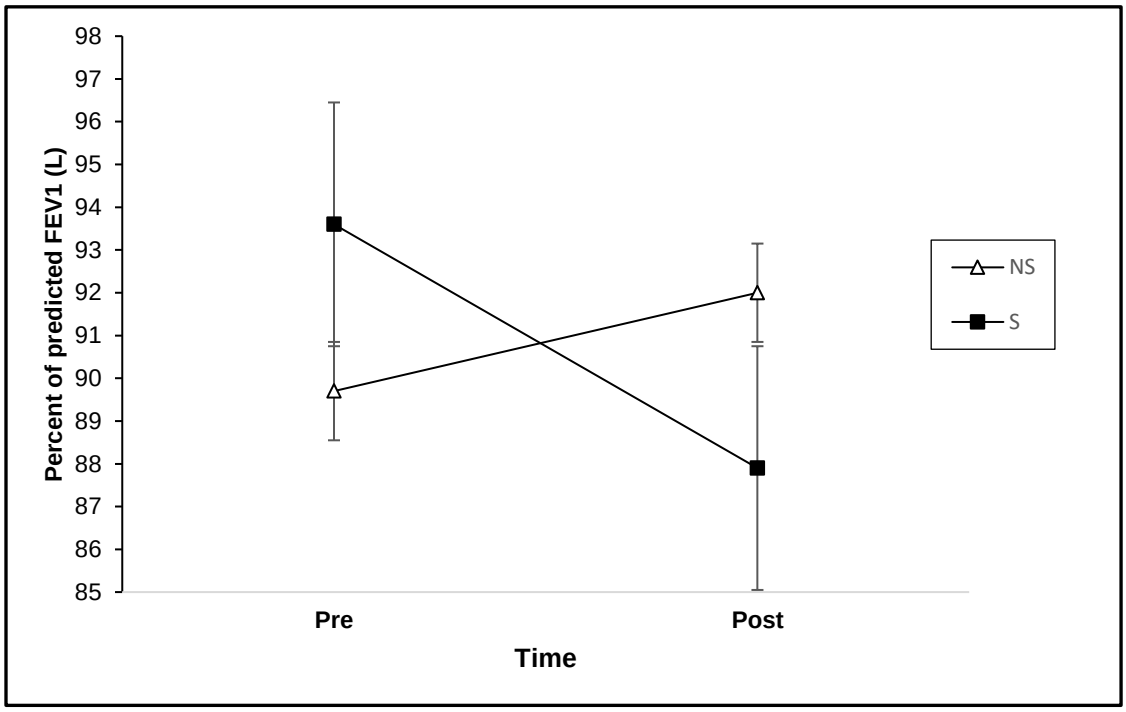

Figure 1a. Mean percent of predicted FEV_1 (L) pre- and post-exercise in the NS and S groups ($p = 0.18$).

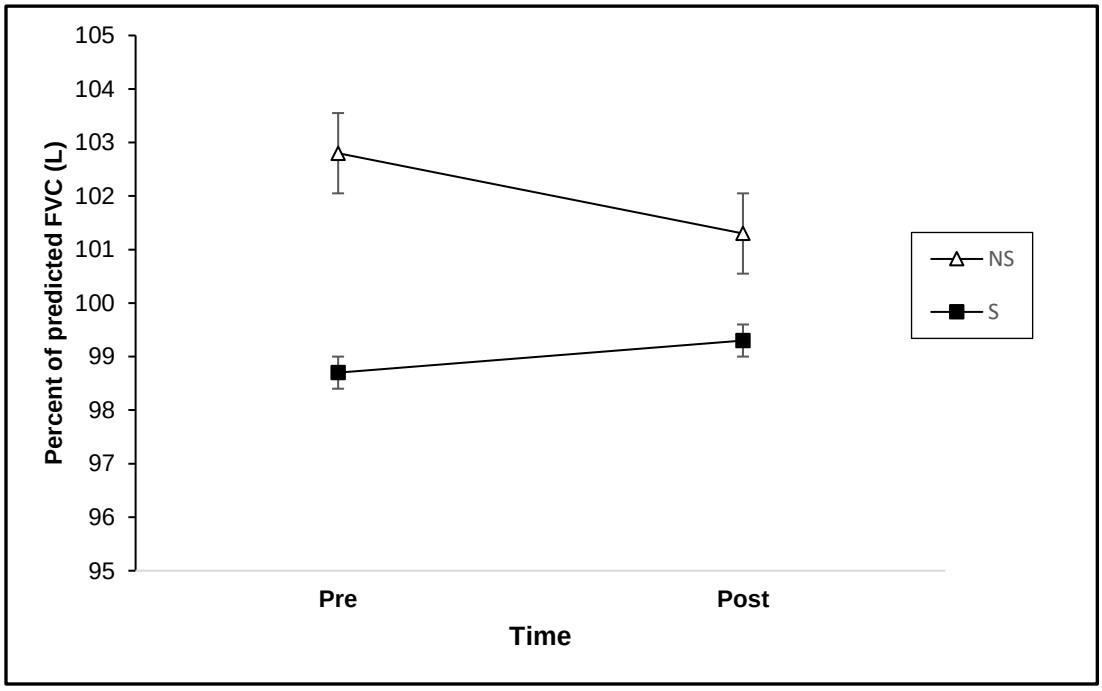

Figure 1b. Mean percent of predicted FVC (L) pre- and post-exercise in the NS and S groups (p >0.05).

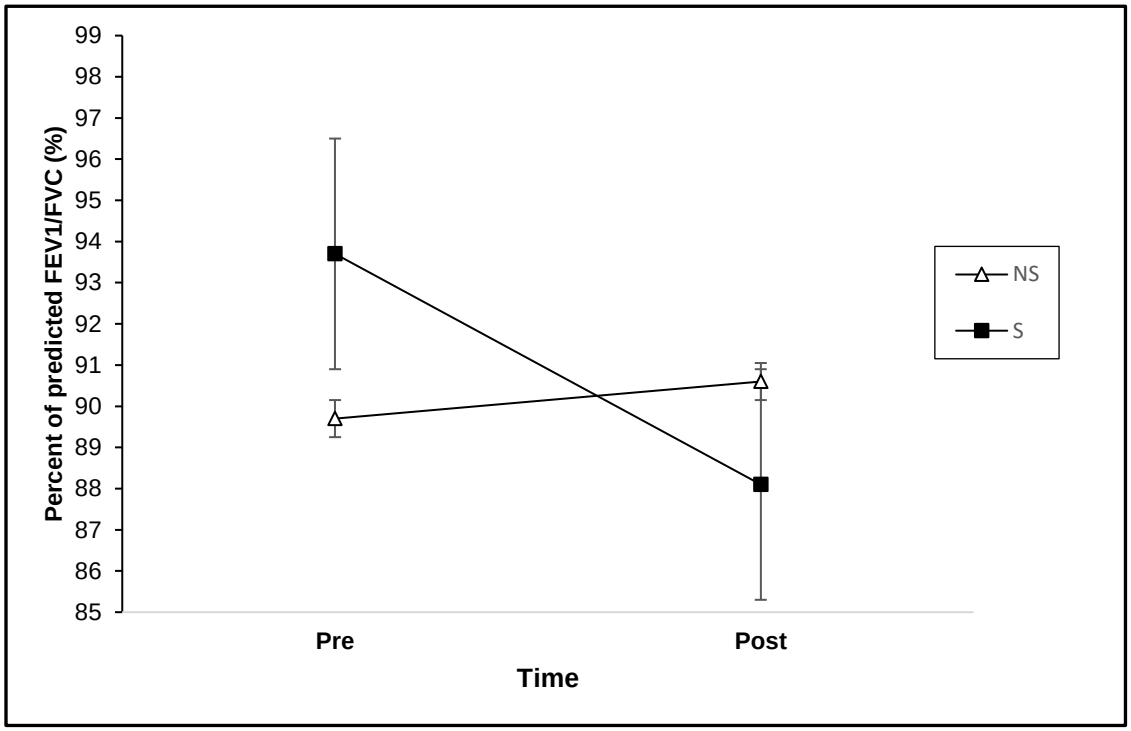

Figure 1c. Mean percent of predicted FEV_1/FVC (%) pre- and post-exercise in the NS and S groups ($p = 0.081$).

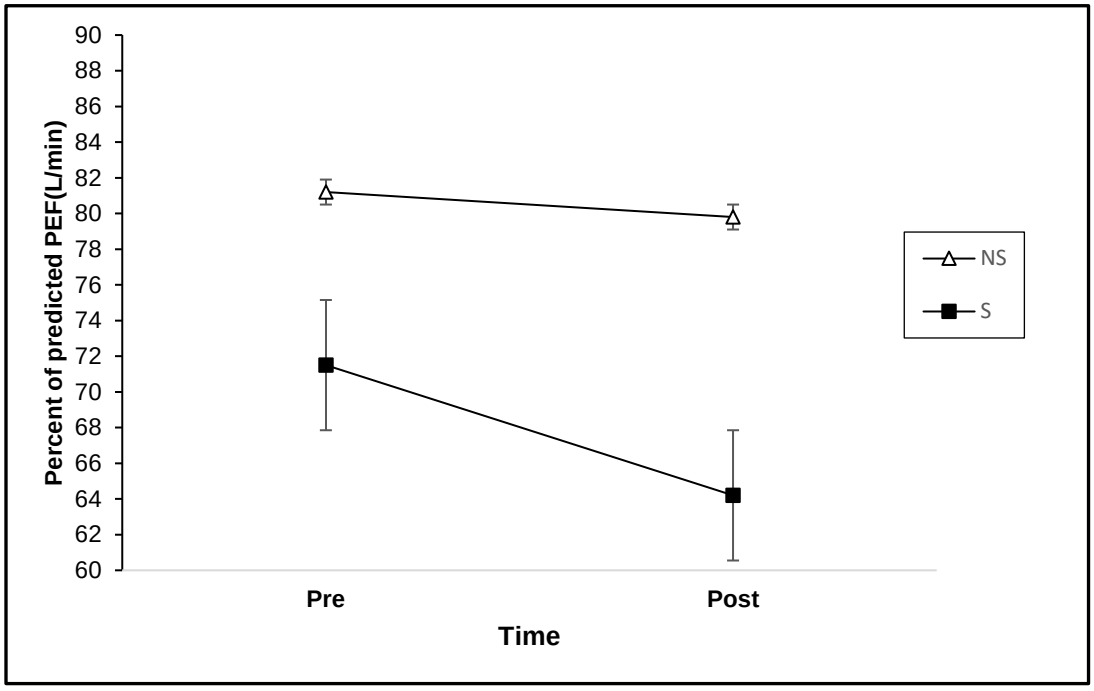

Figure 1d. Mean percent of predicted PEF (L/min) pre- and post-exercise in the NS and S groups ($p > 0.05$).

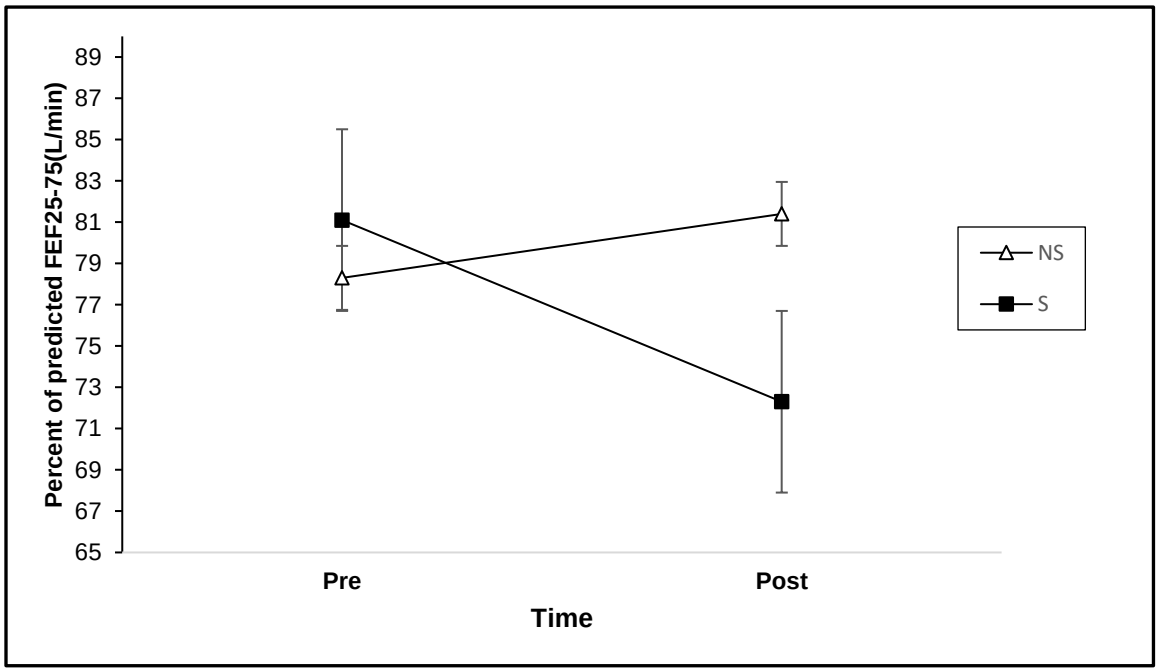

Figure 1e. Mean percent of predicted $FEF_{25-75\%}$ (L/min) pre- and post-exercise in the NS and S groups. (p = 0.082)

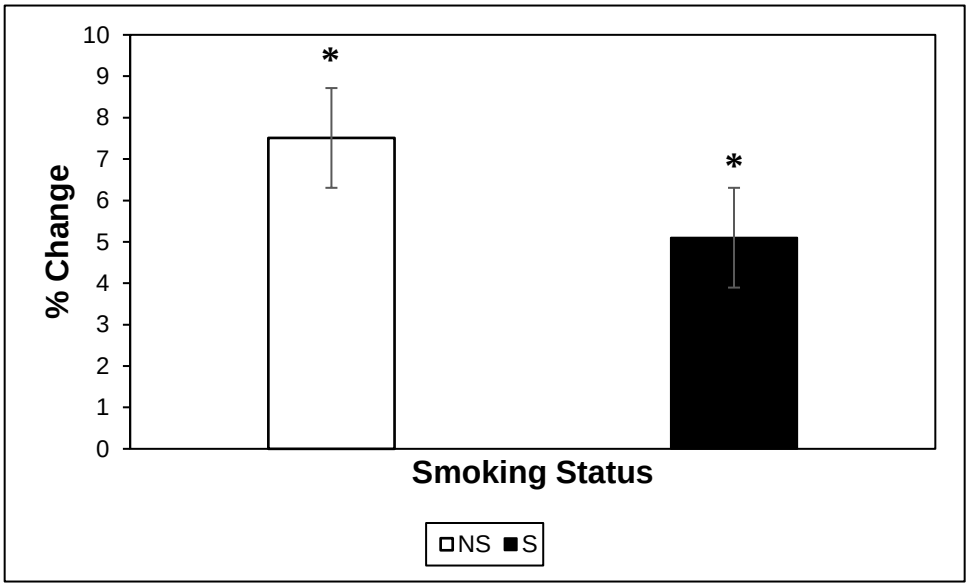

Figure 2. Percent change in eNO for the NS and S groups from pre- to post exercise. (*Significant difference from pre- to post-exercise, $p = 0.049$)

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